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Author(s) **Page**

Abbas Rashidi, Faezeh Fatemi, Samaneh Jahani 286
Abdolhossein Zare, Ramin Yaghobi, Mohammad Hossein Karimi, Afsoon Afshari, Ahmad Rashki, Hamid Reza Miri 201
Abdollah Derakhshandeh, Roya Firouzi, Mohammad Motamedifar, Sina Arabshahi, Aytak Novinrooz 110
Abed Atena, Ali-Reza Ahmadi, Naeimeh Najafi, Lamerad, Behzad 56
Abolfazl Jafari Sales, Haedeh Mobayyen 86
Aboozar Ramezani, Iraj Nikokar, Mohammad Esmaeilpour Bandboni, Behrad Rohi 290
Adel Spotin, Soheila Rouhani, Parviz Parvizi, Parnazsadat Ghaemmaghami, Ali Haghighi, Aref Amirkhani, Ali Bordbar, Amir Yaghoubinezhad 268
Afagh Mohammadi, Abbas Akhavan Sepahy, Reza Hoseinidoust 126
Afrouz Ali- yari, Ali Asghar Deldar, Neda Mokari Bonabi, Ehsan Vafadar Nejad, Ayman Bazzaz 290
Afsaneh Karmostaji, Shahin Najar Peerayeh 33
Afshari Asma, Abdollah jamshidi, Jamshid Razmyar, Ali Kargar 64
Aghil Sharifzadeh, Sepideh Abbaszadeh, Saeid Razzaghi 276
Ahmad Nasser 87
Aida Darvishi, Nima Bahador, Bahman Kholdebarin 126
Aidin Azizpour, Yashar Azizpour 292
Akbar Mohammadzadeh, Alka Hasani, Sajad Zamanlo, Ali Pormohammad 76
Akram Songol, Mandana Behbahani, Fariba Sharifi, Mohammad Hosseini sade 27
Akram Songol, Mandana Behbahani, Mohammad Hosseini sade 241
Ali Akbar Yousefi, Hengameh Zandi, Gilda Eslami 104
Ali Farjah, Parviz Owlia, Seyed Fazlollah Mousavi, Seyed Davar Siadat, Mehdi Shafiee Ardestani 261
Ali Ghorbani Ranjbary 108
Ali Ghorbani ranjbary 116
Ali Ghorbani Ranjbary, Nazanin Ghorbani Ranjbary, Zahra Ghorbani Ranjbary, Ali Bahmani 291
Ali Ghorbani Ranjbary, Zahra Ghorbani Ranjbary, Toba Piro, Fatemeh Juoibar 116
Ali Hashemi 98
Ali Heshmati, Aliasghar Vahidinia, Iraj Khodadai 170
Ali Heshmati, Iraj Khodadadi, Ali Asghar vahidinia 280
ALI JAVADI, Hamid Abdollahi, Mohamad Reza Zand Monfared 182
Ali Madhi, Arash Ghalyanchilangeroudi, Mohahammad Mehdi Soleimani, Seyed Masoud Hosseini 71
Ali Naseri, Abdolmajid Fata, Mohammad Naseri, Mahmoud Parian 275
Ali Pormohammad, Alka Hasani, Forough Shams, Mohammad Reza Nahaie, Akbar Hasani, Akbar Mohammadzade, Behnam Hashemi 91
Ali Raefi, Ebrahim Naghipoor, Ayatollah Nasrollahi Omran, Saba Amiri Kojori, Morteza Soleymanejhad 83
Ali raefi, Saba Amiri Kojori, Ebrahim Naghipoor, Rohallah Hosseini 211
Ali Reza Dehnad, Faramarz Ahmadi, Ali Farzi, Nader Hajizadeh, Najibeh Shirzad 247

Alireza Ahmadzadeh, Hossien Ghalenoi, Masoud Alebouyeh, Nastaran Farzi, Mahsa Molaei, Mohammad Reza Zali **187**
Alireza Japoni-Nejad, Farzam Vaziri, Ehsanollah Ghaznavi-rad **18**
Alireza Mozafarian, Mahnaz Mazaheri Assadi, Ali Abolhassani Soorki **141**
Alireza Sadrebazzaz, Ezzat Mosavinezhad, Majid Farhoodi **193**
Aliyar Pirouzi, Mohammad Mehdi Feizabadi, Mirzakhalil Bahmani, Rouhi Afkari **31**
Amir Eshaghi, Sara Barati **82**
Amir Mirzaie, Jalil Fallah Mehrabadi, Noor Amirmozaffari, Taher Nejadsatari **30**
Amir Moradi, Hamid Moghimi, Javad Hamedi **51**
Amir Peymani, Marzieh Marandi, Reza Najafipour **84**
Amir Peymani, Samaneh Mansouri, Reza Najafipour **85**
Amir Sasan Mozaffari Nejad, Masoud Sabouri **276**
Ania Ahani Azari, Taghi Zahraei Salehi, Bahar Nayeri Fasaei, Masoud Alebouyeh **238**
Aref Shavalipour, Pouya Amiri, Hamidreza Houri **157**
Arezoo Karamimoghadam, Zahra Emam-Djomeh **289**
Arezou Taherpour, Ali Hashemi, Soroor Erfanimanesh, Elahe Taki **298**
Arezzo Omati, Hamid lavakhamseh, Khaled Rahmani **212**
Ariyo Shahin Jafari, Mohammad Hasan Shahhosseiny, Mohammad Hasan Shohhosseiny, Mansour Bayat, Parviz Tajik, Shahla Roudbar Mohammadi **273**
Armin Farhang, Hengameh Zandi, Gilda Eslami, Dariush shokri **41**
Asghar Havaei, Mojtaba Shahin, Sharareh Moghim, Abbas Farahani, Fahimeh Ghanbari, Maryam Afzali **303**
Asiyeh Abdeyazdan, Yahya Tahamtan, M Namavari, M Hayati **127**
Asma Askari, Elham Mohammadi, Somaye Soukhtanloo, Reza Ghanbarpour **117**
Asma Askari, Reza Ghanbarpour **117**
Atefeh Jafarnodehi, Aytollah Nasrollahi Omran, Hamidreza Pordeli **147**
Atefeh Mousavi, Rashid Ramezanzadeh, Fariba Farhadifar, Reza Mirnejad, Omid Zarei, Arash Ganizadeh, Shahoo Menbari **63**
Azam Mokhtari **254**
Azin Baharvand, Pegah Shakib, Gholamreza Goudarzi, Leila Asadpour **99**
Azizeh Shadi Dizaji, Mohammadreza Nahaei, Hayedeh Mobayyen **252**
Babak Elyasifar, Nazila Arbabsolimani, Hatef Ajudanifar, Hamid Nooshiri, Yalda Shini ... **125**
Babak Pourakbari **182**
Babak Soleymani, Saba Amiri Kojouri, Ali Raefi **145**
Bahar, Zali Mohammad Reza **20**
Bahareh Ramin, Noor Amir Mozafari, Leile Asad Pour, Homa Foruhesh Tehrani **95**
Bahman Hormozi, Mahbobeh Barakzahi, Ahmad Rashki, Zahra Rashki Ghalehnoo **100**
Bahram Hasani, Jalal Shayegh, Ali Ameghi **114**
Banafshe Hasanvand, Mahsa Kashef, Amirhooshang Alvandi, Ramin Abiri **42**
Banafsheh Nadalian, Gholamhosein Ebrahimiour, Mohsen Shahriari Moghadam, Bahareh Nadalian **51**
Behin Omidi, Seyed Jamal Hashemi, Mansour Bayat, Kambiz Larijani **249**
Behnam Rafiee, Davoud sadeghi, Sepideh Ghani, Ali Rezaei **100**
Behnam Rafiee, Sepideh Ghani, Davoud Sadeghi, Nader Mosavari, Keyvan Tadayon, Ali Rezaei, Ali Asghar Farazi **269**
Behnam Rafiee, Sepideh Ghani, Davoud Sadeghi, Safieh Sofian, Ali Rezaei, Nader Mosavari **172**

Behrooz Taherijavan, Mhamad Hassan Shahhosseiny 255
Behzad Esfandiari, Hossein Nahravanian, Mohammad Reza Pourshafie, Pejvak Khaki, Ehsan Mostafavi, Mohammad Mehdi Gooya, Jamshid Darvish, Soheila Moradi Bidhendi, Mehdi Gharakhani 23
Bita Bijari, Ali Abassi, Keyvan Karabi 197
Bita Soltanian,Shiva Irani, Hashemi Sarvenaz, Cheraghi Yusef, Alireza Gholami 62
Dariyush Iranpour, Farhad Abbasi, Kamran Mirzayee, Nahid Mohammadzadeh, Alireza Raisi 61
Davood Bastani, Vahid Noaman, Masoud Soltani Alvar 310
Davoud Nikoo, Mohsen Manavian, Mohammad Javad Mehrabanpour, Ali Shirazinezhad, Mohammad Hoseyn Hoseyni 311
Delaram Amini1, Fatemeh Keshavarzi 178
Delsuz Rezaee, Mohammad Ahangar Zade Rezaee, Gholamreza Zarrini 297
Dr.Mohtaram Nasrolahei, Maryam Abdollahi, Zeynab Marzhoseini, Parisa Ebrahimzadeh 204
Ebrahim Hossein Abadi, Nahid Tavakoli 288
Ebrahimzade Azade, Zare Bidaki Majid, Saber Hoseini Seyede Naime 79
Ehsan Kazemi Moghaddam, Atieh Darbandi, Horieh Saderi, Mohammad Reza Jalali Nodoushan 232
Ehsan Zayerzadeh 75
Ehsaneh Shamsolahrari, Farzaneh Firoozeh, Rezvan Moniri 82
Elahe Imani, Saeedeh Saeedi, Shahla Sahraei, Arezoo Azizi, Hamideh Khaje 105
Elahe Mahdizade, Hassan Mohabatkar, Mandana Behbahani 225
Elahe Mahdizade, Hassan Mohabatkar, Mandana Behbahani, Naghmeh PoorinMohammad 235
Elahe Mahdizade, Hassan Mohabatkar, Mandana Behbahani, Naghmeh PoorinMohammad 237
Elahe Tajeddin, Masoud Alebouyeh, Nahid Mansouri, Mohammad Reza Zali 207
Elham Abdollahi, Shahram Hanifian 168
Elham Asl Soleimani, Siamak Gheibi 46
Elham Hassanzadeh, Iraj Rasooli, Ghasemali Mohebali 142
Elham Moazamian, Ahmad Rezazadeh, Nima Bahhador, Manoochehr Rasouli, Negar Azarpira 56
Elham Mohammadi, Ehsanollah Sakhaee, Mohammad Khalili 204
Elhamosadat Ghorbi, Seyed Nezamedin Hosseini, Alireza Harifi Mood 257
Elnaz Afshari, Zahra Amini-Bayat, Setareh Haghighat, Sanaz Jafari 246
Elnaz Azemat Eslamtalab 41
Elnaz Ohadi, Gita Eslami, Hossein Goudarzi, Arezou Taherpour, Soudabeh Taheri 213
Erefanii Soheila, Zare Bidaki Majid, Sadrinia Sajad 227
Faeze Moslemzade Tehrani, Rouha Kasra Kermanshahi, Mohammad Mehdi Feizabadi 224
Faezeh Haghighi, Masoumeh Douraghi, Fereshteh Shahcheraghi, Ebrahim Abbasi, Hossein Asgarian-Omran, Seyyed Saeed Eshraghi, Fazel Shokri 203
Faham Khamesipour, Meysam Khodadadi, Morteza Azhdary Mamoreh 166
Fahimeh Beigi, Majid Baserisalehi, Nima Bahador, Sina Mobasherzadeh, Hamid Najmi Nejad 80
Fahimeh Mohammadi, Daryush Arabian, Rasoul Khalilzadeh, Farzin Abaspour 137
Fahimeh Rahimi Dijvijin, Haedeh Mobaiyen, Mohammad Reza Nahaeii 307

Fahimeh sadat Ghoalm Mostafaei, Parisa Torabi, Masoud Alebouyeh, Mohammad Reza Zali 170
Fahimeh Tehranchi, Mohammad Hassan Shahhoseiny 273
Farhad Abbasi, Bahram Nasri Razin, Simin-Dokht Shoaei, Alireza Family, Mahmood Nabavi 272
Farhad Abbasi, Mansooreh Khalili-azad, Soroor Asadi, Davood Yadegarynia, Sharareh Gholamin, Latif Gachkar 35
Farhad Abbasi, Masoud Mardani, Davood Yadegarynia, Morteza Aghahasani 271
Farhad Abbasi, Mehdi Besharat, Jamile Kiyani 191
Farhad Abbasi, Parviz Vahdani 65
Fariba Asgharpour, Zahra Moulana, Mehrasa Rezaie, Yasaman Alizade..... 279
Fariba Azarbooyeh, Roghaye Gholizadeh Doran Mahaleh 59
Fariba Azarbooyeh,Roghaye Gholizadeh Doran Mahaleh 58
Fariba dastjani farahani, Shahla Mohammad Ganji, Mojtaba sohrabi, Soheil Aghaee, Violet Najafi Shiva..... 223
Fariba Sharifi Teshnizi, Mandana Behbahani, Akram Songol..... 246
Farideh Hayati, Jamshid Fouladi, Sara Gharavi..... 256
Farkhondeh Poursina, Jamshid Faghri, Hajieh Ghasemian Safaei..... 22
Farnosh Gholamhoseinpor, Mohammad Navaderi, Mehdi Mirzaei..... 237
Farshad Nojoomi, Ashraf Mohabbati Mobarez, Seyed Davar Siadat, Ali Hatef Salmanian, Nima Khoramabadi, Ramin Farhoudi..... 221
Farzad Khademi, Arshid Yousefi, Najmeh Ansari..... 177
Farzad Khademi, Arshid Yousefi..... 179
Farzaneh Fakharian, Soheil Aghaee, Mohammad Reza Zolfaghari, Mohammad Soleimani, Ali Javadi..... 301
Farzaneh Firoozeh, Atena Amiri, Rezvan Moniri, Mohammad Zibaei..... 83
Farzaneh Islam-Kish, Taher Mohammadian, Hossein Rassi, Roohollah Solymani..... 184
Farzaneh Islamkish, Taher Mohammadian, Hossein Rassi, Roohollah Solymani..... 185
Farzaneh Nazari, Mohsen Mirzaie, Hossien Fazeli 296
Fateme Mahmoodsaleh, Mohammad Roayaei..... 136
fateme Mahmoodsaleh, Mohammad Roayaei, Abdolkazem Neisi 136
Fatemeh Davoodi 208
Fatemeh Doregiraee, Alebouyeh Masoud, Nayyeri Bahar, Zali Mohammad Reza 159
Fatemeh Doregiraee, Alebouyeh Masoud, Nayyeri 20
Fatemeh Ebrahimi, Zahra Fatahi, Fatemeh Jabbari, Leila Aboali 282
Fatemeh Esmaeil Zadeh, Reza Shapoori..... 213
Fatemeh Esmaeil Zadeh, Reza Shapoori..... 214
Fatemeh Fallah, Maryam Noori, Ali Hashemi, Hossein Goudarzi, Abdollah Karimi, Soroor Erfanimanesh, Shadi Alimehr 40
Fatemeh Ghasemzadeh, Seyed Yahya Salehilisar, Gholamreza Zarrini 142
Fatemeh Honarbakhsh, Mohammad Ali Amoozegar, Shamsozoha Abolmaali, Maliheh Mehrshad 60
Fatemeh Hosseinmardi 154
Fatemeh Izadpanah Qeshmi, Sedigheh Javadpour, Kianoosh Malekzadeh, Mahsa Rahimzadeh, Saeid Tamadoni Jahromi 133
Fatemeh Mihani, Mohammad Yousef Alikhani..... 81
Fatemeh Sadat Zarkesh Isfahani, Bahram Nasr-Esfahani, Nima Bahador, Tooba Radaei .. 270

Fatemeh Saleh, Farnaz Kheirandish, Hossein Azizi, Mahmoud rezazad bari, Maryam Azizi 250
Fatemeh Saleh, Hossein Azizi, Farnaz Kheirandish, Maryam Azizi, Afshin Javanmard..... 215
Fatemeh Yarmohammadi, Mojtaba Mohseni, Mohammadjavad Chaichi..... 129
Fatemeh Zaboli, Ali Dabiri, Samaneh Rouhi..... 162
Fatemeh Zahra Darvishi, Mehran Miroliaei, Mahboobeh Nazarai3..... 263
Fereshteh Rahimipour, Somayeh Heidari, Fatemeh Roudbari, Kiarash Ghazvini, Masoud Youssefi 95
Foozieh Arabzadeh, Fatemeh Keshavarzi, Bahareh Rahimian Zarif..... 79
Foroozan Kavoosinezhad, Esmail Fattahi, Naghmeh Moori Bakhtiari..... 217
Forough Shams, Alka Hasani, Ali Pormohammad..... 99
Forough Yousefi, Seyed Fazlolallah Musavi, Seyed Davar Siadat, Abbas ali Imani Fooladi, Jafar Amani, Mohammad Mehdi Aslani, Alireza Azizi Saraji 43
Ghannad..... 276
Ghasem Rahimi, Masoud Negahdari, Marzieh Ajdary, Ali Roostaei..... 289
Ghazal yazdanpanad, Abolfazl Golshan Tafti, Mohammad Hassan Fooladi..... 149
Ghazale Entezari, Seifkordi Aliakbar, Otadi Maryam 129
Gholamreza Goudarzi, Ali Karami Drikvand 113
Gholamreza Herfehdoost, Zahra Bourbour, Maryam Alemaref, Asgar Emamgholi, Minoo Sadri 230
Gohartaj Rassouli, Masoud Alebouyeh, Elahe Tajeddin2, Bahram Amini, Mohammad Reza Zali..... 163
Hadis Fathizadeh, Mehdi Golchin, Hadi Tavakkoli, Maryam Maazallahi..... 243
Hadis Solyemane, Zahra Eslamirad, Mansour Bayat 299
HajarAghili Dehnavi, Mahnaz Nikaeen, Akbar Hssanzadeh, Mohammd Jalali..... 123
Hamdollah Moshtaghi, Somayeh Yaghoubi, Azade Shefaee..... 161
Hamed Moradi, Raza Ranjbar, Naser Harzandi, Roohollah Kheiri 90
Hamed Moradi, Raza Ranjbar, Naser Harzandi, Roholla Kheyri..... 91
Hamid Badali 77
Hamid Kassiri, Anvar Quaderi 190
Hamid Kassiri, Ezatalldin Javadian 194
Hamid Kassiri, Helia-Sadat Mortazavi, Esmail..... 192
Hamideh Babaei, Masoomeh Shams Ghahfarokhi, Mahboobeh Madani, Razzaghi Abyaneh 278
Hamideh Vaezi, Haiedeh Mobaiyen, Mehdi Ghiyamirad 94
Hamidreza Kazemeini, Ebrahim Rahimi, Fariborz Kianpour 174
Hanar Narenji, Himen Salimizand, Rashid Ramazanzadeh, Babak Shahbazi, Masomeh Khonshah 265
Hanieh Mortazavi, Mahboobeh Nakhaei Moghaddam, Khadijeh Nejad Shahrokh Abadi... 303
Hassan Rokni Zadeh..... 25
Helia Ostad Asadollah Malayeri, Mojdeh Hakemi Vala, Kambiz Davari..... 89
Hesam Alizade, Reza Ghanbarpour, Mojdeh Nekoubin 109
Hesamaddin Shirzad Aski, Mohammad Tabatabaei, Hossein shayegh, Rahem Khoshbakht184
Heshmat Shahi, Marzie Sadeghiani, Majid Mahsa, Somaye Reiisi, Nader bagheri, Sara Shahi, Mohamad sadegh Damavandi, Hedayatollah Shirzad 180
Heshmat Shahi, Marzie Sadeghiani, Rasol Bahreini, Majid Mahsa, Somaye reisi, Gorbanali rahimian, hedayatollah Shirzad 180

Hoda Nazarlotfi, Maryam ghobadidana 57
Homa Farahani, Mohammad Ali Amoozegar, Basaer Asadi, Maliheh Mehrshad, Bagher Yakhchali, Shahrzad Motamedi 147
Homa Forouhesh Tehrani, Maryam Barkhordari, Zahra Safari, Hooman Voosough 199
Hooman Hanifehpour, Vafa Saber 193
Horieh Saderi, Batoul Basatiyan, Shahram Boroumandi,Roxana Sahebnasagh 295
Horieh Saderi, Seyedeh Marzieh Moosavi, Shahram Boroumandi, Roxana Sahebnasagh 73
Hossein Azizi, Mohsen mirzaei, Farnaz KheIrandish 92
Hossein Dabiri, Mahdane Roshani, Neda Yousefi, Mohsen Heidary, Saeed Khoshnood 175
Hossein Dabiri, Neda Yousefi, Mohsen HeidarySaeed khoshnood, Mahdaneh Roshani 188
Hossein Dabiri, SAEED KHOSHNOOD, Mohsen Heidary, Neda Yousefi, Mahdane Roshani 57
Hossein Esmaeili, Afrooz Azarnush 196
Hossein Fazeli, Hooman Sadighian, Bahram Nasr Esfahani, Mohammad Reza Pourmand . 296
Hossein Goudarzi, Shadi Aghamohammad, Ali Hashemi 86
Hossein Khanahmad, Faezeh Sabzehei, Shirin Kohpayeh, SADEGH FEIZOLLAHZADEH, ZAHRA HEJAZI, ILNAZ RAHIMMANESH 252
Hossein Rassi, Behnam Nazari 249
Hossein Rassi, Behnam Nazari 259
Hossein Saghi, Davoud Esmaeili, Abbas Bahador, Azad khaledi, Ferdoes Amiri Dastjerdi ... 36
Hossien Fazeli, Azadeh Taraghian, Farkhondeh Poursina, Razie Kamali 39
Hussein Ali Abdi, Ahmad rashki, Zahra rashki Ghalhnoo 310
Hussein Ali Abdi, Ahmad rashki, Zahra Rashki Ghalhnoo 114
Iradj Ashrafi Tamaei, Aghil Sharifzadeh, Alireza Khosravi 275
Isar Dejban Golpasha, Parviz Owlia, Seyed. Fazlollah Mousavi, Seyed Davar Siadat, Shiva Irani 294
Issa Gholampour Azizi, Moulod Gorgi, Samaneh Rouhi, Mahsa Azimi, Pegah Shakib, Babak Shahbazi, Noushin Abdolmaleki 241
Jafar Panahi, Mohamad Reza Havasian, Iraj Pakzad 36
Jaydari Amin, Khademi peyman, Lorestani Samira, Maleki Shahram 171
Jaydari Amin, Seyfi Abad Shapouri MR 72
Kamal Aramoon, Ania Ahani Azari 128
Kasra Sadeghi, Sina Mirza Ahmadi 291
Keivan Kamali Yazdi, Masumeh Anvari 50
Keyvan Shafienia, Mahjoob Liaghati, Saeid Shirgir Abibeygloo, Ali Mojtahedi, Daryush Asgarpoor 102
Khadijeh Eskandari, Manizheh Ramazani, Mehdi Fasihi, Mehdi Kamali, Fariba Dashtestani 68
Kobra Heidarzadi, Farid Azizi Jalilian, Ali Reza Rekabi, Razieh Amini, Ali Khorshidi, Zahra Ghobadian, Maryam Bouchani, Ali Hematian, Morovat Taherikalani 229
Kobra Salimian Rizi, Shahin Najar Peerayeh, Bita Bakhshi, Mohammad Rahbar 211
Kolsom Asadpour Rahimabadi, Gholamreza Hashemi Tabar, Ali Mojtahedi 112
Ladan Baftechi, Neda Soltani, Fateme Nazari, Mehdi Bolfion 144
Laleh Roomiani, Mehri Roomiani 32
Leila Aliasgharzade Rumiani, Hasan Jalili, Morteza Pahlavan Yali 262
Leila Asadpour 242
Leila Asadpour, Sima Abdollahi, Najmeh Ranji 304

Leila Najafzadeh, Mojtaba Noofeli, Maedeh Samianifard, Ali Nazari Shirvan 44
Leila Shabanimashcool, Gholamreza Zarrini, Iraj Ahadzadeh, Mirghasem Hosseini 138
Leili Akhtari, Abbas Akhavan Sepahy, Kambiz Davari 69
Leili Akhtari, Roohollah Kheiri 268
Lida tavakoli, Fatemeh Keshavarzi, Bahareh Rahimian Zarif 80
Maedeh Shahsanaei, Ali Mohammad Ahadi, Monir Doudi 132
Mahboobeh Nakhaei Moghaddam 19
Mahboubeh Soleimani Sasani 130
Mahdi Askari Badouei, Arash Najafifar 112
Mahdi KhorshidTalab, Mohammad Hassan Shahhosseiny, Fatemeh Keshavarzi 195
Mahdi Marzi, Ali Bahadori, Taylan Bozok, Tulin Gokmen, Begum Kayar, Fatih koksal 93
Mahdi Marzi, Begum Kayar, Taylan Bozok, Gulfer 29
Mahdi Mostafa, Seyed Davar Siadat, Fereshteh Shahcheraghi, Jalil Vand Yousefi, Sare Sadat Hosseini 85
Mahdis zolfaghar, Mohammad Ali Amoozegar, Khosro Khajeh 54
Mahnaz Fatahinia, Marzie Halvaeezadeh 277
Mahsa Rahimzadeh 44
Mahsa Rahimzadeh, Manijeh Poodat, Sadigheh Javadpoor, Fatemeh Izadpanah Qeshmi .. 224
Mahsa Sadri, Nazila Arbabsoleimani, Mahammad Mahdi Forghanifard 227
Mahsa Shams, Bahar Shahnavaz, Kiarash Ghazvini, Toraj Valinasab 46
Majid Alipour Eskandani 59
Majid Alipour, Reza Esmaieli, Maryam Taleb Jannat 119
Majid Zare Bidaki, Mina Arab, Mohtarame Khazaei, Ehsan Afkar 279
Majid Zare Bidaki1, Mohtarame Khazaei, Mina Arab, Ehsan Afkar 280
Mandana Sabour, Mojdeh Hakemi Vala, Helia Ostad Asadollah Malayeri 38
Mandana Sabour, Mojdeh Hakemi Vala, Helia Ostad asadollah malayeri 38
Mansour Amin, Maryam Bieranvand, Abdolrazag Hashemi Shahraki 37
Marjan Estakhr, Negar Azarpira, Elham Moazamian 301
Maryam Afzali, Mojtaba Shahin, Abbas Farahani 176
Maryam Asghari, Roohollah Kheiri 135
Maryam Asghari, Abbas Akhavan Sepahi, Seid Reza Husseini Doost 134
Maryam Bideh, Gholamreza Irajian, Ali Mojtahedi, Nakisa Hooman 218
Maryam Chenari, Mehdy Norouzi, Leila Ghalichi, Abdolrahim Rezaee, Hooshang Rafatpanah, Atefe Yari, Seyed Mohammad Jazayeri1 31
Maryam Ebrahimi, Zahra Moosavi-Nejad 60
Maryam Ekhtelat, Karim Ravaji, Masood Parvari 167
Maryam Farhadi 153
Maryam Golshani, Sima Rafati, Amir Dashti, Saeid Bouzari 26
Maryam Hadian, Mahboobeh Madani 284
Maryam Hadian, Maryam Hadian 285
Maryam Hajihosseiny1, Reza Mirnejad 102
Maryam Hosein pour, Fereshteh Shahcheraghi, Vajiheh Sadat Nikbin, Nazanin Jannesar Ahmadi 25
Maryam Jabbarpour Sattar, Jalal Shayegh 169
Maryam Karimi, Mehdi Hasanshahian 134
Maryam Kaszadeh, Mojgan Bandehpour, Farzaneh Hosseini, Zeinab Soleimanifar, Bahram Kazemi 245

Maryam Makhmalzadeh, Reza Ranjbar, Naser Harzandi, Roohollah Kheiri 309
Maryam Monadi, Mohammad Roayaei 144
Maryam Mortazavi, Behnaz Saffar, Neda Mirakhorli, Mohsen Mobini Dehkordi 258
Maryam Najjari, Zahra Moosavi-Nejad, Ezat Asgaran 61
Maryam Naserinejad, Fereshteh Eftekhar 294
Maryam Nejabat, Abdolah Bazargani, Saeid Amirizadeh 181
Maryam Partovi, Tayebe Bagheri Lotfabad, Azam Haddadi 52
Maryam Poormontaseri, Saeid Hoseinzadeh, Mohammad Vahid Sadeghi Sarvestani 65
Maryam Poormontaseri, Saeid Hosseinzadeh, Atefeh Nasri, Elaheh Sabzevari, Elmira Khatamsaz, Seyed Shahram Shekarforoush 205
Maryam Roohi, Mojgan Bandehpour, Behzad Lamerad, Ameneh Koochaki, Bahram Kazemi 259
Maryam Sadat Soltani, Pejvak Khaki, Soheila Moradi Bidhendi, Mohamad Hassan Shahhosseiny 209
Maryam sadat Soltani, Pejvak Khaki, Soheila Moradi bidhendi, Sama Rezasoltani 257
Maryam Shirzadi, Farzad Mohammadi, Arman Rostamzad, Sohaila Soltanbigi 183
Maryam Yousefi, Nima Bahador, Mahbobeh Cheraghi 124
Marzieh Sadeghiani, Heshmat Shahi, Somayeh Reiisi, Nader Bagheri, Ghorbanali Rahimian, Reza Rashidi, Mohammad Hadi Shafigh, Ghasem Ramezani, Hedayatollah Shirzad 181
Marzieh Sadeghiani, Heshmat Shahi, Somayeh Reiisi, Nader Bagheri, Mohammad Hadi Shafigh, Majid Mahsa, Elahe Shahverdi, Fereshteh Fatollahi, Hedayatollah Shirzad 183
Marzieh Sameni, Mazaher Gholipourmalekabadi, Mojgan Bandehpour, Farzin Sahebjam, Mehrdad Hashemi, Bahram Kazemi 253
Marzieh Shahvarani, Mahnoosh Parsaeimehr, Marzieh Bolandi 164
Masoomeh Parvizi, Mohsen Arzanlou 215
Masoud Dadashi, Gita Eslami, Faezeh Ranjbar, Hossein Goudarzi, Fatemeh Fallah, Soudabeh Taheri, Majid Ayatollahi 308
Masoud Dadashi, Gita Eslami, Hossein Goudarzi, Fatemeh Fallah, Soudabeh Taheri, Ali Hashemi 226
Masoud Dadashi, Gita Eslami, Hossein Goudarzi, Fatemeh Fallah, Ali Hashemi, Soudabeh Taheri, Zohreh Ghlavand 298
Masoud Dadashi, Gita Eslami, Mina Amjadi Kashani, Hossein Goudarzi, Fatemeh Fallah, Soudabeh Taheri 201
Masoud Dadashi, Gita Eslami, Zohreh Ghalavand, Arezou Taherpour, Hossein Goudarzi, Fatemeh Fallah, Zahra Zahirnia 62
Masoud Hamidi, Hossein Nazemyieh, Mohammad Amin Hejazi, Mohammad Saeid Hejazi 242
Masoud Mobini, Maryam Asghar Heydari, Mohammad Salehi 47
Masoud Rahdar, Ahamad Rashky, Hamid Reza Miri 111
Masoud Rahdar, Ahamad Rashky, Hamid Reza Miri 115
Masoude Alebouyeh, Leila Ganji, Abbas Yadegar, Mohammad Hassan Shirazi, Parisa Islami, Mohammad Rahbar, Naser Ebrahimi Daryani, Mohammad Reza Zali 187
Masoume Hadavand, Hojat Es-haghi 76
Masoumeh Aslani Mehr, Davood Darzi Ramandi, Amir Peymani, Taghi Naser Pour-Farivar 118
Masoumeh Aslanimehr, Farnaz Yosefi, Amir Peymani, Taghi Naserpour Fariver 118

Masoumeh Azimirad, Fahimeh Sadat GholamMostafaei, Masoud Alebouyeh, Mohammad Reza Zali..... 66
Masoumeh Khonsha, Rashid Ramazanzadeh, Mohamad Rastegar Khosravi, Hanar Narenji 121
Masoumeh Navidinia, Fatemeh Fallah, Shadi Alimehr, Behnaz Lajevardi, Ashraf Karimi Rahjerdi, Mehdi Shirdoost 305
Masoumeh Navidinia, Shahin Najar Peerayeh, Fatemeh Fallah, Bita Bakhshi 107
Masoumeh Navidinia, Shahin Najar Peerayeh, Fatemeh Fallah 107
Masoumeh Navidinia, Shahin Najar Peerayeh, Fatemeh Fallah 108
Masoumeh Shahbazi, Nasim Kashef 295
Masume Aslanimehr, Amir Peymani, Rezvan Yaghoobfar, Mahnaz Tavakoli 265
Masumeh. Ranjbar, Kavos Solhjoo, MohammadKargar, Raze Parastan, Samaneh Mojahedi Jahromi, Fatemeh. Darughe 222
Mehdi Zolfaghari, Masoud Rezaei,, Ashraf Mohebbati Mobarez, Mehdi Forozandeh Moghadam, Hedayat Hoseyni..... 239
Mehrdad Gholami, Mojdeh Hakemi Vala, Ali Hashemi, Mona Moshiri, Hossein Goudarzi 233
Mehrdad Shamsaddini Bafti, Ezzatkhah, M., Rashidi, H., Amini, M., Mollaei, M., Talebhemmat, M. 66
Mehri Habibi, Mohammad Reza Asadi Karam, Mana Oloomi, Anis Jafari, Saeid Bouzari. 260
Mina Hamidpour, Javad Hamedi, Reyhaneh Papiran, Hamid Moghimi 243
Mina Hassani, Akram Sharifi, Bahram Hassani 166
Mina Jeddi, Gholamreza Zarrini, Farzam Sheikhzadeh..... 164
Mina Jeddi, Gholamreza Zarrini, Seyyed Mehdi Banan Khojasteh..... 163
Mina Mehrara, Nahid Rahimifard, Mojdeh Hakemi-Vala, Jinus Asgarpanah, Mohsen Heidary, Saeed Khoshnood..... 284
Mina Nasiri, Shahram Hanifian 120
Mina Noormohammad, Mehri Khatami, Hosein Tabatabaeian, Kamran Ghaedi, Ardeshir Talebi, ... 23
Miresmael Moosavi, Peymani Amir, Moosavi Raziyeh 74
Mitra Kimiaee Sadr, Roghaye Dehestani, Mahbobeh Mirhosseini..... 110
Moein Aliakbari, Farida Behzadian, Ali Asghar Deldar, Baharak Mahyad, Maryam Bidram, Maedeh Bahreinian..... 263
Moein Hamidi hesari, Jafar Hemmat, Norkhoda Sadegifard 96
Mohaddese Mahboubi, Nastaran Kazempour, Mohammad Hamed Fallah..... 42
Mohadeseh Fadaee, Nasim Kashef..... 219
Mohamad Hassanvand, Gholamreza Godarzi..... 264
Mohamad Mahdi Karimi Yazdi, Sadegh Lotfala Moradi, Parvaneh Khiabani Rad, Mozhgan Esmaeili Benvidi, Gita Eslami, Arezou Taherpour, Soudabeh Taheri 189
Mohamad Mehdi Namvaran, Valiollah Babaeipour, Hossein Vahidi, Firouz Ebrahimi, Fatemeh Abarghooyi..... 28
Mohamad Mehdi Soltan Dallal, Abolfazl Davoodabadi, Rounak Bakhtiari, Zahra Rajabi .. 203
Mohamad Reza Havasian, Jafar Panahi, Iraj Pakzad 272
Mohamad Reza Havasian, Mohamad Ali Ruzegar, Jafar Panahi 78
Mohamadreza Feili, Jafar Panahi, Mohamad Reza Havasian..... 277
Mohammad Ahanjan, Ali Purmohammad, Saeed Ghorbani, Behnam Hashemi..... 104
Mohammad Ahanjan, Fatemeh Abdollahi, Zeynab Esmailpour 105

Mohammad Ahanjan, Mohammad Reza Jabari Amiri, Maryam Abdollahi, Behnam Hashemi 188
Mohammad Amin Abedi, Ghilamhossein Ebrahimipour, Abdolrazagh Marzban 251
Mohammad Faezi Ghasemi, Samaneh Kazemi 123
Mohammad Hajjartabar, Tahereh Kermani Ranjbar 233
Mohammad Hassan Moshafi, Mehdi Rezaeifar, Mehdi Ansari, Forouzan Zare 231
Mohammad Hazhir Alaei, Seyed Hamed Shirazi Beheshtiha 195
Mohammad Khezri, Seyed Ali Pourbakhsh, Abbass Ashtari, Babak Rokhzad 194
Mohammad Latifpour, Abolfazl Gholipour, Mohammad Sadegh Damavandi, Safie Abasi, Pari Kiyani 96
Mohammad Mehdi Attarpour Yazdi 109
Mohammad Mehdi Attarpour Yazdi 197
Mohammad Mehdi Heidari 23
Mohammad Mehdi Pakniat, Mohammad Jafari, Ali Pirouzi, Rouhi Afkari 87
Mohammad Motamedifar, Khoosro Zamani, Yashgin Hassanzadeh, Sajede Pashoutan 214
Mohammad Niakan, Elham esmaeli, Sarah Niakan 232
Mohammad Niakan, Jalayernaderi Nooshin, Jamshidian Hadiseh 220
Mohammad Reza Allahyar Torkaman, Vajihe Sadat Nikbin, Masoumeh Nakhost Lotfi, Fereshteh Shahcheraghi 71
Mohammad Reza Asadi Karam, Mehri Habibi, Saeid Bouzari 24
Mohammad Reza Haeri, Kenneth White, Mohammad Qarebegloo 206
Mohammad Salehi, Masoud Mobini, Maryam Asghar Heydari 266
Mohammad Yousef Alikhani, Mohammad Reza Arabestani, Iraj Sedihghi, Hassan Mahmoudi, Hamed Farhadi Kohan, Mohammad Molavi 127
Mohammad Yousef Memar, Maryam Hajizadeh, Zahra Tabatabaei, Naser Alizadeh 228
Mohammad Yousef Memar, Mohammad Taghi Akhi, Naser Alizadeh, Vida Sorayaei Sowmesarayi, Abed Zahedi Bialvaee 175
Mohammadjavad Eivani, Sajjad Abdi Nowroozani, Bahare Sarmadi, Maryam Hassani Jafari 155
Mohammadreza Mahzounieh, Azam Mokhtari 192
Mohammadsadegh Damavandi, Abolfazl Gholipour, Safiyeh Abbasi, Heshmat Shahi, Mohammad Latifpour 84
Mohmmad Bokaeian, Shahram Shahraki-Zahedani, Morteza Soltanian-BajgIran 88
Mohsen Fathi Najafi, Mohsen Mehrvarz, Behjat Majidi. 262
Mohsen Tabasi, Mohammad Reza Asadi Karam, Mehri Habibi, Saeid Bouzari 101
Mohsen Tabasi, Mohammad Reza Asadi Karam, Mehri Habibi, Saeid Bouzari 236
Mohsen Tabasi, Mohammad Reza Asadi Karam 293
Mojdeh Hakemi-Vala, SAEED KHOSHNOOD, Mojgan por ramezan, mohammad hossein salehie, Mohsen heidary 285
Mojtaba Asadollahi, Ákos P. Molnár, Erzsébet Fekete, Levente Karaffa, Michel Flipphi, Erzsébet Sandor 309
Mojtaba Khedmatgozar, Ehsan Afshari Safavi, Mohsen Solgi 77
Mojtaba Mashhadi Mohammadzadeh Vazifeh, Seyad Masuad Hosseini, Javad Fakhari 153
Mojtaba Mashhadi Mohammadzadeh Vazifeh, Seyad Masuad Hosseini, Javad Fakhar 154
Mojtaba Raeisi, Mojtaba Raeisi 283
Mona Basmechi, Saeed Mirdamadi, Farzaneh Azizmohseni, Fatemeh Bagheri 258
Mona Basmechi, Saied Mirdamady, Fatemeh Bagheri, Farzaneh Azizmohseni 251

Mona Davarpanah, Shahram Hanifian 160
Mona Pakdel, Parisa Mohammadi, Mahbobeh Zarrabi, Asgarani Ezat 254
Mona Rahmani, Mirsaed Mirinargesi, Masood GHane 225
Monadi Maryam, Roayaei Mohammad, Najaf Zadeh Hossein 138
Monire Bana, Zoheir Heshmatipour 143
Morteza Khani, Farshid Kafilzade 125
Morteza Teyhoo, Shila Seyyedi, Khosro Issazadeh 121
Moslem Neyriz Nagadehi, Farzad Gaderi 157
Moslem Neyriz Nagadehi, Kaveh Rahmanpour 165
Moslem Neyriz Nagadehi, Shahram Etemad 156
Moulood Barzavar, Nahid Rahimifard 21
Moulood Barzavar, Nahid Rahimifard 98
Mozhgan Ghasemian dastjerdi, Hojat Ahmadi, Mehdi Nejati, Vajihe Karbasizade 219
Mushtaq talib shawi Al Rubaye, Maryam Hosseini, Javad Fakhari, Mastafa Heilo Jabber Al-Musawi 58
Nader Mohammadzadeh Gheshlaghi, Simin Norouzi Fard 22
Nader Motaali, Hossein Rassi, Hamid Reza Honarmand 220
Naghmeh Moori Bakhtiari, Saad Gooraninejad, Mehdi Zareid, Maryam Farahmandfar ... 169
Najibeh Shirzad, Ali Farzi, Ali Reza Dehnad, Nader Hajizadeh 43
Najmeh Soveizi, Abbas Akhavan sepahi, Parviz Pakzad 18
Narges Bodaghabadi, Majid Sadeghizadeh, Farhood Najafi 191
Narjes Ramezany pour, Arastoo Badoei-Dalfard, Abdolhamide Namaki-Shoushtari, Zahra Karami 146
Naser Alizadeh, Mohammad Taghi Akhi, Reza Gottaslou, Mohammad Yousef Memar, Abed Zahedi Bialbaei 223
Naser Nazari, Houshang Jamali, Ali Mikaeili 196
Nashmin Fayazi Hosseini, Jamshid Fooladi, Khadije Kiarostami 49
Nasim Afshari abarghan, BitaBakhshi 176
Nasim Karimipour, Dr Asghar Tanomand 39
Nasim Maghbooli, Parisa Mohammadi 124
Nasim Safaei, Gholamhossein Ebrahimipour, Mohsen Shahriari Moghadam 48
Nasin Kheshti, Roghieh Ashrafi 168
Nasrin Nassiri Kopaii 50
Nasrin Nassiri Kopaii 226
Nasrin Sheykhi, Shahin Pourazar Diziji, Ensiyeh Ketabchi, Mehdi Shekarabi 267
Nasrollah Vahedi Nouri 198
Nasrollah Vahedi Nouri 198
Nastaran Ashoori, Fereshteh Eftekhar, Morteza 29
Nastaran Farzi, Masoud Alebouyeh, Mohammad Reza Zali 185
Neda Amidi Fazli, Shahram Hanifian 120
Neda Mokari Bonabi, Ali Asghar Deldar, Ehsan Vafadar Nejad, Afruz Ali-Yari,, Baharak Mahyad 240
Neda Mokari Bonabi, Ali Asghar Deldar, Reza Khalili Darmani, Abbas Karimi Fard 145
Neda Nazarzadeh Zare, Mansour Amin 216
Neda Samei, Parviz Parvizi, Adel Spotin, Mohammad Reza Khatami Nezhad, Narmin Najafzadeh, Amir Yaghoubinezhad 287
Neda Soleimani, Ashraf Mohabati Mobarez 234

Neshat Etedali, Monir Doudi, Ali Mohammad Ahadi 140
Nikta Sajjadi, Reza Mirnejad 103
Noeman Ardalan, Rashid Ramazanzadeh 88
Nooshin Changiz, Abbas Akhavan Sepahy, Kambiz Tahvildary 53
Nooshinkaramatvash, Mahmod Rezazade Bari 302
Nooshinkaramatvash, Morteza Abasi 300
Pantea Mohammadi, Ali Mostafaie, Saboor Afzali, Ahmad Tajehmiri, Mariam Chalabi 179
Paria Motahari, Saeed Mirdamadi 245
Parichehr Pezeshknejad, Reza Ranjbar, Kumars Amini, Roohollah Kheiri, Fatemeh Pourali 267
Parinaz Lordifard, Seyyed Mansour Seyyednejhad, Hosein Motamedi, Seyed Soheil Rahmatabadi, Mostafa Amopour Bahnamiry 281
Parinaz Lordifard, Seyyed Mansour Seyyednejhad, Hosein Motamedi, Seyed Soheil Rahmatabadi, Fatemeh Ghazbanzadeh, Mostafa Amopour Bahnamiry 282
Parisa Badiee, Hadis Jafarian 274
Parisa Badiee, Hadis Jafarian 274
Parisa sadeghi, Azar Dokht Khosravi 35
Parnazsadat Ghaemmaghami, Parviz Parvizi, Adel Spotin 287
Parvaneh Khiabani Rad, Gita Eslami 122
Parviz Mohajeri, Abbas Farahani 34
Parviz Mohajeri, Abbas Farahani 34
Parviz Mohajeri, Abbas Farahani, Gita Darfarin, Mojtaba Shahin, Maryam Afzali 106
Parviz Mohajeri, Baharak Norozi, Abbas Farahani 269
Parviz Mohajeri, Hadis Sadri, Abbas Farahani 28
Parviz Mohajeri, Hadis Sadri, Baharak Norozi 78
Parviz Mohajeri, Hosna Khademi, Abbas Farahani, Roya Ebrahimi 106
Parviz Mohajeri, Samira Azizkhani, Abbas Farahani 30
Parviz Mohajeri, Zhaleh Rezaei, Abbas Farahani, Saba Sharbati 33
Pegah Hashemi Kani, Samira Salmanpor, Hossein Rassi 205
Pegah Yazdanpanah Abdolmalaki 45
Peyman Khademi, Mohammadreza Mahzounieh, Mahmoud Esmaeili Koutamehr 292
Pouya Nezhadshamsi, Kambiz Forghan-parast, Zahra Sahranavard 32
Raheleh Karimiravesh, Gita Eslami, Sudabeh Taheri 210
Raheleh Sheikhi, Mansour Amin 72
Rahimeh Sanikhani, Amir Peymani, Mohamad Moeinirad 101
Ramazan Rajabnia, Aynaz Khademian 236
Ramazan Rajabnia, Maryam Salehi 189
Ramin Rostami-Taghi Dizaj, Nasrin Mollania, Farangis Gharib-Bibalan 256
Rashaid Ramazanzadeh, Babak Shahbazi, Samaneh Rouhi, Abbas Manafi 230
Rashid Ramazanzadeh, Hanar Narenji, Samaneh Rouhi, Babak Shahbazi, Pegah Shakib, MohammadHossein Darehshiri, Amin Hoshyar 234
Rashid Ramazanzadeh, Mehran Ahmadizad, IIFatemeh Keshavarzi 19
Rasoul Yousefi Mashouf, Seyed Masoud Mousavi, Mohammad Reza Arabestani, Soghra Rabiee, Mohammad Yousef Alikhani 69
Rasoul Yousefi Mashouf, Mina Momeni, Mohammad Yousef Alikhani 103
Rasoul Yousefi Mashouf, Simal Lary, Javad Bayat 146

Razieh Kimiavimoghadam, Ghader Khalili, Mahnaz Seifi, Esmaeal Jabarzadeh, Sahar Teimury Tafreshi, Sara Khoshmagham..... 202
Razieh Sadati, Amin Barghi 149
Razieh Zabihi, Mohammad Soleimani, Keivan Majidzadeh 244
Reyhane Akherati, Mahmoud Shavandi, Ghasemalii Mohebali, Mohammad Reza Soudi ... 131
Reyhane Akherati, Mohammad Reza Soudi, Mahmoud Shavandi, Ghasem Ali Mohebali... 131
Reyhane Kashefi, Ramezan Ali Ataee..... 305
Reyhaneh Hamidi, Saeid Hosseinzadeh, Seyyed Shahram Shekarforosh, Maryam Poormontaseri, Abdollah Derakhshandeh..... 308
Reyhaneh PapIran, Hamid Moghimi, Javad Hamedi, Fatemeh Khajemohammadi lar..... 27
Reyhaneh PapIran, Javad Hamedi, Hamid Moghimi 26
Reza Azizian, Azar Hasanvand, Saeed Hemati, Mostafa Akbari Qomi, Mohammad Masbi, Lida Bimannad, Nourkhoda Sadeghifard, Mahnaz Shafiee 293
Reza Kamali Kakhki, Mohamad Yousef Alikhani, Rasoul Yousefi Mashouf, Amir Roointan, Malek Abazari..... 74
Reza Khashei, Mehrdad Zalipour, Jamal Sarvari, Marzieh Taheripour..... 307
Reza Mohebi, Sobhan Ghafurian, Aref Shavalipour, Nabi Karimikhah, Shahab Mansori .. 172
Reza Ranjbar, Hedieh Rahmati, Mehdi Ghiyamirad..... 70
Reza Sharafati chaleshtori, Elham Salehi, Ali Sharafati chaleshtori..... 148
Reza Soltani, Saba Belgheisi..... 297
Reza Tabatabaii Qomi, Naser Kalhor, Mohsen Sheykh Hasan, Hoda Fazaely, Mahdieh Ghiasi 200
Rezvan Heidary Tabar, Hamid Mighmi, Javad Hamedi..... 48
Rohollah Taghadosi, Mohammad Reza Shakibaie, Shalaleh Masoumi..... 115
Ronak Bakhtiari, Shahin Ahmadian, Jalil Fallah Mehrabadi 248
Roya Ghaderpanah, Ezat Asgarani, Ozra Sabura 253
Roya Ghaderpanah, Ozra Sabura, Ezat Asgarani, Vahid Yavarpoor..... 247
Roya Rafiee, Fereshteh Eftekhar, S. Ahmad Tabatabaei, Dariush Minaee-Tehrani..... 221
Roya Salimnejad, Seyyed yahya Salehi, Golamreza Zarrini, Hamide Bakhshayeshian 135
Saba Amiri Kojouri, Zoheir Heshmatipour, Esmaeil Ghorbanalinejad..... 68
Saba Belgheisi, Mozhgan Heydari..... 151
Saba belgheisi, Reza Soltani 150
Sadaf sadat Rafati, Bahar Shahnavaz, Hossein Eshghi, Ali Makhdomi-Kakhaki 47
Saeed Alamian, Majid Esmaelizad, Afshar Aetemady 67
Saeed Alamian, Taghi Zahraei Salehi, KhosroAghaiipoor, Majid Esmaelizad, Bahar Nayeri Fasaii, Afshar Aetemady, Ramin Bagheri Nejad..... 67
Saeed Zaker Bostanabad, Abdolrazagh Hashemi-Shahraki,, Parvin Heidarieh..... 266
Saeid Dashti Agjeh, Asieh Aramvash, Zeinab Akbari Shahabi, Mohammad Davood Ghafari 52
Sahar Afzali, Saeed Moradi, Nasrollah Sohrabi, Reza Karami Matin, Taraneh Bahmani, Soraya Bilvayeh, Kamal Farhang, Shiva Farhadian behrooz, Fatemeh Sheykhi 207
Sahar Amirpour-Rostami, Bagher Amir-Heidari, Seyed Mostafa Mousavi 143
Sahar Bagheri, Rasoul Roghanian 63
Sahar Bahmani, Amir Hossein Mohseni..... 150
Sahar Teimury Tafreshi, Ghader Khalili, Mahnaz Seifi, Esmaeal Jabarzadeh, Kambiz Davari, Razieh Kimiavimoghadam, Sara Khoshmagham..... 202
Sajjad Sadrinia, Soheyla Erfani, Majid Zare, Nahid Ghanbarzade 222

SalehiMahiintaj, MohseniMojtaba, NazariOmeleila 128
Samaneh Rahmde, Negar Azimzadeh, Maryam Poormontaseri, Saeid Hosseinzadeh 208
Samaneh Ramezani, Hassan JAhandideh, Masomeh Anvari 49
Samar Jamali, Ali Mojtahedi, Mohammad Shenagari 97
Samin Zamani, Mohammad Javad Nasiri, Mohammad Mehdi Feizabadi 271
Samira Kariminejad, Jinous Asgarpanah, Seyed Reza Hosseini Doust 218
Samira Shirazi, Neda Kianpour 94
Sanei Mahdiyeh, Roayaei Mohammad, Soudi Mohhamadreza 137
Sara Ahmadi Badi, Jamileh Norouzy, Abbas Akhavan Sepahi 210
Sara Moradi, Abbas Ali Eskandarian, Saeed Abedi 231
sara moradi, Abbas Ali Eskandarian, Saeed Abedi 235
Saresadat Hosseini, Mohammad Niakan, Horieh Saderi, Mohammad Emaneini 229
Sarvenaz Falsafi, Saeed Zaker Bostanabad, Mohammad Mehdi Feizabadi, Ramezan Ali Khavari-Nejad, Abdolrazagh Hashemi Shahraki, Mostafa Ghalami, Nasrin Sheykhi 270
Sedigheh Taghinezhad-S, Amirhossein Mohseni, Hossein Keyvani 81
Sepide Sagharidooz, Nahid Bakhtiari 45
Sepideh Abbaszadeh, Aghil Sharifzadeh, Mahboube Bagheri, Narjes Cheraghi 174
Sepideh Hasanzadeh, Mohamadreza Pourmand, Rahil Mashhade, Sanaz Dehbashi 70
Setareh Ameri, Reza Hosseini Doust, Ashraf Mohabati Mobarez 119
Setareh Saeb, Reza Ranjbar, Azar Sabokbar, Roohollah Kheiri, Fatemeh Pourali 159
Seydeh Fatem Najafi Mosavi, Daryush Arabian, Rasoul Khalilzadeh, Farzin Abaspour 139
Seyed Ebrahim Hossaini, Zahra Asadnezhad, Hamed Ahari, Seyed AmirAli Anvar, Fatemeh Abdi, Iraj Toumari, *Farhad Dastmalchi* 160
Seyed Javadi Nasrin Hagi 148
Seyed Mohammad Gheibi hayat, Hekmat allah Morady mogarmon, Babak Elyasifar, Sajjad Yazdansetad 260
Seyed Mohammad Gheibi hayat, Hekmat allah Morady mogarmon, Babak Elyasifar, Sajjad Yazdansetad, Tahereh Azimi 261
Seyed Mostafa Hosseini, Rasuol Yousefi Mashouf 75
Seyed Mostafa Hosseini, Rasoul Yousefi Mashouf, Mohammad Reza Arabestani 156
Seyede Maryam Jafari, Mohammad Jahantigh, Ahmad Rashki, Saeed Salari 173
Seyede marzieh Hosseini, Saeedeh Shojaee-Aliabadi, Ramin Khaksar, Hedayat Hosseini 20
Seyede Marzieh Hosseini, Saeedeh Shojaee-Aliabadi, Ramin Khaksar, Hedayat Hosseini 155
Seyedeh Anahita Hosseini, Seyed Masoud Hashemi, Hadi Fadavi Hosseini 130
Seyedeh Sara Shokraei, Nahid Rahimifard, Maryam Moslehi shad 152
Seyedeh Sara Shokraei, Nahid Rahimifard, Maryam Moslehi Shad 167
Seyedeh Sara Shokraei, Seyedeh Rezvan Shokraei 173
Seyedeh Zahra Bakhti, Saeid Latifi-Navid, Saber Zahri 186
Seyedeh Zahra Bakhti, Saeid Latifi-Navid, Saber Zahri 186
Seyyed Amin Ayatollahi Mousavi 278
Seyyed Soheil Aghaei, Ali Javadi, Abbas Morovvati, Yaser Sharifi 228
Seyyed Soheil Aghaei, Nima Bahador, Majid Baserisalehi 300
ShabaniMaria, MohseniMojtaba, AhmadiSalman, RoohiAbolghasem 132
Shabnam Jeibouei, Mojgan Bandehpour,, Ali Haghighi, Bahram Kazemi 206
Shahin Najar Peerayeh, Ali Jazayeri Moghadas, Mehrdad Behmanesh 306
Shahin Najar Peerayeh, Ali Jazayeri Moghadas, Mehrdad Behmanesh 306
Shahram Hanifian 21

Shahrzad Tavanania, Reza Ranjbar, Azar Sabokbar, Roohollah Kheiri..... 92
Shahrzad Tavanania, Reza Ranjbar, Azar Sabokbar, Roohollah Kheiri, Fatemeh Pourali . 158
Shervin Shokoohi, Sasan Sazgar, Farhad Abbasi, Bahareh Hajikhani, Mohtaram sadat Kashi, Shahram Sabeti, Parvin Sarbakhshi 304
Shima Asadi, Keivan Majidzadeh Ardebili, Mohammad Soleimani 240
Shima Farazandeh Mehr, Javad Hamedi, Hamid Moghimi, Meysam Tabatabaei..... 55
Shima Rashidian, Reza Ghanbarpour, Babak Kheirkhah..... 212
Shirin Ghanooni, Abolfazl Dadkhah, Mohammad Reza Zolfaghari 283
Shirin Shahsavari, Siavosh Salmanzadeh-Ahrabi, Tahereh Falsafi, Gholam Reza Irajian..... 73
Shiva Emami, Iraj Nikokar, Yusuf Ghasemi, Afshin Araghian..... 299
Shohreh Yarizadeh, Gholamreza Zarrini 139
Shokoofeh Amirzadeh Shams, Anita Khanafari..... 133
Shokufeh Rafieyan, Mohammad Ali Amoozegar, Mahmoud Shavandi, Mohammad Reza Mehrnia 53
Sholeh Alivand, Maryam Ebrahimian, Mohammad Roayaei Ardakani 55
Sima Moradi, Abas Ali Motalebi, Noordahr Rokni, Hamed Ahari, Zahra Asadnezhad, Amir Ali Anvar, Alireza Mokhtari 162
Soleiman Mirshekari, Daryush Arabian, Rasoul Khalilzadeh, Farzin Abaspour..... 54
Somaye Mazkour, Seyyed Shahram Shekarforoush, Saeid Hosseinzadeh 141
Somayeh Mikaili, Azam Haddadi, Mahmoud Shavandi, Farzaneh Asadi 217
Somayeh Rahmani, Reza Ranjbar, Taghi zahraie salehi, Roohollah Kheiri, Fatemeh Purali 111
Somayeh Rezaei Kalla, Mehdi Assmar 190
Somayeh Talebi, Azar Sabokbar, Majid Riazipour, Mohsen Saffari 286
Somayeh Yaghoubi, Seyyed Siavash Saei-Dehkordi, Hassan Momtaz 165
Sorayya Hosseini, Reza Ranjbar, Taghi zahraie salehi, Roohollah Kheiri, Fatemeh Pourali 238
Sorayya Hosseini, Reza Ranjbar, Taghi Zahrayi Salehi, Roohollah Kheiri, Fatemeh Pourali 161
Soudeh Kholdi..... 40
Susan Doostvandi, AmirhooshangAlvandi, Ramin Abiri, Parviz Mohajeri 90
Taban Hashemi, Jafar Hemmat, Nourkhoda Sadeghifard 177
Taban Hashemi, Jafar Hemmat, Nourkhoda Sadeghifard 178
Tahereh Moshtaghi Maleki, Shahram Hanifian, Jalal Shayegh 171
Tahmine Kandelouei, Seyed Masoud Hossein, Latif Gachkar, Hossein Keyvan, Foad Davoodbeglou, Maryam Vaezjalali 200
Talayeh Tavakoly, Ali Mojtahedi, Mohammad Shenagari 113
Talayeh Tavakoly, Ali Mojtahedi, Mohammad Shenagari 97
Targol Sheibani, Mehdi Rajabi, Setareh Haghighat 89
Tokhanbigli Samaneh, Kazemi Bahram, Bandehpour Mojgan..... 248
Vahid Nasiri, Abdolhossein Dalimi asl, Fatemeh Ghaffarifar, Azam Bolhassani 199
Vahid Nasiri, Abdolhossein Dalimi asl, Gholamreza karimi, Habibollah Paykari, Fatemeh Ghaffarifar 288
Vahid Sharifzadeh Peyvasti, Ashraf Mohabati Mobarez, Nima Khoramabadi, Naghme Razaz Rahmati 122
Waldemar Poplawski, Masoud Ghane..... 140
Waldemar Poplawski, Masoud Ghane..... 158
Yakici, Ali Bahadori, Ayse Karacali, Fatih Koksal 29

Yasaman sadat Nabipour, Arman Rostamzad, Salman Ahmadi 250
Yousefzadi 29
Zahra Bahman, Mohammad Reza Zolfaghari, Ali Almasi 152
Zahra Firozi Javaheri, Hengame Zandi, Gilda Eslami 93
Zahra Khavas, Seyed Masoud Hosseini, Javad Fakhar 151
Zahra Khoshkamz, Mahboubeh Zarrabi, Zargham Sepehrizadeh 255
Zahra Meshkat, Mahboubeh Naderi Nasab, Elnaz Harifi Mood, Nafiseh Izadi 209
Zahra Mirzaii, Peyman Zare 239
Zahra Zangeneh, Fereshteh Eftekhar 37
Zakiyeh Ghadimi, Mohammad Soleimani, Keyvan Majidzadeh, Ali Mahmoudi 244
Zarrintaj Valadkhani, Mir Mostafa Ghamiloui, Firozeh Rahimi, Nayyereh Hassan, Zohreh Aghighi 264
Zaynab Joneydi, Maryam Niyyati, Ali Haghighi, Mohammad KamaliNejad, Fatemeh Memari, Mahmood Reza Behravan 281
Zeinab Rakhisi, Nabi Jomehzadeh, Mansour Amin 302
Zohreh Abedinifar, Fatemeh Fallah, Fahimeh Asadiamoli, Farahnoosh Doustdar, Hossein Goudarzi 64
Zohreh Ghalavand, Hossein Goudarzi, Mona Ghazi, Bahareh Hajikhani, Masoumeh Foroumand..... 216

SAFETY AND QUALITY OF MEAT PRODUCTS

Saba Belgheisi[1], Mozhgan Heydari[2]
1.Department of Food science & Technology, Faculty of Food Industry and Agriculture, Standard Research Institute (SRI), Karaj, Iran.
2.Tehran Standard Office, Iranian National Standard Organization, Tehran, Iran.

Background: The first consumer right is to have a product of good quality and not constituting any health hazard.Quality products are those that meet some need or expectation of consumers and are safe and wholesome as well. Further processing of meat involves of raw meat carcasses into value products. Unfortunately, such products offer ideal medium for microbial growth for they are highly nutritious, have favorable pH and are normally salted or not salted at all.
Methods: Eighty-eight samples of 5 meat products were collected from Tehran markets. The samples were processed products as sausages, burgers, niggets and kebabs. Samples were collected for microbial analysis to assure their quality and safety according to national standard.
Results: Bacteriological analysis revealed that the mean of total bacterial count was ranged from 8×10^4 cfu/gr for kebabs to 8×10^2 cfu/gr for nuggets and the other products were in the range of 10^3 to 10^5 cfu/gr. While clostridium, *Salmonella* and *S. aureus* mean counts were negative for all samples.
Conclusion: All of samples comply with national microbial standards for meat products.

Keywords: Meat, Safety, Microbial Properties, Quality

DETECTION AND ENUMERATION OF COAGULASE-POSITIVE STAPHYLOCOCCUS IN SOHAN

Zahra Khavas[1], Seyed Masoud Hosseini[1], Javad Fakhar[1]
1.Department of Microbiology, Faculty of Biological Sciences, Shahid Beheshti University,Tehran, Iran.

Background: Sohan, an Iranian traditional confectionery product is prepared from wheat flour, wheat sprout flour, sugar, oil, eggs and other free material decorated with nuts. It comes in various shapes, forms and sizes, and can be stuffed with nuts. Since this confection is produced in traditional manufactories, it seems to increase the risk of contamination. The aim of this study was to determine the level of staphylococcal contamination in Sohan.
Methods: Totally, 7 Sohan samples were obtained from shops manufactured by different producers in Qom city. Samples were tested for the presence of coagulase-positive *Staphylococcus* by the methods described in Iran National Standards (ISIRI 6806-3, 1st. Edition). Sohan samples serial dilution started with adding 4.0 g of samples into 36 ml of buffered peptone water. In this way, two types of selective culture mediums were used including Modified Giolitti and Cantoni broth and Baird Parker agar; and a Non-selective enrichment medium Brain heart broth. Then coagulase confirmatory test using the Rabbit plasma was performed. The MPN method was used for enumeration.
Results: Staphylococcal counts ranged from 0 to $0.36x10^1$ MPN/g. One out of 7 samples exceeded the acceptable level which indicates 14.2% of tested Sohan products are considered contaminated.
Conclusion: Since isolated bacteria often find their way into foods by personnel, the necessity of increasing Good Hygienic Practice (GHP) in manufacturing line should be considered.

Keywords: Sohan, Confectionery Product, Coagulase-Positive *Staphylococcus*.

POPULATION OF MICROORGANISMS THAT IN THE AEROBIC-ANOXIC BIOREACTOR PLAY A ROLE IN TREATMENT OF INDUSTRIAL WASTEWATER

Zahra Bahman[1], Mohammad Reza Zolfaghari[1], Ali Almasi[2]
1.Department of Microbiology, Qom Branch, Islamic Azad University, Qom, Iran.
2.Department of Environmental Health Engineering, Kermanshah University of Medical Sciences, Kermanshah, Iran.

Background: The aim of this study was to identify a domain microbial habitat in a sequential aerobic-anoxic bioreactor for the treatment of industrial wastewater.
Methods: The degradation of organic maters was evaluated using laboratory scale aerobic-anoxic up flow bioreactor (UFBR) with an alternatively aeration action in sequence. Bacteriological study and detection of domain bacteria in the aerobic-anoxic conditions were carried out.
Results: The aerobic-anoxic treatment process resulted in the 45-95% BOD and 75-95% COD removal. Bacterial habitat were found as biomass assessment followed by cultural and biochemical differentiation tests, which revealed 10^9-10^{12}CFU/g of flock forming in sludge built up, the majority of enumerated bacteria were facultative such as coli forms, micrococcaceae, *Staphylococcus* and *Streptococci* and absolutely anaerobe such as *Clostridium*. The small portion of the defined population, less than 10% was detected as aerobic genera such as nitrifyers.
Conclusion: Anoxic condition is affected the aerobic bacteria population which is in appeared after aeration switching off.In opposite, facultative and anaerobic bacteria were able to flourish in building existing biomass inside the bioreactor.

Keywords: Industrial Wastewater, Bacterial Consortium, Aerobic-Anoxic Sequential Bioreactor

EVALUATION OF THE ANTIMICROBIAL EFFECT OF LACTOBACILLUS ACIDOPHILUS ISOLATED FROM PROBIOTIC INFANT FORMULA ON ESCHERICHIA COLI

Seyedeh Sara Shokraei[1], Nahid Rahimifard[2], Maryam Moslehi shad[1]
1.Department of Food Science and Technology, Pharmaceutical Science Branch, Islamic Azad university, Tehran, Iran.
2.Associate Professor in Microbiology, Food and Drug Control Laboratories (FDCL).

Background: Lactic acid bacteria are used as the beneficial bacteria in the production of dairy products. Probiotic dairy products due to their therapeutic effects and special nutritional value have been a subject of the extensive research and also there have been so much interest for studying of probiotic dairy products technologies. The most important aspect of probiotic bacteria is their health effects. Study of the antimicrobial properties of isolated *Lactobacillus acidophilus* from infant formula performed against *E. coli* as common gastrointestinal pathogen agent.
Methods: In this study, 5 samples were collected from companies, Nestle probiotic milk (Nan2 and Nan2 H.A) and Milupa probiotic milk (Bebejunior and Bblak). Antimicrobial activities were determined using common bacteria causing gastroenteritis in children (*E. coli* ATCC 8739) using three microbiological methods, disk diffusion agar, well diffusion agar and agar spot test.
Results: The highest inhibitory effect achieved by well diffusion agar with an inhibitory zone diameter of 17.17 ± 1.64 mm and the minimum belonged to Agar spot test with an inhibitory zone diameter of 7.83 ± 1.19 mm ($P < 0.05$). By comparison above mentioned three methods, the well diffusion agar method was shown more sensitive than the other methods.
Conclusion: The obtained results indicated the antimicrobial activity of *Lactobacillus acidophilus* against *E. coli*. Possibly, probiotic bacteria, *Lactobacillus acidophilus* in infant formula can prevent pediatric gastrointestinal *E. coli* infections particularly in infants that are an important approach and strategy for pediatric health.

Keywords: Infant Formula, *Lactobacillus acidophilus*, *E. coli*

BACTERIAL EXAMINATION OF COOKIE AND FRENCH FRIES BOXES

Mojtaba Mashhadi Mohammadzadeh Vazifeh[1], Seyad Masuad Hosseini[1], Javad Fakhari [1]
1.Department of Microbiology, Faculty of Biological Sciences, University of Shahid Behehshti, Evin Tehran, Iran

Background: The aim of this study was to investigate the bacteriological contamination of paper and paperboard, that are used for food packaging. In this way, two testing methods were recruited in order to estimate the number of bacteria.
Methods: Two methods employed including; Agar flooding and smear methods. In the first method, samples of 1×1 cm2 of packaging material were directed to the bottom of the petri dishes and the nutrient medium Tryptone Glucose Extract Agar (TGEA) was added by pour plate procedure. For the second method, 20 cm2 of samples surface were washed out by means of a swab wet¬ted in Ringer solution. The wetted swab was further put into the flask holding Ringer fluid. Then 2 ml of each sample pour onto 2 Petri dishes with TEAG using pour plate procedure. All Petri dishes were incubated at 37 ± 1 °C for 72 hours.
Results: The number of bacteria was enumerated using two mentioned methods. In agar flooding method the number of bacteria was high and uncountable that is not in standard acceptance level (<5×102cfu/g, ISIRI 4782-1), whereas, bacterial count was <1.0 cfu/1cm2 using smear method.
Conclusion: Preparation method for isolation of bacteria from paper and board using for cookie and French fries disposable packaging is critical factor for enumeration. Packaging standards regarding to contamination of food packaging materials with microorganism, should be taken to serious consideration.

Keywords: Food Packaging, Paper, Paperboard, Bacteria

INVESTIGATION OF EFFECT OF ADDITION ESSENTIAL OILS ON THE GROWTH OF YEAST IN GRAPE JUICE

Maryam Farhadi[1]
1.Food Industry, Vocational and Technical College of UrmiaGirls Branch, Vocational and Technical Universit

Background: Most of the Essential oils extracted from plants contain compounds such as phenols, flavonols and Flavonoids, glycosides, alkaloids and polyacetylenes wich have anti microbial and fungicidal characteristics. Anti microbial effects of essential oils have been considered as a viable alternative to chemical preservatives in the food industry.
Methods: In this study, anti-yeast effects of lemon, cumin and oregano essential oils in grape juice were investigated. The concentration of./6ml essential oils per 130 ml grape juice was inoculated with 5/1 × 108 yeast (derived from fermentated grape juice) for microbial tests and kept at room temperature for two weeks.
Results: During the first week of storage, yeast count in grapes - cumin, grape - Lemons and Grapes - oregano was decreased to 2.11, 2.30, and 3.2 cfu / ml and during second week 0.3, 4.7 cfu / ml and uncountable
Conclusion: According to the results, cumin and lemon in grape juice had an anti yeast effect during of two weeks storage at room tempreture

Keywords: Essential Oils, Anti Yeast, Phenols, Flavonoids

STUDY ON THE THE METHODS OF MYCOTOXINS ELIMINATION FROM FOOD AND FEEDS

Fatemeh Hosseinmardi[1]
1.Department of Food Science and Technology,College of Agriculture,Shahr-e-Qods Branch,Islamic Azad University,Tehran,Iran

Background: The worldwide contamination of foods and feeds by mycotoxins is a significant problem.often more than one mycotoxin is found in a contaminated substrate.To control the toxins, correct agricultural operations,chemical and biological control and elimination of them(use of chemical compounds and different adsorbents)are effective.in this paper the function of different adsorbents has been studied.
Methods: The adsorbents mixed with feeds(aluminosilicates, active charcoal and some polymer compounds)were assayed.adsorption of aflatoxin B1 by natural montmorillonite and montmorillonite modified by Cu+2 ions in PH=3,7,9,adsorption of zearalenone by cholestyramine and other adsorbents and adsorption of sterigmatocystine in concentrations of 5,10,50 μgr/ml by montmorillonite in concentrations of 2,1,0.5,4mg/l were studied.
Results: In assaying adsorbents of feed mycotoxins,aluminosilicates were the best. Adsorption of aflatoxin B1 by montmorillonite was high(more than%93).between the adsorbents of zearelenone,cholestyramine had the highest power of adsorption.the amount of sterigmatocystine adsorption by montmorillonite was %93.1-%97.8.
Conclusion: Between adsorbents of feed mycotoxins,active charcoal and polymer compounds were useful after aluminosilicates.although the function of them depended on other factors.the adsorption of aflatoxinB1 in different pH values was not very different,and in pH=3 non-linear isotherm was seen,and modified montmorillonite was more suitable.between the adsorbents of zearalenone, cholestyramine was the best, chrospovidone and montmorillonite were second and third suitable adsorbents.the amount of sterigmatocystine adsorption was high and the mixture of adsorbent and mycotoxin, at different pH values at 37°c, in various solvents were stable.

Keywords: Mycotoxin,Elimination,Food,Aflatoxin

BACTERIAL ISOLATION FROM DISPOSABLE FOIL CONTAINERS FOR FOOD PACKAGING

Mojtaba Mashhadi Mohammadzadeh Vazifeh[1], Seyad Masuad Hosseini[1], Javad Fakhar[1]
1.Department of Microbiology, Faculty of Biological Sciences, University of Shahid Behehshti, Evin Tehran, Iran

Background: In this study, the bioburden of bacteria on foil was investigated for the first time.
Methods: Two methods employed including; Agar flooding and defibering methods. In the first method, 11 cm × 20 cm of foils were prepared (1 g), which 1cm × 1cm of foil were processed for examination. The tested surface was directed to the bottom of the Petri dishes. Incubation was done at 37 °C for 72 hours. After the incubation had finished, the amount of grown colonies of bacteria was counted for each sample. The number of bacteria was calculated per 1 g of tested sample, per of the foil. Also, defibering method used to enumerate the isolated bacteria. In brief, 1 g of sample in 10-2 and 10-3 dilution were prepared in Ringer liquid. 2 ml of each dilution was put onto 3 Petri dishes and flooded with nutrient medium Tryptone Glucose Extract Agar (TGEA). The petri dishes were incubated at 37 ± 1 °C for 72 hours. Each sample examined in 3 replicates.
Results: In study by agar flooding method, no bacteria growth in nutrient medium was found.The number of bacteria that enumerated using defibering method was in the range of 0.9×103 cfu/g to 6×103 cfu/g with the average number of 2.5×103 cfu/g. There is no microbial standard acceptance level to compare.
Conclusion: Preparation method for isolation of bacteria from foil disposable packaging is a critical factor for enumeration. Packaging standards regarding contamination of food packaging materials with microorganism should be taken to serious consideration.

Keywords: Food Packaging, Aluminium Foil, Bacteria

ANTI-LISTERIAL ACTIVITY OF MICROENCAPSULATED NISIN IN DIFFERENT BIOPOLYMERS MICROPARTICLES

Seyede Marzieh Hosseini[1], Saeedeh Shojaee-Aliabadi[1], Ramin Khaksar[1], Hedayat Hosseini[1]
1.Department of Food Science and Technology, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: The aim of this study was to evaluate the anti-listerial activity of nisin microencapsulated in three polymeric systems including alginate, alginate-resistant starch and alginate-high methoxy pectin.
Methods: Nisin loaded microparticles were prepared by a w/o-emulsion external cross-linking procedure with initial nisin concentration of 600 ppm. Antilisterial activity of nisin encapsulated in alginate, alginate-resistant starch and alginate-high methoxy pectin microparticles was detected by agar diffusion assay.*Listeria monocytogenes* ATCC 19117 was used as a test microorganism. Three wells of 6 mm diameter were punched into the agar on each plate (that seeded by 100µl of an overnight broth culture containing 107-108 CFU/ml of the test organisms) and lyophilised microparticles were loaded into wells. Plates were incubated at 37 ºC for 24 h. The diameter of inhibition zone (mm) was then measured and reported as anti-listerial activity of microparticles containing nisin.
Results: Nisin loaded in alginate, alginate-resistant starch and alginate-high methoxy pectin exhibited anti-listerial activity with inhibition zone of 11, 17 and 13 mm, respectively. As our results showed, the nisin loaded in alginate-resistant starch microparticles demonestrated maximum inhibition zone and thus anti-listerial activity. The results showed that the addition of resistant starch in alginate matrix significantly increased the anti-listerial activity of microparticles. Higher anti-listerial activity with the mixture of alginate and resistant starch was interpreted to be due to the effect that starch had in the stabilization of the alginate matrix and thus higher amount of nisin can be encapsulated in micrpoparticles.
Conclusion: These results indicate that alginate microparticles reinforced with resistant starch with improved alginate networks are a promising means to protect anti-listerial activity of nisin into food products.

Keywords: Nisin, Microemcapsulation, *Listeria monocytogenes*

EFFECT OF GINGER JUICE ON THE VIABILITY OF PROBIOTIC BACTERIA IN PROBIOTIC YOGURT

Mohammadjavad Eivani[1], Sajjad Abdi Nowroozani[2], Bahare Sarmadi[3], Maryam Hassani Jafari[4]
1.Kermanshah University of Medical Sciences, Food and Drug Administration, Kermanshah, Iran.
2.Master of Food Science and Technology Student, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2.Student Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
3.Master of Food Science Student, Islamic Azad University, Science and Research Branch, Tehran, Iran.

Background: Probiotics play an important role in helping the body protect itself from infection, especially along the colonized mucosal surfaces of the gastrointestinal tract. Probiotic products are available in many different forms worldwide, including pills, powders, foods, and infant formula. On the other hand, ginger has 114 volatile components from; it smells good and has many functional effects which can improve human health.
Methods: In this study, the effect of ginger juice addition on probiotic yogurts during a 20-day storage period of was studied. That ginger juice was added to the milk by the rate of 0/5 to 2 grams per liter. Afterward, the milk was fermented by probiotic bacteria. PH and the viability of probiotic bacteria in yogurt were measured at periods of 7, 14 and 20 days.
Results: Results revealed that the effect of the extract has no effect on probiotic bacteria and there is no significant difference in the rate of pH and titratable acidity between our sample and the control sample, which contains no ginger juice.
Conclusion: The viability of probiotics affected by the presence or absence of ginger juice did not show any significant changes. It is suggested that other beneficial herbal extract should be tested.

Keywords: Probiotic Bacteria, Ginger Juice, Yogurt.

FREQUENCY DISTRIBUTION OF MECA IN STAPHYLOCOCCUS AUREUS ISOLATED FROM DAIRY PRODUCTS AND RAW MEAT

Seyed Mostafa Hosseini[1], Rasoul Yousefi Mashouf [1], Mohammad Reza Arabestani [1]
1. Department of Microbiology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, IR Iran

Background: The aim of current study was to investigate antibiotic resistance and detect *mecA* in *S.aureus* isolated from dairy products and meat.
Methods: A total of 1050 food samples including 620 dairy products and 430 raw meat were purchased and were collected from September 2013 to June 2014, in Hamadan, Iran. Food samples have been analyzed differentially according to Iran national standard (number= 1194) for *S.aureus* identification. The antibiotic resistance of 98 *S. aureus* isolates from dairy products and raw meat were determined by disk agar diffusion (DAD) and polymerase chain reaction (PCR) methods.
Results: The using of DAD method, 23.4% (23.98) of the *S. aureus* isolates were resistant to Cefoxitin and also by PCR method, 24.4% (24.98) of the isolates had the *mecA* gene. The MRSA isolates showed a high resistant to Erythromycin (30.61%), Tetracycline (29.59%), Gentamicin (27%), Clindamycin (26.53%), Ciprofloxacin (24.4%), Rifampin (54.4%) and Trimethoprim/Sulfamethoxazole (15.3%), Respectively.
Conclusion: The result of present study showed that the MRSA is increasing in food. Hence, the prevalence of consumedly CA-MRSA infection and antibiotic resistant to it could be a serious problem for public health.

Keywords: *Staphylococcus aureus*, Food, Antibiotic-Resistance, MRSA

A SURVEY ON THE PREVALENCE RATE AND ANTIBIOTIC SUSCEPTIBILITY OF ESCHERICHIA COLI IN CONSUMED KOOZEH CHEESES OF URMIA CITY –Iran

Moslem Neyriz Nagadehi[1], Shahram Etemad[2]
1. Assistance Professor of Food Hygiene and Quality Control Department, Veterinary Medicine Faculty, Islamic Azad University, Urmia Branch, Urmia, Iran
2. Graduated Veterinary Medicine, Veterinary Medicine Faculty, Islamic Azad University, Urmia Branch, Urmia, Iran.

Background: Koozeh cheese is of the local traditional cheeses of Azerbaijan and Kurdistan areas of Iran. This cheese is mainly produced with sheep milk, and curdles with rennet. For ripening, it is placed in the jar and buried in ground for 2 to 3 months in cold seasons of year. *Escherichia coli* is one of the common pathogenic organisms of humans, and also cause spoilage in some foods especially cheese. In the present research, prevalence rate of *E. coli* in consumed Koozeh cheeses of Urmia city and antibiotic susceptibility of isolates were studied.
Methods: 100 samples of Koozeh cheese were collected randomly from Urmia city retails in the spring of 2013. At first, samples were enriched in lactose broth, and then transferred to EC broth tube containing duram and incubated at 45℃ for 24-48 h. Positive tubes (gas formation) streaked on EMB agar, and for confirmation of *E. coli* related standard biochemical tests were done. Antibiotic susceptibility of *E. coli* isolates were determined by disk diffusion method using ampicillin (AM), ceftriaxone (CRO), chloramphenicol (C), erythromycin (E), gentamicin (GM) and sulfadiazine trimethoprim (SXT) antibiotics.
Results: Fromtotal 100 tested samples, *E. coli* was isolated from 22 samples (22%). Susceptibility of isolated *E. coli*strains to tested antibiotics was following: CRO > SXT > C > AM > GM > E.
Conclusion: It can be concluded that the contamination rate of consumed Koozeh cheeses of Urmia city to *E. coli* is high (22%) and ceftriaxone is the most effective antibiotic in inhibiting the growth of isolates.

Keywords: *E. coli*, Antibiotic Susceptibility, Koozeh Cheese, Urmia City (Iran)

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THE EFFECTS OF MONOLAURIN IN COMBINATION WITH R-704 STARTER CULTURE ON THE GROWTH AND SURVIVAL OF LISTERIA MONOCYTOGENES IN IranIAN WHITE FRESH CHEESE DURING REFRIGERATED STORAGE

Moslem Neyriz Nagadehi[1], Farzad Gaderi[2]
1.Assistance Professor of Food Hygiene and Quality Control Department, Veterinary Medicine Faculty, Islamic Azad University, Urmia Branch, Urmia, Iran.
2.Graduated Veterinary Medicine, Veterinary Medicine Faculty, Islamic Azad University, Urmia Branch, Urmia, Iran.

Background: The harmful effects of many chemical food preservatives are well established, so this has triggered interest in natural methods of preservation. Monolaurin, a monoester of lauric acid, occurs naturally in some foods especially coconut, and has various antiviral and antibacterial activities. *Listeria monocytogenes* is a food borne pathogen, and because of its growth at refrigeration temperatures has great importance in public health. Evaluation of the effects of monolaurin separately and in combination with R-704 starter culture (*Lactococcus lactis* subsp cremoris and *Lactococcus lactis* subsp lactis) on the growth and survival of *Listeria monocytogenes* PTCC 1163 in manufactured cheeses during refrigerated storage was the purpose of this research.
Methods: In this study, the number of *L. monocytogenes* and the amount of pH were determined on the manufactured cheeses during storage days (0, 4, 5, 10, and 14) in refrigerator temperature (+4℃).
Results: Monolaurin separately and in combination with starter culture decreased the number of *L. monocytogenes* significantly ($p<0.01$). The number of *L. monocytogenes* affected by monolaurin in cheeses with starter culture was lower than cheeses without starter culture. However, the number of *L. monocytogenes* in both group cheeses only in concentration of ≤ 400 ppm of monolaurin had difference significance ($p<0.05$). The amount of pH affected by monolaurin in cheeses with starter culture was lower than cheeses without starter culture.
Conclusion: It can be concluded that monolaurin only in concentration of ≤ 400 ppm significantly decreases the number of *L. monocytogenes* in cheeses containing starter culture.

Keywords: Monolaurin, R-704 Starter Culture, *Listeria Monocytogenes*, Iranian White Fresh Cheese

BACTERIAL CONTAMINATION OF TRADITIONAL ICE CREAM IN TEHRAN

Aref Shavalipour[1], Pouya Amiri[1], Hamidreza Houri[2]
1.Department of Medical Microbiology, Shahid Beheshti University of Medical Sciences,Tehran, Iran
2.Department of Medical Microbiology,School of Medicine, Shahid Beheshti University of Medical Sciences,Tehran, Iran

Background: From hygienic quality aspect, most of the present traditional ice cream has non-conforming with extant standards. Pasteurization of primary ice cream mixture with sufficient thermal processing increase hygienic quality level and causes control of primary infection. Origins of infection for traditional ice cream consist of: use of infected primary materials (milk or cream) or non-pasteurization of them. The aim of this study wasto evaluate and to detect the pathogenic and opportunistic bacteria in traditional ice cream of Tehran city.
Methods: Thirty ice cream samples from different regions of Tehran city were collected. Ten grams from each ice cream sample were removed aseptically and homogenized in 90 ml of 1.5% peptone water, using a stomacher. The homogenized samples were inoculated onto the following media; MHA incubated at 37°C for 24h and MacConkey agar incubated at 37°C for 24-48h.Colonies with characteristic greenish metalic color were confirmed as *E. coli* with the indole, methyl-red, Voges Proskauer, and citrate utilization (IMVIC) tests. For the identification of *S. aureus*, typical and atypical presumptive *Staphylococci spp.* colonies were examined by Gram stain, coagulase, catalase,Dnase and manitol fermentation. To identify *B. cereus*, presumptive pink-purple, opaque *B. cereus* colonies were subjected to confirmatory tests of Gram stain, catalase, motility, anaerobic fermentation of glucose, Voges Proskauer reaction and production of acid from mannitol. Antibiogram were down for all isolates.
Results: From 30 samples, 83% *E.coli*, 83% *Enterobacter*, 66% *Citrobacter*, 50% *Klebsiella*, 50% *Serratia*, 16% *Proteus*, 50% *S. aureus*, 50% *Bacillus cereus* were isolated. 50% of *Enterobacter* were resistant to amoxicillin- clavulanic and 60% to Erythromycin. 40% of *Klebsiella* were resistant to amoxicillin- clavulanic acid. 40% of *S. aureus* were resistant to oxacilin that it was considerable.
Conclusion: In conclusion, the current investigation has indicated a poor overall level of hygiene in the service of openly sold ice cream in Tehran. The counts of microorganisms abovethe recommended criteria and the presence of some groups of pathogenic bacteria may pose a risk for public health particularlyfor children and vulnerable elderly people. This study supports the necessity of providing hygienic precautions by producers and retailers and their control periodically in Tehran.

Keywords: Traditional Ice Cream, *E.coli*, Antibiogram

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RELATIONSHIP BETWEEN THE PREVALENCE OF CAMPYLOBACTER SPP. IN CASPIAN SEA'S WATER AND VARIOUS TYPES OF FISHES USING PCR TECHNIQUE

Waldemar Poplawski[1], Masoud Ghane[1]
1.Faculty of Microbiology, Tonekabon Islamic Azad University Branch, Iran

Background: The major purpose of this study was isolation, identification and characterization of *Campylobacter* spp. from Caspian Sea's water in north of Iran and various types of fishes obtained from this sea.*Campylobacter*is one of the most common causes of diarrhea world wide. According to world health organization estimated 2-35% of diarrhea induced by *Campylobacter*. So that, annually 400-500 million peoples in the word are infected with these bacteria. Disease in human being occurs through consumption of contaminated water and animal products viz., meat and milk.
Methods: In total, 70 water samples and 165 samples were obtained from south coastal Caspian Sea and various types of fishes obtained from this area in one year.The isolates were characterized by using standard *Campylobacter* phenotypic identification tests recommended by Atabay and Corry. At the end, the PCR method was carried out in order to confirm the phenotyping results.
Results: After conducting of the Phenotyping methods and their confirmation with molecular technique, three strain of *Campylobacter jejuni* were identified. With regard to the obtained results, prevalence of this bacterium in the coastal waters of the Caspian Sea's was evaluated as 3 percent but no *Campylobacter* species were found in the 165 fish samples obtained from the Caspian Sea.
Conclusion: Considering the report of the contamination of this region`s waters with *Campylobacter* presented by the previous researchers and the lack of this bacterium in the samples of fishes, it can be claimed that digestive system of fishes has not appropriate conditions for the growth of *Campylobacter*. The reason could be the temperature of the fish body that is much lower than the temperature that *Campylobacter* need for survive and growth, or the low ability to compete with other bacteria in the high competitive condition of the intestinal system.

Keywords: *Campylobacter*, Caspian Sea, Fish, Iran

THE FREQUENCY OF QNRB AMONG FLUORO-QUINOLONE-RESISTANT ESCHERICHIA COLI STRAINS ISOLATED FROM DIFFERENT SURFACE WATER SOURCES

Shahrzad Tavanania[1], Reza Ranjbar[2], Azar Sabokbar[3], Roohollah Kheiri[4], Fatemeh Pourali[5]
1.Department of Microbiology, Faculty of Science, Islamic Azad University, Karaj Branch, Karaj, Iran
2.Molecular Biology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.
3.Department of Microbiology, Faculty of Science, Islamic Azad University, Karaj Branch, Karaj, Iran
4. Alborz province Water and wastewater Company. Karaj, Iran
5. Molecular Biology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.

Background: The aim of this study was to investigate the *qnrB* associated antibiotic resistance among *E. coli* strains isolated from surface water sources in Alborz province.
Methods: In this study, we examined all *E. coli* strains isolated from different surface water sources in Alborz province in 2013. Bacterial isolates were detected and identified by standard microbiological and biochemical tests. To screen the fluoroquinolone-resistant isolates, the antimicrobial susceptibility testing was determined according to Kirby Baur assay. Total genomic and plasmid DNA were extracted by boiling method. Polymerase chain reaction (PCR) was carried out on screened fluoroquinolone-resistant isolates to detect the presence of *qnrB* genes.
Results: One hundred *E. coli* strains were isolated and included. Antibiotic susceptibility testing showed that 22.44 and 7.14 percentages of the isolates were resistant to Nalidixic acid and Ciprofloxacin. *qnrB* gene was detected in 10 fluoroquinolone-resistant isolates. The remained fluoroquinolone-resistant isolates and all fluoroquinolone-susceptible isolates didn't contain *qnrB* gene.
Conclusion: This study reflects an increasing prevalence of fluoroquinolones resistant *E. coli* strains circulating in water sources. Dissemination of these resistance genes is of particular concern. Antibiotic resistance can be transmitted via direct contact between animals and humans and environment, especially water sources so this problem needs much more proficiency and management.

Keywords: Antibiotic Resistance, *E. coli*, *Qnrb*, Water Sources.

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THE STUDY OF BLA-CTX ASSOCIATED ANTIBIOTIC RESISTANCE AMONG ESCHERICHIA COLI STRAINS ISOLATED FROM DIFFERENT WATER SOURCES IN ALBORZ PROVINCE

Setareh Saeb[1], Reza Ranjbar[2], Azar Sabokbar[3], Roohollah Kheiri[4], Fatemeh Pourali[5]
1.Department of Microbiology, Faculty of Science, Islamic Azad University, Karaj Branch, Karaj, Iran
2.Molecular Biology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
3.Department of Microbiology, Faculty of Science, Islamic Azad University, Karaj Branch, Karaj, Iran
4.Alborz Province Water And Wastewater Company. Karaj, Iran
5.Molecular Biology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.

Background: The aim of this study was to investigate the prevalence of *bla-CTX* associated antibiotic resistance among *E. coli* strains isolated from different water sources in Alborz province.
Methods: The study included all *E. coli* strains isolated from different surface water sources in Alborz province in 2013. Bacterial isolates were all detected and identified by standard microbiological and biochemical tests. The antimicrobial susceptibility testing was determined according to Kirby Baur assay. Total genomic and plasmid DNA were extracted by boiling method. Phenotypic assay for *bla-CTX* was performed by synergic disc then all phenotypically positive ESBL-producing strains were examined for the presence of *CTX-M* genes by PCR. The PCR amplicons were visualized after electrophoresis and staining with ethidium bromide.
Results: One hundred *E. coli* strains were isolated and included in the study. Of these, 38 and 52 isolates were resistant to Ceftazidime and Cefazolin, respectively. Thirty eight percentages of the strains were phenotypically ESBL positive. *CTX-M* gene was detected in 32% of phenotypically ESBL positive strains.
Conclusion: The study revealed a high prevalence of *CTX-M* gene among *E. coli* strains circulating in surface water sources. This finding raises a concern about distribution of such threatening agents in these types of water sources and horizontal gene transfer between other waterborne bacterial species. Our results underline the need for enhanced laboratory capacity and coordinated surveillance strategies to control the further spread of these threatening pathogens.

Keywords: Antibiotic Resistance, *E. coli, CTX*, Water Sources.

DISTRIBUTION OF MAIN E. COLI PHYLOGROUPS ENCODING CTX-M EXTENDED–SPECTRUM BETA-LACTAMASE (ESBLS) IN FECAL ISOLATES OF POULTRY: A SURVEILLANCE STUDY IN FIVE POULTRY HOUSES IN TEHRAN

Fatemeh Doregiraee[1], Alebouyeh Masoud[2], Nayyeri Bahar[1], Zali Mohammad Reza[2]
1.Department of Microbiology and Immunology, Veterinary Medicine,Tehran University, Tehran, Iran
2.Gastroenterology and Liver Disease Research center, Shahid Beheshti Unversity of Medical Sciences,Tehran, Iran

Background: Association of avian *E. coli* phylogroups with human diseases and role of these strains in dissemination of resistance genes to human ones are of major health concerns. This study was aimed to investigate phylogroup diversity and prevalence of genes encoding ESBL among the avian fecal *E. coli* isolates in five different poultry houses.
Methods: A total of 500 avian cloacal swab samples from five different poultry houses were collected over a six month period in 2013 in Tehran, Iran. Antimicrobial susceptibility testing was done against 13 antibiotics from 9 different classes according to the last CLSI guideline. ESBLs phenotypes were detected by using ceftazidime and ceftazidime-clavulanic acid disks. The presence and type of *blaTEM, blaSHV*, and *blaCTX-M* were determined by PCR and sequencing of these isolates. Diversity of phylogroups A, B1, B2 and D were analyzed among the isolates with confirmed ESBLs phenotype by PCR. The plasmid extracts were screened for carriage of β-lactamase genes.
Results: A total of 444 *E. coli* isolates were obtained from the studied samples (88.8%). Nearly 92.6% displayed MDR phenotype. A frequency of 5.8% (26) was confirmed for the isolates presenting ESBL phenotype, which *blaCTX-M, bla TEM* and *blaSHV* were detected in 6, 10, and 5 strains, respectively. Coexistence of *blaTEM* and *blaCTX-M* (3 isolates) and *blaTEM, blaCTX-M*, and *blaSHV* (2 isolates) was also detected in these strains. The phylogrouping results showed prevalence of main phylogroups as follow: D (42.3%, 11), B1 (34.6%, 9), A (15.4%, 4), and B2 (7.7%, 2). Plasmid mediated transmission of the β-lactamase genes were found in 42% of the strains presenting ESBL phenotypes.
Conclusion: The colonization of the avian intestine with *E. coli* strains is related to phylogroups D, as a source of extraintestinal pathogenic *E. coli* strains responsible for human diseases, and carriage of β-lactamase genes in these strains proposed them as a source of pathogenic strains in human food chain.

Keywords: *E. coli*, Multidrug Resistance, Phylogenetic Groups, Extended-Spectrum B-Lactamase, Poultry.

PREVALENCE OF LISTERIA SPECIES IN RAW MILK IN EAST-AZERBAIJAN PROVINCE

Mona Davarpanah[1], Shahram Hanifian[2]
1.Department of Food Science and Technology, College of Agriculture, Tabriz Branch, Islamic Azad University, Tabriz, Iran
2.Department of Food Science and Technology, College of Agriculture, Tabriz Branch, Islamic Azad University, Tabriz, Iran

Background: The genus *Listeria* consists of six species amongst *L. monocytogenes* and *L. ivanovii* are pathogenic to humans and cause listeriosis. Listeriosis may be transmitted through direct contact with infected animals or by consumption of contaminated raw milk and dairy products. This study aimed to investigate the prevalence of *Listeria* spp. in bulk raw milks of East-Azerbaijan province.
Methods: A total sample of 85 bulk-raw milks was collected from six distinct parts of East-Azerbaijan province. The samples were analyzed for the presence of *Listeria* spp. using conventional culture methods. For primary enrichment, 25 ml of each sample was added to 225 ml of *Listeria* enrichment broth (UVM I) and incubated at 37°C for 24 h. Afterwards, for secondary enrichment 0.1 ml of UVM I was transferred to 9 ml of UVM II and incubated at 37°C for 24 h. Both primary and secondly enrichment broths were streaked on PALCAM *Listeria* Selective agar and were incubated at 37°C for 24-48 h. Green-gray colonies with beige halo were subjected to differential morphological/biochemical analysis including, Gram staining, catalase, MR/VP, motility at 25°C and 37°C, acid production from glucose, manitol, rhamnose, xylose, α-methyl-D-manoside, nitrare reduction, hydrolysis of esculin, β-hemolytic activity and CAMP test.
Results: According to the results, the overall prevalence of *Listeria* spp. in bulk-raw milks was estimated at 3.52%. Results also revealed a different prevalence rates in six regions of East-Azerbaijan. That is to say, the prevalence rates in northwest, southwest and one of regions located in the center of the province was determined as 7.14%. Meanwhile, no positive sample was detected in the entire three regions.
Conclusion: The results suggested a relative high prevalence of *Listeria* spp. in bulk-raw milks which can be a potential risk from the public health point of view, particularly for the individuals consuming unpasteurized milks and/or traditional dairy products manufactured from raw milk.

Keywords: *Listeria* Spp., *Listeria monocytogenes*, Bulk-Raw Milk, East-Azerbaijan, Iran

SURVEY OF INCREASING THE SHELF-LIFE OF DRY SALAMI BY NANO PACKAGINGS

Seyed Ebrahim Hossaini [1], Zahra Asadnezhad[1], Hamed Ahari [1], Seyed AmirAli Anvar [2], Fatemeh Abdi [2], Iraj Toumari [2], *Farhad Dastmalchi* [2]
1.Department Of Food Science And Technology, Science And Research Branch, Islamic Azad University, Tehran, Iran
2.Food Microbiology Laboratory, Zakaria Razi Complex, Science and Research Branch, Tehran, Iran
3.Pars Installed Nano Company, Iran

Background: Today, considering the increase in per capita consumption of ready to eat products such as sausage in country, the importance of food security and enhance the shelf life of such products using covers that in addition to maintaining a quality coating that reduces the microbial load and concern to prevent loss of product is important.
Methods: In this study, the coatings containing silver nanoparticles (3% and 5%) was used for packing dry salami.In the first part of the study, the total count of microbial load of packing samples at protein stores in Tehran (Region 2) was performed in food microbiology laboratory. In the second part of the study,*Staphylococcus aureus* and *E. coli* bacterial tests in accordance with ISO Standard No. 2303, National Organization of Iran on products packaged in 3% and 5% nano silver coating and the control samples without nano-coating was performed.
Results: Analysis results indicate that the use of coatings containing silver nanoparticles of 5% compared to the coverage of 3% control, Bacterial counts of *Staphylococcus aureus* and *E. coli* levels were reduced 78%.
Conclusion: Nano coating of 5% as efficient coverage in order to reduce the microbial load and increase the shelf life of dry salami was found. The results showed an increase in survival time was 2 times the amount of dry salami.

Keywords: Coated Nano Particles, Shelf-Life, Salami

NANOPARTICLES IN FOOD INDUSTRY AND THEIR EFFECT ON FOOD CHARACTERISTICS

Hamdollah Moshtaghi[1], Somayeh Yaghoubi[1], Azade Shefaee[1]
1.Department of Food Hygiene and Quality Control, Faculty of Vet. Medicine, Shahrekord University, Shahrekord, Iran

Background: Nanotechnology is having an impact on several aspects of the food industry, from how food is grown to how it is packaged. Nanosensors are being developed that can detect bacteria and other contaminates such as *Salmonella* on the surface of food at a packaging plant. This will allow for frequent testing at a much lower cost than is incurred by sending samples to a lab for analysis
Methods: This point-of-packaging testing, if conducted properly, has the potential to dramatically reduce the chance of contaminated food reaching grocery store shelves. There are also nanosensors being developed to detect pesticides on fruit and vegetables
Results: Nanoparticles are being used to deliver vitamins or other nutrients in food and beverages without affecting the taste or appearance. These nanoparticles actually encapsulate the nutrients and carry them through the stomach into the bloodstream.
Conclusion: For many vitamins this delivery method also allows a higher percentage of the nutrients to be used by the body because, when not encapsulated by the nanoparticles, some nutrients would be lost in the stomach.

Keywords: Nanoparticles, Food, Characteristics

THE STUDY OF PREVALENCE OF GENE ESTA IN ESCHERICHIA COLI STRAINS ISOLATED FROM DIFFERENT SURFACE WATER SOURCES IN ALBORZ PROVINCE

Sorayya Hosseini[1], Reza Ranjbar[2], Taghi Zahrayi Salehi [3], Roohollah Kheiri[4], Fatemeh Pourali[2]
2.Department of Microbiology, Faculty of Science, Islamic Azad University, Saveh Branch, Saveh, Iran
2.Molecular Biology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.
3.Department of Microbiology, Faculty of Science, Islamic Azad University, Saveh Branch, Saveh, Iran
4.Alborz province Water and wastewater Company. Karaj

Background: *Escherichia coli* is a member of the normal intestinal microflora of humans and of many animal. Although most strains of *E. coli* are harmless but some types of *E. coli* especially ETEC pathotypes cause intestinal diseases and are transmitted through water and foods. The aim of this study was to investigate the prevalence of *E. coli* strains carrying virulence gene, encoding enterotoxin (*estA*) isolated from different water sources in Alborz province.
Methods: This study was carried out in 2013. The research included all *E. coli* strains isolated from different surface water sources in Alborz province. *E. coli* strains were isolated, detected and identified by standard microbiological and biochemical tests. The strains were evaluated for presence of virulence gene *estA* by PCR using specific primers. The PCR amplicons were visualized after electrophoresis and stained with ethidium bromide.
Results: Ninety nine *E. coli* strains were isolated and included in the study. Approximately an equal isolation rate of ETEC was seen among different water source. The PCR results showed that %48 of the strains harbored *estA* gene. Most of bacterial strains harboring *estA* gene were multi drug resistant.
Conclusion: Our finding showed the high prevalence rate of virulence gene *estA* among strains isolated from different surface water sources in Alborz province. Considering its plasmid borne nature, the risk of transmission of this gene between other bacterial species could pose a high threat for public health.

KEYWORDS: *E. coli, ESTa*, pcr, WATER.

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INCREASING THE SHELF-LIFE OF BELUGA CAVIAR BY NANO–SILVER PACKAGING

Sima Moradi [1], Abas Ali Motalebi [2], Noordahr Rokni [2], Hamed Ahari [3], Zahra Asadnezhad [4], Amir Ali Anvar[3], Alireza Mokhtari [5]

1.Veterinary Student, Science And Research Branch, Islamic Azad University, Tehran, Iran
2.Member Of Faculty, Department Of Food Hygiene, Science And Research Branch, Islamic Azad University, Tehran, Iran
3.Assistant Profesor, Department Of Food Science And Technology, Science And Research Branch, Islamic Azad University, Tehran, Iran
4.Msc Student, Department Of Food Science And Technology, Science And Research Branch, Islamic Azad University, Tehran, Iran
5.Science And Research Branch, Islamic Azad University, Tehran, Iran

Background: Use of new technologies in food industries has been a modern attitude well noticed recently. Utilizing new technologies such as the Nano- technology or biotechnology could solve many of the drawbacks in food industries. Nano-technology,in fact, covers the control or manipulation of a material in atomic, molecular or macromolecular scales in which the particle affecting the materials' function has a size of around 100 Nano- meter or less.
Methods: The present study tried to explore the effective dose of nanosilver film for preserving, the high quality and luxury product, Beluga Caviar with food safety and health care considerations. In this regard, six treatment groups were introduced by applying 500, 1000,2000, 3000, 4000ppm nanosilver cover and also Japanese plastic cover and kept and examined for a period of six and twelve months.
Results: Microbial analyses were shown that applying 1000 ppm of nanosilver film is effective in preserving the Beluga Caviar.
Conclusion: Use of 1000 ppm of nanosilver is safe for consumers because it has less liberation in comparison with the other treatments ($p<0.05$) during six months of shelf-life.

Keywords: Nanosilver Film, Shelf-Life, Beluga Caviar

CAMPYLOBACTER JEJUNI ISOLATED FROM MILK SAMPLES IN WARM MONTHS

Fatemeh Zaboli[1], Ali Dabiri[1], Samaneh Rouhi[2]

1.Department of Agriculture Engineering, Food Engineering, Sciences and Research Branch, Islamic Azad University, Ayatollah Amoli, Amol, Iran
2.Cellular&Molecular Research Center and Microbiology Department, Member of Student Research Committee, Kurdistan university of Medical Sciences, Sanandaj, Iran

Background: Several pathogens can be transmitted through food such as *Campylobacter jejuni*. This bacterium causes gastroenteritis, diarrhea, recurrent arthritis, urinary tract infection, meningitis, cholecystitis, endo-carditis and GBS.Since *C. jejuni* infection is common in summer, the purpose of this study was to isolate *C. jejuni* from milk samples in Amol city (north of Iran).
Methods: Samples were collected in 2013 warm months: July (12 samples), August (12 samples), September (12 samples).Phenotypic test was used to detection of *C. jejuni* in milk samples: Grown in nutrient broth, enrichment, microscopic identification, catalase and cytochrome oxidase tests which are positive for *C. jejuni*, TSI, hydrolysis of hyporate.
Results: The results of this study showed that the 1 (8.3%) sample in the first half of July, 3 (25%) in the second half of July and in the first half August the 2 (16.66 %) were positive for the presence of *C. jejuni.*
Conclusion: The determination of contamination of raw milk and making the necessary arrangements are prominent, because human infection can occur through milk or its products.

Keywords: *Campylobacter Jejuni*, Milk, Warm, Months

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IDENTIFICATION OF MULTI-DRUG RESISTANT STAPHYLOCOCCUS AUREUS ISOLATES IN SAMPLES OBTAINED FROM MEDICAL FOODS, KITCHEN UTENSILS AND FOOD HANDLERS IN A HOSPITAL IN TEHRAN

Gohartaj Rassouli[1], Masoud Alebouyeh[2], Elahe Tajeddin2[3], Bahram Amini[1], Mohammad Reza Zali[3]
1.Department of Microbiology, School of Mediicne, Zanjan University of Medical sciences, Zanjanm, Iran.
2.Gastroenterology and Liver diseases Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
3.Gastroenterology and Liver diseases Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Background: Multi- drug resistant (MDR) *S. aureus* is one of the most important etiological factors in food-borne diseases in predisposed patients at medical centers. This study was carried out to determine the frequency of MDR- *S. aureus* isolates in samples collected from hospital foods and association of their drug resistance pattern with those of samples obtained from kitchen utensils and food handlers.
Methods: A total of 144 samples were collected from hospital foods, kitchen utensils and hands of the food handlers during food preparation in 2012-2013. The samples were transported to the microbiology laboratory in order to detect *S. aureus* contamination. Culture in mannitol salt agar media, morphological evaluation of the grown colonies, specialized tests such as catalase, oxidase, hemolysis, coagulase, DNase, as well as PCR testing (for *femA* and *nucA* genes) were done for more identification of the isolates. Drug-resistance pattern of the *S. aureus* isolates were also analyzed with the disk diffusion method in accordance with CLSI, 2012 guidelines.
Results: *S.aureus* bacteria were isolated in 12 out of 72 food samples (16.7%), 15 out of 18 samples from food handlers (83%) and 24 out of 54 utensil samples (44.4%). The most frequently drug resistance in *S.aureus* isolates obtained from food, utensil and food handlers' samples were seen against ampicillin (55%), ceftazidim (48%) and ampicillin (51.8%), respectively. MDR patterns were also found in all three kinds of the samples. The most similar drug-resistance patterns were found among food and food handlers' samples.
Conclusion: The high level of *S. aureus* contamination in the prepared foods and the high level of bacterial resistance to broad-spectrum antibiotics, such as ceftazidim suggest the involvement of hospital foods in distribution of drug-resistant strains in hospitals.

Keywords: Hospital, Food, *S. Aureus*, Multi-Drug Resistance

BIOCONTROL OF MILK CONTAMINATION TO ESCHERICHIA COLI BY USING OF BACTERIOPHAGE

Mina Jeddi[1], Gholamreza Zarrini[1], Seyyed Mehdi Banan Khojasteh[1]
1.Department of Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran;

Background: Milk is a complete food for humans, and it is also a good media for bacteria because it contains all the nutrients such as proteins, fat and lactose. Entry of food-borne pathogens via contaminated raw milk into dairy products processing plants can lead to contamination of processed products. *Escherichia coli* is a pathogen often found in milk and can also be considered as corruption agent of milk. High levels of bacteria in milk can reduce the shelf life and quality of milk that cause economic losses. Bacteriophages are entities that have ability of elimination bacteria. The aim of this study was isolation suitable phages against *Escherichia coli* in dairy products and biocontrol effects of phages to reduce milk contamination.
Methods: For this purpose, the first specific phages were isolated against *Escherichia coli* via two-layer agar method from environmental samples of water and sewage.Then evaluation of the bacteriophages' effect on the bacteria was performed,*in vitro.* Finally, the phages be added in various concentration (0, 4 log10 and 6 log10) to milk that included *Escherichia coli* . The milk was plated in various time for assessing the number of phage and bacteria. Plate was incubated in 37℃ for 24 hr.
Results: The bacterial count showed that phages have significant effect on *Escherichia coli* in milk. There were no bacteria after 2 hrs in high concentration.
Conclusion: According to the results of this research phages have capable of proliferation in milk and killing the bacteria.

Keywords: Bacteriophage, Biocontrol, Milk Contamination, *Escherichia coli.*

THE EFFECT OF BACTERIOPHAGE ON THE STAPHYLOCOCCUS AUREUS IN MILK

Mina Jeddi[1], Gholamreza Zarrini[1], Farzam Sheikhzadeh[1]
1.Department of Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran;

Background: The aim of this study was to assess the ability of staphylococcal bacteriophage to inhibit *Staphylococcus aureus* in milk.
Methods: The effect of bacteriophage was studied against*S. aureus* in sterile and pasteurized milk as biocontrol agent. Phages were collected from environmental sewage samples by the soft-layer agar method. Isolated lytic phages were tittered and then were added in various initial concentrations (0, 102, 104, 106pfu/ml) to milk samples which consist of 104 and 106cfu/ml. The milk samples were stored in 24 ℃ for 8 hrs. The milk samples are used as a source for colony count and plaque assay.
Results: The results were showed that isolated phages had the ability of elimination *S.aureus* in milk. There was significant reduction in bacterial count in high concentration of phages and bacteria, but in low concentration was not observed significant decrease in the number of bacteria.
Conclusion: The bactericidal potential of bacteriophages can be used in dairy industry to prevent from product contamination.

Keywords: Bacteriophage, Milk Contamination, *Staphylococcus aureus*, Biocontrol

QUALITATIVE STUDY ON HYGIENIC MICROBIAL INDICATORS OF SEMNAN TRADITIONAL CHEESE (KHIKI)

Marzieh Shahvarani[1], Mahnoosh Parsaeimehr[2], Marzieh Bolandi[3]
1.MSc student of Food Science and Technology, Faculty of Agriculture, Islamic Azad University, Damghan Branch, Damghan- Iran
2.Food Hygiene Department,Faculty of Veterinary Medicine, Semnan University, Semnan Iran
3.Food Science and Technology, Faculty of Agriculture, Islamic Azad University, Damghan Branch, Damghan- Iran

Background: The aim of this study was to determine the hygienic condition of processing of a traditional, starter-free cheese of Semnan province (khiki) produced from raw ewe's milk.
Methods: The diversity and dynamics of the microbial populations in ripening cheese samples were assessed by culturing. In this study, 30 cheese samples were collected randomly from different regions of Semnan and examined.
Results: The result indicated that the coliform, enterobacteriacae, *Staphylococcus aureus*, molds and yeasts of cheese were 6.78, 5.7, 1.12, 5.51 log cfu/g, respectively. Also, in some samples, the number of coliform and enterobacteriacae were higher than the limits allowed by the national standard for Iranian ripened cheese.
Conclusion: Generally, we may claim that in ripening khiki cheese a large number of different microbes were involved and its hygienic matters call for more attention.

Keywords: Microbial Quality, Traditional Cheese,

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THE EFFECTS OF LACTIC STARTER CULTURES ON THE GROWTH AND SURVIVAL OF ESCHERICHIA COLI O157: H7 IN IranIAN WHITE FRESH CHEESE DURING REFRIGERATED STORAGE

Moslem Neyriz Nagadehi[1], Kaveh Rahmanpour[2]
1.Assistance Professor of Food Hygiene and Quality Control Department, Veterinary Medicine Faculty, Islamic Azad University, Urmia Branch, Urmia, Iran.
2.Graduated Veterinary Medicine, Veterinary Medicine Faculty, Islamic Azad University, Urmia Branch, Urmia, Iran.

Background: Lactic starter cultures are used for increasing the shelf life and producing favorable organoleptic properties in the food industry. *Escherichia coli* O157: H7 is a pathogenic microorganism and cause hemorrhagic colitis and hemolytic uremic syndrome (HUS) in humans. Study on the effects of various lactic starter cultures on the growth and survival of *E. coli* O157: H7 in manufactured cheeses during maintenance times were the objectives of this research.
Methods: Manufacturing of cheeses containing *E. coli* O157: H7 and various lactic starter cultures (YF-L811: *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. bulgaricus, R-704: *Lactococcus lactis* subsp. cremoris and *Lactococcus lactis* subsp. lactis, FRC-65: *Streptococcus thermophilus, LactoBacillus delbrueckii subsp. bulgaricus, Lactococcus lactis subsp. cremoris and Lactococcus lactis subsp. lactis*, CHN-22: *Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. lactis biovar diacetylactis, Leuconostoc.,* and ABT-2: *Bifidobacterium, LactoBacillus acidophilus* and *Streptococcus thermophilus*),count of *E. coli* and measurement of pH in produced cheeses during storage days (0, 1, 5, 10 and 14) in refrigerator (+4℃) were the methods used in this study.
Results: FRC-65 starter cultures decrease almost 2 logarithms of *E. coli* counts, and Yf-L811 starter culture decrease about one logarithm of *E. coli* counts in comparison to control group. The effects of FRC-65, Yf-L811 and R-704 starter cultures on decreasing the pH value of manufactured cheeses in comparison to the control group were significant ($p<0.05$). Also by increasing cheese storage time in cheeses produced with different starter cultures, *E. coli* counts and cheese pH decreased. Moreover, by reducing the pH value of cheeses produced under influence of different starter cultures, the number of *E. coli* also decreased.
Conclusion: Therefore,it can be concluded that FRC-65 starter cultures are the most effective starters against the growth of *E. coli* and recommended to be used in the production of Iranian white cheese.

Keywords: Iranian White Fresh Chesses,Different Lactic Starter Culture,*Escherichia coli* O157: H7

OCCURRENCE OF ENTEROTOXIN GENE PROFILE OF STAPHYLOCOCCUS AUREUS ISOLATED FROM RAW MILK OF CATTLE, SHEEP AND GOAT AND PASTEURIZED MILK MARKETED IN SHAHREKORD

Somayeh Yaghoubi[1], Seyyed Siavash Saei-Dehkordi[1], Hassan Momtaz[2]
1.Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran
2.Department of Microbiology, Faculty of Veterinary Medicine, Islamic Azad University, P.O. Box166, Shahrekord Branch, Shahrekord, Iran

Background: Nowadays, milk has been considered as one of the most important supplies to provide nutritional requirements of human. However, milk and dairy products could be a source of contamination by different foodborne pathogens such as *Staphylococcus aureus*. This study was aimed to determine the occurrence of genes encoding the staphylococcal enterotoxins (SEs), sea, *seb, sec, sed, see, seg, seh, sei* and *sej* in *S. aureus* strains isolated from raw or pasteurized milk of cattle, sheep and goats marketed in Shahrekord.
Methods: A total of 256 samples of raw milk of cattle, sheep and goat and 10 samples of pasteurized milk were collected from Shahrekord market, in Chaharmahal and Bakhtiari province, during 8 months. Multiplex PCR was performed to detect different kinds of the SEs genes of isolated strains.
Results: *S. aureus* was isolated from 123 (50 %) of 246 raw milk samples. The number of isolated strains of raw milk of cattle, sheep and goat were 89, (54.93 %), 18, (56.25 %) and 16, (30.76 %), respectively. The microorganism was not isolated from pasteurized milk samples. Of 123 *S. aureus* examined samples, 42.27 % were positive for one or more genes encoding the enterotoxins. The gene encoding enterotoxin A, sea, was the most abundant (26 strains, 50%), followed by *seb* (20 strains, 38.46 %), *sec* and *sed* (3 strains of each, 5.76%). None of the isolates carried other enterotoxion genes (*see, seg, seh, sei* and *sej*).
Conclusion: The high occurrence of enterotoxin genes, *sea* and *seb* in*S. aureus* and the presence of other enterotoxin genes, *sec* and *sed*, in isolates from raw milk, may be important as it is relevant to food hygiene. Therefore, the marketing of raw milk could result in public health problems and it should be prohibited by governmental authorities.

Keywords: *Staphylococcus aureus*, Milk, Enterotoxin Genes, Multiplex PCR

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THE EFFECT OF FUNCTIONAL PROPERTIES OF BARBERRY AQUEOUS EXTRACT ON SURVIVAL OF LACTOBACILLUS ACIDOPHILUS AND BIFIDOBACTERIUM BIFIDUM IN PROBIOTIC STIRRED AND SET YOGURT SAMPLES

Mina Hassani[1], Akram Sharifi[1], Bahram Hassani[2]
1.Department of Food Science and Microbiology, Islamic Azad University, Quchan Branch, Quchan, Iran.
2.Department of Food Science and Technology, Ferdowsi University of Mashhad, Khorasan, Iran.

Background: The main objective of this study was to investigate the survival of probiotic bacteria including *LactoBacillus acidophilus* and *Bifidobacterium bifidum* and an equal mixture of these two strains in set and stirred probiotic yogurt samples enriched by barberry extract.
Methods: Seedless barberry (Berberis vulgaris) extract was obtained through a reflux system and filtered in vacuum conditions. The probiotic stirred and set yogurts were produced using a standard yogurt manufacturing process. Then, different concentrations of barberry extract (4% and 5% w/w) were added to the probiotic yogurt samples over the duration of storage at 4C°. Direct colony counting was done by using an automatic colony counter. All the culture media were tested previously to ensure their selectivity for the specific microorganisms. Microbiological analyses were carried out in triplicate and the results were expressed as cfu/gr.
Results: The result represented that *L. acidophilus* and *B. bifidum* counts generally decreased during the storage period. Moreover, the durability of *L. acidophilus* in yogurt samples was significantly greater than the durability of *B. bifidum*during during 21 days of storage. In fact, the highest *L. acidophilus* count was related to stirred and set yogurt containing 5% barberry extract (1.15×108 and 1.1×108 cfu/gr, respectively) on the first day ($p<0.05$). In addition, the highest *B. bifidum* count was also recorded in set and stirred yogurt containing 5% extract of barberry ($p<0.05$) in monoculture and mixed yogurt samples on the first day.
Conclusion: The growth of *L. acidophilus* was substantially higher than the growth of *B. bifidum* by the barberry extract. Furthermore, the much higher survival was considered in stirred and set probiotic yoghurt containing 5% extract of barberry.

Keywords: Barberry Extract, *Bifidobacterium bifidum, Lactobacillus acidophilus*, Probiotic Yogurt

MOLECULAR DETECTION OF SALMONELLA SPP. IN RAW CHICKEN MEAT IN THE ISFAHAN CITY, Iran BY POLYMERASE CHAIN REACTION (PCR) TECHNIQUE

Faham Khamesipour[1], Meysam Khodadadi[2], Morteza Azhdary Mamoreh[2]
1.Young Researchers and Elite Club, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
2.Veterinary Medicine, College of Veterinary Medicine, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran

Background: *Salmonella* species are one of the primary foodborne pathogens that cause infections in both animals and humans, worldwide. Foods from animal Sources such as beef, poultry meat, egg and milk have been proved to carry these pathogens. This study was aimed at comparing polymerase chain reaction (PCR) and culture in detecting *Salmonella* spp. in raw chicken meat samples in Isfahan province, Iran.
Methods: From February 2013 to March 2013, a total of 50 samples of raw chicken meat were collected at Isfahan city, Iran. Samples were sent to the Biotechnology Research Center of Islamic Azad University of Shahrekord and Samples were tested by bacteriological and PCR tests.
Results: In this study, 1 of 50 raw chicken meat samples (2%) were positive for *Salmonella* by PCR method.
Conclusion: To establish the zoonotic significance of chicken meat *Salmonella*, isolates need to be future characterized and compared with those of human, and that consumption of undercooked or cooked contaminated poultry products presented a possible risk for consumers. Make more confident that the detection of thesebacteria can be used for rapid diagnosis of *Salmonella* infections in poultry and other hosts by molecular techniques.

Keywords: *Salmonella*, Chicken, Meat, Iran

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EFFECT OF DIFFERENT CONCENTRATION OF ZIZIPHUS HONEY ON GROWTH OF SOME FOODBORNE PATHOGENS

Maryam Ekhtelat[1], Karim Ravaji[2], Masood Parvari[2]
1.Department of Pharmacognosy, Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
2.Agricultural Jahad of Applied Science and Technology University of Ahvaz, Iran

Background: Honey exhibits antibacterial properties. The growth of bacteria is inhibited due to the low water activity, high acidity, and the hydrogen peroxide activity in honey. Also honey contains natural antioxidant properties. With regard to these properties,the present investigation was performed to determine the antibacterial activity of Ziziphus honey against *L.monocytogenes, S. typhimorium, E. coli* and *S. aureus*.
Methods: In the present research, a natural Ziziphus honey in 10%, 20%, 30% and 40% dilutions (v/v) provided in nutrient broth medium. Then about 106 CFU/ml of each of bacteria separately was added to experimental trials and incubated at 35 degree centigrade for 120 hours. Viable count enumeration of the sample was investigated after 0, 24, 72 and 120 hours post inoculation.
Results: The results showed that antibacterial activity of Ziziphus honey against *S. aureus* and *L.monocytogenes* was more than *S. typhimorium* and *E. coli*. In a comparative trial, antibacterial activity of Ziziphus honey was higher after 120 hours incubation for each four bacteria in most dilutions.
Conclusion: The statistical analysis by SPSS 16 showed no any significant difference between 24, 72 and 120 hours incubation on *S. aureus* in 40% dilution. Also it showed no any significant difference between 30% and 40% dilution on *Salmonella*. For the fourth bacteria, antibacterial activity was increased with addition concentration of honey. The microbial count showed 3 to 7/5 log reduction comparing with control after 120 hours. Therefore it is recommended that Ziziphus honey is used as a natural preservative, antioxidant and antibacterial agent.

Keywords: Antibacterial Activity, Ziziphus Honey, Foodborne Pathogens

COMPARISON OF THREE METHODS FOR EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF LACTOBACILLUS ACIDOPHILUS BACTERIA AGAINSTESCHERICHIA COLI AND SALMONELLA ENTERICA SEROTYPE ENTERITIDIS

Seyedeh Sara Shokraei[1], Nahid Rahimifard[2], Maryam Moslehi Shad[1]
1.Food science and Technology Dept., pharmaceutical Science Branch, Islamic Azad university Tehran- Iran
2.Associate Professor in Microbiology; Food and Drug Control Laboratories(FDCL); Food And Drug Laboratories Research Centre(FDLRC

Background: In this study, the comparison of three methods was accomplished for evaluation of the antimicrobial activity of *Lactobacillus acidophilus* bacteria against *Escherichia coli* and *Salmonella enterica* serotype enteritidis as common gastrointestinal pathogen agent.
Methods: In this study, 5 samples were collected from companies, Nestle probiotic milk (Nan2 and Nan2 H.A) and Milupa probiotic milk (Bebejunior and Bblak). Antimicrobial activities were determined using common bacteria causing gastroenteritis (*E. coli* ATCC 8739 and *Salmonella enterica* serotype enteritidis ATCC 13311) using three microbiological methods, disk diffusion agar, well diffusion agar and agar spot test.
Results: The maximum inhibitory effect on *E. coli*was achieved by well diffusion agar with an inhibitory zone diameter of 17.17 ± 1.64 mm and the minimum inhibitory effect on *Salmonella enterica* serotype enteritidis was achieved using Agar spot test with an inhibitory zone diameter of 5/33±1/64mm ($P < 0.05$). In this study, by comparing the three above mentioned methods, we found that the well diffusion agar method was the best method for the evaluation of antimicrobial effects of used probiotic bacteria.
Conclusion: The obtained results indicated that *Lactobacillus acidophilus* had a good antimicrobial activity against *E. coli* and *Salmonella enterica* serotype enteritidis. Possibly, probiotic bacteria, *Lactobacillus acidophilus* can prevent pediatric gastrointestinal *E. coli* and *Salmonella enterica* serotype enteritidis infections.

Keywords: Pathogens, *Lactobacillus acidophilus, E. coli,* Antimicrobial Activity, Probiotic.

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BEHAVIOR OF DIFFERENT STRAINS OF YERSINIA ENTEROCOLITICA DURING MANUFACTURE, RIPENING AND STORAGE OF IranIAN ULTRA-FILTERED WHITE CHEESE

Elham Abdollahi[1], Shahram Hanifian[1]
1.Department of Food Science and Technology, College of Agriculture, Tabriz Branch, Islamic Azad University, Tabriz, Iran

Background: This study aimed to evaluate the behavior of different strains of *Y. enterocolitica* during manufacture, ripening and storage of ultra-filtered white cheese.
Methods: Three strains of *Y. enterocolitica* (PTCC 1480, PTCC 1478 and PTCC 1151) with approximate inoculum level of 3-4 log cfu/g were inoculated in pasteurized retentate (72 °C for 15 s). Cheese samples manufactured according to the standard protocol were used for the preparation of Iranian ultra-filtered white cheese. The variation of *Y. enterocolitica* number was monitored at the time of inoculation (0 h), after incubation (27 °C for 24 h) and throughout the 60 days of storage at 8°C. The number of *Y. enterocolitica* in cheese samples were enumerated by means of surface-plating on CIN agar supplemented with *Yersinia* selective antibiotics. Enumerated colonies were confirmed by PCR targeting ail gene.
Results: Data suggested that the number of *Y. enterocolitica* in all three strains was significantly increased after incubation time. However, during storage period the number of *Y. enterocolitica* was decreased. Decreasing rate for the strains of PTCC 1478 and PTCC 1151 was significant. Meanwhile, high load of PTCC 1480 strain of *Y. enterocolitica* was detected at the end of storage period.
Conclusion: According to the results, various strains of *Y. enterocolitica* showed different behavior in the same environmental condition. In addition, ultra-filtered white cheese supported the growth and survival of *Y. enterocolitica*. Activity of starter culture did not entirely eliminate *Y. enterocolitica* during the 60-days of storage period. It seems that short incubation period cannot provide sufficient time for the proper activity of lactic starter bacteria and consequently, incapable to eradicate *Y. enterocolitica*.

Keywords: *Yersinia enterocolitica*, Ultra-Filtered Cheese, Ail-PCR

MICROBIOLOGY OF IranIAN PROBIOTIC WHITE CHEESE MADE WITH DIFFERENT FAT CONTENTS

Nasin Kheshti[1], Roghieh Ashrafi[2]
1.Department Of Food Science And Technology,Faculty Of Agriculture,Tabriz University,Iran
2.Department Of Food Science And Technology,Technical And Vocational University Of Iran

Background: Iranian White cheeses were produced in three different fat content with Freeze-dried mixed mesophilic-thermophilic culture (blend of *Lactococcus lactis* subsp. Lactis strain 227 and Lc. lactis subsp. cremoris strains 223) and probiotic strains(Lb. acidophilus 4962, Lb. casei 279, B. lactis LAFTIs B94) to study the survival of the starter and probiotic during ripening period of 2 months at 6 ◦C.
Methods: Data analysis was carried out with Minitab Statistical Package. One-way analysis of variance was used to establish differences between means, with a significant level at a ¼ 0: 05. A total of 21 batches of cheeses were produced from the seven variations and all analyses were carried out at least in duplicate.
Results: Enumeration of starter and Probiotics in experimental cheeses showed that all samples contained approximately 1 × 109 starter cfu/g 1 and 14 day after manufacture.After 1 mo, starter numbers were still greater than 1 × 108 cfu/g in SFC and LFC cheeses. Starter viability declined in SFC and LFC versus FFC cheese were also noted in 1-mo-old samples.By 2 mo, starter numbers in most experimental cheeses could not be quantified because they had fallen to levels below those of the probiotic population. Differences were also noted in levels of probiotics in FFC varieties versus SFC and LFC cheeses. Probiotic populations in FFC cheese rarely exceeded 1 × 103 cfu/g within first day of producing, but were rarely below 1 × 105 cfu/g in corresponding SFC and LFC cheeses. Moreover, probiotic levels exceeded 1 × 107 cfu/g in LFC samples by 14 day of ripening, whereas only a few FFC cheeses contained more than 1 × 106 probiotic cfu/g after even 1 to 2 months of aging (Table 2). The probiotic populations in SFC cheese during ripening were essentially intermediate to those of their FFC and LFC experimental counterparts.
Conclusion: We found that all probiotic adjuncts survived the cheese-making process at a high level without alteration of cheese-making process.

Keywords: *Lactococcus lactis*, Probiotic Strains

QUANTITY AND DIVERSITY OF AEROBIC SPORE-FORMING BACILLI IN RAW MILK AND INDUSTRIAL DAIRY PRODUCTS IN EAST-AZERBAIJAN PROVINCE

Maryam Jabbarpour Sattar[1], Jalal Shayegh[2]
1.Department of Microbiology, College of Dentistry, Borojerd Branch, Islamic Azad University, Borojerd, Iran
2.Department of Veterinary Medicine, Shabestar Branch, Islamic Azad University, Shabestar, Iran.

Background: The aim of this study was to evaluate the load and diversity of spore-forming bacilli in bulk raw milk and industrial dairy products in East-Azerbaijan province.
Methods: Samples were heat-treated at 80°C for 30 min. Afterwards, the samples were serially diluted and cultured on Tryptic Soy Agar plates with 1% glucose. The plates were incubated aerobically at 37°C and 55°C for 24-48 h. Based on colony morphology, several colonies were selected and subjected to various biochemical examinations.
Results: According to the results, mean number of mesophilic and thermophilic spore-formers in bulk raw milks was estimated at 11.43 and 3.84 log cfu/ml, respectively. Meanwhile, the number of mesophilic and thermophilic spore-formers in heat-treated dairy products was ranged from 6.11 to 9.70 and 2.75 to 4.95 log cfu/ml or g, respectively. A variety of *Bacillus* species including *B. cereus*, *B. macerans*, *B. pantothenticus, B. stearothermophilus, B. badius, B. amyloliquefaciens* and *B. laterosporus* were identified.
Conclusion: It was concluded that environmental contamination of raw milk could be the major reason for the presence of high loads of spore-formers in raw milk. In addition, occurrence of different species of spore-forming bacilli in heat-treated dairy products could be considered as a potential health threat since some of these species are able to produce toxins.

Keywords: Spore-Formers, *Bacillus* Spp., Raw Milk, Dairy Products

DETECTION OF ESCHERICHIA COLI O157: H7 IN MILK BY DIRECT PCR

Naghmeh Moori Bakhtiari[1], Saad Gooraninejad[1], Mehdi Zareid[1], Maryam Farahmandfar[2]
1.Department Of Microbiology, Faculty Of Veterinary Medicine, Shahid Chamran University Of Ahvaz, Iran
1.Graguated Student Of Veterinary Faculty, Shahid Chamran University Of Ahvaz, Iran

Background: *Escherichia coli* O157 is the most common member of a group of pathogenic *E. coli* strains known variously as enterohaemorrhagic or Shiga-toxin-producing organisms. Domestic and wild ruminants are regarded as the main natural reservoirs. In developed countries, serotype O157: H7 represents the major cause of human gastrointestinal infections. The present study was conducted to investigate the presence of *E. coli* O157: H7 strains and the presence of virulence genes (*stx1, stx2*), in isolates derived from collected milk of dairy industry.
Methods: A total number of 150 milk samples were collected from dairy industry in Khuzestan, over a period of 6 months and were evaluated by cultivation in pre-enrich media tryptic soy broth contain of novobiocin and direct PCR on it.
Results: According to direct PCR on milk samples, 45 samples were containing of different genomic patterns based on investigated genes (*O157, H7, stx1, stx2*).With direct PCR only 2 of 150 milk samples were positive for the presence of O157: H7 which were toxigenic.
Conclusion: *E. coli* O157: H7 presents in this region and so the necessity for strict compliance of health standards is recommended in province.

Keywords: Milk, *Escherichia coli* O157: H7, Polymerase Chain Reaction

MOLECULAR FINGERPRINTING AS A TOOL FOR SURVEILLANCE STUDY OF FOODBORNE INFECTIONS IN HOSPITAL SETTINGS

Fahimeh sadat Ghoalm Mostafaei[1], Parisa Torabi[1], Masoud Alebouyeh[1], Mohammad Reza Zali[1]
1.Gastroenterology and Liver Diseases Research Center Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Background: Hospital acquired infections are still a major healthcare problem in the world. Hospitals have been identified as high food safety risk institutions because they serve potentially hazardous foods to vulnerable people. The aim of this study was to investigate role of hospital foods in transition ofand *S. aureus* strains into the hospital environment.
Methods: A total of 322 samples from hospital's personnel, patients' clinical and environmental samples, and 210 samples from the hospital kitchen, including raw and cooked foods, food handlers and food processing devices were collected in a hospital in Tehran- Iran. Standard swab and food sampling methods were used in each case. Colony count of each culture and biochemical or molecular identification of each isolate was determined according to standard methods. Antimicrobial susceptibility of each bacterial isolate was determined according to the latest clinical laboratory standard guideline. RAPD-PCR molecular typing method was used as a biotyping method to analyze genetics convergence among the clinical and food isolates. Gelcompare II software was used for analysis of the RAPD patterns. Possible correlation between the strains from different samples was detected by comparing both RAPD patterns and resistance profiles.
Results: Among the studied samples, *S. aureus* showed the highest frequency among the obtained isolates from the hospital kitchen (16%), while *E. coli* represent 8% of the contaminations. Frequency of *S. aureus* and *E. coli* was 8.7% and 1.55% among the samples from the intensive care unit, respectively. Close relationship was seen among resistance patterns of some *S. aureus* and *E. coli* isolates from the clinical and food related samples. Methicillin resistant *S. aureus* (18.7%) and multidrug resistant *E. coli* (5.8%) were detected among the samples from hospital kitchen. Similar RAPD patterns were seen among *E. coli* isolates from clinical samples, utensils, kitchen environment and food products.
Conclusion: Outbreaks of foodborne disease in hospitals are reporting in developing and developed countries. Frequency of clinically important bacteria in studied hospital foods and their processing units emphasis control of their dissemination into hospital environment.

Keywords: Hospital Acquired Infections, Foodborne Disease

THE INFLUENCE OF MICROCAPSULATION ON SURVIVAL INCREMENT OF PROBIOTIC BACTERIA IN DAIRY PRODUCTS

Ali Heshmati[1], Aliasghar Vahidinia[1], Iraj Khodadai[1]
1.Nutrition Department, Medicine Faculty, Hamadan University of Medical Sciences And Health Services, Hamadan, Iran.

Background: The goal of this research was to investigate if microcapsulating probiotic bacteria could enhances their viability in Iranian white cheese and yoghurt
Methods: Probiotic bacteria (*Lactobacillus acidophilus* and *Bifidobacterium*) were encapsulated in alginate to study the effect of encapsulation on their survival in yogurt and cheese. Yogurt and Iranian white cheese were manufactured according industrial procedure and incorporating encapsulated and free probiotic bacteria. The survival and the effect of encapsulated probiotic bacteria on the growth of starter lactic acid bacteria were assessed during storage of yogurt (21 days) and cheese (6 months).
Results: We observed probiotic bacteria decrease during storage. Results showed that microcapsulated probiotic bacteria count (107 cfu/g in cheese and 106 cfu/g in yoghurt) was significantly ($P<0.05$) greater than that of free bacteria (106 cfu/g in cheese and 104 cfu/g) at the end of shelf life. Also, addition of encapsulated probiotic bacteria did not change the population of starter lactic acid bacteria
Conclusion: Microcapsulation of various bacterial cultures including probiotics has been a common practice for extending their storage life and converting them into a powder form for ease of their use.

Keywords: Probiotc, Micocapsulation, Alginat

OCCURRENCE AND DIVERSITY OF MOLD SPECIES IN RAW MILK AND DAIRY PRODUCTS

Tahereh Moshtaghi Maleki[1], Shahram Hanifian[2], Jalal Shayegh[3]
1.Department of Microbiology, College of Biology, Ahar Branch, Islamic Azad University, Ahar, Iran
2.Department of Food Science and Technology, College of Agriculture, Tabriz Branch, Islamic Azad University, Tabriz, Iran.
3.Department of Veterinary Medicine, Shabestar Branch, Islamic Azad University, Shabestar, Iran

Background: This study aimed to investigate the occurrence and diversity of mold species in bulk-raw milks and different dairy products in East-Azerbaijan province.
Methods: A total sample of 105 milk and dairy products were analyzed using conventional culture methods. After preparation of sample suspensions/emulsions, they were cultured on Saboraud Dextrose agar and incubated at ~25°C for 1–2 weeks. In order to identify the mold species, the isolates were examined using slide-culture method followed by microscopic observation.
Results: According to the results, mold contamination was determined in 92% (46/50) of bulk-raw milks. Mold species were identified as *Aspergillus* spp., *Penicillium, Geotrichum, Alternaria* and *Fusarium*. In the case of dairy products, the contamination was found in 24% (12/55) of the samples. The most isolated species were *Aspergillus, Penicillium, Mucor* and *Scopulariopsis*.
Conclusion: Results suggested that milk production and collection in this area was performed in non-hygienic conditions since almost all of the bulk-milk samples were contaminated with various mold species. It seems that hand-milking rather than using milking machines as well as using non-hygienic utensils for the storage of bulk milks was resulted in environmental contamination. Presence of molds in heat-treated dairy products might be due to the thermo-resistance nature of the isolates and/or cross contamination through the dairy plants environment.

Keywords: Mold Diversity, Bulk-Raw Milk, Pasteurized Milk, Dairy Products

DEVELOPMENT OF NESTED-PCR METHOD FOR DETECTING COXIELLA BURNETII IN GOAT MILK SAMPLES

Jaydari Amin[1], Khademi peyman[2], Lorestani Samira[3], Maleki Shahram[4]
1.Department Of Microbiology,Faculty Of Veterinary Medicine, Lorestan University, Khorramabad, Iran
2.Master Of Science In Bacteriology, Faculty Of Veterinary Medicine, Shahrekord University, Shahrekord, Iran
3.Bacteriology, Faculty Of Veterinary Medicine, Lorestan University,Khorramabad, Iran
4.Department Of Large Animal Medicine, Faculty Of Veterinary Medicine, Lorestan University, Khorramabad, Iran

Background: This study was conducted to determine the seasonal prevalence rate of *Coxiella burentii* in raw samples obtained from different goats in Khoramabad.
Methods: In this cross-sectional study (from January 2013 to May 2014), 51 Goat milk samples were collected random. These samples were tested for the presence of *Coxiella burnetii* by Nested PCR method.
Results: In this study, from a total of 51 Goat milk samples, 21 samples (41/17%) were found to be positive for the presence of *Coxiella burnetii*.The prevalence of *C.burnetii* varied during different seasons.The highest incidence of *C. burnetii* observed in winter (11.11%).
Conclusion: The season of sample collecting affects the amount of bacteria excerted, and that goat milk can be one of the potential sources of *Coxiella burnetii* in Iran.

Keywords: Q Fever, *Coxiella burnetii*, Nested- PCR, Goat Milk

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PREVALENCE OF BACTERIAL ISOLATES FROM MEAT AND MEAT PRODUCTS IN ILAM CITY

Reza Mohebi [1], Sobhan Ghafurian[1], Aref Shavalipour[1], Nabi Karimikhah[1], Shahab Mansori[1]
1.Clinical Microbiology Research Center, Ilam University of Medical Sciences, Ilam

Background: The aim of this study was to evaluatethe drug susceptibility pattern of bacteria isolated from raw meat in Ilam city, Iran.
Methods: Total 30 samples (raw meat and meat products) were collected during 2013 collected from butchers and slaughterhouse of Ilam city. Of 20 g of each sample was placed in a sterile bag and placed in Cool Box with 4 ° C and then were transported to the laboratory. The samples were cultured immediately and then biochemical and serological survey was conducted for detection of any bacteria. Finally drug susceptibility testing was performed for all samples.
Results: The most important isolated bacteria were *E. coli* O157 H7 3.3%,other serotypes of *E. coli* 24.47%,*Pseudomonas aeruginosa* 3.47%,*Proteus mirabilis* 3.47%,*Klebsiella pneumoniae* 1.47%, *Morganella morganii* 4.47% and *Serratia marcescens* 3.47% respectively. The most resistances bacteria including *E. coli* were resistant to Co-amoxiclav (88%) and Imipenem (33%). All *Klebsiella pneumoniae* isolates were resistant to Co-amoxiclav. *E. coli* O157 H7 was susceptible to all of the tested antibiotics.
Conclusion: The findings of the current study report a important prevalence (3.3%) of *E. coli* O157: H7 in meat and meat products. It must be noted that the presence of these pathogens in foods meant for human consumption is of great concern owing to the very low infectivity dose (10–100 cells) of *E. coli* O157: H7. On the other hand, the resistance of the isolated bacteria such *E. coli* and *Klebsiella* was considerable.

Keywords: Meat And Meat Products, *E. coli* O157: H7, Foodborne Illness

PREVALENCE OF COAGULASE POSITIVE PATHOGENIC STAPHYLOCOCCUS AUREUS IN MAYONNAISE SAUCE

Behnam Rafiee[1], Sepideh Ghani[2], Davoud Sadeghi [3], Safieh Sofian [4], Ali Rezaei[5], Nader Mosavari[6]
1.Department of Biology, Payam-e-Noor University, Arak, Iran
2.Young Researchers Club and Elite, Arak Branch, Islamic Azad University, Arak, Iran
3.Department of Biology, faculty of basic science, Imam hosein University, Tehran, Iran
4.Department of Biology, Payam-e-Noor University, Arak, Iran
5.Department of Biology, Arak University, Arak, Iran
6.Razi Vaccine and Serum Research Institute, Karaj, Iran

Background: This study was done to determine the prevalence of coagulase positive pathogenic *Staphylococcus aureus* strains isolated from mayonnaise sauce and drug sensitivity pattern.
Methods: In this experimental study, we used of 120 packets (sachet) of mayonnaise sauce and enriched samples were streaked on Baird Parker Agar (BPA) and the plate was incubated at 37 °C for 24–48 hrs. Appearance of jet black colonies surrounded by a white halo were considered to be presumptive *Staphylococcus aureus* and were further analyzed by gram's reaction and biochemical tests (catalase test, oxidase test, indole, methyl red, voges-Proskauer, nitrate reduction, coagulase test) and*S.aureus* was confirmed by the coagulase test. Then samples were examined and antibiogram was performed by disc diffusion method on Mueller Hinton agar with 12 antibiotics
Results: We identified 42 coagulase positive *Staphylococcus aureus* strains from mayonnaise sauce packets. Antibiotic sensitivity testing revealed that 40 (95.2%)of isolates were sensitive to vancomycin, 36 (85.5%) and 33 (78.5%) were resistant to penicillin and cotrimoxazole, respectively. Our result showed that 12 (28%) of strains were resistant at 10 antibiotics.
Conclusion: This study showed that the prevalence of coagulase positive *Staphylococcus aureus*in mayonnaise sauce was 35% of samples, and the highest sensitivity was toward to vancomycin. It seems the health standards of workers in sauce factories do not follow and probably the *Staphylococcus aureus* entering by the mouth and nose secretions or skin ulcer workers to mayonnaise sauce.

Keywords: Antibiotic, Coagulase, Mayonnaise Sauce, *Staphylococcus aureus*

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INHIBITORY EFFECTS OF GARLIC EXTRACT ON THE GROWTH OF STAPHYLOCOCCUS AUREUS AND YERSINIA ENTEROCOLITICA

Seyedeh Sara Shokraei[1], Seyedeh Rezvan Shokraei[2]
1. Food Science And Technology Dept., Pharmaceutical Science Branch, Islamic Azad University Tehran- Iran.
2. Zenetic Dept., Branch Islamic Azad University Zanjan- Iran.

Background: Nowadays, plant extracts as antimicrobial additives have an important role in maintaining the quality of the food products. Previous studies revealed that garlic has antimicrobial effects Therefore, in this study the effect of different concentrations of garlic extracts and powder against pathogenic bacteria *Staphylococcus aureus* and *Yersinia enterocolitica*has been evaluated.
Methods: In this research, fresh garlic from Hamadan and the garlic tablet prepared from Exir pharmaceutical company were tested. The minimum inhibitory concentration and minimum concentration of microbial from the extract and powder of garlic on the growth of two microorganisms were measured using standard methods like tube.
Results: The minimum inhibitory concentration (MIC) of the extract of garlic powder against the microorganisms tested was about 20 mgmL-1 and the MBC about 40 mgmL-1was obtained.While the MIC of garlic extract on the growth of the microorganism was about 30 mg/dl and the MBC was about 60 mg/dl, respectively.
Conclusion: Based on the results,garlic extract inhibits the growth of both bacteria, but the minimum inhibitory concentration of garlic powder, were much stronger than the garlic tablet (1.5 times).The finding revealed the biopreservative role of garlic extract to prevent microbial contamination and pathogenic microorganisms.

Keywords: Garlic Extract, Minimum Inhibitory Concentration, Antimicrobial Activity

STUDY OF ANTIBIOTIC RESISTANCE IN SALMONELLA SPP. ISOLATED FROM TURKEYS FARMED IN ZABOL

Seyede Maryam Jafari[1], Mohammad Jahantigh[2], Ahmad Rashki[3], Saeed Salari[3]
1.Veterinary Medicine, Faculty of Veterinary Medicine, University of Zabol, Iran
2.Department of Clinical Science, Faculty of Veterinary Medicine, University of Zabol, Iran
3.Department of Pathobiology, Faculty of Veterinary Medicine, University of Zabol, Iran

Background: *Salmonella* is a gram negative bacteria, caused problematic disease in human and poultry and could be considered as a food born pathogen. The detection of antibiotic resistance patterns among *Salmonella* is a serious global concern. The objective of the presented work was to study the antibiotic resistance of *Salmonella* isolated from turkey farms in Zabol.
Methods: Antibiotic resistance phenotypes of 37 *Salmonella* isolates of turkey origin, farmed in Zabol, were investigated. All samples were assessed by culture method and standard biochemical tests for identification of *Salmonella* strains. The sensitivity of the *Salmonella* isolates was tested towards: Lincospectin, Gentamicin, Chloramphenicol, Tetracyclin, Cephalexin, Streptomycin, Trimethoprim-Sulfamethoxazole, Furazolidone, Ciprofloxacin, and Colistin according to Bauer-Kirby method. Interpretation of the results was done according to CLSI guidelines M100-S17.
Results: All isolates were resistant to Cephalexin (100%). The most common resistant phenotypes belonged to Colistin (89.18%), Tetracyclin (86.48%), and Furazolidone (72.97%). Most isolates were sensitive to Gentamicin (86.48 %). Sensitivity of isolates to Ciprofloxacin and Streptomycin was found 83.78% and 40.54%, respectively.
Conclusion: Antibiotic resistance can be resulted from mutations and acquisition of resistance encoding genes. Proper administrations of antibiotics lead to decrease the side effects and treatment period of antibiotic therapy. It is achievable by knowing about the antibiotic resistance pattern in a special area and could lead to minimize the spreading of antibiotic resistance genes.

Keywords: *Salmonella*, Antibiotic Resistance, Zabol, Turkey

ANTIBACTERIAL ACTIVITY OF FOUR ESSENTIAL OIL COMPONENTS ON ERWINIA CAROTOVORA

Sepideh Abbaszadeh[1], Aghil Sharifzadeh[2], Mahboube Bagheri[3], Narjes Cheraghi[3]
1.Department Of Nutrition And Food Hygiene, Health School, Baqiyatallah University Of Medical Sciences, Tehran, Iran
2.Mycology Research Center, Faculty Of Veterinary Medicine,University Of Tehran,Tehran, Iran
3.Department Of Food Hygiene, Faculty Of Veterinary Medicine,University Of Tehran,Tehran, Iran

Background: Antibacterial activity of four essential oil components including thymol, carvacrol, eugenol and menthol has been evaluated towards the vegetable spoilage bacterium, *Erwinia carotovora*.
Methods: The above mentioned compounds were screened for antibacterial activity using micro broth dilution assay and disc diffusion method against *Erwinia carotovora* isolated from vegetables. The minimum inhibitory concentration (MIC) values of these components were determined by micro broth dilution assay using a 96-well microtitre plate and MICs were defined as the lowest concentration of the compound that inhibits bacterial growth after 24-48 hours. For the disk diffusion method, coated discs were positioned in the center of inoculated agar plate and were incubated at 35±2°C for 24-48 hours; the inhibition zone diameter surrounding the disc was measured in millimeters. Each component was assayed in duplicate.
Results: The MIC value of thymol was the lowest (75 ppm) showing its high potential of bioactivity while eugenol MIC value was the highest (125 ppm) and possessed the weakest effect. The mean inhibitory zone diameters have been reported 6.50, 6.83, 5.83 and 7.17 millimeters for thymol, carvacrol, eugenol and menthol respectively.
Conclusion: All applied EO individual components, especially thymol and menthol represented strong antibacterial efficacy, having a great potential for application as natural antimicrobial agents in food preservation.

Keywords: *Erwinia carotovora*, Thymol, Carvacrol, Eugenol And Menthol

PREVALENCE OF HELICOBACTER PYLORI IN BUFFALO MILK IN Iran

Hamidreza Kazemeini[1], Ebrahim Rahimi[2], Fariborz Kianpour[3]
1.Department of Food Hygiene and quality Control, Shahrekord Universiy, Shahrekord, Iran
2. Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.
3.Clinical Microbiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.

Background: *Helicobacter pylori* infection in human is one of the most common infections worldwide. However, the origin and transmission of this bacterium has not been clearly explained. One of the suggested theories is transmission via raw milk from animals to human beings. This study was conducted to determine the prevalence rate of *H. pylori* in bulk milk samples from dairy buffalo herds in Iran.
Methods: In the present study, 210 bulk milk samples from 71 dairy buffalo herds were collected and tested for *H. pylori* by cultural method and polymerase chain reaction for the detection of the *ureC* gene.
Results: Using cultural method, any of 210 milk samples were found to be contaminated with *H. pylori*. *H. pylori ureC* gene was detected in 24 (11.4%) of milk samples.
Conclusion: Using PCR method, there were significant differences ($P < 0.05$) in the level of contamination with *H. pylori* between milk samples collected from different provinces.

Keywords: Buffalo, *Helicobacter pylori*, Milk

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MICROBIAL ANALYSIS OF VEGETABLES IN Iran DURING PAST 5 YEARS

Hossein Dabiri[1], Mahdane Roshani[1], Neda Yousefi[1], Mohsen Heidary [1], Saeed Khoshnood[1]
1.Department of Medical Microbiology, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: We aimed the Meta analysis study on prevalence of microbes transmitted by vegetables in Iran during 5 past years.
Methods: In the current Meta-analysis study, by using keywords such as Iran, vegetable, microbial contamination, and using search engines SID, Iranmedex, Web of Science, PubMed and Google scholar. We did comprehensive data collection. All articles with no row data were excluded. According to inclusion, criteria out of 24 articles18 studies data were analyzed by using Meta analysis software.
Results: In this systematic review, of 30 studies, totally 20 articles were included based on inclusion criteria which all had been performed during 2008 to 2013 in Iran. Overall 3408 Samples had been studied in these studies in which *Trichinella, Trichostrongylus, Ascaris, Taenia, Entamoeba coli, Giardia Free-living larvae, Escherichia coli,Echinococcus, Staphylococcus aureus,* and *Salmonella* were isolated. Forty seven percent of all studied vegetables in Iran was contaminated. Regardless to type of vegetable Entamoeba(12.6%) was the prodominat parsite which followed by *Giardia* (8.5%), Taenia (5.5%) Ascaris(3.3%). The lowest rate of contamination was contributed to the bacteria such as *E. coli* and *Salmonella* spp with less than one percent of all vegetable. Lettus and radish was the most contaminated vegetables using in Iran
Conclusion: Based on our Meta analysis results in Iran, contamination rate of vegetables in Iran was high (47%) and parasites were more likely to contaminate vegetable compare to bacteria. Regard to the fact that entamoba and *Giardia* were the most frequent parasites found in vegetables in Iran during past 5 years, thereby the gastroenteritis in summer due to consumption of vegetables may relates to parasite rather than bacteria.Using guideline for vegetable sanitation and food safety is highly recommend for prevention of food borne diseases due to the consumption of contaminated vegetables.

Keywords: Vegetable,*Giardia*, Entamoeba,Ascaris

PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF ANAEROBIC BACTERIA ISOLATED FROM SURGICAL SITE INFECTIONS

Mohammad Yousef Memar[1], Mohammad Taghi Akhi[1], Naser Alizadeh[1], Vida Sorayaei Sowmesarayi[1], Abed Zahedi Bialvaee [1]
1.Department Of Bacteriology And Virology, School Of Medicine, Tabriz University Of Medical Sciences, Iran

Background: The aim of this study was to investigate anaerobic bacteria prevalence in SSIs and determination of antibiotic susceptibility pattern in these isolates.
Methods: Between October 2012 and July 2012, 100 surgical site infections obtained by needle aspiration of purulent material in depth of infected site. These specimens were processed for detection of anaerobe bacteria in bacteriology lab. For isolation of anaerobic organisms pre-reduced vitamin K enriched *Brucella* blood agar, Bacteroides Bile Esculin (BBE) agar, kanamycin Vancomycin lacked blood(KVLB) and phenyl ethyl alcohol(PEA) agar were inoculated and incubated under anaerobic condition implicated by Anoxomat system at 370 C and examined at 72 and 96h. For antimicrobial drug susceptibility assay in gram negative anaerobic bacteria isolated from these infections, the minimum inhibitory concentration (MIC) of Imipenem, Chloramphenicol, metronidazole, Clindamycin, Cefoxitin, and penicillin G was determined by the agar dilution method. MIC of penicillin, metronidazole, Clindamycin, Cefoxitin for gram positive anaerobic bacteria were determined by E-test strip according to CLSI guideline for anaerobic susceptibility testing.
Results: In this study, *Bacteroides fragilis*was isolated from 28% of specimens and *Clostridium perfringens*was isolated from 2% of specimens. *Bacteroides fragilis* isolates were 100% resistant to penicillin followed by Clindamycin (46.2), Cefoxitin (38.5), Chloramphenicol (30.8), Metronidazole (30.8) and Imipenem (7.6). All *Clostridium perfringens* isolated in this study were susceptible to penicillin, Cefoxitin, Clindamycin and Metronidazole.
Conclusion: *Bacteroides fragilis* was predominant anaerobic bacteria isolated from SSIs. *Bacteroides fragilis* have shown tendency to development resistance to antibiotic drugs. Presence of antibiotic resistance anaerobic bacteria in SSIs can causes failure in antibiotic therapy.

Keywords: Anaerobic Bacteria, Surgical Site Infections, *Bacteroides fragilis, Clostridium perfringens*,

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THE PREVALENCE OF TEM-1, SHV-1 GENES AND THE PATTERNS OF SUSCEPTIBILITY ANTIBIOTIC IN ESBL- PRODUCING KLEBSIELLA PNEUMONIA OF CLINICAL ISOLATES OBTAINED FROM UNIVERSITY HOSPITALS OF KERMAN, 2011

Maryam Afzali[1], Mojtaba Shahin[1], Abbas Farahani[1]
1.Dept. of Microbiology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Background: The aim of this study was to determine the prevalence of SHV-1 and TEM-1 genes and the patterns of antibiotic susceptibility among *K. pneumoniae* ESBL producing isolated from clinical samples obtained from university hospitals of Kerman city 2011.
Methods: Totally 110 isolates were identified as*K. pneumonia* from clinical samples hospitals. *K. pneumonia* was identified using standard Methods. The ESBL status was determined by double disk diffusion test (DDDT). The DNA extraction was taken by boiling and PCR detected the samples producing ESBL. The susceptibility of the isolates to 11 different antibiotics was examined by disk diffusion test.
Results: The maximum antibiotic resistances were recorded against Cefalotin (52.7%) and maximum antibiotic susceptivity were recorded aginst Imipenem (99.1%). Among 110 isolates, 44 isolates (40%) were found ESBL positive by DDDT. From 44 isolates ESBL positive, 38 isolates were positive for SHV-1 and TEM-1.27.2% had SHV-1 gene alone, 9.1% had TEM-1 gene alone and 50% had both of TEM-1 and SHV-1 genes. 13/6%isolates did not show either TEM-1 or SHV-1 genes.
Conclusion: The emergence and spread of 40% of ESBL *K. pneumonia*in the study is worrisome and usage of cephalosporins against these isolates is in effective. Therefore, it is necessary to have continuous control of *K. pneumonia*, which produces ESBL in hospitals and community.

Keywords: *Klebsiella pneumonia*, ESBL, SHV-1 Gene, TEM-1 Gene

MOLECULAR DIAGNOSIS OF SHIGELLA SONNEI ISOLATES WITH WBZG GENE IN STOOL SAMPLE OF CHILDREN WITH DIARRHEA IN TEHRAN

Nasim Afshari abarghan[1], BitaBakhshi[2]
1.Science & Research Azad Islamic University,Department of Microbiology,Saveh,Iran.(MSC
2.Tarbiat Modares University, Faculty of Medical Sciences, Department of Bacteriology, Tehran, Iran.(PHD)

Background: The objective of this study was to identify *Shigella sonnei*among isolates by means of Polymerase Chain Reaction (PCR) with specific gene *wbzg* and serology method. *Shigella* spp.wre isolated from stool samples of patients with diarrhea.
Methods: During summer and autumn of 2012, 160 *Shigella* spp. isolates were collected from children under 10 years old with diarrhea and clinical signs such as fever and cramp and weakness from 2 major hospitals in Tehran, Iran. The presence of *ipaH* gene was investigated by PCR method with specific pairs of primers and serogrouping of isolates was done by monospecific antisera.Then primer oligonucleotides specific for *Shigella sonnei*(wbzg) was used for distinguishing one of the most commonly found *Shigella* species.
Results: Among 160 isolates having the pathogen gene *ipaH*, 50 isolate were positive for *wbgZ* gene indicative of *Shigella sonnei*. All of the 50 isolates confirmed by PCR method were identified as *Shigellasonnei* by serogrouping.
Conclusion: In conclusion, results of this study introduce the PCR amplification of *ipaH* as a promising method for identification of *Shigella* spp..Comparing PCR method for detecting *wbgZ* gene for identification of *Shigella sonnei* with serogrouping method, the former one has the same or even better results.

Keywords: *Shigella sonnei*, PCR, Wbzg

STUDY OF FREQUENCY OF LUXS GENE IN CLINICAL ISOLATES OF KLEBSIELLA PNEUMONIA

Taban Hashemi[1], Jafar Hemmat[2], Nourkhoda Sadeghifard[3]
1.Department of Biology, Faculty of Sciences, Science and Research Branch, Islamic Azad University, neyshabour, Iran
2.Department of Biotechnology, Iranian Research Organization for Science and Technology (IROST), P.O. Box 15815-3538, Tehran, I.R. Iran
3.Clinical Microbiology Research Center, Ilam University of Medical Sciences, Ilam 693917134, Iran

Background: In this study, the frequency of *luxS* gene in clinical isolates of *Klebsiella pneumoniae* was investigated.
Methods: In this study, 80 clinical isolates of *Klebsiella pneumonia* were isolated from in hospital samples from Shirvan, in North Khorasan and Ilam providences and identified by biochemical tests such as TSI, SIM, Simmons citrate and urea.
Results: 100% of clinical isolates of *Klebsiella* have *luxS* gene and consequently the quorum-sensing mechanisms are involved in the regulation of different factors involved in virulence and biofilm formation.
Conclusion: According to 100% frequency of *luxS* genes in clinical samples it can be a important target for synthesis of new antibiotics.

Keywords: *Klebsiella pneumoniae, Luxs Gene*

ANTIBIOTIC RESISTANCE PATTERNS OF KLEBSIELLA PNEUMONIAE FROM PATIENTS IN GHAEM HOSPITAL, MASHHAD UNIVERSITY OF MEDICAL SCIENCES

Farzad Khademi[1], Arshid Yousefi [1], Najmeh Ansari[1]
1.Mashhad University of Medical Sciences, Mashhad, Iran

Background: *Klebsiella pneumoniae* is gram-negativebacilli, non-motile and with capsule. These bacteria are important human pathogens in hospital infections and intrinsically resistant to many antimicrobial agents.The purpose of this study was the determination of antibiotic resistance patterns of *Klebsiella pneumonia* isolated from patients in Ghaem hospital.
Methods: A total of 274 isolates of *Klebsiella pneumoniawere* collected fromMashhad University of Medical Sciences Ghaemteaching hospitalfrom in 2013. MDDM(Modified disk diffusion method) was then used to detect the susceptibility of the isolates to amoxicillin, nalidixic acid, ceftizoxime, ceftazidime,cefotaxime, ceftriaxone, ciprofloxacin, imipenem, gentamicin and nitrofurantoin.
Results: In this study, 175 specimens were urine and others 99 specimens were included: blood, CSF, wound andascitic fluid.*Klebsiella pneumoniae* isolates were resistance to ciprofloxacin,46.71%, imipenem, 56.9%, nitrofurantoin, 39.3% and gentamicin, 37%.In this study these antibiotics were most effective.
Conclusion: The results of this study indicate that significant percentage of *Klebsiella pneumonia* strains have shown various antibiotics resistance.Therefore, more research is needed on the use of new drugs.

Keywords: *Klebsiella pneumonia*, Antibiotic Resistance, Modified Disk Diffusion Method

STUDY OF THE ABILITY OF BIOFILM FORMATION OF ISOLATED KLEBSIELLA FROM TWO GEOGRAPHIC REGIONS OF Iran

Taban Hashemi[1], Jafar Hemmat[2], Nourkhoda Sadeghifard[3]
1.Department of Biology, Faculty of Sciences, Science and Research Branch, Islamic Azad University, neyshabour, Iran
2.Department of Biotechnology, Iranian Research Organization for Science and Technology (IROST), P.O. Box 15815-3538, Tehran, I.R. Iran
3.Clinical Microbiology Research Center, Ilam University of Medical Sciences, Ilam 693917134, Iran

Background: In this study, the ability of the biofilm formation of *Klebsiella* species isolates from two geographic regions of West North and West South of Iran was investigated.
Methods: *Klebsiella* species were screened and isolated from clinical samples of Shirvan (in North Khorasan providence) and Ilam cities. Then, the biofilm formation ability of isolates was studied.
Results: According to this study, 53 percent of total isolates had ability to biofilm formation. The highest and lowest percentages of biofilm formation among isolates were related to *Klebsiella pneumoniae* and *Klebsiella ozaenae*, respectively, with 40/6% and 1/6% frequency in the total population.
Conclusion: Significant percentage of the biofilm formation of *K. pneumoniae* isolates is noticeable in biological, genetic, ecological and treatment aspects. The studies are ongoing.

Keywords: Biofilm, *Klebsiella*

DETECTION OF METHICILLIN/OXACILLIN RESISTANCE OF STAPHYLOCOCCUS AUROUS IN CLINICAL ISOLATES IN SANANDAJ CITY

Delaram Amini1[1], Fatemeh Keshavarzi[1]
1.Department Of Biology, Kurdistan Science And Research Branch, Islamic Azad University, Sanandaj, Iran

Background: *Staphylococcus aureus* is one of the important agents of many infections in hospitals and society. Increasing *Staphylococcus aurous* resistance to antibacterial drugs is one of the major health concerns.therefore studying antibiotic resistance of *Staphylococcus aurous* is very important and it has a main role in preventing creation of resistant strains.
Methods: The antibiotic resistance of 150 *Staphylococcus aurous* isolates from various clinical specimens was determined by disk agar diffusion (DAD) and polymerase chain reaction (PCR) methods. Statistical analysis of data has performed by SPSS and Microsoft Office Excel software.
Results: Using the DAD method, 77/33% (116/150) of the *Staphylococcus aurous* isolates were resistant to Methicillin and 47/33% (71/150) were resistant to oxacillin. The results indicated that primer MR3,4 was more appropriate than primer MR1,2 for the detection of *mecA* gene in MRSA.
Conclusion: The results of this study can provide guidance for physicians toward a more appropriate treatment of *Staphylococcus aurous* infections in Iran, thereby preventing the emergence of further antibiotic resistance among *Staphylococcus aurous*. Our results also revealed the need for further investigations using a higher number of specimens representing a wider variety of locations to determine the antibiotic resistance patterns in our state more precisely.

Keywords: *Staphylococcus aurous*, Antibiotic Resistance, PCR.

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HELICOBACTER PYLORI ANTIBIOTIC RESISTANCE IN Iran: A SYSTEMATIC REVIEW

Farzad Khademi[1], Arshid Yousefi[1]
1.Department of Microbiology, Mashhad University of Medical Sciences, Mashhad, Iran

Background: *Helicobacter pylori* (*H. pylori*) is a pathogenic bacterium that colonizes the stomachs of approximately 50% the world's population. Resistance of *H. pylori* to antibiotics is considered as the main reason for the failure to eradicate these bacteria. The aim of this study was to determine the rate of resistant *H. pylori* strains to various antimicrobial agents in different Iran areas.
Methods: A systematic review of studies on antibiotic resistance of *H. pylori* in Iran that published in various magazines from 1997 to 2013 was performed. Information gathered from various studies in Iran were analyzed according to geographical area, year, methods and Number of strains and patients
Results: During 1997 - 2013, a total of 21 studies on *H. pylori* antibiotic resistance has been done in different parts of Iran. In this study, *H. pylori* resistance to various antibiotics, including metronidazole, clarithromycin, amoxicillin, tetracycline, ciprofloxacin, levofloxacin and furazolidone were 61.6% (95% CI: 59.18% to 64.02%), 22.4% (95% CI: 20.37% to 24.43%), 16.0% (95% CI: 14.21% to 17.79%), 12.2% (95% CI: 10.6% to 13.8%), 21.0% (95% CI: 17.89% to 24.11%), 5.3% (95% CI: 1.71% to 8.89%) and 21.6 % (95% CI: 18.48% to 24.72%) respectively. However, study on *H. pylori* resistance to rifabutin has not been tested in Iran.
Conclusion: This study showed that in order to determine an appropriate drug regimen against *H. pylori*, we are needed to determine the antibiotic susceptibility.

Keywords: *Helicobacter pylori*, Antibiotic Resistance, Iran

PURIFICATION OF CATALASE FROM HELICOBACTER PYLORI ISOLATES USING A SIMPLE TWO-STEP PROCEDURE

Pantea Mohammadi[1], Ali Mostafaie[1], Saboor Afzali[1], Ahmad Tajehmiri[1], Mariam Chalabi[1]
1.Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran.

Background: We have developed a two-step simple and efficient method for the purification of *Helicobacter pylori* catalase.
Methods: *Helicobacter pylori* isolates obtained from gastric biopsies of patients were cultured on modified Columbia agar and then disrupted by sonication. The unbroken cells were removed by centrifugation and the supernatant was collected and directly applied to a CM ion-exchange column of Sepharose CL-6B. Then the enzyme fractions was precipitated with polyethylene glycol for more purity.
Results: The results indicated that recovery, folds of purity and degree of purity are 60%, 41 times and 98%, respectively.
Conclusion: The main advantages of this study are the efficiency and short purification procedure that results in production of homogenous catalase used for specific immunoassay and vaccine development.

Keywords: Catalase, Chromatography, *Helicobacter pylori*, Purification

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THE RELATION BETWEEN HELICOBACTER PYLORI INFECTION AND ABO BLOOD GROUP AND RHESUS FACTOR

Heshmat Shahi[1], Marzie Sadeghiani[1], Majid Mahsa[2], Somaye Reiisi[3], Nader bagheri[4], Sara Shahi[5], Mohamad sadegh Damavandi[6], Hedayatollah Shirzad[1]

1. Department of Immunology, School of Medicine, ShahrekordUniversity of Medical Sciences, Shahrekord, Iran.
2.Department of Internal Medicine, School of Medicin, Shahrekord University of medical sciences, Shahrekord, Iran.
3. Department of Medical Genetics, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran.
4. Department of Immunology, school of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
5. Islamic Azad University, Astara branch, Gilan, Iran.
6. Department of Bacteriology, School of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran.

Background: *Helicobacter pylori* infection is currently endemic worldwide health problem. The infection causes chronic gastritis, peptic ulcer, and gastric adenocarcinoma. It is clear that blood group (BG) antigens are important to the development of intestinal disorders. This study determines the relationship between *H.pylori* and ABO blood group, Rh factor and bacterial infection.
Methods: The study included 160 patients (18-80 years) with dyspepsia symptoms referred to Hajar hospital in Shahrekord. Demographic data were collected. Patients were checked by 16sRNA gene polymerase chain reaction for *H. pylori*. Severity of *H.pylori* infection was graded according to the number of HP bacteria counted in light microscopy by (x1000 magnified). Blood groups were detected by a standard hemagglutination test.
Results: We observed that 61.87% of patients (99 of 160) were positive for *H. pylori*, and 38.13% were negative (61of 160). In the positive patients, 37.74% were male and 62.26% were female; also in the negative patients, 50.05% were male and 49.95% were female. The frequency of the ABO blood groups among positive patients was (A = 33.05%, B = 20.23%, AB = 6.06%, O = 40.66%) and was (A = 32.32%, B = 25.51%, AB = 8.84%, O = 33.33%) in negatives. HP was found in 20/40 Rh- and in 77/120 Rh+ patients without statistical difference (P>0.05). So this study showed the frequency of the ABO blood groups among sever infection was (A=18.2%, B=0%, AB=0%, O=81.8%). A significant difference (P< 0.05) was observed when we compared severity of infection with ABO blood group phenotypes.
Conclusion: Severity of *H.pylori* infection can be related by ABO blood group phenotypes.

Keywords: *Helicobacter pylori*, Blood Group Antigens, Rh-Hr Blood-Group System

THE RELATION BETWEEN DENSITY OF HELICOBACTER PYLORI IN BIOPSY WITH SERUM LEVEL OF METALLOPOROTEINASE IN INFECTED PATIENTS

Heshmat Shahi[1], Marzie Sadeghiani[1], Rasol Bahreini[2], Majid Mahsa[3], Somaye reisi[4], Gorbanali rahimian[2], hedayatollah Shirzad[1]

1. Department of Immunology, School of Medicine, ShahrekordUniversity of Medical Sciences, Shahrekord, Iran.
2. Department of Internal Medicine, School of Medicin, Shahrekord University of Medical Sciences, Shahrekord, Iran.
3. Department of Internal Medicine, School of Medicin, Shahrekord University of Medical Sciences, Shahrekord, Iran.
4. Medical Genetics Department, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran.

Background: This study determines the relationship between *H.pylori* density in biopsy and concentration of mmp-3 in serum.
Methods: The study was performed on 217 patients (20-80 years) both sexes, with dyspepsia symptoms referred to Hajar hospital in Shahrekord. Demographic data were recorded, such as age and sex. Blood sampling were done and the serum was separated immediately and stored at -70 degree of centigrade until measuring the concentration of mmp-3 in serum by ELISA methods. Patient's biopsies were checked by 16sRNA and *glm* genes by polymerase chain reaction for *H. pylori*. Density of *H.pylori*was graded according to the number of HP bacteria counted in light microscopy by (x1000 magnified).
Results: We observed that 69.12% of patients (150 of 217) were positive for *H. pylori*, and 30.87% were negative (67 of 217). In the positive patients, 46% were male and 54% were female; also in the negative patients, 41% were male and 59% were female. A significant difference (P<0.05) was observed when we compared density of bacteria by serum concentration of mmp-3.
Conclusion: High level of mmp-3 concentration in serum can be related by *H.pylori* sever density.

Keywords: Matrix Metalloproteinase, Elisa, *Helicobacter pylori*

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FREQUENCY OF HELICOBACTER PYLORI AND PRESENCE OF BAB B GENE IN POSITIVE SAMPLES OF HAJAR HOSPITAL PATIENTS IN SHAHREKORD

Marzieh Sadeghiani[1], Heshmat Shahi[1], Somayeh Reiisi[2], Nader Bagheri[3], Ghorbanali Rahimian[4], Reza Rashidi[4], Mohammad Hadi Shafigh[4], Ghasem Ramezani[5], Hedayatollah Shirzad[1]

1. Department of Immunology, School of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran.
2. Department of Medical Genetics, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran.
3. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
4. Department of Internal Medicine, School of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran.
5. Department of Nursing, Azad University of Shahrekord, Shahrekord, Iran.

Background: The aim of this study is to determine the frequency of *Helicobacter pylori* and *bab B* gene in patients of Shahrekord.
Methods: In this descriptive study, demographic data were recorded from 230 patients with gastrointestinal disorders who were referred to the endoscopy ward Hajar hospital of Shahrekord for upper endoscopy. one gastric biopsy sample of antrum were taken, Patient's biopsies were checked by rapid urease test (RUT) and by polymerase chain reaction (PCR) of 16srna.In the laboratory DNA was extracted, using bioflux tissue kit and examined for *bab B* genes by PCR.
Results: Among 230 patients (mean age 44 years); there were 109(47.4%) men and 121(52.6%) female. RUT and 16srna was examined for samples, 102(44.3) samples were positive for RUT and 16sRNA gene evaluation, and 128(55.7%) were negative for *Helicobacter pylori*. *Bab B* gene was examined for positive samples (infected patients), and 77(75.49%) were positive, ($p<0.001$).
Conclusion: Our results showed that the frequency of *Helicobacter pylori* is lower than 50% in Shahrekord and it can be related to genetic, host factors, environment and virulence factors of bacterium in Shahrekord, and *Helicobacter pylori* strains with bab B are common in patients of Shahrekord.

Keywords: *Helicobacter pylori*, DNA, Bab B, Polymerase Chain Reaction (PCR), Gastrointestinal Disease

ONE YEAR STUDY ON THE PREVALENCE OF CAMPYLOBACTER JEJUNI AND THEIR VIRULENCE HIPO AND FLAA GENES AMONG DIARRHEAL PATIENTS IN SHIRAZ, SOUTHWEST Iran

Maryam Nejabat[1], Abdolah Bazargani[1], Saeid Amirizadeh[2]

1. Shiraz University of Medical Sciences, Shiraz, Iran.
2. Gastroentro hepathology Research Center, Shiraz University of Medical Sciences.

Background: Our aim was to investigate the prevalence of these bacteria and the frequency of some of their important genes among gastrointestinal patients in Shiraz (Southern of Iran).
Methods: A total of 300 non-repeated diarrheal specimens (bloody, watery, mucosal) from patients in Shiraz teaching hospitals were included in the study from March 2013 to March 2014. Isolated organisms from stool specimens were identified as *C. jejuni* by modified Gram staining and positive oxidase, catalase and hydrolysis of sodium hiporate tests. Molecular study for detection *flaA* gene (flagella) and *hipo* gene (sodium hypurate hydrolysis) was done by PCR with specific primers on *C. jejuni* isolates.
Results: Of 300 stool specimens, 58 isolates (19.3%) were identified as *C. jejuni*. All of the isolates (100%) were positive for *flaA* gene and the *hipo* gene was detected in 94% of the isolates.
Conclusion: The prevalence of *C. jejuni* among the studied patients with gasterointestinal infection in Shiraz was19.3 %.Considering *C. jejuni* as an important cause of diarrheal syndromes in clinical settings is essential and conducting sanitary and preventive measures in food production and consumption is necessary.

Keywords: *Campylobacter, Campylobacter Jejuni, Hipo Gene, Flaa Gene*

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GOLD NANOPARTICLES EFFECT ON IMPROVING THE DELIVERY OF METRONIDAZOLE AGAINST METRONIDAZOLE RESISTANT HELICOBACTER PYLORI

ALI JAVADI[1], Hamid Abdollahi[1], Mohamad Reza Zand Monfared[2]
1. Department of Medical Microbiology, Kerman University of Medical Sciences, Kerman, Iran.
2. Department of Chimistry, Qom Branch, Islamic Azad University, Qom, Iran.

Background: Our aim in this research was to investigate the impact of gold nanoparticels conjugated with metronidazole on a metronidazole - resistant *H.pylori.*
Methods: The gold nanoparticles were synthesized by Turkevich method. Their characteristics were determined, using spectrophotometery, transmission electron microscopey (TEM) methods. Then, the conjugation of metronidazole with gold nanoparticles was carried out by metronidazole then analyzed by spectrophotometry, electron imaging and FT-IR. Anti-*H. pylori* activity of pure gold nanoparticles and metronidazole was compaired with their conjugate using agar disc diffusion method (according to CLSI).
Results: The size of gold nanoparticles was found to be between 9-12 nm. The wavelength of maximum absorbance for nanoparticles and metronidazole were 522 nm and 320nm respectively. In the conjugated state, the size was larger (about 2-3 times and in an aggregate form). The wavelength of maximum absorbance of their conjugate was changed to 540 nm. The FT-IR spectrum, of conjugated metronidazole was also completely changed. There was no inhibition zone around metronidazole and gold nanoparticles discs in their free state, but in conjugated state, The inhibition zone around the impregnated disc was 17 mm.
Conclusion: The antimicrobial activity of metronidazole on a resistant strain of *H.pylori* was significantly increased when it was conjugated with gold nanoparticle.

Keywords: *Helicobacter pylori*, Metronidazole Resistant, Gold Nanoparticles, Drug Delivery

INTRAFAMILIAL TRANSMISSION OF HELICOBACTER PYLORI: GENOTYPING OF FECAL SAMPLES

Babak Pourakbari[1]
1. Pediatric Infectious Diseases Research Center, Tehran University of Medical Sciences, Tehran, Iran.

Background: *Helicobacter pylori* (*H. pylori*) commonly acquired in early childhood. After more than 20 years of research, there is a little information about the details routes of this bacterium transmission. The aim of this study was to explore intrafamilial transmission of *H. pylori* in children who had indication for upper gastrointestinal endoscopy and their parents, using RAPD-PCR.
Methods: Children (aged up to 15 years) attending Children Medical Center Hospital for upper gastrointestinal endoscopy of suspected *H. pylori* infection during September 2012 to October 2013 was studied. Then, the parents of those with positive urea breath test (UBT) results were asked to provide fecal and blood samples after informed parental consent. Noninvasive tests such as immunoassay for serological antibodies against *H. pylori* and detection of its antigen in feces were measured. The genetic similarity of the family strains was investigated by amplification of the random amplification of polymorphic DNA (RAPD-PCR) genotyping method.
Results: According to the genotyping results of 30 families (children with their parents), in 10 (33.3%) children related *H. pylori* genotypes to their mothers was found, while only 2 children (6.7%) had similar genotypes to their fathers. Children with similar *H. pylori* genotyping with their parents were younger than the other infected children. Interestingly, children with similar *H. pylori* genotype with their mothers had higher IgA (35.7± 10.8) and IgM antibody titers (87.23± 19.15) than other children. In addition, in these children lower titers of IgG antibodies (9.93±3.31) were found rather than children who had no *H. pylori* in their faces or had no similarities with their parents (mean (30.28±6.15).
Conclusion: In conclusion, our data provide further evidence that mother-to-child transmission is the main route of intrafamilial transmission of *H. pylori* in Iranian families. Molecular typing of *H. pylori* can help to control the spread of the infection. In addition, it can be useful in identifying a high-risk population in order to reduce the cost risk of its complications.

Keywords: *Helicobacter pylori*, Intrafamilial Transmission, Genotyping

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MOLECULAR DETECTION AND SPECIATION OF CAMPYLOBACTER SPP. IN CHILDREN GASTRO-ENTERITIS USING PCR-RFLP METHOD IN KA-RAJ HOSPITALS.

Maryam Shirzadi[1], Farzad Mohammadi[2], Arman Rostamzad[3], Sohaila Soltanbigi[2]
1.Payame Noor University of Sarable, Iran.
2.Payame Noor University of Ilam, Ilam, Iran.
3.Ilam University, Ilam, Iran.

Background: In this survey, PCR-RFLP technique was used for detection and simultaneous speciation of *Campylobacter* isolates in stool samples and determination of the frequency of *Campylobacter* contamination in the cases of children gastroenteritis in Bahonar hospital of Karaj city. To this, two hundred stool samples were obtained from neonates and children up to the age of 8 years with gastrointestinal disorders during June to September of 2011.
Methods: DNA of the samples were extracted using DNG plus kit (Cinnagen) and PCR was optimized to amplify a 491 bp fragment of the 23s rRNA gene of *Campylobacter* genus and *C. jejuni* RTCC 1097 reference strain as positive control in the clinical samples, then RFLP technique using AluI and TasI was performed to differentiate jejuni, coli, lari and upsaliensis species
Results: Evaluation of PCR positive samples (21 out of 200/10.5% of the samples) showed the jejuni species electerophertic pattern in 11(5.5%) and coli species pattern in 7(3.5%) of the samples, three out of 21 positive samples (1.5% of total samples) showed both of the patterns and mixed infection
Conclusion: PCR-RFLP technique can be used as a rapid, sensitive and specific method for detection and simultaneous differentiation of *Campylobacter* species in clinical samples.

Keywords: (*Campylobacter*,Children Stool,PCR,PCR-RFLP)

PREVALENCE AND ASSOCIATION OF HELICO-BACTER PYLORI INFECTION WITH GASTROIN-TESTINAL DISEASE

Marzieh Sadeghiani[1], Heshmat Shahi[1], Somayeh Reiisi[2], Nader Bagheri[3], Mohammad Hadi Shafigh[4], Majid Mahsa[4], Elahe Shahverdi[5], Fereshteh Fatollahi[5], Hedayatollah Shirzad[1]
1.Department of Immunology, School of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran.
2.Department of Medical Genetics, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran.
3.Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
4.Department of Internal Medicine, School of Medicin, Shahrekord University of Medical Sciences, Shahrekord, Iran.
5.Department of Nursing, Shahrekord University of Medical Sciences, Shahrekord, Iran.

Background: *Helicobacter pylori* is one of the most infections in worldwide, it can colonize the gastric mucosa for years. The effect of *Helicobacter pylori* to cause gastrointestinal diseases is proved and also it can be associated with an increased risk of gastric cancer. This bacterium can have roles in making different disease such as gastritis, duodenal and peptic ulcer.
Methods: In this study, 230 patients who referred to Hajar hospital of Shahrekord with complain of gastric disorders, diagnosed for undergoing upper endoscopy, demographic data were recorded. 1 biopsy of antrum was collected; biopsies were checked by 16sRNA and *glm* genes by polymerase chain reaction for *H. pylori*, and type of disease was diagnosed by Gastroenterologist.
Results: Of 230 patients, 109 were men (47.4%) and 121 of them were women (52.6%) and the mean age was 44years old. Endoscopic reports showed, 101 patients had gastritis (43.9%), 31patients had peptic ulcer (13.5%) and 14 patients had duodenal ulcer (6.1%), and 81 patients didn't have ulcer disorders (35.2%). The prevalence of hp was 44.3 %(102of 230) in patients. High Frequency of hp positive was in age 31To 50years old (49.6%). The prevalence of *H. pylori* infection was 28.57% in NUD cases (18 of 63), 62.9%GU cases (39 of 62), 55.55% in DU cases (5 of 9) and 100% in combined GU and DU cases (3 of 3)
Conclusion: Our study reveals that prevalence of gastritis (43.9%) is more common than other gastrointestinal diseases ($p<.001$).

Keywords: *Helicobacter pylori*, Gastritis, Peptic Ulcer, Duodenal Ulcer, Polymerase Chain Reaction

OCCURRENCE OF PUTATIVE VIRULENCE GENES IN ARCOBACTER SPECIES ISOLATED FROM ANIMAL

Hesamaddin Shirzad Aski[1], Mohammad Tabatabaei[1], Hossein shayegh[2], Rahem Khoshbakht[3]
1.Department of Pathobiology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran.
2.DVM student, School of Veterinary Medicine, Shiraz University, Shiraz, Iran.
3.Department of Food Hygiene, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran.

Background: The aim of this study was to evaluate the presence of six putative *Arcobacter* virulence genes (*cadF, ciaB, cj1349, mviN, pldA,* and *tlyA*) in *A. butzleri, A. cryaerophilus,* and *A. skirrowii* isolates, isolated from food of animal origins obtained from slaughterhouse in Shiraz, Southern Iran, using PCR.
Methods: The isolates (113 *A. butzleri*, 40 *A. cryaerophilus*, and 15 *A. skirrowii* isolates) were confirmed on the basis of polymerase chain reaction (PCR) amplification of genus and species specific PCR for determining three species. For confirmed isolates, PCR was carried out for the presence of virulence genes using specific primers.
Results: All *A. butzleri* isolates carried all six genes. In*A. cryaerophilus* and *A. skirrowii*, the *cadF* gene was detected just in 55 and 53.3%, *ciaB* in 97.5 and 86.6%, *cj1349* in 45 and 60%, *mviN* in 90 and 80%, *pldA* in 32.5 and 13.3%, and *tlyA* in 37.5 and 40%, respectively. In*A. cryaerophilus* and *A. skirrowii*, the genes *ciaB* and *mviN* were significantly more prevalent than other virulence markers ($p < 0.05$). In addition, the occurrence of all six virulence genes among *A. cryaerophilus* isolated from cattle were significantly more present than other isolates. The *pldA* gene was significantly more detected in cattle isolates compared with sheep isolates ($P < 0.05$).
Conclusion: The findings revealed that many of the important *Arcobacter* strains (86%) have these putative virulence genes which can be potential pathogenic properties for humans.

Keywords: *Arcobacter* Species, Virulence Genes, Iran

RELATION OF HELICOBACTER PYLORI INFECTION WITH ELEVATED SERUM LIPID

Farzaneh Islam-Kish[1], Taher Mohammadian[2], Hossein Rassi[3], Roohollah Solymani[4]
1.Department of Microbiology, Islamic Azad University of Kerman, Iran.
2.Department of Microbiology, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran.
3.Department of Microbiology, Karaj Branch, Islamic Azad University, Karaj, Iran.
4.Pathology Laboratory of Fatemeh Al-Zahra Hospital, Kerman, Iran.

Background: The aim of study was to examine serum lipid level in patients infected with *Helicobacter pylori.*
Methods: The total number of 700 subjects who underwent health check-up were included in this study. Patients receiving statin and fibrate therapy and diabetic patients were excluded. *H. pylori* –specific IgG antibody was determined from serum samples by an enzyme-linked immunosorbent assay kit. Patients were divided into 2 subgroups which were *H. pylori* negative (group 1) and *H. pylori* positive (group 2).Total cholesterol (TC), LDL-cholesterol and HDL-cholesterol and triglyceride levels were measured in all subjects. Then, the obtained results analyzed statistically by SPSS (V.20) software.
Results: Among 433 (172=36.7% female, 261=63.3% male) studied patients, 113 were in group 1 and and 320 were in group 2. Total cholesterol (178 ± 40 vs 196 ± 44 mg/dl), LDL-C (110 ± 36 vs 128 ± 64 mg/dl) and triglyceride (178 ± 40 vs 196 ± 44 mg/dl) was significantly higher in group 2 than group 1. HDL-C (52±21 vs 40± 20 mg/dl) was significantly lower in group 2 than group 1.
Conclusion: LDL-cholesterol, triglyceride and total cholesterol levels were higher in subjects with *H. pylori* infection, and HDL cholesterol level decreases in subjects with *H. pylori* infection. These findings suggest that *H. pylori* infection may cause lipid alteration and, at least partially contribute to the atherosclerotic process.

Keywords: *Helicobacter pylori*, Cholesterol, Triglyceride, LDL, HDL, Atherosclerosis

SEROEPIDEMIOLOGICAL STUDY OF HELICOBACTER PYLORI IN KERMAN

Farzaneh Islamkish[1], Taher Mohammadian[2], Hossein Rassi[3], Roohollah Solymani[4]

1. Department of Microbiology, Kerman Branch, Islamic Azad University, Kerman, Iran.
2. Department of Microbiology, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran.
3. Department of Microbiology, Karaj Branch, Islamic Azad University, Karaj, Iran.
4. Pathology Laboratory of Fatemeh – Al - Zahra Hospital, Kerman, Iran.

Background: Due to the interference caused by the bacteria of, the aim of the present study was to determine the extent of pollution in the Iranian population
Methods: The seroepidemiological study of 433 serum samples from individuals 15-75 years old, to perform lab tests to check the health of Kerman hospital patients who were tested. Samples were tested for determination of *Helicobacter pylori* infection for serum IgG measured by ELISA kits.
Results: Total of 433 serum samples tested, 172 patients with the 36.7 % women and 261 men of the 63.3 % of men surveyed and tested. A total of 113 cases of normal serology and 320 cases had high titers of antibodies against *H.pylori.*
Conclusion: Statistical analysis using SPSS software showed that 73.9% of the population of Kerman are *Helicobacter pylori* infected. Furthermore, this study showed that the prevalence is higher in older people, according to the test statistical significant difference between prevalence and age were found to have (P = 0.01).

Keywords: *Helicobacter pylor*i, Seroprevalence,Igg

HOMOLOGY OF MULTI LOCUS SEQUENCE TYPES OF H. PYLORI STRAINS WITH DIFFERENT GENOTYPES IN A SINGLE HOST

Nastaran Farzi[1], Masoud Alebouyeh[1], Mohammad Reza Zali[1]

1.Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran and Basic Science and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: The purpose of this study was the investigation of multiple sequence types of single isolates of *H. pylori* presenting diverse genotypes but identical RAPD patterns in a single host by MLST method.
Methods: The gastric biopsy specimens were obtained from the antrum of 98 patients with gastritis and peptic ulcer disease undergoing endoscopy in a gastroenterology unit in Tehran. The biopsy specimens were homogenized and cultured. The isolates were identified by PCR for detection of the glmM and also conventional biochemical tests. Six single colonies were isolated from each individual. RAPD-PCR typing (1283 and 1254) and genotyping (*vacAs1/s2, vacAm1/m2, iceA1/iceA2*) were performed for each single isolate. Multi locus sequence typing (MLST) of seven gene loci (*atpA, efp, mutY, ppa, ureI, trpC, yphC*) were done for strains with identical RAPD patterns but diverse genotypes.
Results: Out of the 98 patients, 39 patients were *H. pylori* positive (39.8%). Mean age of the subjects was 35 years old. Males and females constituted 36% and 63% of these patients, respectively. Analysis of the obtained RAPD-PCR patterns among the single strains in a host showed identical RAPD patterns in 23% of the infected patients. Diverse genotypes were observed among 44.4% of the patients presenting identical RAPD patterns. Changes of the allelic variants including vacAm2+: vacA m2- (25%), cagA+: cagA- (50%), and cagA+/ ice A1 A2+: cagA-/ ice A1 A2- (25%) was observed among these patients. Results of the MLST analysis established existence of the same allelic types for strains with identical RAPD patterns but different genotypes in each patient.
Conclusion: Our results confirmed the occurrence of genotype conversion in the *vacA, cagA*, and *iceA* gene alleles in a single *H. pylori* strain during chronic infection. We can conclude that MLST and RAPD typing methods can be regarded as appropriate tools for confirmation of genetic variations among *H. pylori* strains during long-time colonization of the studied patients.

Keywords: *Helicobacter pylori*, RAPD-Fingerprint, Genotyping, MLST, Genotype Genotype Conversion

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RELEVANCE OF HELICOBACTER PYLORI CAGA STATUS AND GENOTYPES OF VACA GENE TO PEPTIC ULCER DISEASE (PUD) IN Iran

Seyedeh Zahra Bakhti[1], Saeid Latifi-Navid[1], Saber Zahri[1]
1.Department of Biology, Faculty of Sciences, University of Mohaghegh Ardabili, Ardabil, Iran.

Background: *Helicobacter pylori* (*H. pylori*) is the main cause of peptic ulcer disease (PUD) and gastric adenocarcinoma. There is a close relationship between *H. pylori*-specific factors and different gastroduodenal diseases. The aim of present study was to clarify the roles of the *H. pylori* vacA-s, -m, -i, and -d regions, and *H. pylori cagA* status in relation to PUD risk.
Methods: A total of 171 *H. pylori* isolates from ten districts in Iran were used for genotyping.
Results: The subjects included 114/171 with non-atrophic gastritis (NAG) and 57/171 with PUD. The frequency of vacA s1 was 92.4%, s2 8.8%, m1 26.3%, m2 73.7%, i1 43.3%, i2 57.3%, d1 40.9%, d2 59.1%, and cagA 67.8%. Statistical analysis showed that frequency of the vacA i1 and cagA genotypes in patients with PUD (58.6% and 81.0%, respectively) was higher than in those with NAG (35.4% and 61.1%, respectively) ($P < 0.05$). The vacA-s, -m, and -d genotypes were not independently associated to PUD risk. The vacA i1 and cagA genotypes were significantly associated with the risk of PUD, the OR was 2.735 (95% confidence interval (CI): 1.422- 5.261, $P = 0.002$) and 2.629 (95% CI: 1.231-5.611, $P = 0.011$), respectively.
Conclusion: We have proposed that the presence of *cagA* gene and *vacA* i1 genotype in Iranian *H. pylori* strains could be considered as a benefit biomarker for prediction of risk of PUD in Iran.

Keywords: *H. pylori, Vaca, Caga*, PUD, Iran

RELEVANCE OF HELICOBACTER PYLORI VACA 3′-END REGION POLYMORPHISM TO GASTRIC CANCER

Seyedeh Zahra Bakhti[1], Saeid Latifi-Navid[1], Saber Zahri[1]
1. Department of Biology, Faculty of Sciences, University of Mohaghegh Ardabili, Iran.

Background: Significance of the 3'-end region polymorphisms (denoted c1/c2-c2: no deletion) of *Helicobacter pylori vacA* gene in determining risk of gastroduodenal diseases has still not been understood. The aim of present study was to analyze the relationship between *H. pylori vacA* 3′-end region polymorphism (c1/-c2) and risk gastric cancer (GC).
Methods: A total of 160 *H. pylori* isolates from different regions of Iran were recruited and genotyped. Data were collected and analyzed using SPSS version 19.
Results: The subjects included 114/160 with non-atrophic gastritis (NAG) and 46/160 with GC. The frequency of vacA s1 was 84.4%, s2 11.3%, m1 32.5%, m2 67.5%, i1 48.1%, i2 56.3%, d1 44.4%, d2 54.4%, c1 41.3, c2 65.6, and cagA 66.9%. Statistical analysis showed that frequency of the vacA m1, vacA i1, vacA d1, vacA c1, and cagA genotypes in patients whit GC (54.3%, 80.4%, 65.9%, 84.8%, and 82.2%, respectively) was higher than in those with NAG (23.7%, 35.1%, 36.8%, 23.7%, and 61.4%, respectively). The vacA m1, i1, d1, c1, and cagA genotypes was significantly associated with the risk of GC, the OR was 3.836, 7.606, 3.314, 17.952, and 2.907, respectively ($P< 0.05$). There was not a significant difference between the frequencies of vacA s1a/-s1b/, and -s2 genotypes in isolates from GC and those from gastritis ($P > 0.05$).
Conclusion: The present study is the first report regarding the relevance of *H. pylori vac*A 3′-end region polymorphism to GC. This association is independent of and larger than the associations of vacA m-, i-, and d-type or cagA status with GC. We have proposed that the vacA c1 genotype could therefore be the strongest predictor of GC in Iran.

Keywords: *H. pylori*, Genotypes, Gastric Cancer, Iran

HELICOBACTER PYLORI AND OVERWEIGHT STATUS IN IranIAN PATIENTS WITH DIFFERENT GASTROINTESTINAL DISORDERS

Alireza Ahmadzadeh[1], Hossien Ghalenoi[1], Masoud Alebouyeh[2], Nastaran Farzi [3], Mahsa Molaei[2], Mohammad Reza Zali[2]

1. Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2. Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
3. Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Background: Obesity is an important public health problem among Iranian people. Because of its potential effects on gastric homeostasis, *Helicobacter pylori* may play a role in regulating body weight. The main aim of this study was to examine the association between *H. pylori* colonization and BMI status in patients with gastrointestinal disorders in Tehran, Iran.
Methods: *H. pylori* from patients with different gastrointestinal disorders were detected after culture and identification by phenotypic and genotypic methods. In continue association between pathological finding and BMI were analyzed by using SPSS version 19.0 software. χ 2 test and Fisher's exact test were used to assess relationships between categorical variables.
Results: A total of 67 people (35 male and 32 female) from 424 patients with different gastrointestinal disorders were recognized as *H. pylori* positive. The mean age of patients was 46.6. *H. pylori* infection showed an inverse association with obesity. A significant association was observed between overweight status and occurrence of sever active gastritis (P value =0/0001).
Conclusion: Increase in body mass in infected patients with *H. pylori* promotes sever inflammation of gastric tissue that is consider as a risk factor for gastric cancer.

Keywords: *H. pylori*, Overweight, Pathological Finding

PREVALENCE OF CDTB OF CAMPYLOBACTER SPP. IN PATIENTS WITH GASTEROENTERITIS

Masoude Alebouyeh[1], Leila Ganji[2], Abbas Yadegar[1], Mohammad Hassan Shirazi[2], Parisa Islami[3], Mohammad Rahbar[3], Naser Ebrahimi Daryani[4], Mohammad Reza Zali[1]

1. Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2. Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
3. Department of Medical Microbiology, Research Center of Reference Laboratories of Iran.
4.Department of Gastroenterology and Hepatology, Imam Khomeini Hospital, Tehran University of Medical Sciences, Tehran, Iran

Background: Our objective was to detect the prevalence of *cdtB* gene in patients with acute gasteroenteritis.
Methods: This study was conducted on stool samples that obtained from patients with acute gasteroenteritis. Genomic DNA was extracted with the Qiagene (QIAamp DNA Mini Kit) according to the manufacture´s instruction. Detection of *cdtB* was determined by PCR using specific primers. The suspected PCR products were subjected to sequencing by using an automated sequencer (source Bioscience, UK). Presence of *Campylobacter* spp. DNA in positive samples was also detected by PCR with genus specific primers.
Results: We assessed a total of 50 stool samples. The PCR results showed that 32% of the samples were positive for *cdtB*. Also the PCR reaction for *Campylobacter* genus was positive in these samples. These results were finally confirmed by sequencing.
Conclusion: The results of this study showed high prevalence of *Campylobacter* spp. among patients with gastritis. Presence of *cdtB* among fecal DNA samples of these patients proposes possible role of this virulence factor in pathogenesis of gastroenteritis in human. However further studies will increase our knowledge about role of CDT in human intestinal disorders.

Keywords: Cytolethal Distending Toxin, *Campylobacter* Spp., Gasteroenteritis.

DISTRIBUTION OF CLINICAL SYMPTOMS IN PATIENTS WITH DIARRHEA CAUSED BY CAMPYLOBACTER SPECIES REFERRED TO TEHRAN HOSPITALS

Hossein Dabiri[1], Neda Yousefi[1], Mohsen Heidary[1]Saeed khoshnood[1], Mahdaneh Roshani [1]
1. Department of Microbiology, Shahid Behehsti University of medical sciences, Tehran, Iran.

Background: The purpose of this study was to evaluate the prevalence of clinical symptoms in patients with diarrhea caused by *Campylobacter* referred to Tehran hospitals.
Methods: Demographic and clinical data of patients with diarrhea admitted to hospitals during 2012 to 2013, in Tehran, Iran were collected. Stool samples were obtained from all patients and then transported to the microbiology laboratory using medium such as CARY-BLAIR and were cultured in specific media. Bacterial isolates were identified by biochemical and microbial tests and results were analyzed with SPSS software.
Results: Of 434 studied patients; 239 (45%) women and 195 (55%) male, 33 (6.7%) case; 18 female and 15 male were positive for *Campylobacter* spp. In *Campylobacter* positive groups, 93% nausea, 100% anorexia and fever, 94% abdominal pain, 25% Headache, 89% weakness, bloody stool 89% and 95% mucosal stool was seen whereas this data for non-*Campylobacter* positive group was 87% nausea, 90% anorexia, 94% fever, 86% abdominal pain, 31% Headache, 40% weakness, 56% bloody stool and 85% mucosal stool. There was not any statically significant among clinical finding and *Campylobacter* presence.
Conclusion: A large family of bacteria involve in the gastrointestinal tract infections. Family Campylobacteriaceae is Common causes of gastrointestinal infections. Although anorexia, nausea, abdominal pain, fever and bloody stool was slightly more frequent in *Campylobacter* positive patients, but since we did not find any statically relationship among clinical symptoms and sign with *Campylobacter* infection, so we strongly recommend to perform microbial culture for microbial enteritis.

Keywords: *Campylobacter*,Gastroenteritis, Iran, Clinical Symptoms

GASTRIC BACTERIUM HELICOBACTER INFECTION IN PATIENTS WITH SEROLOGIC TESTS AND FECAL ANTIGEN PICKUP

Mohammad Ahanjan[1], Mohammad Reza Jabari Amiri[2], Maryam Abdollahi[3], Behnam Hashemi[3]
1. Associate Professor in Microbiology, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.
2. MSc Student in Mycology, Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran.
3. MSc Student in Microbiology, Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran.

Background: *Helicobacter pylori* is a major cause of gastric and duodenalulcersis considered. One way to detect infections caused by bacteria is using non-invasive methods, such as the study of antigens in feces and review of specific antibodies in serum.
Methods: In this study, patients referred to internal medicine wards and clinics of medical internist digestion by gastrointestinal symptoms, their indicative of gastritis were enrolled.20 patients at an average age of 20 to 66 years old stool antigen IgG and IgA antibodies were pickups.
Results: 20 patients studied, only 1 stool was antigen negative and three suspected cases and 16 cases were positive. The person who had negative stool antigen also showed negative IgA antibody. But all of them suspected fecal antigen were IgG positive. All antigen-positive patients were positive for IgG antibodies.In 14 patients who had positive antigen, only 2 of them were positive for both IgG and IgA.
Conclusion: According to the research results of stool antigen of *H. pylori* infection as anon-invasive procedure which is acceptable

Keywords: *Helicobacter pylori*, Fecal Antigen, Antibody

OUTBREAK OF CAGA AND ICEA IN H. PYLORI STRAINS ISOLATED FROM PATIENTS WITH GASTRO DUODENAL DISEASES IN BABOL CITY

Ramazan Rajabnia[1], Maryam Salehi[1]
1.Department of Microbiology, Babol University of Medical Sciences, Babol, Iran.

Background: *Helicobacter pylori* is one of the most common bacteria that afflicts world human paulation.Virulance factors of*Helicobacter pylori* have been associated with clinical outcome of the infection including gastriris, gastric ulcer (GU),duodenal ulcer(DU) and gastric cancer.The aim of the present study was to determine the outbreak of cagA and iceA genotypes of *H.pylori* in patients with gastroduodenal disease in Babol,north of Iran.
Methods: Fifty patients with dyspepsia underwent gastroscopy and antral biopsy for histologic study. CagA, iceA, iceA1 and iceA2 genotypes were determined by PCR.
Results: Of 30(60%) HP positive strain,80% were positive for cagA gene.CagA genotype was positive in 91.7 % (11 of 12),7303%(11 of 15) and 66.7% (2 of 3) of patients with gastritis, DU and GU, respectively. IceA was positive in 21 (70%) of all patients and iceA1 was found in 14(66.7%) isolates and iceA 2 was found in 23.8% isolates.IceA 1 and iceA 2 was not found in 2 (9.5%).IceA 1 outbreak in patient with gastritis,DU and GU was 88.9%, 4505% and 50% respectively.
Conclusion: Positive cagA strain was more than negative cagA and,iceA1 was predominant strains in our patients with HP infection. We found that two genes other than icea1 and iceA2 in this study.It seems that new allele other than ice A1 and ice A2 genes are present in *H.pylori.*

Keywords: *Helicobacter pylori, Caga, Icea*, Gasteroduodental

INVESTIGATING THE PRESENCE OF HELICOBACTER PYLORI IN ATHEROMA PLAQUES OF ARTERIES IN PATIENTS WITH ATHEROSCLEROSIS

Mohamad Mahdi Karimi Yazdi[1], Sadegh Lotfala Moradi[1], Parvaneh Khiabani Rad[1], Mozhgan Esmaeili Benvidi[1], Gita Eslami[1], Arezou Taherpour[1], Soudabeh Taheri[1]
1.Department of Microbiology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Background: The aim of this study was to investigate the role of *H.pylori* in atherosclerosis plaque.
Methods: Our study is descriptive, we studied 90 patients with atherosclerosis. Specimen of atheroma plaques in the arteries of each patient sampled by physician aseptically. Some samples saved on -70 degrees refrigerator and some others were used for microbial culture. For detection of *H.pylori* from the specimen of atheroma plaque in arteries, direct smear were prepared, gram stained, then investigated with microscope. Also for culture of *H.pylori Brucella* agar media (contain 7-1-% defibrinated sheep blood, 1% starch and vancomycin, trimethoprim, pilymixin B, amphotericin antibiotics) was used. The plates were cultured in microaerophilic condition and were placed in 37 degrees incubator and investigating after 2-3 weeks and finally differential biochemical tests were performed.
Results: On the 90 specimen of patient's atheroma plaques, culture and PCR were performed.70% of patients were male and maximum age that have vessels involvement were between 40-59 ages. Cultures were positive in only one case.
Conclusion: The result of this study showed the presence of *H.pylori* in atheroma plaques of patients. Also the percentage of *H.pylori* was 33% that was similar to the research performed in Turkey.

Keywords: Atheroma Plaques, Atherosclerosis, *Helicobacter pylori*

ASSESSMENT OFTHE VIABILITY OF THE COCCOID FORM OF HELICOBACTER PYLORI IN MICE

Somayeh Rezaei Kalla[1], Mehdi Assmar[1]
1.Department of Microbiology, Lahijan Azad University, Lahijan, Iran.

Background: In this investigation, the transmission of the infection by *Helicobacter pylori* coccoid form was assessed.
Methods: In this study, the suspension of coccoid form *Helicobacter pylori*was prepared in PBS. Mice were fed with suspension coccoid form. Then transmission of the infection by *Helicobacter pylori* coccoid form was assessed.
Results: In this study, the results showed that mice were infected. So, non-culturable coccoid forms of *H. pylori*which could resist environmental stresses were alive.
Conclusion: *Helicobacter pylori* is one of the most common infectious diseases in the world. It colonizes about 50-60% of the world's population. This study showed that non-culturable coccoid forms of *H. pylori* which could resist environmental stresses were alive. Thus might be responsible for bacterial transmission and failure in disease treatment.

Keywords: Coccoid Forms,*Helicobacter pylori*, Transmission

DETECTION AND PREVALENCE RATE OF AMERICAN COCKROACHES' BACTERIAL INFECTIONS IN HUMAN DWELLINGS, SOUTH WESTERN Iran

Hamid Kassiri[1], Anvar Quaderi [1]
1.Health Faculty, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Background: This descriptive study was carried out to confirm that Periplaneta americana L. (Dictyoptera; Blattidae) carry pathogenic and potentially pathogenic bacteria in residential areas by bacteriological analysis on the cuticles of this insect.
Methods: In this research, we caught 25 American cockroaches from human dwelling localities of Ahvaz County in 2008-2009. Cockroaches were collected using sticky traps, vacuum cleaner and direct collection. The collected cockroaches were brought to the laboratory. Captured cockroaches were identified as P. americana. They were examined for the presence of bacteria in their external surfaces using specific standard methods for bacterial infection.
Results: Twenty- five American cockroaches were randomly selected and were detected by bacteriological examination. Medically important bacteria were isolated from external surface of 100% of cockroaches. This finding suggests that almost all the cockroaches in the residential environments carry medically important pathogenic bacteria. Eleven bacterial species were isolated from cockroaches. The most common detected bacteria were found to be *Escherichia coli, Staphylococcus aureus, Proteus* spp., *Klebsiella* spp., *Citrobacter* spp. and *Enterobacter* spp. At which, 100%, 72%, 60%, 60%, 56% and 52% of cockroaches were infected, respectively. The minimum contamination was approved to *Serratia* spp. (20%), *Micrococcus* spp. (32%) and *Enterococcus* spp. (40%). The other detected bacteria were *Psudomonas* spp. (44%) and *Sterptococcus* spp. (48%). Most of the bacterial isolates from the external surface of cockroaches were Gram positive. All of American cockroaches examined in this research were infected with at least one bacterium.
Conclusion: The results showed that *P. americana* is a possible reservoir and potential vector of some pathogenic agents.

Keywords: Periplaneta Americana; Bacterial Infection; Houses; Iran

NOVEL TREATMENT FOR BRUCELLA INFECTION

Narges Bodaghabadi[1], Majid Sadeghizadeh[1], Farhood Najafi[2]
1.Molecular Genetics Department, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran.
2.Department of Resin and Additives, Institute for Color Science and Technology, Tehran, Iran.

Background: We aimed to increase the efficacy of antibiotics in eradicating *Brucella* (through augmenting their capacity to penetrate in to cells) and reducing consuming dose.
Methods: Loading of antibiotics, Rifampin and Cotrimoxazole in nanocarrier MTT resulted in declaring loaded antibiotics toxicity on phagocytic cells, investigating the loaded-antibiotics efficacy on reduction of *Brucella.*
Results: Encapsulation efficiency for Rifampin=1200/16000×100=7.5 Encapsulation efficiency for Cotrimoxazole=1355.215/16000×100=8.47 LD50 for Nanocarrier~217mM LD50 for Cotrimoxazole~1954.20mM LD50 for Cotrimoxazole in nanocarrier~196.66mM LD50 for Rifampin>243mM LD50 for Rifampin in nanocarrier~61.63mM
Conclusion: We are able to conclude that loaded antibiotics are more efficient than free antibiotics to reduce *Brucella.*

Keywords: Intracellular Bacteria, Mouse Phagocytic Cells J774, Nano Carrier

INFECTIVE ENDOCARDITIS AMONG INTRAVENOUS DRUG USERS

Farhad Abbasi[1], Mehdi Besharat [2], Jamile Kiyani[1]
1.Bushehr University of Medical Sciences, Bushehr, Iran.
2.Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Background: Infective endocarditis is a common infection among intravenous drug users and can cause high mortality and morbidity.
Methods: In a retrospective study, we evaluated 33 intravenous drug users with documented infective endocarditis in two tertiary care hospitals in Tehran, Iran; Imam Hosein and Loghman Hakim hospitals during 6 years. Demographic information, clinical manifestation, radiologic, laboratory and echocardiographic data were evaluated.
Results: Eighty two percent of patients were in 20-40 year old age group. The most common symptoms were fever, cough, dyspnea and lower extremities edema. The most common involved valve was tricuspid with frequency of 45%, followed by mitral, aortic and pulmonary valves. Anemia was observed in 95%, leukocytosis in55%, thrombocytopenia in 35%, leukopnea in 10% and elevated ESR in 70% of patients. Of our patient 3 were HIV and 2 were HCV positive. Positive blood culture was observed only in %40 of cases that may due to sampling error or previously antibiotic disuse by patients. The most common isolated organism was *Staphylococcus aureus*. Mortality rate was 55% in our study.
Conclusion: Endocarditis in intravenous drug users was with high mortality rate in this study. Positivity of blood culture was low.

Keywords: Endocarditis, Intravenous Drug Users, Mortality

STUDY OF EPIDEMIOLOGICAL ATTRIBUTES OF VISCERAL LEISHMANIASIS IN MESHKIN-SHAHR COUNTY, ARDEBIL PROVINCE, NORTH-WEST OF Iran

Hamid Kassiri[1], Helia-Sadat Mortazavi[1], Esmail Ghorbani [1]
1.School of Health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Background: This study aimed to review the epidemiological characteristics of kala-azar cases in hospitalized patients and cases reported to health center in Meshkin-Shahr County.
Methods: This retrospective descriptive study was performed over a period of 20 years, from 1986 until 2005. Collected epidemiological data of visceral leishmaniasis in human were analyzed by SPSS.
Results: The total number of patients from the year 1988 to 2005 that affected by this disease and were reported in Emam-Khomeini hospital and health center of Meshkin-Shahr county were 2623. With respect of age division, 98.1% were below 10 years old. About 54.8% cases were male and 45.2% were female based on sexuality and the male to female ratio of disease is 1.2 respectively. The maximum out breaking of kala-azar disease with 32.6% were in spring and winter, the minimum with 17.5% in summer and 17.1% in autumn. Most of patients lived in rural areas.
Conclusion: Visceral leishmaniasis is major health problem in Meshkin-Shahr County, particularly within the rural people. Education programs alongside ecological studies and more intense control is recommended.

Keywords: Epidemiology; Visceral Leishmaniasis; Iran

THE PREVALENCE OF BOVINE IMMUNODEFICIENCY VIRUS IN WEST-CENTRAL Iran AND ITS ASSOCIATION WITH BRUCELLOSIS RATES AS A PREDISPOSING FACTOR

Mohammadreza Mahzounieh[1], Azam Mokhtari[2]
1.Associate Professor in Microbiology, Department of Pathobiology, Shahrekord University, Shahrekord, Iran.
2.Assistant Professor in Virology, Department of Pathobiology, Shahrekord University, Shahrekord, Iran.

Background: Bovine immunodeficiency virus (BIV) is an immunosuppressive pathogen. In recent years, it has been hypothesized that infection with BIV, might predispose cattle to be infected by other agents. The aim of this study was to investigate the effect of BIV infection on the susceptibility to *Brucella* infection and also changing some cattle health indices.
Methods: In this study, a total of 2290 blood samples collected from cattle housed in non-industrial (n=490) and industrial (n=1800) dairy farms respectively from Isfahan and Chaharmahal va Bakhtiari provinces, Iran. The BIV positive animals were detected by Lab- ELISA and nested PCR tests.
Results: In this study, the overall prevalence of BIV in Iran was 1.61% (4.5% and 0.83% in non- industrial and industrial dairy farms, respectively). There was a statistically significant relationship between BIV status and *Brucella* infection using Chi square and Pearson's correlation coefficient test for all of the samples (p=0.0001, r=0.24), samples from Chaharmahal va Bakhtiari (p=0.044, r=0.13) and from industrial farm in Isfahan (p=0.001, r=0.074).
Conclusion: It seems that BIV infection increases the susceptibility to brucellosis.

Keywords: Bovine Immunodeficiency Virus, *Brucella*, Seroprevalence

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MOLECULAR CHARACTERIZATION OF CRYPTOSPORIDIUM SPP. FROM CHILDREN WITH DIARRHEA IN MASHHAD CITY, Iran

Alireza Sadrebazzaz[1], Ezzat Mosavinezhad[2], Majid Farhoodi[1]
1. RAZI Vaccine and Serum Research Institute,Mashhad,Iran.
2. MSc in Biochemistry,Payam Noor University,Mashhad,Iran.

Background: *Cryptosporidium* species are intracellular and extra cytoplasmic protozoan parasites that have emerged as an important cause of diarrhea among humans and animals. *Cryptosporidium* also causes an important number of sporadic cases of diarrhea worldwide especially in children and the immunocompromised patients with widely differing prevalence rates among regions.
Methods: In this study, for molecular detection of this parasite from children under 6 years by RFLP-PCR methods in Mashhad city,Iran, a total of 250 diarrheal fecal samples from children under 6 years age average were collected in 2013 year and examined for *Cryptosporidium* oocyst using the cold Ziehl-Neelson staining method.
Results: Of the 250 fecal samples examined, there were 7 specimens positive for *Cryptosporidium* sp. Oocyst. *Cryptosporidium* oocysts presented in the 7 specimens were genotyped initially by nested PCR amplification of an approximate 830-bp fragment of the small subunit (SSU) rRNA gene and restriction fragment length polymorphism (RFLP) analysis of the secondary PCR products using restriction enzymes Ssp I and Vsp I and Dde I. All restriction patterns identified *C.parvum* and *C. andersoni* in children.
Conclusion: This study identified *C. parvum* and *C. andersoni* suggesting that they played more important role in the epidemiology of *Cryptosporidium* in this area.

Keywords: *Cryptosporidium*, Rflp-Pcr,Children, Iran

SURVEY PREVALENCE OF INTESTINAL PARASITIC INFECTIONS IN CHILDREN REFERRING TO MOFID HOSPITAL OF TEHRAN

Hooman Hanifehpour[1], Vafa Saber[1]
1. Department of Medical Parasitology, School of Medicine, Shahrekord University of Medical Sciences, Shahrekord,Iran.

Background: Today in many parts of the world the infectious disease created many problems. However injuries and damages caused by these diseases are more affected in developing countries compared with industrialized countries. Almost share of parasitic diseases in creating social and economic damages equal to some disease such as tuberculosis, sexually transmitted disease, vaccine preventable disease and acute respiratory infections.Parasitic infection is a common problem in children that the diseases are more important than other diseasessinceaffect the diagnosis time and treatment of diseases in growth and development of children.
Methods: In this study, 250 stool samples of children referring to Mofid hospital were collected. Then samples were tested by method of Formalin-Ether Concentration and then studied with light microscope.
Results: From 250 examined samples, stool collected from 165(66.25%) male and 85(34.2%) female. 43(17.16%) cases were positive for intestinal parasites.The rate of contamination with *Blastocystis hominis* was (12.5%) *Giardia lamblia* (3.40%)-*Iodamoeba buetschlii*(1.2%) – *Enterobius vermicularis* (0.04%) – *Entamoeba histolytica* (0.02%). No significant difference was found between infection the rate in different ages & sexes.
Conclusion: Results of this study showed that the infection rate of intestinal pathogenic protozoa especially protozoa *Blastocystis huminis* in children referring to Mofid hospital are more compared to intestinal pathogenic worms and this may be for various reasons including failure to comply cases of child health and also autoinfection of children and simple cycle of parasite transmission between people.

Keywords: Intestinal Parasites – Children –Autoinfection

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NOCTURNAL ACTIVITY OF SAND FLIES (DIPTERA: PSYCHODIDAE) AND THEIR MONTHLY LEPTOMONAD INFECTION IN CHABAHAR COUNTY, SISTAN-BALUCHISTAN PROVINCE, SOUTHEAST OF Iran

Hamid Kassiri[1], Ezatalldin Javadian [2]
1.Health School, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
2.Health School, Tehran University of Medical Sciences, Tehran, Iran.

Background: Cutaneous Leishmaniasis (CL) is one of the most important vector-borne diseases in Iran. Phlebotomus papatasi and P. salehi are reported as vectors of the disease in southeast of the country. The nocturnal activity of these species and their monthly leptomonad infection rates was studied in Chabahar County.
Methods: Sand flies were collected using sticky-paper traps at 2 - hours intervals from rodent burrows during May-October.
Results: A total of 9367 sand flies from four species of *Phlebotomus* and *Sergentomyia* genera were collected in the study of nocturnal activity. The most sand flies were collected in the first third of the night (7: 00-11: 00 PM), although there was seasonal variation in the nocturnal activity of different species. A total of 1132 female sand flies due to two species of *P. papatasi* and *P. salehi* were dissected. Totally 2.1% of *P. papatasi* and 1.07% of *P. salehi* were found to have leptomonad infection in their midgut, pharynx or head. The infection was observed in September and October in *P. salehi*, but in June, August, September and October in *P. papatasi*. Knowledge about nocturnal activity and biting rhythms of sand flies and therefore the risk of disease transmission is important for planning control programs for CL.
Conclusion: Decision makers in health system can do their best for control of vector-borne diseases, when different particles of transmission puzzle are defined. An important particle in the case of CL in the study area is clarified and helps for managing control program well.

Keywords: *Phlebotomus Papatasi; Phlebotomus Salehi*; Nocturnal Activity; Monthly Leptomonad Infection; Iran

THE PREVALENCE OF MYCOPLASMA AGALACTIAE IN SHEEP HERDS WITH AND WITHOUT SINGS OF CONTAGIOUS AGALACTIA IN KURDISTAN PROVINCE

Mohammad Khezri[1], Seyed Ali Pourbakhsh[2], Abbass Ashtari[2], Babak Rokhzad[1]
1.Veterinary Department, Agricultural and Natural Resources Research Center, Sanandaj, Kurdistan, Iran.
2.Reference Mycoplasma Laboratory, Razi Vaccine and Serum Research Institute, Karaj, Alborz, Iran.

Background: The aim of the present study was to detect *Mycoplasma* species and *M. agalactiae* in conjunctival, synovial fluid, nasal, ear and milk samples in sheep herds with or without CA sign in Kurdistan province by PCR.
Methods: Between 2012 and 2013, one hundred and seventy three samples analyzed were taken from sheep herds reared in a CA endemic area with (113 samples) and without (60 samples) signs of CA syndrome. The PCR with *Mycoplasma* 16S rRNA was applied for detection of a variety of *Mycoplasma* species. In this study two primers (forward and reverse) amplify 163bp region of 16S rRNA gene of *Mycoplasma* genus and amplify 375bp region of 16S rRNA gene of *M. agalactiae* species were used.
Results: A total number of 113 samples were analyzed, of which 105 (93.0%) proved positive for the presence of *Mycoplasma* species in sheep with signs of CA syndrome. From these positive samples, 13 isolates (12.4%) was positive for *M. agalactiae. M. agalactiae* was detected in conjunctival (4/50), synovial fluid (1/7), and milk (8/17) samples. Ear and nasal swap samples were free of *M. agalactiae.* The results of 60 samples in sheep without signs of CA syndrome showed that *Mycoplasma* species was detected in 25 samples (41.7%), and of these, 6 (24%) showed a positive result for *M. agalactiae. M. agalactiae* was detected in conjunctival (3/9), and milk (3/4) samples. Synovial fluid, ear and nasal swap samples were free of *M. agalactiae.*
Conclusion: Our findings indicate that in Kurdistan province; *M. agalactiae* was not the main etiological agents of the CA syndrome. Also, this species can be isolated from animals without clinical signs of disease.

Keywords: Contagious Agalactiae, *Mycoplasma agalactiae, Mycoplasma* Species, PCR,

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RAPID IDENTIFICATION OF CLINICALLY RELEVANT NOCARDIA SPP. BY 16S RRNA GENE PCR IN AIDS PATIENTS

Mahdi KhorshidTalab[1], Mohammad Hassan Shahhosseiny[2], Fatemeh Keshavarzi[3]
1.Department of Biology, college of Cellular & Molecular Biology, kurdistan Science and Research Branch, Islamic Azad University, Sanandaj, Iran.
2.Iranian Gene Fanavar Institute (IGF), Tehran, Iran.
3.Department of Biology, Islamic Azad University, Sanandaj, Iran.

Background: In this study, we developed an alternative PCR-based identification strategy targeted at the gene coding for 16S rRNA. Although the sequence for this gene is largely phylogenetically conserved, there may be variable sequences characteristic of particular organisms. The detection of these variable regions can therefore allow bacteria to be identified and differentiated from each other.
Methods: Primers NG1 and NG2 were used to amplify a *Nocardia* genus-specific 595-bp fragment of 16S rRNA. PCR test was optimized by *Nocardia brasiliensis* standard strain. Limit of detection and specificity test evaluated and then 30 AIDS patient serum samples was extracted by DNG kit. Amplicon was cloned in *E. coli* JM107 by pTZ57R plasmid and sequenced by Dideoxy chain termination.
Results: PCR testing was performed on 30 samples and 595-bp amplified product was observed by agarose gel electrophoresis. 2(6.6%) of 30 samples were positive for *Nocardia* PCR. In specificity test any products were observed, which indicates the high specificity of the test. Sensitivity of the test was obtained at least 100 CFU.
Conclusion: *Nocardia* spp. is an opportunistic bacterium that can produce severe infection and brain abscess in people with immune deficiency like AIDS patients. According to accurate and fast detection of *Nocardia* spp. can help AIDS patients to survive. Molecular identification methods such as PCR are accurate, cheap and fast methods.

Keywords: Aids; *Nocardia*; Pcr; Rapid Detection

AKI IN ZOONOTIC LEPTOSPIROSIS INFECTION

Mohammad Hazhir Alaei[1], Seyed Hamed Shirazi Beheshtiha[2]
1.Faculty of Veterinary Medine, Karaj Branch, Islamic Azad University, Karaj, Iran.
2.Department of Clinical Sciences, Faculty of Veterinary Medine, Karaj Branch, Islamic Azad University, Karaj, Iran.

Background: In the past decade, leptospirosis has emerged as a globally important infectious disease in human medicine specially in travelers that returning from endemic contries;butchers,veterinerian and farmers too. Leptospirosis is a risk factor for swine producers and slaughterhouse workers.
Methods: The incidence of positive agglutination tests in humans in contact with infected cattle(Dairy Cow) is surprisingly low and clinical cases in humans in which the infection is acquired from animals are not common. Symptoms can range from none to mild such as headaches, muscles pains, and fevers to severe with bleeding from the lungs or meningitis.
Results: If the infection causes the person to turn yellow, have kidney failure and bleeding it is then known as Weil's disease. Several factors are involved in acute kidney injury (AKI) in leptospirosis, including direct nephrotoxic action of the *Leptospira*, hyperbilirubinemia, rhabdomyolysis and hypovolemia.
Conclusion: The major histological finding is acute interstitial nephritis and acute tubular necrosis (ATN). Leptospirosis-induced AKI is usually nonoliguric and hypokalemic.tubular function abnormalities precede a decline in the glomerular filtration rate (GFR) which could explain the high frequency of hypokalemia.

Keywords: Aki, Leptospirosis, Zoonosis

COMPARISON OF THE MORBIDITY OF THRICHOMONAS VAGINALIS WITH THREE METHODS: DORSET, DIAMOND AND PCR

Naser Nazari[1], Houshang Jamali[2], Ali Mikaeili[1]
1.Department of Parasitology & Mycology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Ira.
2.Islamic Azad University, Jahrom Branch, Jahrom, Iran.

Background: *Trichomonas vaginalis* is a flagellate cell, which lives in the genital- urinary area. It is one of the most common factors, which results in the antivirus and venereal disease cause trichomoniasis. The object of this study is to evaluate the level of infection of *Trichomonas vaginalis* by the method of culture like Dorsse diamond and comparison with molecular method PCR in the Kermanshah Province.
Methods: 150 women referred to health center of Kermanshah from farvardin 1390, were sampled after taking information and questionnaire by speculum and cotton swab which were maintained in soluble Glucose from vagina secretions by a trained midwife. They were used for performing Dorsse culture and Diamond culture and another part soluble like a suspension for specific polymerase chain reaction(PCR) for trichomonas vaginalis.
Results: From 150 women suspecting to have trichomonas by Dorsse method, 8 subjects were positive. By diamond method, 10 subjects were positive and by PCR method, 14 subjects were positive. Our findings indicated that 10 subject of positive samples were 20 to 35 years old. This group of women had little education or illiterate and it was seen more percentage of infection.
Conclusion: Molecular method, PCR, campared with the Dorsse culture and Diamond culture showed high sensitivity while the culture of Dorsse or Diamond are time consuming. The culture of diamond costs more. So, we recommend the use of molecular method, PCR, for diagnosing the infection of *Trichomonas vaginalis* in the health centre.

Keywords: *Trichomonas vaginalis*, Dorset, Diamond, PCR, Infectious Diseases

SURVEY OF *SALMONELLA* SEROTYPES IN REPTILE PETS

Hossein Esmaeili[1], Afrooz Azarnush[1]
1.Department of Microbiology, Faculty of Veterinary Medicine, Tehran University, Tehran, Iran.

Background: The aim of this study was the detection of *Salmonella* serotypes in feces of some domestic reptiles.
Methods: Swab samples were taken from feces and environmental water of 4 snakes, 5 turtles and 3 iguanas existences. Samples were pre-enriched in lactose broth and then cultured in selenite cysteine enrichment media and tetrathionate broth. Finally, culture results transferred to XLD, SS and BG agar. Suspected colonies were confirmed by biochemical tests, then, *Salmonella* serotypes were determined by using kits supplied from Difco.
Results: *Salmonella typhimurium* was isolated from 1 turtle, 2 iguanas and 2 snakes and *Salmonella havana* was isolated from only 1 turtle. In total, 50% of the animals studied had *Salmonella* infection.
Conclusion: The high rate of *Salmonella* in reptiles increases the risk of transmission of this organism to people, especially children who use these unusual animals as pets and are in near contact with them.So reptile owners should be informed about the potential risks of owning these animals and appropriate preventive education should be provided for them.

Keywords: Iran, Reptiles, *Salmonella*, Zoonotic Risk

NOSOCOMIAL INFECTIONS AND RELATED FACTORS IN SOUTHERN KHORASAN HOSPITALS

Bita Bijari[1], Ali Abassi[2], Keyvan Karabi[3]
1.Department of Community Medicine, School of Medicine, Birjand University of Medical Sciences, Birjand,Iran.
2.Health Department, Birjand University of medical Sciences, Birjand, Iran.
3.Birjand University of medical Sciences, Birjand, Iran.

Background: This study aimed to determine the prevalence of nosocomial infections, the responsible microorganisms, and to identify the patients at high risk in hospitals with more than 100 beds in south Khorasan Province.
Methods: In three hospital with more than 100 beds in south Khorasan, an investigator-administered questionnaire was completed for each patient with nosocomial infections diagnosis from 20 Mar 2012 to 19 Mar 2013. This questionnaire conation demographic characteristic of patients, name of admission department, duration of admission, kind of pathogen and risk factors that was designed according to standard questionnaire of Iranian nasocomial infections surveillance system (INIS) of Center for communicable Disease Control, Ministry of Health that its validity and stability has been confirmed. Data were analyzed with SPSS 16 software.
Results: Number of patients with nosocomial infection was 358. The incidence of nosocomial infection was 0.9%. ICU had the highest incidence rate (17.3%) The mean age of patients was 37.6 ± 31.2. With an age range 1month -91 year. The most common nosocomial infection was pneumonia (43%), while the second one was urinary tract infection (UTI) (15.1%) and septicemia (11.7%). In 33.5% culture result were negative. In other cases, culture results showed that *Klebsiella*(12.8%) and *Pseudomonas aeruginosa*(9.8%) were the most prevalent bacteria. Pneumonia in ICU and PICU, wound infection in gynecology and surgical department and septicemia in NICU were the most common nosocomial infections. Most factors associated with nosocomial infection in patients were urinary catheters(70.4%), suction(66.8%) and Tracheal tube (54.2%).Ventilator-associated pneumonia (VAP) was found in 63.6% of patients. Mean length of hospitalization was 20.65 ± 18.76 days. 24% of patients expired.
Conclusion: The results of this study showed lower ratio of nosocomial infection in comparisonto other studies. The main reason is the failure to detect and report actual cases of nosocomial infection. Considering the importance of nosocomial infection, promoting detection and reporting system for Prevention and control of nosocomial infection was recommended.

Keywords: Nosocomial Infections, Related Factors,

ANTIBACTERIAL ACTIVITY OF TERMINALIA CATAPPA EXTRACT AGAINST PSEUDOMONAS AERUGINOSA ISOLATED FROM DIFFERENT INFECTIONS

Mohammad Mehdi Attarpour Yazdi[1]
1.Department of Microbiology, Faculty of Medicine, Shahed University, Tehran, Iran.

Background: The purpose of this study was to determine the antibacterial activity of methanolic extract from fruit of Terminalia catappa against *P.aeruginosa* isolated from different infections and to compare with effects of selected antibiotics in vitro.
Methods: This research is a descriptive analytic study. First, a sample of methanolic extract of the plant fruit was prepared by maceration method. Then its antibacterial activity against 126 isolates of *P.aeruginosa* from 185 samples of different infection was evaluated by well diffusion and then agar serial dilution method. Also, the MIC (Minimum Inhibitory Concentration) of the extract was determined. The effect of selected antibiotics was tested by disk diffusion method.
Results: The frequency distribution tables, diagrams, onava test were used to describe and compare the results. The results demonstrated that the plant extract had been effected against 126 of *P.aeruginosa* isolated(98.4%).The MIC of the extract for this bacteria was 20 mg/ml, while they were often resistant to selected antibiotics(100% resistant to Ceftazidime and 97.6% resistant to Tobramycin).There was significant difference between the effects of plant and antibiotics on *P.aeruginosa* ($P<0.001$).
Conclusion: This study demonstrates that a methanolic extract of Terminalia catappa is effective on *P.aeruginosa* isolated from different infection and its effect is even better than that of selective antibiotics. Further investigations will be necessary.

Keywords: *Pseudomonas aeruginosa*, Terminalia Catappa, Methanol Extract, Infection

CRYPTOSPORIDIUM INFECTION IN LAMBS AND CALVES: A THREAT TO PUBLIC HEALTH IN Iran

Nasrollah Vahedi Nouri[1]
1.Center of Agricultural Research and Natural Resource of Mazandaran, Mazandaran, Iran.

Background: This study investigates parasitic infections in lambs and calves country that can be a serious threat to the public health community.
Methods: Stool samples were collected from animals with apparently healthy and animals with diarrhea. These samples were transported to the laboratory. The diagnosis of *Cryptosporidium* Spp infections in these samples were determined by examining for the presence of oocysts in the fecal samples, using modified Ziehl-Neelson staining method.
Results: Results obtained from several studies in Different regions of the country shows that infection rate in animals (calves – Lambs) are variable from 4% in apparently healthy animals to 100% in diarrhea animals.
Conclusion: Considering that in many cases, infected animals, do not show symptoms, so oocysts excreted in the feces of these animals and can contaminate food and water and thus the threat to human health and public health.

Keywords: *Cryptosporidium* - Lambs – Calves- Threat - Public Health

Leptospira Infection In Cattle: A Threat To Public Health

Nasrollah Vahedi Nouri[1]
1.Center of Agricultural Research and Natural Resource of Mazandaran, Mazandaran, Iran.

Background: This study deals with the role and significance of infected animals a serious threat to the public health community.
Methods: A total of 1000 blood samples were collected from cattle without clinical signs. These samples were submitted to the *Leptospira* Research Laboratory. Serum was separated by centrifugation of blood at 3 000 g for 10 minutes at room temperature, the sera were transferred into 1.5 mL sterile micro tube (Eppendorf) and were kept at -20 °C until use. Seroconversion in microscopic agglutination test (MAT) was performed in *Leptospira* Research Laboratory.
Results: Antibodies were detected at least against one serovar of *Leptospira interrogans* in 116 sera (11.6 %) among 1000 samples at a dilution 1: 100 or greater.
Conclusion: Considering the apparently healthy cattle and Accordingly they can excrete infectious agents through the urine, this they can contaminate water or food and thus the threat to human health and public health.

Keywords: *Leptospira*, Cattle,Threat, Public Health

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PANTOEA AGGLOMERANS, A PLANT PATHOGEN CAUSING HUMAN DISEASE

Homa Forouhesh Tehrani[1], Maryam Barkhordari[2], Zahra Safari[2], Hooman Voosough[2]
1.Iran University of Medical Sciences, Tehran, Iran.
2.Department of Bacteriology, Bou-ali hospital, Tehran, Iran.

Background: *Pantoea agglomerans* is a Gram-negative aerobic *Bacillus* in the family Enterobacteriaceae. All species can be isolated from feculent material, plants and soil. Within the genus, *P.agglomerans* is the most commonly isolated species in humans, resulting in soft tissue or bone/joint infections. *P.agglomerans* bacteremia has also been described. Here we present a *P.agglomerans* in a patient who involved Abdominal abscesses.
Methods: The case was a 77 years old woman with the history of previous cholecystitis. Fallowing surgery, she was referred again to hospital with abdominal abscess. The aspirated specimen submitted to laboratory and evaluated by bacteriological procedure using Blood agar plate, Chocolate agar, Macconkey agar and Thioglycolate broth.
Results: Gram strained showed Gram negative bacilli. On Macconkey agar, Blood agar and chocolate agar yellow pigmented colonies about 4 diameters isolated. Differential test were negative for decarboxylaion of amino acids such as lysine,ornithin,arginine.
Conclusion: *P.agglomerans* is an uncommon cause of infection that can cause bacteremia, abscess, CVL infection, joint, bone. One limitation of diagnosis is that this bacterium is misidentified or incorrectly reported by inadequate differential test. The organism is susceptible to multiple antimicrobial agents. We have highlighted the need for another molecular-based technique.

Keywords: *Pantoea agglomerans*, Infection, Abscess

PROTECTIVE EFFICACY OF LIVE RECOMBINANT LEISHMANIA TARENTOLAE EXPRESSING KMP-11 – NTGP96-GFP FUSION COMBINED WITH NALOXONE AS A VACCINE CANDIDATE AGAINST VISCERAL LEISHMANIASIS

Vahid Nasiri[1], Abdolhossein Dalimi asl[1], Fatemeh Ghaffarifar[1], Azam Bolhassani [2]
1.Department of Parasitology, Faculty of Medicine, Tarbiat Modares University, Tehran, Iran.
2.Department of Hepatitis & AIDS, Pasteur Institute of Iran, Tehran, Iran.

Background: The use of the non-pathogenic *Leishmania tarentolae* as a live vaccine vector to deliver specific *Leishmania* antigens is a recent approach.
Methods: *L.infantum* KMP-11 and NT-Gp96 cloned into the pJET1.2/blunt cloning vector and then into pEGFP-N1 expression vector and then the KMP-11, NT-Gp96 and GFP fused in pEGFP-N1 and subcloned into leishmanian pLEXSY-neo vector. Finally this construct was transferred to *Leishmania tarentolae* by electroporation. Tranfection was confirmed by SDS-PAGE, WESTERN blot, flowcytometry and RT-PCR. Protective Efficacy of recombinant *Leishmania tarentolae* Combined with Naloxone were tested in susceptible BALB/c mice. Both humoral and cellular immune responses were assessed before challenge and at 4 weeks after *Leishmania* infection.
Results: KMP- NT-Gp96-GFP Fusion was cloned successfully into pLEXSY-neo vector and this construct was successfully transferred into *Leishmania tarentolae*. The strong protective effect was observed with live recombinant *L. tarentolae* with Naloxone.
Conclusion: The present study is the first to use a combination of live recombinant *L. tarentolae* with Naloxone and this vaccination regime as described in the present study could provide a potent strategy for future vaccine development.

Keywords: KMP-11, GP96*, Leishmania infantum*, Vaccine.

THE STUDY OF BACTERIAL INFECTION ASSOCIATED WITH MALE INFERTILITY IN QOM CITY

Reza Tabatabaii Qomi[1], Naser Kalhor[1], Mohsen Sheykh Hasan[1], Hoda Fazaely[1], Mahdieh Ghiasi[1]
1.Jihad Daneshgahi Infertility Center, Qom, Iran.

Background: An understanding of the link between infection of the accessory sex glands and reduced male infertility has been scientifically acquired and diagnostic tools are available, but the result of antibiotic treatment in terms of fertility remains disappointing. Acute and chronic infections and consequent inflammation in the male reproductive system may compromise the sperm cell function and the whole spermatogenetic process, causing qualitative and quantitative sperm alterations. The aim of this study was to investigate the frequency of semen bacterial infection in infertile men in Qom city.
Methods: The microscopic analyses and microbial culture of 76 semen specimens- using blood agar, EMB and muller mediums- collected over 3 months from male investigated for infertility were done.
Results: We find that 30.26% of evaluated samples were infected by different bacterial genus including: *Diplococcus* and *Staphylococcus*.
Conclusion: As a prerequisite step in treating male infertility, it seems to be necessary to detect bacterial infection of semen and curing it.

Keywords: Male Infertility, Bacterial Infection, Microbial Culture

MOLECULAR CHARACTERISTICS OF HEPATITIS B VIRUS ISOLATED FROM INTRAVENOUS DRUG USERS IN TEHRAN-Iran, 2013

Tahmine Kandelouei[1], Seyed Masoud Hossein[1], Latif Gachkar [2], Hossein Keyvan[3], Foad Davoodbeglou[4], Maryam Vaezjalali [1]
1.Department of Microbiology, Faculty of Biological Sciences, Shahid Beheshti University, Tehran, Iran.
2.Infectious Diseases and Tropical Medicine Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
3.Department of Virology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran.
4.Department of Immunology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Background: We investigated the possible genotypes in HBV-infected IVDU in Tehran.
Methods: This study was conducted on 229 intravenous drug users who referred to three Drop- in-Centers and Loghman hospital in Tehran. Their serum samples were tested for the presence of HBsAg (Hepatitis B surface Antigen) by Enzyme-Linked Immunosorbent Assay (ELISA). Then HBV DNA was extracted from HBsAg positive samples and a fragment of the S gene amplified by Nested PCR. HBV genotype, subgenotypes were determined by direct sequencing.
Results: Out of 229 individuals, 5 (2.1%) subjects were found to be serological positive for HBsAg, and HBV DNA was found in 3 HBsAg positive cases. Phylogenetic tree drawn by neighbor- joining method in HBV DNA positive drug users confirmed the existence of genotype D (subgenotype D1/ D2, subtype ayw2).
Conclusion: Classifying HBV into genotypes has to be cost-effective and clinically relevant, specially in high risk groups such as intravenous drug users. The most important finding of our study was that the only HBV genotype D was detected in all patients; our study concurs with other reports from Iran, all showing that genotype D is the only detectable genotype in Iran up to now.Drug injection behavior as the greatest risk factor, may have the leading role in the presence of genotype D. IVDU should be at a prime importance as the targets of disease preventation and control programs in Iran, as they do not stay permanently at a particular or specified area and they considered as mobile source of disease transmission.

Keywords: Drug Users, Enzyme-Linked Immunosorbent Assay, HBV, Genotype

HBV INFECTION AND IL-27 GENE EXPRESSION IN LIVER TRANSPLANT PATIENTS

Abdolhossein Zare[1], Ramin Yaghobi[2], Mohammad Hossein Karimi[2], Afsoon Afshari[2], Ahmad Rashki[3], Hamid Reza Miri[1]

1.Department of Biology, Faculty of Sciences, Zabol University, Zabol, Iran.
2.Shiraz Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
3.Department of Physiopathology, Faculty of Veterinary Medicine, Zabol University, Zabol, Iran.

Background: In this study the possible association between IL-27 expression level in HBV-infected liver transplant patients was evaluated.
Methods: In a cross sectional study, 50 liver transplant patients sub grouped to each 25 HBV infected and non-infected ones were enrolled between years.The 25 healthy controls were also evaluated. Screening and sub grouping patients to HBV infected and non-infected ones were analyzed using immunologic and molecular diagnostic methods.The expression of IL-27 gene was evaluated in three studied groups using in-house Syber Green Real Time PCR method: The GAPDH gene was used as internal control for Real-Time PCR reactions. The rate of increased expression was calculated using the Livak(2–ΔΔCT) method.
Results: The expression level of IL-27 gene was significantly higher in HBV infected liver transplant patients compared with non-infected ones. The expression level of IL-27 gene was also significantly higher in HBV infected liver transplant patients compared with healthy controls. The expression level of IL-27 gene relative to GAPDH gene was increased 10.27 times in HBV-positive liver transplant patients compared to healthy controls.
Conclusion: Based on these findings, HBV infection can lead to overexpression of IL-27 gene in liver transplant patients. Thus, it may possible to control post inflammatory outcomes of IL-27 by use of anti-HBV strategies in liver transplant patients. However, confirmation of these results needs to confirm in further completed studies

Keywords: Hepatitis B Virus, Interleukin 27, Liver Transplantation

EVALUATION OF ANTIBACTERIAL EFFECT OF GREEN TEA EXTRACT ON BACTERIAL CAUSE OF VAGINAL INFECTION

Masoud Dadashi[1], Gita Eslami[1], Mina Amjadi Kashani[1], Hossein Goudarzi[1], Fatemeh Fallah[1], Soudabeh Taheri[1]

1.Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Background: In this study, we examined the possible antimicrobial effects of Green tea on bacteria isolated from vaginit infection.
Methods: This study was conducted on samples obtained from 60 patients with genital tract infection in age from 20 to 40 years that referred to medical centers of Shahid Beheshti University of Medical Sciences.After sampling from patients by gynecologist, the samples were inserted in the transport media and were transferred to the microbiology laboratory of University.Then incubated samples after were cultured in specific media.with observing colonies and confirmation, Were isolated 4 types of microorganisms responsible for vaginitis detected that Including, *Listeria, Lactobacillus, Candida, Streptococcus* Group B.
Results: This experimental study was conducted on 60 patients with vaginitis the amount of the antimicrobial effect of green tea extract has been studied compared with six common antibiotics in the treatment of vaginitis on three microorganisms.Antimicrobial effects of green tea extract on *Lactobacillus* and *Listeria* was greater than other commonly used antibiotics studied except ciprofloxacin.In the case of group B *Streptococcus* antimicrobial effects of green tea extract was equivalent to ampicillin and gentamicin antibiotics.
Conclusion: Green tea extract can be used as an effective antimicrobial besides other commonly used antibiotics for the treatment of vaginal infections in women.

Keywords: Bacterial Vaginitis,Green Tea Extract,Bacterial Infection

EVALUATION OF HUMORAL IMMUNE RESPONSE IN TUBERCULOSIS PATIENTS THAN RECOMBINANT TB10.4 ANTIGEN BY ELISA METHOD

Razieh Kimiavimoghadam[1], Ghader Khalili[2], Mahnaz Seifi[3], Esmaeal Jabarzadeh[3], Sahar Teimury Tafreshi[4], Sara Khoshmagham[1]

1.Department of Microbial Biotechnology, Rabe Rashid University, Tabriz, Iran.
2.Department of Immunology, Pasteur Institute, Tehran, Iran.
3.Department of Tuberculosis and Lung Research, Tehran, Iran.
4.Department of Microbiology, Islamic Azad University of Kurdistan, Science and Research Branch, Kurdistan, Iran.

Background: The aim of this study was to determine the antibody level measurement among patients with tuberculosis in comparison with the control group against recombinant TB10.4 antigen by ELISA method.
Methods: Sixty eight serum samples from TB suspicious patients were referred to the Pasteur Institute during 2 years (91-92), 44 serum samples from healthy individuals and 5 samples from individuals infected with atypical mycobacteria were collected. In order to assess IgG class antibodies against recombinant TB10.4 antigen, Indirect ELISA method was applied. After optimization of all components, data analyzed in comparison with the absorbance of negative control and the range of results interpretation was determined.
Results: The recombinant TB10.4 antigen, on average with 96% of all serum samples from TB patients showed a positive reaction which shows sensitivity over 100%. On the other hand, among negative control serum samples with a history of BCG vaccination or isolation of environmental *Mycobacteria* also positive results were observed.
Conclusion: These findings indicate that despite the high sensitivity of TB10.4 antigen for screening of tuberculosis infection, because the high similarity of the genome sequence of this antigen among the most of mycobacterial species, including BCG vaccine strain, application of this antigen in separate for the rapid diagnosis of tuberculosis, do not have a high accuracy and should be used in combination with other specific antigens of *Mycobacterium tuberculosis* to meet an acceptable sensitivity and specificity.

Keywords: TB10.4 Antigen, ELISA, Atypical Mycobacteria, Humoral Immune

DESIGNING OF ELISA KIT FOR RAPID DETECTION OF MYCOBACTERIUM TUBERCULOSIS INFECTION WITH RECOMBINANT CFP10 PROTEIN.

Sahar Teimury Tafreshi[1], Ghader Khalili[2], Mahnaz Seifi[3], Esmaeal Jabarzadeh[3], Kambiz Davari[1], Razieh Kimiavimoghadam[4], Sara Khoshmagham[4]

1.Department of Microbiology, Islamic Azad University of Kurdistan, Kurdistan, Iran.
2.Department of Immunology, Pasteur Institute, Tehran, Iran.
3.Department of Tuberculosis and Lung Research, Tehran, Iran.
4.Department of Microbial Biotechnology, Rabe Rashid University, Tabriz, Iran.

Background: In the present study using Indirect ELISA, we evaluated IgG class antibodies among tuberculosis patients against recombinant CFP10 antigen.
Methods: In this study, Indirect ELISA method was used to investigate the serum reactivity to CFP10 antigen in 4 patient groups. Seventy serum samples of smear, culture and/or PCR positive cases, 5 serum samples from patients who have infections with non-tuberculosis mycobacteria, 20 cases of smear, culture and PCR negative, and 46 serum samples from healthy individuals (as negative control) were studied for IgG antibody levels during one year study (91-92) in mycobacteriology department of Pasteur institute of Iran.
Results: On average, recombinant antigen CFP10, reacts with 90% of all serum samples from patients with tuberculosis, by ELISA method. This issue shows accepted sensitivity of this antigen, especially for serum samples from tuberculosis cases with TB culture, smear and PCR positive (100% sensitivity). Among serum samples from healthy individuals, about 84% of the cases were negative. Antigen test results for sera from individuals infected with Non-*Mycobacterium tuberculosis* was also positive.
Conclusion: Application of the CFP10 antigen in rapid diagnostic kits for primary screening of TB infection meets acceptable sensitivity and specificity and using of this method could be more effective in accurate diagnosis, control and effective treatment of TB infection.

Keywords: Cfp10, ELISA, *Mycobacterium tuberculosis*

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ANALYSIS OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) IN PERTUSSIS TOXIN PROMOTER OF VACCINE AND CLINICAL ISOLATES OF BORDETELLA PERTUSSIS

Faezeh Haghighi [1], Masoumeh Douraghi [2], Fereshteh Shahcheraghi [3], Ebrahim Abbasi [4], Hossein Asgarian-Omran [5], Seyyed Saeed Eshraghi [2], Fazel Shokri [6]

1.Division of Microbiology, Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
2.Division of Microbiology, Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
3.Department of Bacteriology and Microbiology, Pasteur Institute of Iran, Tehran, Iran.
4.Department of Bacterial Vaccines, Razi Vaccine and Serum Research Institute, Karaj, Iran.
5.Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
6. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.

Background: The aim of this study is to identify the single nucleotide polymorphisms (SNPs) of pertussis toxin promoter (ptxP) in clinical isolates and vaccine strains.
Methods: Five clinical isolates of Bp, Tohama I as the reference strain, and 2 vaccine strains employed in Iran were used in this study. All of the isolates and strains were confirmed to be Bp by phenotypic and molecular tests. *ptxP* gene was amplified by PCR, cloned in TA vector and sequenced. The sequences were aligned and analyzed via BioEdit and ClustalX.
Results: Three distinct ptxP alleles were identified. The clinical isolates displayed the ptxP3 allele, whereas both vaccine strains and Tohama I was found to have ptxP1 and ptxP2 alleles, respectively.
Conclusion: In contrast to the vaccine strains, Bp strains presently circulating in Iran have the ptxP3 allele type. This change is a global phenomenon, which seems to be associated with resurgence of pertussis. The ptxP SNPs are mostly located in a region involved in regulation of transcription. This region is involved in binding to BvgA gene, which regulates the expression of the virulence genes in Bp. Presumably, changes in this region of promoter affects pertussis toxin gene expression.

Keywords: Pertussis, *Boredetella pertussis*, Single Nucleotide Polymorphism, Vaccination

ANTAGONISTIC ACTIVITY OF LACTOBACILLUS STRAINS ISOLATED FROM FECAL MICROFLORA OF HEALTHY BREAST-FED INFANTS AGAINST SHIGELLA FLEXNERI

Mohamad Mehdi Soltan Dallal[1], Abolfazl Davoodabadi[2], Rounak Bakhtiari[2], Zahra Rajabi[1]

1.Food Microbiology Research Center, Tehran University of Medical Sciences, Tehran, Iran.
2.Division of Bacteriology, Department of Pathobiology,School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.

Background: *Shigella flexneri* is a Gram-negative facultatively intracellular pathogen responsible for bacillary dysentery in humans. More than one million deaths occur yearly due to infections with *Shigella* spp. and the victims are mostly children of the developing world. The aim of this study was to investigate antagonistic activity of Lacto*bacillus* strains isolated from fecal microflora of healthy breast-fed infants against *Shigella flexneri.*
Methods: Fecal samples were collected from 90 healthy breast-fed infants younger than 18 months and cultured on MRS agar under anaerobic condition. Lacto*bacillus* strains were identified with phenotypic and biochemical tests. Then probiotic potential of isolated strains was evaluated in MRS broth with pH 2.5 and oxbile 3%. Finally antagonistic activity of *Lactobacillus* strains with probiotic potential evaluated against *Shigella flexneri* ATCC 12022 by well diffusion method.
Results: Out of 90 infants' stool samples, 72 (80%) samples were positive for *Lactobacillus*, and 238 isolates were identified as *Lactobacillus*. Twenty nine strains have probiotic potential. Twenty seven of 29 *lactoBacillus* strains inhibit the growth of *Shigella flexneri.*
Conclusion: This study suggests that these *Lactobacillus* strains could be used for prevention of *Shigella flexneri* infections.

Keywords: *Shigella flexneri, Lactobacillus*, Probiotic

URINARY TRACT INFECTION AMONG INTELLECTUAL DISABILITY INDIVIDUALS "ETIOLOGY AND ANTIBIOTIC RESISTANCE PATTERNS" IN REHABILITATION CENTERS OF MAZANDARAN PROVINCE, NORTHERN Iran 2014

Dr.Mohtaram Nasrolahei [1], Maryam Abdollahi [1], Zeynab Marzhoseini[1], Parisa Ebrahimzadeh[1]
1.Department of Microbiology and Virology, School of Medicine, Mazandaran University of Medical Sciences, Mazandaran, Iran.

Background: Urinary tract infection (UTI) is one of the major problems in rehabilitation centers for mentally retarded. The aim of this study was to determine the prevalence rate and etiologic agents of UTI in inhabitants of rehabilitation centers of Mazandaran province in northern Iran and to evaluate the antimicrobial susceptibility patterns of the uropathogens isolated.
Methods: Clean catch midstream urine sample was collected from each of 314 participants (163 males, 151 females) residing in 12 rehabilitation centers of Ramsar, Nowshahr, chalows, Amoul, Sari and behshahr. Urine specimens were cultured and bacterial isolates were identified by conventional methods. All urines fulfilling the criteria for the presence of pyuria and ≥104 cfu / ml urine were included in the study. Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method.
Results: The rate of urinary tract infection was30.9 %. In the present study, the highest rate of UTI were identified in pediatrics ($p<0.0001$).The prevalence of UTI were shown to be higher in female subjects than in male subjects with the rate of 46.3% in young aged females (20-29 years), 60% in middle aged females (40-49 years) and 50% in elderly (>50years). Bacteria most frequently isolated from urine specimens were *Escherichia coli* (39.2%), and *Enterobacter spp*(30.9%), followed by *Kelebsiella pneumoniae*(9.3%), *Enterococcus fecalis* (8.2%), *Proteus spp* (6.2%) and *Staphylococcus aureus*(6.2 %). The highest rate of infection caused by *E. coli* in female subjects age group <10 years ($p<0.001$) were observed. Among the antibiotics tested against the isolated organisms for sensitivity test, ceftriaxone and gentamicin maintain good activity against the majority of gram negative bacteria that cause UTIs recovered from intellectual disability individuals. vancomycin was effectiveagainst*S.aureus*.
Conclusion: This survey shows that the prevalence of UTI among intellectual disability individuals residing in 12 rehabilitation centers of northern Iran and the susceptibility pattern of uropathogens to antimicrobial agents are similar to many other reports in normal population.

Keywords: Urinary Tract Infection, Rehabilitation Center, Antimicrobial Resistance

SEROPREVALENCE OF LEPTOSPIRA INTERROGANS GRIPPOTYPHOSA BY MICROSCOPIC AGGLUTINATION TEST IN DAIRY FARMS IN SOUTHEAST OF Iran

Elham Mohammadi[1], Ehsanollah Sakhaee[1], Mohammad Khalili[1]
1.Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.

Background: Leptospirosis is a worldwide zoonosis caused by bacteria of the genus *Leptospira*. This study was conducted to detect antibody against serovar grippotyphosa of bovine leptospirosis by Microscopic Agglutination Test (MAT), in Jiroft suburb dairy farms, Kerman, Iran.
Methods: A total of 167 serum samples from 8 semi-industrial and 40 traditional dairy farms in Jiroft suburbs, were collected.
Results: Antibody was detected by microscopic agglutination test at least against one serovar of *Leptospirainterrogans* in 29 sera (17/36%) among 167 samples at a dilution 1: 100 or higher. Positive titers against more than one serovar were detected in 6 sera of the positive samples. A total of 11 samples were recorded positive against *Leptospira grippotyphosa*.
Conclusion: This study showed*Leptospira grippotyphosa* was the prevalent serovar in Jiroft suburbs dairy farms.

Keywords: *Leptospira grippotyphosa*, Serology, MAT, Dairy Farm, Iran

Investigation Of HBV Genotypes In Iranian HBS Ag Positive Blood Donors By Nested PCR

Pegah Hashemi Kani[1], Samira Salmanpor[2], Hossein Rassi[3]
1.Department of Microbiology, College of Basic Sciences,Shahre Qods Branch, Islamic Azad University, Alborz, Iran.
2.Biotechnology Academy of Gene Gostar, Iran
3.Department of Microbiology, Karaj Branch, Islamic Azad University,Tehran, Iran.

Background: Hepatitis B virus (HBV) is estimated to cause chronic infection in more than 350 million people worldwide and death in 1 million per year.
Methods: In this study, the serum samples of HBS Ag positive blood donors were collected from Emam Hospital in Tehran. Sera of 52 blood donors who were positive for HBS Ag were selected and viral load confirmed by Real Time PCR. DNA was extracted using commercial kit and the S gene sequence was amplified by nested-PCR. Finally, the PCR products were then analyzed for agarose gel electrophoresis that would be genotype specific.
Results: Overall prevalence of HBV-DNA infection was 50 out 52: 38 (76%) HBV D, 6 (12%) HPV A, 4 (8%) HPV F and 4 (8%) HBV C. The most frequent HBV genotypes were D and A.
Conclusion: Our results demonstrate that infection with HBV D was prevalent among HBS Ag positive blood donors, which is consistent with Iran and the Middle East dominant genotype. This assay system is considered to be a useful tool for the molecular diagnosis of HBV infection and for large-scale surveys.

Keywords: HBV Genotypes, HBV D In Iran,Nested PCR

IDENTIFICATION OF COXIELLA BURNETII (Q-FEVER) IN THE VARIOUS TISSUES OF THE SLAUGHTERED ANIMALS, IN SHIRAZ

Maryam Poormontaseri[1], Saeid Hosseinzadeh[1], Atefeh Nasri[1], Elaheh Sabzevari[1], Elmira Khatamsaz[1], Seyed Shahram Shekarforoush[1]
1.Department of Food Hygiene, School of Veterinary Medicine, Shiraz University, Shiraz Iran.

Background: The current study was aimed to investigate the frequency of Q fever agent using a PCR assay in the slaughtered animals in Shiraz. Therefore, DNA was extracted from various tissues including bronchial, mediastinal lymph nodes, mammary gland and kidney samples of sheep, goat in order to perform the PCR assay.
Methods: A total of 100 specimens including 50 kidney and 50 lymph node samples (from 15 sheep, 10 goat, and 25 cattle) were collected, DNA was purified followed by a Trans-PCR assay to detect IS1111 genome.
Results: A pair of species-specific oligonucleotide primers (Cox-F: GTCTTAAGGTGGGCTGCGTG, Cox-R: CCCCGAATCTCATTGATCAGC) was employed to detect a 295 bp fragment corresponding to IS1111 genome of C. burnetii. Our results showed a prevalence of 4% in all the specimens, in which, kidney samples from cattle were mainly involved.
Conclusion: Based on our observations, we can conclude that measures are to be implemented for the control of *C. burnetii* and Q fever in Iran.

Keywords: *Coxiella burnetii*, Trans-PCR, Slaughtered Specimens, Q Fever

PREPARTION OF A GENE CONSTRUCT FROM PROTECTIVE DOMAINS OF LEISHMANIA MAJOR

Shabnam Jeibouei[1], Mojgan Bandehpour, [2], Ali Haghighi[3], Bahram Kazemi[2]
1.Department of Medical Parasitology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2.Department of Biotechnology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
3.Department of Parasitology,School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Background: Leishmaniasis is an infectious disease affecting millions of people worldwide. The treatment of the disease is hampered due to high cost, toxicity and the crisis of drug resistance. Polytope approaches of genetic immunization could be a strategy for prevention of infectious diseases. Furthermore, the identification of leishmania genome sequence and the application of bioinformatics assist us to devise an effective vaccine's candidate.
Methods: In current study, we have designed a linear sequence from three predicted epitopes of GP63, LACK and CPC antigens by "T-cell prediction" server. The synthesized sequence regarded as "LAKJB93" was ligated to pEGFP-N1 plasmid. In current study, we have designed a linear sequence from three predicted epitopes of GP63, LACK and CPC antigens by "T-cell prediction" server. The synthesized sequence regarded as "LAKJB93" was ligated to pEGFP-N1 plasmid. In current study, we have designed a linear sequence from three predicted epitopes of GP63, LACK and CPC antigens by "T-cell prediction" server. The synthesized sequence regarded as "LAKJB93" was ligated to pEGFP-N1 plasmid.
Results: The synthesized construct consisting of 264 bp was confirmed through PCR amplification using specific primers. "LAKJB93" was cut by specific restriction enzymes, ligated to "pEGFP-N1", subcloned in "TOP10", and continual investigation is in progress.
Conclusion: After confirming the desired clone, later stages in producing the recombinant protein vaccine will be in progress.

Keywords: *Leishmania*, Epitope, Plasmid

ANTIMICROBIAL EFFECT OF STATINS IS SUPPRESSED BY CHOLESTEROL

Mohammad Reza Haeri[1], Kenneth White[2], Mohammad Qarebegloo[1]
1.Department of Clinical Biochemistry, School of Medicine, Qom University of Medical Sciences, Qom, Iran.
2.Londonmet University, London, England.

Background: Isoprenoid biosynthesis is a key metabolic pathway producing a wide variety of biomolecules such as cholesterol and carotenoids, which target cell membrane. In the other hand it has been reported that statins known as inhibitors of isoprenoid biosynthesis and cholesterol lowering agent may have direct antimicrobial effect on some bacteria. The exact action of statins in microbial metabolism is not clearly understood. It is possible that statins inhibit synthesis or utilization of some sterol precursor, which is necessary for bacterial membrane integrity.In order to test if statins inhibit the production of a compound, which can be used in membrane, cholesterol, would replace it and rescue bacteria from toxic effect of statins.
Methods: To examine that possibility, we assessed the antibacterial effect of statins with different classes; lovastatin, simvastatin and atorvastatin, alone and in combination with cholesterol on two gram-positive (*Staphylococcus aureus* and *Enterococcus faecalis*) and two gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli)* bacteria using gel diffusion assay.
Results: Our results showed that all of the statins except for lovastatin had significant antibacterial property in S. aureus, E coli and E faecalis. Surprisingly cholesterol nullified antimicrobial action of the effective statins in statin-sensitive bacteria.
Conclusion: It is concluded that statins may deprive bacteria from a metabolite responsible for membrane stability which is effectively substituted by cholesterol.

Keywords: Statin, Bacteria, Sterol, Hydroxymethylglutaryl Coenzyme A

THE PREVALENCE AND THE ANTIBIOTIC SUSCEPTIBILITY PATTERN OF BACTERIAL AGENTS ISOLATED FROM PATIENTS WITH BURN INFECTIONS IN IMAM KHOMEINI HOSPITAL IN KERMANSHAH 2009-20012

Sahar Afzali[1], Saeed Moradi[1], Nasrollah Sohrabi [2], Reza Karami Matin[3], Taraneh Bahmani[1], Soraya Bilvayeh[1], Kamal Farhang[4], Shiva Farhadian behrooz[5], Fatemeh Sheykhi[1]

1.Student of Laboratory Sciences, School of Paramedicine, Kermanshah University of Medical Sciences, Kermanshah, Iran.
2.Department of Medical laboratory Sciences, School of Paramedicine, Kermanshah University of Medical Sciences, Kermanshah, Iran.
3.Specialist of Infectious Diseases,Emam Khomeini Hospital, Kermanshah, Iran.
4.Laboratory Expert, Taleghani Hospital, Kermanshah, Iran.
5.Nurse, Taleghani Hospital, Kermanshah, Iran.

Background: The burn wounds provide bacteria with an enriched environment to grow and multiplication. Bacterial infections are responsible for more 50% of mortality associated with burned patients. The aim of this study was to determine the prevalence and the antibiotic susceptibility pattern of bacterial agents isolated from patients with burn infections in Imam Khomeini Hospital in Kermanshah from 2009 to 2012.
Methods: In this descriptive study, 200 samples were taken from patients and tested for bacterial infections. The samples were cultured on EMB and Blood agar. The bacterial agents then were identified using bacteriological and biochemical tests. Their susceptibility to antibiotics was assessed using disk diffusion method. The data were analyzed using SPSS software.
Results: Of 200 tested samples, 123 cases (61.5%) were positive culture at least for one bacteria. The high frequency infections were wound and urinary tract infections. The most common isolated bacteria were *Citrobacter freundii* (45.1%), *Pseudomonas aeruginosa* (18.7%) and *Klebsiella pneumoniae* (13.2%). The results of antibiotic susceptibility testing showed the maximum resistance for cephalexin and minimum for imipenem, ciprofloxacin and gentamycin.
Conclusion: Results of this study showed the high frequency of gram negative bacilli such as *Citrobacter* and *Klebsiella*. It is necessary to use appropriate control measures to prevent of these infections in this hospital. It is also important to use effective antibiotics based on results of antibiotic susceptibility testing.

Keywords: Bacterial Agents, Burn Infections, Antibiotic Susceptibility Pattern

INVESTIGATION OF SHIGA TOXIN PRODUCING ESCHERICHIA COLI AND CITROBACTER SPP. IN INTESTINAL DISORDERS OF PATIENTS IN THREE SEPARATE FOODBORNE OUTBREAKS IN TEHRAN, Iran

Elahe Tajeddin[1], Masoud Alebouyeh[1], Nahid Mansouri[1], Mohammad Reza Zali[1]

1.Gastroenterology and Liver diseases Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Background: The aim of this study was investigation of the involvement of Shiga toxin producing *Escherichia coli* and Citrobacter spp. in intestinal disorders of patients in three separate foodborne outbreaks in Tehran, Iran.
Methods: A total of 62 Stool samples from patients with diarrhea related to three outbreaks were cultured on Mac-Conkey agar and SMAC agar plates at 37°c under aerobic conditions. The plates were examined for bacterial growth at 24 hours (CFU/gram). All the isolates were characterized based on standard biochemical tests. DNA of the isolates was extracted by boiling method and screening of the *stx-1* and *stx-2* genes were performed by PCR.
Results: Out of the 62 diarrhetic samples, 33 (53.23%) samples were positive for *E. coli* and 3(4.84%) for *Citrobacter* spp. Shiga toxin 1 (*stx1*) gene was detected in 1 out of 3 *Citrobacter* spp.; but *stx 2* was not detected in any of these isolates. None of the stx1 and stx2 genes were detected among the *E. coli* isolates.
Conclusion: Role of the Stx1-producing *Citrobacter* spp. in human diarrhea was initially provided by our results in Iran. While this bacterium was not considered as responsible agent for occurrence of the studied outbreaks, major concern should be to control its dissemination in the community. Further studies are needed to understand role of this toxin and its carriage by Citrobacter spp. in human health.

Keywords: Shigatoxigenic *E. coli* (STEC), *Citrobacter* Spp., *Escherichia coli*

PREVALENCE OF Q FEVER IN DAIRY CATTLE BASED ON BULK- MILK ANALYSIS, IN SHIRAZ

Samaneh Rahmde[1], Negar Azimzadeh[1], Maryam Poormontaseri[1], Saeid Hosseinzadeh[1]
1.Department Of Food Hygiene, School of Veterinary Medicine, Shiraz University, Shiraz, Iran.

Background: The objective of this study was to estimate the prevalence of exposure to *C. burnetii* in bulk-milk samples of cows in Shiraz.
Methods: Bulk-tank milk samples from 100 dairy herds (included 80 industrial and 20 traditional dairy herds) were tested by Trans-PCR to detect IS1111 genome.
Results: A pair species-specific oligonucleotide primers was employed to detect a 295 bp fragment corresponding to IS1111 genome of *C. burnetii*. According to our results, 6 herds (6%) were positive by a PCR assay in this study which involved 3% positive samples corresponding to the industrial and traditional dairy herds.
Conclusion: Apparently based on our results, the proportion on the infection is higher in the traditional herd compared with the industrial one, so more epidemiological study is advised.

Keywords: *Coxiella burnetii*, Q Fever, Bulk-Tank Milk

HERD-PREVALENCE OF COXIELLA BURNETII (Q FEVER) ANTIBODIES IN DAIRY CATTLE FARMS BASED ON BULK TANK MILK ANALYSIS

Fatemeh Davoodi[1]
1.Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.

Background: To determine the prevalence of *Coxiella burnetii* (*C. burnetii*) antibody positive randomly selected dairy herds in southeast Iran (Kerman).
Methods: Bulk tank milk samples were collected randomly from 44 sufficiently large commercial dairy herds, included near 12000 dairy cattle, in Kerman (the largest province of Iran),southeast Iran. The samples were tested for antibodies against *C. burnetii* using the commercial CHEKIT Q fever antibody ELISA Test Kit (Idexx, Liebefeld-Bern, Switzerland).
Results: The prevalence of positive, negative and intermediate herds was 45.4%, 43.2% and 11.4%, respectively.
Conclusion: The result supports the hypothesis of high prevalence and endemic pattern of Q fever in Iran. The investigation highlights the importance of further studies on Q fever in Iran.

Keywords: Q Fever, *Coxiella burnetii*, Bulk Tank Milk, Dairy Cattle, ELISA, Iran

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MOLECULAR ANALYSIS OF THE LIPL41 GENE WITHIN LEPTOSPIRA INTERROGANS STANDARD SEROVARS IN Iran

Maryam Sadat Soltani[1], Pejvak Khaki[2], Soheila Moradi Bidhendi[2], Mohamad Hassan Shahhosseiny[1]
1.Department of Biology, Science and Research Branch, Islamic Azad University,Tehran,Iran.
2.National Reference Laboratory for Leptospira, Department of Microbiology, Razi Vaccine & Serum Research Institute, Karaj, Iran.

Background: In order to homological (polymorphism) analysis we sequenced and compared lipl41 genes cloned from standard pathogenic serovars of *Leptospira* prevalent in Iran.
Methods: Eleven pathogenic serovars and one saprophytic species were inoculated into the selective culture medium EMJH and the genomic DNA was extracted. The lipl41 gene was amplified by specific primers and cloned into a pTZ57R/T vector and transformed in competent *E. coli* Top10 cells. The extracted recombinant plasmid were sequenced. The percentage identity and divergence among different leptospiral serovars was deduced using the Blast and MegAlign program.
Results: PCR amplification of the lipl41 gene resulted in a 1065bp lipl41 gene product in all eleven pathogenic serovars tested. No PCR products were amplified from the non-pathogenic *L.biflexa*. In our study, nucleotide-sequencing results showed that the lipl41 gene has high identity between (96%-100%)
Conclusion: lipl41 gene was highly conserved among various pathogenic *Leptospira* strains and Lipl41 was a genus specific protein antigen, hence the cloned gene may be a good candidate for recombinant vaccine against leptospirosis.

Keywords: Leptospirosis; Sequencing ;Lipl41 ; Molecular Analysis

DETECTION OF MAGA GENE AMONG ESBL-POSITIVE AND ESBL-NEGATIVE KLEBSIELLA PNEUMONIAE ISOLATED FROM CLINICAL SAMPLES

Zahra Meshkat[1], Mahboubeh Naderi Nasab [1], Elnaz Harifi Mood[1], Nafiseh Izadi[2]
1.Antimicrobial Resistance Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
2.Student Research Committee (SRC), Mashhad University of Medical Sciences, Mashhad, Iran.Antimicrobial Resistance Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.

Background: The present study was performed to determine the prevalence of magA gene among extended-spectrum betalactamase (ESBL)-positive and ESBL-negative *K. pneumonia*e.
Methods: The current cross-sectional study was conducted on 130 *K. pneumonia*e isolates collected from patients at Imam Reza hospital and its associated clinics in the city of Mashhad (Iran) from May 2011 to July 2012. The presence of *K. pneumonia*e species were confirmed by conventional microbiological methods. Samples were tested for production of ESBLs by double disk diffusion (DDS) test. PCR was performed to detect magA gene. The hypermucoviscosity (HV) phenotype of *Klebsiella* isolates was characterized by string test.
Results: MagA gene was detected in 11(8.5%) isolates of K. pneumoniae. Of 11 isolates with positive result for magA gene, three were HV + and 8 were HV – phenotype. Of 11 isolates with magA gene, 7.14% (4 of 56) were ESBL-positive and non-HV phenotype9.46% (7 of 74) were ESBL-negative.
Conclusion: In the present study, HV phenotype were not found among ESBLs. Also, the presence of magA gene among non- HV phenotype strains suggests the role of other genes in the expression of HV phenotype.

Keywords: *Klebsiella pneumoniae*; Extended-Spectrum Beta-Lactamase (ESBL) Gene

INHIBITORY EFFECT OF LACTOBACILLUS REUTERI ON PATHOGENIC BACTERIA ISOLATED FROM WOMEN WITH BACTERIAL VAGINOSIS

Raheleh Karimiravesh[1], Gita Eslami[1], Sudabeh Taheri[1]
1.Department of Microbiology, Faculty of Medicine, Shahid Beheshti University, Tehran, Iran.

Background: The purpose of this study was to investigate the inhibitory effect of *Lactobacillus reuteri* on pathogenic bacteria isolated from women with bacterial vaginosis, respectively.
Methods: 96 samples from women with bacterial vaginosis discharge were taken by a gynecologist with a dacron swab and put in sterile tubes containing TSB broth and Thioglycollate broth and were immediately sent to the lab location in cold chain for the next stages of investigation. From Thioglycollate and TSB medium was cultured on blood agar and EMB and Palkam and Differential diagnosis environments. Then incubated for 24 h at 37 ° C. Strains of *Lactobacillus reuteri* were cultured in MRSA environment and were transfered to the lab. After purification of pathogenic bacteria, MIC methods and antibiogram, *Lactobacillus reuteri* inhibitory effect on pathogenic bacteria is checked. Statistical analysis was done by SPSS software v.16.
Results: The results of this study demonstrate the inhibitory effect of *Lactobacillus reuteri* on some pathogenic bacteria that cause bacterial vaginosis, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Streptococcus agalactiae*, *Entrococcus*, *Listeria monocytogenes* and *E. coli*. Microscopic examination of stained smears of the large number of *Lactobacillus* and pathogenic bacteria showed reduced. The prevalence of abnormal vaginal discharge, history of drug use means of preventing pregnancy and douching, respectively, 61%, 55%, 42% and 13% respectively. Significant difference was observed between the use and non-use of IUD in women with bacterial vaginosis infection.
Conclusion: Our findings indicated the inhibitory effect of *Lactobacillus reuteri* on the pathogenic bacteria that cause bacterial vaginosis.

Keywords: *Lactobacillus reuteri*, Probiotics, Bacterial Vaginosis

DETECTION RSBA GENE'S BAND & EFFECT OF MIRISTIC ACID IN VIRULENCE OF PROTEUS MIRABILIS ISOLATED FROM URINARY TRACT INFRCTION

Sara Ahmadi Badi[1], Jamileh Norouzy[1], Abbas Akhavan Sepahi[1]
1.Islamic Azad University, North Tehran Branch, Tehran, Iran.

Background: Bacteria communicate with each other by using molecular chemical signal, which are called autoinducer. By the increase of concentration of these signals which is the result of increase of cellular density, they coordinate genes, expersion in a microbial community. This process is called Quorum sensing. Considering the importance of urine tract infections prominent role of Quorum sensing and external signals in regulation of bacteria's genes' expression, in the present paper these consepts were studied: luxS, and rsbA genes' band in *Proteus mirabilis* isolated from urinary tract infections and the effect of Miristic Acid as an external signal in regulation of genes' expression.
Methods: In this study, 100 urine samples were collected. 10 *Proteus mirabilis* were isolated from these samples by standard bacteriologic methods. Then, by the extraction of genome, the presence of luxS & rsbA genes' band was studied in 10 P. mirabilis. At the end, the effect of Miristic Acid on the swarming & the biofilm formation ability of the P. mirabilis is assessed.
Results: In 100 urine sample studied, 10 *Proteus mirabilis* (10%) were isolated. 70% studied P.mirabilis, contained luxS & rsbA genes' band. Miristic Acid decreased the swarming of P. mirabilis in the concentration of 40 to 80 µl/100 ml of LB culture and the ability of p. mirabilis in the biofilm formation in all concentrations added to LB culture increased
Conclusion: The results obtained from the presence study indicative the presence of luxS,& rsbA genes' band in the most of *P. mirabilis* isolated from urinary tract infections, which demonstrate the presence of Quorum sensing regulatory systemes in these bacteria.

Keywords: Urinary Tract Infections, Quorum Sensing, Rsba Gene, *Proteus mirabilis*, Miristic Acid

OCCURRENCE OF INTEGRONS AND ANTIMICROBIAL RESISTANCE GENES AMONG CLINICAL ISOLATES OF ENTEROBACTER SPP. FROM HOSPITALS OF TEHRAN

Kobra Salimian Rizi[1], Shahin Najar Peerayeh[1], Bita Bakhshi[1], Mohammad Rahbar[2]
1.Department of Bacteriology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.
2.Department of Microbiology, Iranian Reference Health Laboratory, Ministry of Health and Medical Education, Tehran, Iran.

Background: The objectives of this study were to determine the prevalence of three classes of integrons in isolated *Enterobacter* spp. and to find out the association between the presence of integrons and antibiotic resistance.
Methods: A total of 110 clinical isolates of *Enterobacter* were isolated from several hospitals in Tehran between 2012 to 2013. *Enterobacter* species were identified by using API 20E system. The existence of integron classes was investigated by PCR assay through the amplification of integrase genes. In isolates with one of the integrons, antibacterial susceptibility the fourteen antibiotic disks were determined by disk diffusion and ESBL phenotype was confirmed by Combined Disk. Then, the bla groups, blaTEM, blaSHV, blaCTX-M-1 and aminoglycoside modifying enzymes genes were identified by PCR with specific primers.
Results: The prevalence of *Enterobacter* species were *E. cloacae* (78.2 %), E. aerogenes (13.6 %) and E. sakazakii (8.2%). They were from different clinical sources. Forty four of *Enterobacter* isolates have integron but there was not detected class 3 of integrons among isolates. All isolates with integron were susceptible to imipenem. In this 44 isolates, the rate of resistance to ceftazidime, cefotaxime, tobramycin, amikacin and gentamicin was 27.3 %, 22.7 %, 22.7 %, 11.4 % and 20.5 % respectively. Nine isolates of *Enterobacter* with integron showed ESBL phenotype that the frequency of blaTEM, blaSHV and blaCTX-M-1 genes between them are 20.5 %, 0 % and 16 %, respectively. The frequency of genes encoding ANT (2?)-Ia, APH (3?)-Ia, AAC (6?)-Ib and AAC (3)-IIa were 9.1%, 11.4%, 20.5 % and 13.6 %, respectively.
Conclusion: This is the first report of the emergence of AMEs and ESBLs with integrons in *Enterobacter* in Iran.

Keywords: *Enterobacter*, Integrons, Esbls, Aminoglycoside Resistance, API 20 E

PREVALENCE OF MALASSEZIA SPECIES ISOLATED FROM SKIN OF PATIENTS WITH SEBORRHEIC DERMATITIS REFFERED TO TONEKABON CLINICS BY PCR- SEQUENCING METHOD.

Ali raefi[1], Saba Amiri Kojori[1], Ebrahim Naghipoor[1], Rohallah Hosseini[1]
1. Department of Microbiology, Faculty of Biology Sciences, Islamic Azad University, Tonekabon Branch, Tonekabon, Iran.

Background: In this study, the yeast were studied by using real-time PCR method for the samples of skin scales from patients with seborrheic dermatitis and dandruff, skin clinic Tonekabon city
Methods: In this study, 65 collected from behind the ears, scalp, nails,dandruff are transferred onto selective media (SDA,Mdixon) to avoid dehydration of the yeasts After performing diagnostic tests to determine the biochemical and fungal species.Genomic DNA extraction by phenol-Chloroform method was done.for real-time PCR,ITS1-4 universal primers were used. PCR products for identification of *Malassezia* were sequenced
Results: Isolates obtained by cultured and biochemical test included: 4 case *Malassezia* globosa, 2case *Malassezia* restricta. Three colonies of fungus were not identified by culture and biochemical test. Cultural and biochemical identification was confirmed by molecular test. The result for 9 samples PCR-sequencing fungus,4 case *Malassezia globosa*,2case *Malassezia restricta*,2 case *Cryptococcus albidus* and 1case *Cryptococcus albidusmillis* were identified
Conclusion: The conventional identification of *Malassezia* species by phenotypic methods is complicated and time-consuming, and the result based on cultured methods is difficult to interpret. The PCR-sequencing method, could provide a sensitive and rapid detection and identification system for *Malassezia* sp., which may be applied to epidemiological survey and routine laboratory detection

Keywords: *Malassezia*, Real-Time PCR, Seborhiec Dermatitis

DETERMINE AND AND PREVALENCE OF CTX-M AND CTX-M-15 IN KLEBSIELLA PNEUMONIA ISOLATES FROM CLINICAL CASES OF KERMAN

Shima Rashidian[1], Reza Ghanbarpour[2], Babak Kheirkhah[1]
1.Science and Research Branch, Islamic Azad University of Kerman,Kerman,Iran.
2.Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman,Kerman,Iran.

Background: The purposes of this study were to determine the presence and prevalence of CTX-M and CTX-M-15 genes in *Klebsiella pneumoniae* isolates from clinical cases of Kerman
Methods: One hundred thirty two clinical samples were obtained during six month from different parts of Kerman hospitals and for identification of *Klebsiella pneumoniae* were referred to laboratory of veterinary faculty of shaheid Bahonar university of Kerman. Samples were cultured for isolation of *Klebsiella pneumoniae*. The suspected isolates were confirmed according to the biochemical tests. DNA was extracted from all of the confirmed isolates and reference strains by lysis method. PCR assay was used for detection of mentioned genes.
Results: Among the examined samples 92 *Klebsiella pneumoniae* were confirmed, which least 20 isolate was possessed one of the examined genes. Among 20 positive isolates 19 isolates (20.65%) were positive for CTX-M-15 and the one isolate (1.08%) was positive for CTX-M and CTX-M-15 genes
Conclusion: The CTX-M-15 gene has the highest prevalence among CTX-M genes and reported in different geographic regions of the world. In this study, the prevalence of CTX-M and CTX-M-15 genes among clinical isolates of *Klebsiella pneumoniae* indicated which the most prevalence was CTX-M-15 gene.

Keywords: Ctx-M, Ctx-M-15,*Klebsiella pneumoniae*

ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIAL PATHOGENS CAUSING URINARY TRACT INFECTION IN BESAT HOSPITAL OF SANANDAJ, 2012-2013

Arezzo Omati[1], Hamid lavakhamseh[2], Khaled Rahmani[3]
1.MSc student in Microbiology, Rasht Sciences and Researches University, Rasht,Iran.
2.MSc student in Microbiology, Kurdistan University of Medical Sciences, Sanandaj, Iran.
3.PhD student in Epidemiology, Department of Epidemiology, School of Public Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Background: The present study aimed to ascertain the current situation of antimicrobial resistance of Urinary Tract Infections (UTIs) caused by human pathogens in the Besat hospital of Sanandaj to provide data to help direct empirical therapy
Methods: This was a cross-sectional study, Referring to National Guide to Clinical Laboratory Procedures, all isolates of pathogenic bacteria causing urinary tract infections in besat hospital of Sanandaj in 2012 and 2013 were cultured and identified; the Antimicrobial susceptibility testing was analyzed by disc diffusion method using different antibiotics and their zone of inhibition was measured.
Results: From January 2012 to December 2013, 782 patients with UTIs identified that 596 (76.2%) of them were female and 379 (48.46%) were fromin-patients. 717 and 65 from them were Gram-negative and Gram-positive, respectively. Among 700 case of Gram-positive Pathogens (except Pseudomonas) 606 cases (86.4%) were *E.coli*. Percent of *E. coli* sensitivity to Antibiotics as follows: Ampicillin 18.7%, NalidixicAsid 45.86%, Nitrofurantoin 81.23%, Cotrimoxazole 41.22%, Amicacin 84.69%, Norfloxacin 70.33%, Cephotaxime 67.89%, Ciprofloxacin 75.30%, Gentamicin 73.74%, Cefrizoxime 80.93%, Tetracycline 39.21%, Ceftriaxone 69.29%, Ceftazidime 75.87%, Imipenem 74.14%, Cefixime 63.01%. There was no statistical association between Sex and Antimicrobial Susceptibility (P=0.7). In addition, 17 case of pseudomonas were Identified that most Sensitivity Related to Amikacin 92.3% followed by Gentamicin 84.6%, Ceftazidime 76.92%, Ciprofloxacin 62.5%, and Cefrizoxime 50%. In persons with Gram-positive Pathogens(65 case), antibiogram test results showed that the sensetivity to different antibiotics were as follows: Ampicillin 41.93%, Nitrofurantoin 75%, Cotrimoxazole 26.78%, Cephalotin 53.84%, Gentamicin 61.11%, Norfloxacin 38.88%, Oxacillin 31.19%, Vancomicine 59.25%, Tetracycline 14.28%.
Conclusion: By considering these results it seems clinicians should reasonably use antibiotics based on the results of drug susceptibility testing so as to restrain the increasing tendency of bacterial resistance.

Keywords: Urinary Tract Infection (UTI), Antimicrobial Sensitivity, Disc Diffusion

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IDENTIFICATION OF LISTERIA MONOCYTOGENES VIRULENCE FACTORS IN WOMEN WITH ABORTION BY PCR REFER TO MEDICAL CENTERS IN Iran

Elnaz Ohadi[1], Gita Eslami[2], Hossein Goudarzi[1], Arezou Taherpour[1], Soudabeh Taheri[1]
1.Department of Microbiology, International branch of Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2.Department of Microbiology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Background: The purpose of this study is the detection of virulence factors (hlyA and PLCA) *L. monocytogenes* in women with abortion using PCR method.
Methods: In this study, 96 patients with abortion were surveyed for *L. monocytogenes* by PCR and culture methods. Some varies like age, job, history of abortion and education was considered for all patients. Vaginal swabs and secretions were transferred to Trypticase Soy Broth as a transport media and then all samples transferred to Microbiology Laboratory. The tubes were incubated in 4o C and specimens were cultured on PALCAM media. Isolates were verified by gram staining, catalase and oxidase test, MR-VP, sugar fermentations and motility in 20 -25 o C. Then, PCR method was done for extracted DNAs. Data were analyzed by SPSS software.
Results: Out of 96 sampels, 16 isolates of *L. monocytogenes* by PCR (plcA and hlyA) and 4 isolates *Listeria monocytogenes* by culture have been identified. There was a significant differences between PCR and culture methods (p= 0.003). The results of this study showed that the PCR method is more sensitive and specific than culture method. There was also a significant association between bacteria and hlyA and plcA genes with human abortion and between patients with abortion precedent and education.
Conclusion: Based on our study, plcA and hlyA have played key role in the virulence determination of *L. monocytogenes*.

Keywords: *L. monocytogenes*, Abortion; Polymerase Chain Reaction; Culture.

EVALUATION OF INDUCED ANTIBODIES BY MICROPARTICLES CONTAINING CONJUGATED DETOXIFIED LPS FROM SALMONELLA PARATYPHI C TO TETANUS TOXOID

Fatemeh Esmaeil Zadeh[1], Reza Shapoori[1]
1.Basic Sience Faculty, Zanjan Branch, Islamic Azad University, Zanjan, Iran

Background: The purpose of this study is to design new candidate vaccine and assess its immunogenicity against *Salmonella* paratyphi C.
Methods: LPS extracted by hot phenol procedure and LPS Conjugation to TT was performed using amidation procedure, and for purification conjugated molecules column chromatography with 2B-Cl have been used and microparticles was prepared by emulsion technique in iso-octane. To evaluate the immunogenicity of prepared antigens, purified LPS, pure tetanus toxoid, normal saline and LPS-TT microparticles either injected intraperitoneally with a final volume of 0/5 mg to female mice BALB/c (6-8 weeks), and three times injection was conducted at two-week intervals and heart blood of mice was performed two weeks after injection. Collected serum stored at a temperature of 70 ℃ and specific lipopolysaccharide antibodies titers were determined by indirect ELISA.
Results: In this study mice IgM, IgG, IgA and IgG1, IgG2a, IgG2b and IgG3 subclasses against LPS of *Salmonella* paratyphi C were determined. All groups received LPS - TT microparticles and LPS rather than controlled groups showed a significant differences in all three course of injections in terms of serum antibodies. IgG titers for LPS-TT microparticles were 400, 1350 and 1890 EU in received mice at three times of vaccination, predominantly and mot induced IgG subclasses were IgG1 and IgG3.
Conclusion: Results indicated that microparticle molecules have a better ability than pure LPS to stimulate of humoral immunity. Also tetanus toxoid has appropriate capabilities as for protein carried conjugated vaccines and conjugation has been conducted more stable.

Keywords: Microparticles, *Salmonella paratyphi* C, LPS, Tetanus Toxoid

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PREPARATION OF DETOXIFIED LOADED ALGINATE MICROPARTICLES LPS SALMONELLA PARATYPHI C WITH TETANUS TOXOID

Fatemeh Esmaeil Zadeh[1], Reza Shapoori[2]
1.MSc Student in Microbiology, Basic Science Faculty, Zanjan Branch, Islamic Azad University, Zanjan, Iran.
2.Department of Microbiology,Basic Science Faculty, Zanjan Branch, Islamic Azad University, Zanjan, Iran.

Background: The goal of this research is to design vaccine against *Salmonella* paratyphi C.
Methods: LPS extraction was performed using phenol-water and LPS Conjugation to the TT was applied by amidation method. For purification of conjugated molecules Sepharose Cl-2B column chromatography was used. Collected fractions at two wavelengths 210 and 280 nm were read out and tubes with the highest absorbance at both wavelengths have been collected and are being merged. Then the alginate microparticles were prepared by emulsification in the iso-octane organic phase.
Results: The results of SDS-PAGE electrophoresis containing urea with 4M silver staining was shown that healthy LPS were isolated from bacteria. Fractions 83 and 84 have highest absorb by both wavelengths of 210 and 280 and have merged and after alginate microparticles were prepared and photographed by light microscopy, which showed the formation of solid microparticles and particle size analyzer was used to measure the diameter of microparticles that the diameter of the particle was 89.36 μm.
Conclusion: The results show that TT Conjugation with LPS *Salmonella* paratyphi C can be done by amidation technique and the conjugated can be absorbed in the form of alginate microparticles.

Keywords: Microparticle, Detoxified LPS, *Salmonella paratyphi* C

ANTIBIOTIC SUSCEPTIBILITY PROFILE OF COMMON BACTERIAL PATHOGENS IN THE PEDIATRIC WARD OF DASTGHEIB HOSPITAL, SHIRAZ, Iran: A CROSS SECTIONAL STUDY

Mohammad Motamedifar[1], Khoosro Zamani[2], Yashgin Hassanzadeh[2], Sajede Pashoutan[3]
1.Bacteriology and Virology Department, Shiraz Medical School. HIV/AIDS Research Center (SHARK), Shiraz University of Medical Sciences, Shiraz, Iran.
2.Bacteriology and Virology Department, Shiraz Medical School, Shiraz University Of Medical Sciences, Shiraz, Iran.
3.Student of research committee, Shiraz University of Medical Sciences, Shiraz, Iran.

Background: The aim of this study was determination of etiological agents of bacterial infection and their antimicrobial susceptibility profiles in the pediatric ward of Dastgheib hospital, Shiraz.
Methods: A cross-sectional study was carried out on 4500 hospitalized patients with diagnostic of nosocomial infections and range age between 1 month to 14 years old in pediatric ward of Dastgheib hospital of the Shiraz University of Medical Sciences from July 2009 to September 2011. Samples were included urine, blood and cerebrospinal fluid (CSF). Bacterial pathogens were identified by using conventional bacteriology methods (culture and biochemical tests). Antimicrobial susceptibility testing was done using the disc diffusion technique (Kirby Bauer method).
Results: out of 720 positive samples, 460 samples related to urine culture (63.9%), 180 samples related to blood cultures (25%) and 80 samples related to cerebrospinal which the most prevalent isolated bacteria included *Escherichia coli* (65.2%), coagulase negative Staphylococcus (55.6%) and *Streptococcus pneumoniae* (50%). *Escherichia coli* isolates were showed the highest and the lowest resistance rate to cephalexin (60.5%) and Imipenem (11.6%) respectively. Vancomycin was most active agent against coagulase negative Staphylococcus and *Streptococcus pneumoniae* isolates, with susceptibility rates of 96.5 and 85.7%, respectively. Cotrimoxazol (92%) and Ampicillin (91.2%) were the least effective agents against Staphylococcus coagulase negative. Also Penicillin and Ampicillin with resistance rate of 93.6% and 91.5% were the least effective agents against *Streptococcus pneumoniae* respectively.
Conclusion: *Escherichia coli*, Coagulase negative staphylococcus and *Streptococcus pneumoniae* were the most prevalent cause of infections in Dastgheib hospital. The rates of antibiotic resistance among pathogens in this study were high.

Keywords: Pediatric, Antibiotic Susceptibility, Bacterial Pathogens

ISOLATION AND MOLECULAR IDENTIFICATION OF BASILLUS PUMILUS FROM WOUND INFECTION FOR THE FIRST TIME IN IRAN

Fatemeh Saleh[1], Hossein Azizi[2], Farnaz Kheirandish[3], Maryam Azizi[4], Afshin Javanmard[5]

1.MSc student in Microbiology, Razi Herbal Medicines Research Center. Lorestan University of Medical Sciences, Khorramabad,Iran.
2.MSc student in Microbiology, Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences,Khorramabad,Iran.
3.Department of Parasitology and Mycology, Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran.
4.Department of Microbiology,Yazd Branch, Islamic Azad University, Yazd,Iran.
5.Department of Laboratory Science, Broujerd Branch, Islamic Azad University, Broujerd Iran.

Background: *Bacillus pumilus* is used for transferring genetic materials and its infecting cases have been rarely reported among humans. In human, it mostly induces secondary infection.
Methods: In October 2012 a male patient with a sore back at the age of 41 years was sampled by sterile swab. Then the wound specimens were cultured on Blood Agar medium. After incubation, the culture medium put at 37 ° C in aerobic conditions and 5% CO2. PCR was used to determine the molecular identity.
Results: After various biochemical tests, PCR, and sequencing of the 16S rRNA gene analysis showed 99% homology with the target gene based on the gene sequence. It was a strain of *Bacillus pumilus*. Target gene in the NCBI GeneBank database access numbers were AB894358. This is the first report of isolation of *Bacillus pumilus* of bedsores in Iran.
Conclusion: *Bacillus pumilus* as a human pathogen, has been paid little.

Keywords: *Bacillus pumilus*, PCR, Bedsores, Hospital Infections

DISTRIBUTION OF PSPC, PHTD, PHTE, RRGA AND LYTA GENES AMONG STREPTOCOCCUS PNEUMONIAE ISOLATES RECOVERED FROM HEALTHY CHILDREN IN ARDABIL, Iran

Masoomeh Parvizi[1], Mohsen Arzanlou[2]

1.Islamic Azad University of Ahar, Ahar, Iran.
2.Ardabil University of Medical Sciences, Ardabil, Iran.

Background: The objective of this study was to determine the distribution of gens for four protein antigens: Pneumococcal histidine triad D and E (PhtD; PhtE), RIr-regulated gene A (RrgA) and Autolysin (LytA) among pneumococcal isolates from healthy nasopharyngeal carriers.
Methods: A total of 43 pneumococcal isolates, were collected from nasopharyngeal specimens of healthy children attending the kindergartens in Ardabil province, Iranin 2013. The strains were identified using optochin susceptibility and bile solubility tests and further confirmed by amplification of capsular polysaccharide A gene(cpsA). PCR was used to screen for the carriage of phtD, phtE, rrgA and lytA genes.
Results: 81.4% of isolateswere found to contain at least one of the tested genes.lytA, phtE, phtD and rrgA were detected in 70%, 39.5%, 35% and 25.5% of isolates respectively. In total, 9.3% of isolates were found to harbor all of the 4 genes.
Conclusion: The results showed that the genes were not distributed consistently among the isolates and for obtaining of a full coverage pneumococcal vaccine, multiple of these antigens should be included

Keywords: *Streptococcus pneumonia*, Phtd, Phte, Rrga, Lyta

SPREAD OF MULTIDRUG-RESISTANT ACINETOBACTER BAUMANNII ISOLATES IN ICU PATIENTS

Zohreh Ghalavand[1], Hossein Goudarzi[1], Mona Ghazi[1], Bahareh Hajikhani[1], Masoumeh Foroumand[2]
1.Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Science, Tehran, Iran.
2.Imam Hossein Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: The aims of this study were to evaluate the OXA-type carbapenemase genes in clinical isolates of *A. baumannii* and investigate genetic relationship among the isolates.
Methods: One hundred and twenty-five clinical isolates of *Acinetobacter baumannii* were obtained from hospitalized patients from June to Desember 2010 in Imam Hossein Hospital, Tehran. Isolates were identified by conventional biochemical tests such as oxidase test, motility, growth at 44°C and then were confirmed by amplification of blaoxa-51-like gene using PCR. Susceptibility to antimicrobial agents was assessed by the disk diffusion method. DNA extraction was carried out by boiling method. The presence of OXA-type carbapenemase genes detected by Multiplex PCR. Molecular typing of isolates was performed using repetitive extragenic palindromic sequence based polymerase chain reaction (REP-PCR) method. Then, REP-PCR fingerprints were analysed with GelCompar II software.
Results: Our results showed that *A. baumannii* strains were isolated more frequently from patients hospitalized in ICU (74.4%) other than wards and the most rate of isolation was from respiratory secretions (53.6%). Although all of the clinical isolates were susceptible to colistin, the resistant rate to piperacillin- tazobactam, cefteriaxone, ceftazidim, gentamicin, ciprofloxacin, cotrimoxazole, imipenem, levofloxacin, was more than 90%. We found that 74 (59.2%) isolates carried the blaOXA-23-like gene. Among the isolates, 14(11.2%) harbored the blaOXA-58-like and 3(2.4%) the blaOXA-24-like genes. All of the isolates harbored the blaOXA-51-like gene. Molecular typing results showed the presence of different REP patterns among isolates in different wards of hospital.
Conclusion: This study shows a high distribution of blaOXA-23 -like gene in clinical isolates of *A.baumannii.*

Keywords: *Acinetobacter baumannii*, Multidrug-Resistant, REP-PCR

FREQUENCY OF LISTERIA MONOCYTOGENES IN 2TH AND 3TH TRIMESTERS PREGNANT WOMEN REFERRED TO EMAM KHOMEINI AND RAZI HOSPITALS IN AHVAZ -Iran

Neda Nazarzadeh Zare[1], Mansour Amin[1]
1.Department of Microbiology, School of Medicine,Infectious Diseases and Tropical Medicine Research Center, Ahvaz Jundishapour University of Medical Sciences, Ahvaz, Iran.

Background: The aim of this study was to detect the bacteria in vaginal specimens by culture and PCR methods, and performing a comparison between them.
Methods: A total of 354 women (mean age 32.3 ± 4.6 years) were included in this study. Their vaginal specimens were cultured on PALCAM agar medium. Grams staining of grown colonies were then performed. The motility of suspected colonies was assessed on SIM medium at 25 and 35 degrees. In addition to SIM medium, the colonies were cultured on bile sculin medium, as well. Blue- brown colonies were produced through sculin hydrolysis by *Listeria monocytogenes*. Catalase, Oxidase and MR-VP tests were performed.The suspected colonies were evaluated by the PCR technique.
Results: Eight samples out of 354 samples were positive for *Listeria monocytogenes* by culture(2.3%). All positive samples confirmed by PCR. Four women with history of abortion, had positive samples for *L. monocytogenes* by both culture and PCR methods (50%).
Conclusion: The present study revealed that the culture method is still as the gold standard technique in detecting *Listeria monocytogenes*; however, it is time- consuming. The 50% aborted women who had culture and PCR positive, confirms the important role of *Listeria monocytogenes* in the abortion in pregnant women.

Keywords: Vaginal Specimens; *Listeria monocytogenes*; Culture; PCR

STUDY OF RESISTANCE PATTERN OF ANTIBIOTIC OF STAPHYLOCOCCUS AUREUS STRAINS TAKEN FROM CLINICAL SAMPLES OF SOME AHVAZ HOSPITALS BY DISK DIFFUSION METHOD.

Foroozan Kavoosinezhad[1], Esmail Fattahi[1], Naghmeh Moori Bakhtiari[2]
1.Department of Biology, Ayatollah Amoli branch, Islamic Azad University, Amol, Iran.
2.Department of Pathobiology, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Background: This project was conducted along with purpose of studying antibiotic resistance pattern of *Staphylococcus aureus* strains taken from clinical samples of Ahvaz hospitals.
Methods: In this descriptive study, *Staphylococcus aureus* strains in clinical samples (urine, blood, abscesses, ulcers) of patients visited to medical centers of Ahvaz during the whole year of 92 were separated and after diagnosis and confirmation by using biochemical test, their antibiotic resistance was analyzed by Kirby-Bauer method.
Results: The findings of this research by disk diffusion showed that 50 achieved isoles out of 230 clinical samples, 100 % of isoles (50 items) were resistant against methicillin, ampicillin and penicillin. After that the most resistance in isoles against Erythromycin about 48 % (24 isoles), next resistance against Gentamicin and ciprofloxacin and clindamycin by 34% was seen. The resistance to Vancomycin was seen in only one of the isoles by 2 % abundance.
Conclusion: By considering high resistance of antibiotics, strict care must be taken over *Staphylococcus aureus* and the unnecessary use of antibiotics must be avoided.

Keywords: Antibiotic Resistance, *Staphylococcus aureus*, Clinical Samples, Disk Diffusion.

ASSAY FOR INTEGRONS AND CHARACTERIZTION OF ANTIMICROBIAL RESISTANCE IN E.COLI STRAINS ISOLATED FROM URINARY TRACT INFECTIONS

Somayeh Mikaili[1], Azam Haddadi[2], Mahmoud Shavandi[1], Farzaneh Asadi[1]
1.Department of Biology, College of Sciences, Karaj branch, Islamic Azad University, Alborz, Iran.
2.Microbiology and Biotechnology Group, Research Institute of Petroleum Industry, Tehran, Iran.

Background: The aim of this study was to determine the prevalence of multidrug resistant *Escherichia coli* in clinical specimens. In addition, the existence of integrons in resistant isolates was assessed by amplification of integrase genes.
Methods: Susceptibility of 111 isolates from Karaj hospitals and laboratories was determined to 20 antibiotics by the Kirby-Bauer disk diffusion method and integrons were detected with degenerated primers to conserve regions for integron-encoded integrase genes. Then the frequency of multi-drug resistance was assessed.
Results: Isolates showed the highest resistance to amoxicillin. Nitrofurantoin by the highest susceptibility was the most effective drug in vitro. Ninety-eight isolates out of 111 were multi-drug resistance. Frequencies of MDR to three, four, five and six or more antibiotics were 79.2%, 74.7%, 72% and 59.4%, respectively. The existence of integrons was confirmed for 23.4% of isolates by PCR. Integrons play an important role in antibiotic resistance of clinical *E.coli* strains because they are able to capture, integrate and express gene cassettes encoding antibiotic resistance. It is well known that integrons carry and transfer MDR genes in bacteria.
Conclusion: Multi- drug resistance suggests that strategy for treatment of patients with *E.coli* infections needs to be revised. The possibility transmission of resistance genes by integrons would be decreased by treatment of patients with the appropriate antibiotics.

Keywords: *E. coli*, Antibiotic Resistance, Integron, PCR.

THE PREVALENCE OF BACTERIAL INFECTIONS IN CHILDREN UNDERGOING DIALYSIS WITH CULTURE METHOD AND THE PCR, AND DETERMINATION DRUG SENSITIVITY AND RISK FACTORS OF INFECTION

Maryam Bideh[1], Gholamreza Irajian[2], Ali Mojtahedi[3], Nakisa Hooman[4]

1.Department of Microbiology,Science and Research Branch, Islamic Azad Universityof Guilan, Guilan, Iran.
2.Department of Bacteriology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran.
3.Department of Microbiology, Faculty of Medicine, Gilan University of medical science, Guilan, Iran.
4.Department of Pediatric Nephrology, Ali-Asghar Childeren Hospital, Tehran,Iran.

Background: Diagnosis of infection caused by bacteria and determining of their antibiotic resistance patterns may be helpful in treatment of patients and increasing of life quality.
Methods: Blood or peritoneum samples of hospitalized children in dialysis ward of Bahrami, Hazrat Ali Asghar and Mofid hospitals with clinical symptoms of peritonitis in peritoneal dialysis or septicemia in the hemodialysis were collected and sent to the laboratory. Microbiological and biochemical tests revealed bacterial infection in 4 samples of 42 and sensitivity test performed for all 4 bacterial isolates. PCR performed for 4 isolates using specific primers and PCR products were electrophoresed in 1.5% agarose gel containing ethidium bromide. DNA ladder was used to detect the molecular weights of observed bands under UV lamp. Finally, results of microbiological and molecular methods were compared.
Results: The isolated bacteria were *E.coli*, *Klebsiella pneumoniae*, *P.aeruginosa* and *Acinetobacter baumannii*. All of them were susceptible to Gentamycin and Ciprofloxacin and resistant to the Ampicillin. As expected, all positive cultures had positive reaction in PCR. None of the negative cultures had band in agarose gel after PCR. So, PCR results confirmed the results of cultures.
Conclusion: Only 9.52% of patients with infection symptoms had blood or peritoneal infections, and similar symptoms in the remaining patients were because of other reasons. Determining of antibiotic resistance pattern leads to fast and appropriate treatment. As identification of bacteria via cultivation usually takes 24 to 48 hours, rapid diagnosis can save patients' life in some cases, so application of molecular methods along with cultivation can be more effective for the diagnosis of bacterial infection.

Keywords: Peritoneal Dialysis, Hemodialysis, Septicemia, Peritonitis, Bacterial Infection, PCR

ANTIFUNGAL ACTIVITY OF TOTAL EXTRACT AND CHLOROFORM, METHANOL AND AQUEOUS FRACTIONS OF AERIAL PARTS OF NEPETA DEPAUPERATA AGAINST 5 FUNGAL STRAINS

Samira Kariminejad[1], Jinous Asgarpanah[2], Seyed Reza Hosseini Doust[1]

1.Department of Microbiology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran.
2.Department of Pharmacognosy, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran.

Background: Increased consumption of synthetic antifungal compounds has created serious side effects and wide spread antifungal resistance. We report the results of evaluation of antifungal properties of different crude extract and relevant fraction of aerial parts of *Nepeta depauperata.*
Methods: Extracts and fractions were obtained according to maceration standard methods. The crude extract and fractions were diluted in different values from 400 to 25 mg/ml. *Candida albicans*(ATCC 10231), *Aspergillus niger* (PTCC 5010), *Aspergillus flavus* (PTCC 5004), *Aspergillus fumigatus* (PTCC 5009), fusarium oxysporium (PFCC 38-115), were used to assay the antifungal activity by disc diffusion method. The drug ketoconazole was used as a control for lack of growth in order to comparing it as a chemical drug with the mentioned extract and fractions. MIC values were determined using micro-dilution method according to CLSI.
Results: The results showed that total extract and chloroform, methanol and aqueous fractions have antifungal effects against *Candida albicans* only.Methanol and chloroform fractions have more inhibition effect than total extract and aqueous fraction on *Candida albicans*.
Conclusion: We conclude that this plant contains antifungal compounds, so the exact experiments and evaluation are prerequirement of any applicable recommendation

Keywords: *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Candida albicans*, *Fusarium oxysporum, Nepeta depauperata*

EVALUATION OF HUMORAL IMMUNITY OF HAEMOPHILUS INFLUENZAE TYPE B PRP POLYSACCHARID CONJUGATE WITH TETANUS TOXOID

Mozhgan Ghasemian dastjerdi[1], Hojat Ahmadi[2], Mehdi Nejati[2], Vajihe Karbasizade[1]
1.Department of Biology,Islamic Azad University, Falavarjan Branch, Falavarjan, Iran.
2.Department of Bacterial vaccine and Antigen Production, Research and Production Complex, Pasteur Institute, Tehran, Iran.

Background: Our aim in this study was to increase immunugenicity of PRP by conjugating PRP to Tetanus toxoid.
Methods: *Haemophilus influenza* type B PTCC1623 was cultivated in 10L of CY broth medium in fermentor, and purified by alcohol precipitation method, and by the addition of Cetavlon, and Hydroxyl Apatite. Tetanus toxoid was provided by Razi Vaccine & Serum Institute. 5mg of PRP was conjugated to 2 mg of tetanus toxoid by chu method. In this method, Adipic Acid Di Hydrazide (ADH) and Cyanogen bromide were used as the spacer, and 1-Ethyl-3,3-di Methyl Amino Propyl (Carbo Di Amid) (EDAC) was used as the linker. The conjugate obtained was purified by flowing through Chromatography Column, using 4B-CL gel. Immunization was done choosing1 2 groups of white New Zealand rabbits. 25µg of pure PRP in 0. 5ml serum physiology and 0.5 of the conjugate were injected to groups 1 and 2, with a 15 day interval, intramuscularly, respectively. The blood was drawn at days 0, 15, 30, 45. The sera were separated, in order to perform Serum Bactericidal Assay.
Results: The results obtained in this study have shown the bactericidal titer of pure PRP was 16. There was no antibody increase in the second injection. The antibody titer of the conjugate was arisen up to 32, by the first injection, and 64 in the second injection. The conjugation rate was 43.3%.
Conclusion: The PRP-TT conjugate and the pure PRP were both able to stimulate the bactericidal antibody.

Keywords: Polyribosyl Ribitol Phosphate, Tetanus Toxoid, Conjugate Vaccine, *Haemophilus influenza* Type B

EVALUATION OF THE EFFECT OF CHITOSAN ON IMPROVING THE EFFICACY OF PHOTODYNAMIC INACTIVATION OF ACINETOBACTER SPP. BIOFILMS

Mohadeseh Fadaee[1], Nasim Kashef[1]
1.Department of Microbiology, School of Biology, College of Science, University of Tehran, Tehran, Iran

Background: We aimed to use chitosan, a polycationic biopolymer, to improve the efficiency of PDI using methylene blue (MB) on *Acinetobacter* spp. growing as biofilms.
Methods: Effect of MB concentration (200 µM) and light dose (47 J/cm2) on PDI of five drug-resistant *Acinetobacter* spp. isolates in biofilm forms was investigated. In vitro bactericidal effect of MB-PDI on *Acinetobacter* spp. biofilms treated with chitosan (1 mg/ml) was also studied.
Results: For this set of PDI parameters, *Acinetobacter* spp. isolates showed 0.1-2.3 log killing. However, MB-PDI applied on biofilms treated with chitosan was significantly able to disrupt pre-formed biofilms (viable count reduction ranging from 3.3 to 4.9 log10-unit in comparison to controls in all tested isolates).
Conclusion: Chitosan/PDI combination had significant ability to eradicate the pre-formed mature biofilms of *Acinetobacter* spp. These results indicate that chitosan may improve the uptake of MB, so it can potentiate the PDI efficacy against biofilm cells.

Keywords: *Acinetobacter* Spp., Biofilm, Photodynamic Inactivation (PDI), Chitosan

INHIBITORY EFFECTS OF CAMELLIA SCIENCES ON THE PERIODONTAL-DISEASE CAUSING ANAEROBIC BACTERIUM PORPHYROMONAS GINGIVALIS.

Mohammad Niakan[1], Jalayernaderi Nooshin[2], Jamshidian Hadiseh[2]
1.Department of Microboilogy, Faculty of Medicine,Shahed University,Tehran,Iran.
2.Oral and Maxillofacial Pathology Department, Faculty of Dentistry,Shahed University,Tehran,Iran.

Background: This study was designed to examine the chemical composition and in vitro antimicrobial potential of methanolic extracts of Camellia sciences (Green tea).
Methods: The inhibitory effect of methanolic extract of Camellia sciences was tested against bacterial *Porphyromonas gingivalis* (ATCC 33227) strains by using the paper MIC and MBC methods.
Results: The methanolic extract exhibited antibacterial activity against *P. gingivalis* with minimum inhibitory concentration (MIC) 20 g/ml, minimum bactericidal concentration (MBC) of 40 ?g/ml, and appreciable antibacterial activity. Preliminary phytochemical analysis of methanolic extract revealed the presence of antimicrobial compounds such as flavonoids, steroids, and tannins, which may contribute for the antimicrobial action of Camellia sciences.
Conclusion: The extract was found to be bacteriostatic and bactericidal effects on periodontal-disease causing anaerobic bacterium *Porphyromonas gingivalis.*

Keywords: Antimicrobial Activity, MIC, MBC, Porphyromonas Gingivalis, Camellia Sciences

RELATION BETWEEN HPV GENOTYPES AND HSV-2 IN CERVICAL LESION

Nader Motaali[1], Hossein Rassi[2], Hamid Reza Honarmand[1]
1.Department of Microbiology, Gilan Science and Research Branch, Islamic Azad University, Gilan, Iran.
2.Department of Microbiology, Karaj Branch, Islamic Azad University, Karaj, Iran.

Background: This study attempts to clarify the relation of HPV genotypes and HSV-2 in cervical lesions.
Methods: One hundred archival samples with cervical lesion retired from Khatam hospital and a simple method was used to detect the simultaneous amplification of the HPV consensus L1 region and HPV-16,-18, -11 and -31 along with the b-globin gene as an internal control. We use Multiplex-PCR for detection of HSV in our lab. After that, we use the Epi Info software for statistical analyse.
Results: Cervix lesions were collected from 100 patients with Squamous metaplasia (SM, n=50), cervical intraepithelial neoplasia (CINI, n=14, CINII, III n=12), and cervical carcinoma (CC, n=24). For paraffin-embedded tissues, DNA extracted by the simple boiling method yielded higher proportions of successful gene amplifications (99 %) for b-actin gene. Overall prevalence of HPV infection was 6% in the SM, 61.53 % in the CIN group, 91.66 % in the CC group. Furthermore, HSV-2 identified 8% in the SM, 26.92 % in the CIN group, 45.83 % in the CC group. In this study HPV types 16, 18, 31, 33 and 35 were considered high risk.
Conclusion: The results demonstrate that the prevalence of HPV infection and HSV-2 was higher in the precancers and cancer samples (P <0.001) compared to the non-cancer samples. Furthermore, the results indicate a causal role for HPV18/16 in cervical cancers, and also offer the possibility of primary prevention of cervical cancer by vaccination against this virus in Iran. The presence of HSV-2, together with the absence of transcriptional activity for high risk HPV types suggest that HSV-2 presence may have a biological significance in cervical carcinogenesis as a contributing factor to tumor development, but not as the tumor driven force.

Keywords: HPV Genotypes, HSV-2 And Cervical Lesions

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THE PRODUCTION AND PRIMARY IMMUNOLOGIC EVALUATION OF PRP AND RECOMBINANT PROTEIN P6 CONJUGATE AS A VACCINE CANDIDATE AGAINST HAEMOPHILUS INFLUENZAE TYPE B, IN MICE

Farshad Nojoomi[1], Ashraf Mohabbati Mobarez[2], Seyed Davar Siadat[3], Ali Hatef Salmanian[4], Nima Khoramabadi[5], Ramin Farhoudi[6]

1.Assistant professor, Department of Medical Microbiology,Faculty of Medicine, AJA University of Medical Sciences,Tehran,Iran.
2.Associate professor, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.
3.Associate professor, Deprtment of Microbiology, Pasteur Institute, Tehran, Iran.
4.Associate professor, Deprtment of Plant Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran.
5.Ph.D in Medical Bacteriology, Researcher,Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.
6.Researcher, Deprtment of Laboratory animals sciences, Pasteur Institute, Tehran, Iran.

Background: Our aim from this study was to conjugation between recombinant protein P6(rP6) and PRP and production of a new vaccine candidate for Hib.
Methods: The standard strain of Hib(ATCC 10211) was cultivated in 50 liters fermentor for large-scale PRP production. *Haemophilus* p6 gene was cloned and expressed in *E.coli.* After purification process, recombinant protein P6(rP6) was conjugated with PRP and PRP-rP6 conjugate was injected to BALB/c mice, intraperitonally (I.P).Specific IgM and IgG response to PRP-rP6 was evaluated 14 and 28 days after injection by ELISA.
Results: Cultivation of Hib in 50L fermentor was resulted to about 790 mg/lit PRP. P6 encoding gene was successfully cloned in both cloning and expression plasmids and expressed in *E.coli* with high level of approximately 4 mg/lit. rP6 was successfully conjugated to PRP. Anti-PRP IgM and IgG titre to PRP-rP6 conjugate was significantly elicited 28 days after I.P injection in mice.
Conclusion: The results of this study indicate the good immunogenisity of PRP-rP6 conjugate to induce specific immune responses against Hib infections.

Keywords: *Haemophilus influenzae* Type B, Capsule, Prp, Rp6, Conjugated Vaccine

EXTENDED-SPECTRUM BETA-LACTAMASE PRODUCING KLEBSIELLA PNEUMONIAE ISOLATES FROM PATIENTS WITH CYSTIC FIBROSIS

Roya Rafiee[1], Fereshteh Eftekhar[1], S. Ahmad Tabatabaei [2], Dariush Minaee-Tehrani[1]

1.Department of Microbiology, Faculty of Biological Sciences, Shahid Beheshti University, G.C., Tehran, Iran.
2.Department of Pediatrics, Shahid Beheshti University of Medical Sciences, M.C., Tehran, Iran.

Background: The aim of this research was to determine the antibiotic resistance profiles as well as the prevalence of β-lactamase production in *K. pneumonia*e isolates from CF patients in Tehran.
Methods: A total of 16 non-repetitive *K. pneumonia*e strains were isolated from 98 children with cystic fibrosis at Mofid Children's Hospital in Tehran between July 2012 and February 2013. Bacterial isolates were identified by standard biochemical tests. Antimicrobial susceptibility of the isolates was determined to 19 antibiotics by disc diffusion and minimum inhibitory concentrations (MIC) for ceftazidime were measured by microdilution. ESBL production was detected by the conventional double disc synergy test (DDST) as recommended by the CLSI. Presence of TEM, SHV, CTX-M, and OXA type β-lactamase genes was shown using specific primers and PCR. Sequence analysis of PCR all products was performed using lasergene software version 6.
Results: Antibiotic resistance rates were: ticarcillin (93.8%), cefotaxime and carbenicillin (75%), ceftazidime, ceftriaxone and piperacilin (68.8%), aztreonam and cotrimoxazole (62.5%), tobramycin (50%), cefepime and co-amoxiclave (37.5%), amikacin and ampicillin-sulbactam (31.3%), cefoxitin and ciprofloxacin (18.8%) and ertapenem (6.3%). All isolates were sensitive to imipenem, meropenem and piperacillin/tazobactam. The MIC results showed 68.8% resistance to ceftazidim. ESBL production occurred in 10 isolates (62.5%), all of which harbored blaTEM, 7 (70%) had blaSHV and blaCTX-M and 6 isolates (60%) carried the blaOXA gene. Among the ESBL producers, 1 isolate carried blaTEM alone, 2 had blaTEM + SHV, 1 harbored blaTEM + CTX-M, 1 hosted blaTEM + CTX-M + blaOXA and finally, 5 isolates carried all 4 genes. The sequencing results identified presence of blaTEM-1, blaSHV-11, blaCTX-M-15 and blaOXA-1 type ?-lactamases.
Conclusion: Our study shows the emergence of ESBL-producing *K. pneumonia*e from CF patients. More importantly, multiple β-lactamase production was observed in the majority of ESBL producers. Colonization of CF lungs by drug resistant *K. pneumonia*e is alarming and can cause complications in treatment of chronic infections CF patients.

Keywords: *Klebsiella pneumoniae*, Cystic Fibrosis, B-Lactamase, Blatem, Blashv, Blactx-M, Blaoxa.

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DETERMINATION OF MDR,XDR AND PDR STRAINS IN ESCHERICHIA COLI ISOLATES CAUSED URINARY TRACT INFECTION IN JAHROM

Masumeh. Ranjbar[1], Kavos Solhjoo[2], MohammadKargar[3], Raze Parastan[1], Samaneh Mojahedi Jahromi[1], Fatemeh. Darughe[1]

1.Drug and Food Department, Jahrom University of Medical Sciences, Jahrom, Iran.
2.Microbiology Department, Faculty of Medicine, Jahrom University of Medical Sciences, Jahrom, Iran.
3.Microbiology Department, Islamic Azad University, Jahrom Branch, Jahrom, Iran.

Background: *Escherichia coli* is one of the most important factors of urinary tract infection. Determination of antibiotic resistance in each region can be useful in the choose effective drug and faster treatment of urinary tract infections caused by these bacteria. Thus, the aim of this study is determination MDR، XDR and PDR strains of *Escherichia coli* isolated from urinary tract infections collected in the jahrom city
Methods: In this cross - sectional study, 100 isolates of *Escherichia coli* urinary tract infection in the first quarter of 1392 laboratories of hospitals Pymanyh and Motahari in jahrom city collected and antibiotic resistance to 14 antibiotics was determined by disk diffusion method. Inhibition zone diameter was based on the CLSI investigated and the results registrated in the form of Sensitive, semi-sensitive and resistant
Results: The evaluation of antibiotic susceptibility test of 100 isolates of *Escherichia coli* urinary tract infection showed MDR,XDR and PDR were in urin samples 60%,14% and 0% inrespectivly.
Conclusion: Antibiotic resistance patterns revealed that seven of antibiotic resistance is more than 50% and recommended nitrofurantoin, imipenem, and chloramphenicol be used for the treatment of urinary tract infections caused by *Escherichia coli* in the Jahrom city.

Keywords: Keywords: *E. coli*, Urine, Antibiotic Resistance, MDR,XDR And PDR Strains

AN EVALUATION AND COMPARISON OF ANTI-BACTERIAL EFFECTS OF HUMAN AMNIOTIC MEMBRANES AND THE AMNIOTIC FLUID IN VITRO

Sajjad Sadrinia[1], Soheyla Erfani[2], Majid Zare [3], Nahid Ghanbarzade [4]

2.School of Dentistry, University of Birjand, Birjand, Iran.
3.Faculty of Medicine, Birjand University of Medical Sciences, Birjand, Iran.
4.Department of Medical Microbiology, Birjand University of Medical Sciences, Birjand, Iran.
5.Department of Obstetrics and Gynecology, Faculty of Medicine, Birjand University of Medical Sciences, Birjand, Iran.

Background: The present study was conducted to evaluate antibacterial effects of human amniotic membranes and amniotic fluid in vitro circumstances.
Methods: Samples, including amniotic membrane and amniotic fluid were taken from 60 pregnant women who had cesarean delivery. After spreading plates by individual microbial suspensions of seven species, each sample was cultured on seven bacterial species. For amniotic fluid, normal saline and probiotic bacterial extract were used as negative and positive control respectively. For amniotic membrane, the umbilical cord and antibiogram discs were used as negative and positive controls respectively. Inhibition zones were measured and compared.
Results: All 61 amniotic fluid samples showed antibacterial effects. However, the effects were highly variable among different bacterial strains. 39 amniotic fluid samples (63.9%) showed inhibitory effects against *Staphylococcus aureus* while no amniotic fluid samples revealed inhibitory effects on *Lactobacillus* plantarum All 61 amniotic fluid samples showed antibacterial effects. However, the effects were highly variable among different bacterial strains. 39 amniotic fluid samples (63.9%) showed inhibitory effects against *Staphylococcus aureus* while no amniotic fluid samples revealed inhibitory effects on *Lactobacillus* plantarum Amniotic fluid shows the most antibacterial effects and chorioalantoic and amniotic membrane were located in second and third levels respectively.
Conclusion: Our findings confirm antibacterial properties of the fluid and the amniotic membranes. These may consequently suggest using the extracts as complementary to antibiotictrapy.

Keywords: Antibacterial Effects, Amniotic Fluid, Amniotic Membrane, Chorioalantoic Membrane

MICROBIOLOGY OF DIABETIC FOOT INFECTIONS

Naser Alizadeh[1], Mohammad Taghi Akhi[1], Reza Gottaslou[1], Mohammad Yousef Memar[1], Abed Zahedi Bialbaei[1]
1.Department of Bacteriology and Virology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.

Background: The aim of this research is bacteriological evaluation of diabetic foot ulcer infections, which is essential to prevent worsening, and deterioration of these lesions.
Methods: Between October 2013 and July 2013, 30 diabetic foot infection specimens obtained by needle aspiration of purulent material in depth of infected foot. Chocolate, sheep blood, phenyl ethyl alcohol and MacConkey agar plate were inoculated for isolation of aerobic organisms. These plates incubated at 370 C under 10% CO2 and examined at 24 and 48h. For isolation of anaerobic organisms pre-reduced vitamin K enriched *Brucella* blood agar, bacteroides bile esculin (BBE) agar, kanamycin vancomycin laked blood(KVLB) and phenyl ethyl alcohol(PEA) agar were inoculated and incubated under anaerobic condition implicated by Anoxomat system at 370 C and examined at 72 and 96h. All isolates were later identified by biochemical tests.
Results: Altogether a total of 37 aerobic and anaerobic bacteria isolated from 30 specimens. Predominant aerobic bacteria isolated from these infections were Staphy-lococcusaureus (24.32%) and Enterobacteriaceae family (24.32%), including *E.coli* (16.21%), citrobacter spp. (5.40%), *Enterobacter* spp.(2.7%) followed by *Enterococcus* spp. (21.62%), Coagulase negative staphylococci spp. (18.92%), pseudomonas aeroginosa (2.7%), *Acinetobacter* spp. (2.7%). Predominant anaerobic bacteria isolated from these infections were bacteroides fragilis group (5.7%).
Conclusion: Our study revealed the poly-microbial and mono-microbial nature diabetic foot infections and importance of both aerobic and anaerobic bacteria in diabetic foot infections. The obvious diversity of bacteria isolated from these infections, emphasize the risk of inappropriate empirical therapy and highlight the required of close collaboration between clinicians and bacteriologists.

Keywords: Diabetic Foot Infection, Aerobic Bacteria, Anaerobic Bacteria

MOLECULAR DETECTION OF PKS + E.COLI AS A NEW RISK FACTOR FOR IBD AND COLORECTAL CANCER IN HUMAN HEALTH

Fariba dastjani farahani[1], Shahla Mohammad Ganji[2], Mojtaba sohrabi[1], Soheil Aghaee[1], Violet Najafi Shiva[1]
1.Department of Microbiology, Qom branch, Islamic Azad University, Qom, Iran.
2.National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran.

Background: The aim of this study was to investigate the frequency of human health with PKs + *E.coli* and this can also help in the prevention of IBD disease and colorectal cancer.
Methods: 66 Colon biopsies were obtained from human whom referred to colonoscopy section of Shahid Beheshti Hospital (in Qom) during Aug_Dec2013. After Isolating the *E.coli* and Extraction of their DNA, Multiplex-PCR performed for 2 genes clbB AND clbN as specific site for PKs Island region.
Results: In this study, Among 47 Isolating *E.coli* it is revealed 2 (4%) Pks-positive strains carrying all of clbB, clbN.
Conclusion: The present data demonstrated that the *E. coli* genotoxin colibactin, there is a lower frequency In the Iranian society. So finding this study may help clinicians decide on the appropriate therapy.

Keywords: Pks + *E.coli*, Double-Strand DNA Breaks, Human Health

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THE EFFECT OF SOME LACTOBACILLUS SPECIES (PROBIOTICS) ON BIOFILM INHIBITION IN ENTEROCOCCUS FAECIUM

Faeze Moslemzade Tehrani[1], Rouha Kasra Kermanshahi[1], Mohammad Mehdi Feizabadi[2]
1.Department of Biology, Faculty of Basic Sciences, Alzahra University, Tehran, Iran.
2.Department of Microbiology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran.

Background: In this study we first assessed the capability of forming biofilm in some *E. faecium* strains and then we evaluated the effect of cell-free culture supernatant of some probiotic bacteria on biofilm inhibition of them.
Methods: Clinical isolates (*E. faecium*) were obtained from Labbafinejad hospital. *Lactobacillus casei* spp. *casei* PTCC 1608, *Lactobacillus rhamnosus* PTCC 1637 were used as probiotics. For Biofilm formation assay a dilution of overnight cultures (5×105 cfu/ml) of 30 strains in TSB+0.025% glucose was used to inoculate wells in microtiter plates. Biofilm inhibition was carried out by adding the same bacterial suspension along with some predetermined concentrations of cell-free culture supernatant of probiotic bacteria to the wells. Observations were quantitated by measuring the OD540 by ELIZA reader.
Results: The capability of forming biofilm among *E. faecium* strains is different. Most of strains can produce strong biofilms. Results from biofilm inhibition test showed that in presence of cell-free culture supernatant of *L.casei* and *L. rhamnosus* the OD540 significantly reduced compared with bacterial suspension only. This means that they can inhibit biofilm formation in *E. faecium*.
Conclusion: Our results implicate that adhesion and biofilm formation in *E. faecium* can be prevented by cell-free culture supernatants of both *L.casei* and *L. rhamnosus*. Because adhesion is a major factor in *E. faecium* virulence, cell- free culture supernatants of these probiotic species may be an effective inhibitor of infections.

Keywords: *E. faecium*, Probiotic, Biofilm,

PURIFICATION AND CHARACTERIZATION OF L- ASPARAGINASE FROM BACILLUS SP. PG- 03

Mahsa Rahimzadeh[1], Manijeh Poodat[1], Sadigheh Javadpoor[1], Fatemeh Izadpanah Qeshmi[1]
1.Molecular Medicine Research Center, Department of Biochemistry, Faculty of Medicine, Hormozgan University of Medical Science, Bandar Abbas, Iran.

Background: The aim of the present work was the discovery of a new L-asparaginase that is serologically different from the previously reported ones, but has similar therapeutic effects.
Methods: A novel *Bacillus* sp.PG-03 was grown in modified M9 medium and incubated in a rotary shaking incubator. After 24 h of inoculation, the cells were removed by centrifugation. Sonication of the cells was carried out to release the intracellular L-asparaginase and DEAE-Cellulose Ion exchange chromatography was used for purification of the enzyme. SDS PAGE analysis was performed and the purity and molecular weight of the enzyme was estimated. The activity of L-asparaginase was evaluated at different pH values and temperature.
Results: A novel bacterial strain, *Bacillus* PG-03 was isolated from the Persian Gulf sediments and screened for the ability of L- asparaginase production. The purified enzyme was eluted in 0.4 M NaCl. SDS-PAGE analysis revealed that the enzyme was purified and its molecular weight was approximately 40 kDa. The optimal pH determined for L-asparaginase activity was 6. Enzyme activity measurments in different temperatures showed that the enzyme was maximally active at 37°C.
Conclusion: The study revealed that the proper activity of the purified L-asparaginase produced by *Bacillus* sp.PG-03 near the physiological condition (e.g. optimum temperature 37°C) makes it extremely valuable in the chemotherapeutic treatment of leukemia.

Keywords: L-Asparaginase, *Bacillus* Sp.PG-03, Leukemia, Enzyme Activity

PREVALENCE OF LISTERIA MONOCYTOGENES INFECTION AMONG PREGNANT WOMEN WITH MISCARRIAGE HISTORY IN AMOL CITY IN Iran.

Mona Rahmani[1], Mirsaed Mirinargesi[2], Masood GHane[2]
1.MSc student in Microbiology, Islamic Azad University, branch of Tonekabon,Tonekabon, Iran.
2.Department of Microbiology, Islamic Azad University, branch of Tonekabon, Tonekabon, Iran.

Background: The aim of this study was to determine the prevalence of infection with this bacterium among pregnant women with a history of spontaneous abortion in Amol city in Northern Iran; to evaluate the possible relationship between infection with *L. monocytogenes* and increased risk of abortion in this area.
Methods: A total of 81 vaginal discharge samples were collected from pregnant women who had a history of spontaneous abortion and were living in Amol city in Northern Iran. All samples were examined for *L. monocytogenes* infection both by cultured and PCR method.
Results: The results obtained by both culture and PCR methods showed that none of the studies samples was infected with *L. monocytogenes*.
Conclusion: The results showed no *L. monocytogenes* infection among pregnant women with a history of spontaneous abortion in Amol city. The results reflect either the lack or a low level of infected with this bacteria in women who live in this region.

Keywords: Vaginal Secretions, PCR, *Listeria monocytogenes*, Listeriosis.

ANTIBACTERIAL ACTIVITY OF PLATELET RICH PLASMA AGAINST SALMONELLA ENTERICA

Elahe Mahdizade[1], Hassan Mohabatkar[1], Mandana Behbahani[1]
1.Department of Biotechnology, Faculty of Advanced Sciences and Technologies, University of Isfahan, Isfahan, Iran.

Background: The aim of this study was to evaluate the microbicidal activity of platelet rich plasma against *Salmonella enterica*.
Methods: The whole blood was obtained from healthy donors and PRP was prepared by two stages of centrifugation. Then, three forms of it, the PRP activated with thrombin, PRP activated with CaCl2 and untreated PRP was prepared. Antibacterial activity of these three samples of PRP was determined by the Kirby-Bauer disc-diffusion method.
Results: Results showed that PRP inhibited the growth of *Salmonella enterica* and antimicrobial activity of three samples tested was not significantly different.
Conclusion: Due to divers bioactive compounds, specially antimicrobial peptides, PRP is suggested to be appropriate candidate to develop novel antibiotics which can be used instead of conventional problematic antibiotics.

Keywords: Platelet Rich Plasma, Antimicrobial Activity, Disk Diffusion Assay.

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PREVALENCE OF BACTERIA IN PATIENTS WITH CORNEAL INFECTION

Masoud Dadashi[1], Gita Eslami[1], Hossein Goudarzi[1], Fatemeh Fallah[1], Soudabeh Taheri[1], Ali Hashemi[1]
1.Department of Microbiology,school of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Background: The aim of this study is determination of etiologic bacterial factors of cornea sore and prevalence of microbes that cause this disease.
Methods: In the present descriptive research, patients were selected randomly in one year.Then, patients with bacterial cornea sore were applied by smear and cultivation. Most of the samples were gathered from operation room and then put from cornea sore by cotton tipped applicator and immediately sent for inoculation on cultivation media(blood agar) and also smear is prepared for positive and negative gram bacteria. In patients,suspecting to fungoid keratit that have distinct sore apparently, confocal scan was used for study of fungoids.
Results: This study include 30 male (48.3%) and 32 female(51.6%).Their age range is from newborn to 105 years old. From among 62 samples,37 cases(59.6%) showed positive smear and 25 cases(40.3%)showed negative smear.43 cases(69.3%) were reported with positive cultivation and 19 cases(30.6%) with negative cultivation. The most bacteria,seperated from cultivations,were *Pseudomonas aeruginosa*,17 cases (27.4%)and the least related to fungoid and *Klebsiella* 1 cases for each one(1.6%),of all the positive cultivation patients.
Conclusion: According to results of this study,gram negative bacteria were the most contaminated portion of the corneal infection.Therefore, to reduce corneal infection in patients should be used antibiotics against gram-negative bacteria especially *Pseudomonas aeruginosa.* Antibiotics such as imipenem, colistin and ciprofloxacin can be helpful.

Keywords: Corneal, Bacterial Infection,*Pseudomonas aeruginosa*

SYNTHESIS AND EVALUATION OF PHENYL-THIOUREA DERIVATIVES WITH POTENTIAL ANTIBACTERIAL EFFECTS

Nasrin Nassiri Kopaii[1]
1.Department of Medicinal Chemistry, School of Pharmaceutical Sciences, Islamic Azad University, Tehran, Iran.

Background: We decided to build new derivatives using benzocaine with more potency to prevent drug resistance, in which hydrazone and thiourea moieties were combined in one structure, have been synthesized starting from 4-amino-phenyl-ethyl ester (benzocaine) for evaluation of their antibacterial potency.
Methods: 4-amino phenyl ethyl ester 2 as a base compound was produced by known methods from benzoic acid 1. 4-amino phenyl hydrazide 3 was prepared by reaction of hydrazine hydrate with compound 2. 4-amino phenyl hydrazone 4, 5 were prepared by reaction of compound 3 with 3-nitrobenzaldehyde and 3-pyridine carbaldehyde. Target compounds were synthesized by the reaction of compound 4, 5 with corresponding aliphatic isothiocyanat.
Results: Thin layer chromatography was used to access the reaction and purity of the compounds synthesized. The melting points were determined in open capillary tubes and presented uncorrected. The structures of target compounds were confirmed by IR, NMR and Mass spectra. FT-IR peak in 1390-1370 there because there is a thiourea C = S and maintain the structure. Protons between the 8.5-8 (ppm) H-NMR spectra indicate hydrogen imin and hydrazone is formed. The activity of target compounds in protein binding sites was evaluated by using the outodock configuration software.
Conclusion: Because of the existence of both hydrazone and thiourea pharmacophores in the target compounds, their antibacterial activity can be considerable. According to the results and presence of active part of thiourea; it is anticipated that the these compounds for the purpose considered, can be acceptable for invitro antibacterial tests.

Keywords: Synthesis, Hydrazone, Thiourea, Antibacterial.

STUDY OF ANTI-BACTERIAL EFFECT OF HUMAN AMNIOTIC FLUID IN VITRO

Erefanii Soheila[1], Zare Bidaki Majid[2], Sadrinia Sajad[3]
1.Faculty of Medicine, Birjand University of Medical Sciences (BUMS), Birjand, Iran.
2.Assistant Professor, Department of Microbiology, Faculty of paramedicine, Birjand University of Medical Sciences, Birjand, Iran.
3.School of Dentistry, Birjand University of Medical Sciences, Birjand, Iran

Background: Studies have shown that the existence of some substances in the amniotic fluid may prevent bacterial infection in the uterus and fetus. The aim of the present study was to evaluate in vitro antibacterial property of amniotic fluid.
Methods: During this study, the amniotic fluid samples from 60 pregnant women with caesarean delivery were collected. Eight standard strains of bacteria were cultured on plates and 50 µL aliqouts of amniotic fluid poured on plates using well method. Physiologic serum and probiotic bacterium were used as negative and positive controls respectively. The comparisons were carried out by measuring the inhibition zone around each well.
Results: Antibacterial effect against some strains was founded in the amniotic fluid. Most anti-bacterial effect was observed on the strains of Streptococcus and *Staphylococcus aureus*.
Conclusion: This study confirmed the antibacterial properties of amniotic fluid. Therefore, the amniotic fluid can be considered as a valuable antibiotic supplement. Its use can be suggested for the treatment of clinical and wound infections.

Keywords: Antibacterial, Amniotic Fluid, In Vitro

THE STUDY OF ANTIMICROBIAL AND ANTI-ADHESIVE EFFECT OF PROBIOTIC LACTOBACILLI ON UROPATHOGENIC ESCHERICHIA COLI (UPEC)

Mahsa Sadri[1], Nazila Arbabsoleimani[1], Mahammad Mahdi Forghanifard[1]
1.Department of Micribiology,Damghan Branch, Islamic Azad University, Damghan, Iran.

Background: The aim of this study was to investigate the antimicrobial and especially anti adhesive characteristics of probiotic bacteria against *Escherichia coli* by using microbial techniques.
Methods: In this study two strains of *Lactobacillus* acidophilus PTCC 1643 and *Lactobacillus* casei PTCC 1608 have been used to investigate the antimicrobial and anti -adhesive effects on 20 selected samples from total of 40 UPEC collected from Semnan province hospitals. To evaluate the antimicrobial activity of complete culture and supernatant of probiotic lactobacilli, modified double layer method and dilution of supernatant were used, respectively. The mechanism of co-aggregation of lactobacilli with pathogens was also examined. The microtitre plate method was used to detect anti adhesion activity of *Lactobacilli* supernatant.
Results: The antimicrobial and anti-adhesive effects of probiotic lactobacilli on Uropathogenic *Escherichia coli* were confirmed in all tests. The growth inhibitory and anti-adhesive effect of L.casei was better than L.acidophilus.
Conclusion: According to the results, the probiotic lactobacilli have spectacular effects to prevent attachment, biofilm formation and pathogenicity of UPEC, so using them to prevent and treat Urinary tract infection is a practical, reasonable and acceptable method.

Keywords: Uropathogenic *Escherichia coli*,Probiotic Lactobacilli, Urinary Tract Infection

STUDY OF BACTERIAL PREVALENCE IN URINARY TRACT INFECTION AND DETERMINATION OF ANTIBIOTIC SUSCEPTIBILITY PATTERN OF GRAM NEGATIVE BACTERIAL ISOLATE IN 2013

Mohammad Yousef Memar[1], Maryam Hajizadeh[1], Zahra Tabatabaei[1], Naser Alizadeh[1]
1.Department of Bacteriology and Virology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.

Background: The aim of this study is determination of bacterial pathogen profile of UTI and antibiotic susceptibility pattern in gram negative isolates.
Methods: In this study performed in Tabriz, We processed 8153 urine sample collected from patients hospitalized in Imam Reza hospital. These specimens were processed for detection of bacteria in bacteriology lab. These specimens were inoculated in sheep blood and MacConkey agar plates and incubated at 370for 24 hours. Gram staining and standard microbiological and biochemical tests performed for identification of colonies. Disk diffusion method on Mueller-Hinton agar medium was performed according to Clinical Laboratory Standard Institute (CLSI) guideline for determination of antibiotic susceptibility pattern. The agents were used for antibiotic susceptibility testing of isolates were Gentamicin(10µg),, Amikacin(30 µg),, Ciprofloxacin(5 µg) Ceftizoxime(30 µg), Nitrofurantoin(300 µg), cotrimoxazole(1.25/ 23.75 µ g) and Nalidixic acid(30µg).
Results: In this study *E.coli* (55.38%) was the comments bacterial pathogen followed by *Enterobacter* spp. (29.61%), *P.aeruginosa* (4.9%), *Staphylococcus aureus* (3.21%), *Enterococcus* spp. (2.3%), Klebsilla spp. (0.48%) and 1.5% of them were fungi. The antibiogram results of gram negative in order of sensitivity have included: Amikacin (95.7%), Nitrofurantoin (91.5%), Gentamicin (64.1%), Ceftizoxime (56.8%), ciprofloxacin (37.6%), cotrimoxazole (31.4%) and Nalidixic acid (23.5%).
Conclusion: *E.coli* was predominant bacterial pathogen isolated from urinary tract infection.. Presence of antibiotic resistant bacteria in UTI can causes failure in antibiotic therapy. Antibiotic susceptibility pattern of these strains should be considered, for appropriate antibiotic therapy of UTIs.

Keywords: Antibiotic Susceptibility Pattern, Urinary Tract Infection, Antibiotic Resistant Bacteria.

DETECTION OF EXOTOXIN (A, Y, T, U, S) GENES OF PSEUDOMONAS AERUGINOSA WITH MULTIPLEX PCR

Seyyed Soheil Aghaei[1], Ali Javadi[1], Abbas Morovvati[1], Yaser Sharifi[1]
1.Department of Microbiology, Qom Branch, Islamic Azad University, Qom, Iran.

Background: We aimed to develop and evaluate a pentaplex PCR assay for the specific identification of Exotoxin (A, Y, T, U, S) genes in isolates of resistant *Pseudomonas aeruginosa* to third generation cephalosporin's from hospitalized patients in hospitals of Qom.
Methods: PCR reactions performed with primers which targeted of the each genes in p.aeruginosa. PCR reactions were optimized using the specific primers. For evaluating the specificity of these primers, PCR reactions were done on negative control bacteria. Presence of suitable DNA for amplification in negative control genomes confirmed with amplification of each Exotoxin genes.
Results: As expected, electrophoresis of PCR products of the Exotoxin S,T,A,Yand U was showed 318,471,664,1018 and 884 bp and bands respectively. Result of amplification using negative control genomes as template was negative
Conclusion: This was achieved by combining primers for five specific genes in a single reaction and done by sensitive, specific diagnostic PCR. The Multiplex PCR test is a suitable option for detection of *P.aeruginosa* drug resistance in clinical samples.

Keywords: *Pseudomonas aeruginosa*,Exotoxin Genes,Multiplex PCR

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SURVEY OF MICROBIAL CONTAMINATION PREVALANCE AND THE EFFECTIVE FACTORS IN ILAM UNIVERSITY OF MEDICAL SCIENCES STUDENT'S TOOTHBRUSHES

Kobra Heidarzadi[1], Farid Azizi Jalilian[1], Ali Reza Rekabi[2], Razieh Amini[3], Ali Khorshidi[4], Zahra Ghobadian[5], Maryam Bouchani[5], Ali Hematian[1], Morovat Taherikalani[1]

1.Department of Medical Microbiology, Ilam University of Medical Sciences, Ilam, Iran.
2.Department of Periodontology, Faculty of Dentistry, Ilam University of Medical Sciences. Ilam, Iran.
3.Department of Micobiology, Faculty of Sciences, Azad Isalmic University, Hamedan Branch, Hamedan, Iran.
4.Department of Epidemiology, Faculty of Medicine, Ilam University of Medical Sciences, Ilam,Iran.
5.Student Research Committee, Ilam University of Medical Sciences, Ilam, Iran.

Background: The most common method of oral hygiene is tooth brushing. However, occasionally brushing becomes a risk factor for health. The main reason is getting infected with various microorganisms. The purpose of this study was to investigate bacterial and fungal contamination of toothbrushes students, and factors affecting the contamination
Methods: Ninety five brushes were assessed in this study. Samples were incubated in test tubes containing nutrient broth for twenty-four hours. Then the microorganisms were isolated using specific nutrient medium containing Blood agar, Chocolate agar MacConkey and Sabouraud Dextrose agar. The inoculated plates were incubated for 24 to 48 hours at 37 C. The identification of micro-organisms was performed based on biochemical tests.
Results: The infection rate was more than 40% of the subject staphylococcus, *Niesseria*, Diphteroids, Actinomycetes and Enterobacteriacae family members were isolated from samples. Microbial contamination was higher in women than men ($P<0.05$).There was found correlation between the number of microorganism on brush with time of use, using mouthwash and gender ($P<0.05$). There was statistically significant relation between cocci microorganisms' infections with sexes as well as between *Bacillus* infections with capped brush.
Conclusion: Based on results, high incidence of bacterial contamination observed in brushes, therefore the proper use, maintenance and timely replacement of toothbrush play an important role in reducing pollution and consequently decreasing oral disease.

Keywords: Microbial Contamination, Student, Toothbrushes, Oral Hygiene

COMPARISION CEFOXITIN DISK DIFFUSION TEST AND PCR FOR MECA GENE FOR DETECTION OF MRSA

Saresadat Hosseini[1], Mohammad Niakan[1], Horieh Saderi[2], Mohammad Emaneini[1]

1.Department of Microbiology, Faculty of Medicine, Shahed University, Tehran, Iran.
2.Molecular Microbiology Research Center, Shahed University, Tehran, Iran.

Background: In our study phenotypic method for detection of methicillin resistant *Staphylococcus aureus* (MRSA) have been compared with PCR method to evaluate the efficacy of cefoxitin disk diffusion test to detect MRSA and compare it with molecular method.
Methods: All the isolates were subjected to cefoxitin disc diffusion test using a 30 µg disc. A 0.5 Mc Farland standard suspension of the isolate was made and lawn culture done on muller hinton agar plate. Plates were incubated at 37 ºC for 18 h and zone diameters were measured according to (CLSI). PCR for amplif cation of the mecA gene was performed.
Results: Out of 101 clinical isolates identified by phenotypic method, in disk diffusion test by cefoxitin disk (30µg), 58 isolates were considered to be MRSA. For these 58 isolates, mecA gene was positive.
Conclusion: Results of cefoxitin disk diffusion test are in concordance with the PCR for mecA gene. Thus, cefoxitin disk diffusion test can be used in the absence of availability of molecular biology techniques for detection of MRSA.

Keywords: MRSA, Cefoxitin, PCR, Mec A

EVALUATION THE SPECIFICITIES OF ANTI BACTERIAL OF CHITOSAN-PEO-HENNA EXTRACT NANOFIBERS

Gholamreza Herfehdoost[1], Zahra Bourbour[1], Maryam Alemaref[1], Asgar Emamgholi[1], Minoo Sadri[2]
1.Neurosince Research center,Baqiyatallah University of Medical Sciences, Tehran, Iran.
2.Department of Biotechnology, Malek Ashtar University of Technology, Tehran, Iran.

Background: Biocompatible polymer nanofibers with Specific antibacterial for Superficial wound coverage as used in burn wounds. The nanofibers can be a suitable alternative to conventional bandage. In this study, the preparation and review of nanofibers for its anti-bacterial properties has been studied.
Methods: Polymer solution of chitosan / polyethylene oxide / henna extract were prepared and using electrospining machine for nanofibers prepared and Its antibacterial properties was evaluated with Hinton agar medium containing the bacteria *Escherichia coli* and staphylococcus.
Results: The prepared nanofibers by SEM microscopy diameter were of 130 to 300 nm. And an antibiogram result from tests with disks containing antibiotics and nanofibers indicates the zone of no growth bacterial growth on medium.
Conclusion: Antibiogram test results indicate that the antibacterial properties of nano-fibers produced This is due to the antibacterial properties of chitosan and extracts Henna.

Keywords: Electrospining, Chitosan/PEO Nanofibers, Henna Extract, Antibiogram Test

SURVEY ON PVL TOXIN GENE IN ISOLATED STAPHYLOCOCCUS AUREUS FROM CLINICAL AND ENVIRONMENTAL SAMPLES

Rashaid Ramazanzadeh[1], Babak Shahbazi[2], Samaneh Rouhi[1], Abbas Manafi[2]
1.Cellular and Molecular Research Center, Microbiology Department, Member of Student Research Committee, Kurdistan University of Medical Sciences, Sanandaj, Iran.
2.Department of Microbiology, Member of Student Research Committee, Kurdistan University of Medical Sciences, Sanandaj, Iran.

Background: *Staphylococcus aureus* is an important human pathogen. *S. aureus* strains produce leukocidin pantonvalentine (PVL) toxin. PVL subunit binds to the leukocyte cell membrane and by inducing trans-membrane pore formation damage cell. *S. aureus* strains that carry pvl toxin are associated with skin and soft tissue infection. The aim of this study was to evaluate the rate of this toxin in Sanandaj hospitals.
Methods: 65 isolates of *S. aureus* strains were isolated from clinical and environmental specimens (urine, wound, abscess, blood and cerebrospinal fluid) that were collected during 2011 and 2012 years in Sanandaj hospitals, Kurdistan Province, Iran. pvl gene were detected by polymerase chain reaction (PCR).
Results: Rate of pvl gene among of 65 isolates of *S. aureus* strains was 13 (20%). Frequency of pvl in the patient's specimens was higher, among these specimens prevalence of pvl was more in the urine samples.
Conclusion: The prevalence of pvl positive *S. aureus* and resistant isolates in hospitals are increasing, so necessary accurate and continuous surveillance should be considered in health programs.

Keywords: Survey, Pvl, Gene, *Staphylococcus aureus*

SOME PLASMODIUM VIVAX DHFR GENE MUTATIONS AMONG ISOLATES FROM SOUTH OF Iran

Sara Moradi[1], Abbas Ali Eskandarian[2], Saeed Abedi[1]
1.Department of Microbiology, Faculty of Biological Sciences, Islamic AzadUniversity, Falavarjan Branch, Isfahan, Iran.
2.Department of Parasitology and Mycology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.

Background: The purpose of this research is studying of mutation causes resistant to SP in *Plasmodim vivax*.
Methods: In the present study, 50 blood samples on slides of patients were collected from 2 endemic regions of south Iran contain Hormozgan and Sistan and balochestan. Pvdhfr gene was sequenced for polymorphism analysis. Studied Point mutations at residues 57and 117 of Pvdhfr gene by the PCR-RFLP method.
Results: Polymorphism at positions 57L, S117 of *P. vivax* dihydrofolate reductase (Pvdhfr) gene has been found in 14% and 12% of isolates, respectively.
Conclusion: The present study shows a limited polymorphism in pvdhfr in isolates. As *P.vivax* is the most prevalent species of human malaria parasites in Iran, monitoring of resistance of the parasite against the drug is necessary. Therefore, continuous surveillance of *P.vivax* molecular markers is needed to monitor the development of resistance to SP in the region.

Keywords: Malaria, *Plasmodium vivax*, Sulfadoxine - Pyrimethamine, Mutation, Iran.

INVESTIGATION OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) PREVALENCE IN BAHONAR HOSPITAL OF KERMAN DURING A SIX MONTHS PERIOD

Mohammad Hassan Moshafi[1], Mehdi Rezaeifar[1], Mehdi Ansari[1], Forouzan Zare[2]
1.Department of Pharmaceutics, School of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran
2.School of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) are known to be the main cause of nosocomial infections for many years. Beside Methicillin, these bacteria became resistant to many other antibacterial agents.
Methods: This study was conducted through 4027 admitted patients in Bahonar Hospital of Kerman during a six months period. Samples from patients were collected and cultured on blood agar. Oxacillin-Screening Plate was used to determine methicillin resistant *Staphylococcus aureus*.
Results: During this study 158 patients (3.92%) were carriers of *Staphylococcus aureus*, of which 67 patients (42.4%) were carriers of MRSA. The prevalence of MRSA 67 patients (1.66%) of the total population was obtained.
Conclusion: In exposure of serious *Staphylococcal* infections, it is preferred to start treatment procedure with antibiotics such as vancomycin, but first we have to think about the control and the precautionary principles and hand hygiene in hospitals.

Keywords: *Staphylococcus aureus*, Methicillin Resistant, Nosocomial

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ANTIMICROBIAL EFFECTS OF ZINC OXIDE AND SILVER NITRATE NANO PARTICLES ON S.AUREUS, A.BAUMANNII AND P.AEROGINOSA

Mohammad Niakan[1], Elham esmaeli[2], Sarah Niakan[3]
1.Microboilogy Department,Medicine Faculty,Shahed University,Tehran,Iran
2.Faculty Of Veterinary Medicine Science And Research Branch,Islamic Azad University, Tehran,Iran
3.Faculty Of Veterinary Medicine, Science And Research Branch, Islamic Azad University, Tehran,Iran

Background: Nano particles have been introduced as novel antimicrobial agents because of their properties that are different from their bulk properties. Present study is aimed to investigate antimicrobial activity of silver nitrate and zinc oxide nano particles against three main bacteria responsible for nosocomial infections *S.aureus, P.aeroginosa* and *A.baumanii.*
Methods: Solutions of nanoparticles with various concentrations were prepared (4000 ppm to 31.25 ppm) in a serial dilution method. Disks with various Concentrations of nanoparticles then placed on bacterial cultures for 24 hours, in Diameter of inhibition was measured after 24 hours of exposure to nanoparticle in incubator. Diameters of inhibition were compared between various concentrations and kinds of bacteria. Analyses of variance were used for comparison of diameter of inhibition between bacteria based on kinds of nanoparticles regardless of their concentration.
Results: Nanoparticles of zinc oxide made an inhibitory diameter of 13.6 mm in highest concentration to 7 mm in lowest concentration of nanoparticle in *S.aureus.* In this bacterium, silver nitrate nanoparticle had a larger inhibitory diameter (16.33 mm to 8.67 mm). Zinc oxide nanoparticle had no inhibitory effect on *P.aeroginosa and A.baumanii.* Maximum Inhibitory diameter of silver nitrate nanoparticle on *P.aeroginosa* and A.baumanii were 13.33 mm and 22.67 mm for P.aeroginosa and A.baumanii, retrospectively. In both bacteria inhibitory area reached to zero at concentration of 125ppm. Inhibitory areas of silver nitrate were greater than zinc oxide ones significantly ($p<0.0001$).
Conclusion: Silver nitrate nano particles have more antimicrobial activity. Antimicrobial activity of zinc oxide nano particles was restricted to Gram-positive bacteria.

Keywords: Nanoparticles, Silver, Zinc, *S.aureus, P. aeroginosa, A. baumanii*

ANTIBIOTIC RESISTANCE OF BACTERIA ISOLATED FROM CLINICAL SPECIMENS IN A UNIVERSITY HOSPITAL IN TEHRAN, Iran

Ehsan Kazemi Moghaddam*, Atieh Darbandi, Horieh Saderi, Mohammad Reza Jalali Nodoushan
1.MSc Student, Microbiology Department, School of Medicine, Shahed University, Tehran, Iran
2.Professor, Molecular Microbiology Research Center, School of Medicine, Shahed University, Tehran, Iran
3.Professor, Pathology Department, School of Medicine, Shahed University, Tehran, Iran

Background: Determination of antibiotic resistance of clinical isolates can be helpful for delegacy of appropriate drug in urgent experimental remedy of infections.
Methods: We tested antibiotic resistance of 696 non-duplicate clinical isolates were obtained from various clinical samples during eight months (from October 2012 to June 2013) in laboratory of Mostafa Khomaini Hospital (Hospital of Shahed University). We used the disk diffusion method to determine antibiotic resistance.
Results: Totally, the abundance rate of gram negative isolates was as much as gram positive isolates and the common sequestrated bacteria were *Escherichia-coli* (39.7%). The extent of resistance to various antibiotics (Ampicillin, Co-amoxiclav, Cephalothin, Ceftriaxone, Cefotaxime, Piperacillin, Carbenicillin, Imipenem, Gentamycin, Tetracycline, Amikacin, Ciprofloxacin, Co-trimoxazole) was different in Escherichia-coli, Klebsiella, Proteuse isolates (8.2% to 96.1%). Despite all isolates of *Pseudomonas* and *Acinetobacter* were sensitive to colistin, all of these isolates were endure to some of the antibiotics. About 80.80% of isolated *Staphylococcus aureus* bacteria were MRSA (Methicillin-resistant *Staphylococcus aureus*) and resistance of these isolates to Penicillin, Ampicillin, Co-amoxiclav, Cephalothin, Ceftriaxone, Cefotaxime, Gentamycin, Tetracycline, Erytromycin, Clindamycin, Ciprofloxacin, Co-trimoxazole was 44.9% to 100%. Resistance to Penicillin was not seen in *Streptococcus* B isolates; however some of these isolates were renitent to Cefotaxime, Tetracycline, Erytromycin, Clindamycin, Ciprofloxacin, Co-trimoxazole antibiotics. 28.6% of *Enterococcus*isolates showed resistance to Vancomycine as well as their resistance to Tetracycline but resistance to Penicillin, Ampicillin, Tetracycline, Erytromycin, and Ciprofloxacin was seen just in some of the *Enterococcus* isolates.
Conclusion: To some extent, the frequency of antibiotic resistance of clinical isolates at this study was similar to other studies which were performed at the other regions of Iran and this frequency is higher as compared to other regions of the world which reveals that the use of antibiotics should be limited and their misuse should be prevented.

Keywords: Antibiotic Resistance, Clinical Isolates, Gram Negative Bacteria, Gram Positive Bacteria, MRSA

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EFFECT OF EFFLUX PUMP INHIBITOR PHENYLALANINE-ARGININE B-NAPHTHYLAMIDE ON THE MINIMUM INHIBITORY CONCENTRATION OF IMIPENEM IN MDR ACINETOBACTER BAUMANNII ISOLATED FROM BURNT PATIENTS

Mehrdad Gholami[1], Mojdeh Hakemi Vala[1], Ali Hashemi[1], Mona Moshiri[2], Hossein Goudarzi[1]
1.Department of Microbiology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2.Department of Pathobiology, Division of Microbiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Background: Efflux pumps of the RND super family such as AdeABC play a significant role in producing multidrug resistance *A.baumannii* as important agents in burn patients. So, the aims of this study were determination the role of efflux pump, effect of β –naphthylamide (PaβN) and also the frequency of adeABC genes in imipenem resistance of *A. baumannii* strains isolated from burnt patients.
Methods: This study was conducted on 60 *A. baumannii* isolates collected from 240 wound samples of burnt patients admitted to the Shahid Motahari Hospital of Tehran during 2013. Isolates identified by using standard methods, Microgen kit and existence of OXA-51gene. Antibiotic susceptibility tests were performed by Kirby-Bauer disc diffusion and MIC by broth microdilution methods according to CLSI 2013. The DNA purification was done by the phenol-chloroform method.Activity of the efflux pumpwas evaluated by using efflux pump inhibitor(PaβN).The frequency of adeABC genes were detected by PCR and sequencing methods.
Results: Of all isolates, 100% were resistant to imipenem. The susceptibility of strains to imipenem was highly increased in the presence of EPI; So that, for 58 (96.6%) of isolates, (PaβN) reduced the MIC by 4 to 64 folds. The existence of *adeA, B and C* genes was detected in 60(100%), 60(100%) and 51 (86%) of isolates, respectively.
Conclusion: By confirmation the role efflux pumpand PaβN in MIC decreasing in imipenem resistance *A. baumannii* isolates. So, new strategies, for instance by development of EPIs for use in combination with antibiotics, are required in order to eliminate the efflux transport activity.

Keywords: *Acinetobacter baumannii*; RND; Phenylalanine-Arginine B-Naphthylamide

POTENTIAL RISK OF ACUTE GASTROINTESTINAL ILLNESS (AGI) IN YOUNG CHILDREN ASSOCIATED WITH SWIMMING POOLS DUE TO PSEUDOMONAS AERUGINOSA

Mohammad Hajjartabar[1], Tahereh Kermani Ranjbar[2]
1.Ofogh St. - Sahel Park - Boulevard Noor - Hakimyeh Tehranpars, Tehran, Iran
2.Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: To assess if these swimming pools were a health risk to young children (age 7 to 11), thirteen public swimming pools, were examined and the exposed children were monitored by completing a questionnaire. An adequate control group was chosen randomly from those who never used the investigated pools.
Methods: Samples of swimming-pools water were examined for colony counts, *Escherichia coli*, coliforms, and *Pseudomonas aeruginosa* according to the Standard Method 20th edition. Routine chemical tests of the samples were performed and other useful information was recorded simultaneously. To investigate the relevance of *P. aeruginosa* and AGI, 375 young children users (age 7 to 11), were asked to participate for a brief interview. The participants were asked to come back to the pools the next couple days after interview. Eventually, 291(77.6%) of the participants could continue and finalize the process.
Results: Results were statistically analyzed and matched for age, and other achieved data such as gastrointestinal symptoms, how many children had ingested pools-water undesirably during swimming in the pools, amount of swimming-pools water they may ingested and other useful information. *P. aeruginosa* was isolated from 11(84.6%) of the pools. *P. aeruginosa* was the only predominant organism isolated from 9(69.2%) of the swimming-pool water samples while in the remained 4(30.7%) other samples, in addition to P. aeruginosa, high rates of total bacterial count, total coliforms and faecal coliform counts were found too. 103(27.4%) of the children announced that they had ingested pools-water undesirably during swimming in the pools. 21(20.3%) of the recent group announced gastrointestinal symptoms.
Conclusion: Comparisons of the results of the swimming-pool water tests and statistical analysis showed 90.4% (19) of the recent children had ingested contaminated swimming pools water with *P. aeruginosa.*

Keywords: Acute Gastrointestinal Illness, *Pseudomonas aeruginosa*, Recreational Water, Swimming Pools

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EVALUATION OF ANTIBACTERIAL EFFECT OF ALGINATE POLY AMIDOAMINE (PAMAM) DENDRIMERS NANOCOMPOSITES G2 "A IN-VIVO STUDY"

Rashid Ramazanzadeh[1], Hanar Narenji[2], Samaneh Rouhi[1], Babak Shahbazi[2], Pegah Shakib[5], MohammadHossein Darehshiri[1], Amin Hoshyar[2]
1.Cellular&Molecular Research Center and Microbiology Department, Member of Student Research Committee, Kurdistan university of Medical Sciences, Sanandaj, Iran
2.Microbiology Department, Member of Student Research committee, Kurdistan University of Medical Sciences, Sanandaj, Iran

Background: Aim of this study was survey on antibacterial activity of Alginate (PAMAM) dendrimer nanocomposite G2 on *S. typhimurium.*
Methods: In this study 45 mice in 3 grups (15 Control non infected mice *S. typhimurium*, 15 infected mice by *S. typhimurium* without treatment by alginate PAMAM dendrimer nanocomposite G2 and 15 infected mice by *S. typhimurium* with treatment by alginate PAMAM dendrimer nanocomposite G2 in dose of 10 and 20 mg/kg) were used. Then about CFU 105 bacteria diluted with buffered phosphate by oral gavage needle was inoculated into mice. Then tissue damage was determined by light microscope.
Results: Based on the images, focal necrosis and accumulation of inflammatory cells in the necrotic area in liver cells were visible in infected mice. Also in infected mice by *S. typhimurium* that treated by alginate PAMAM dendrimer nanocomposite G2, accumulation of inflammatory cells and liver necrosis areas significantly reduced.
Conclusion: The protective effects of alginate PAMAM dendrimer nanocomposite G2 in infected tissues was observed in our study. These results showed this nanocomposite material is a potential antibiotic.

Keywords: Antibacterial Effect, Alginate Poly Amidoamine (PAMAM) Dendrimers Nanocomposites G2

EFFECT OF RECOMBINANT NEUTROPHIL-ACTIVATING PROTEIN (HP-NAP) OF HELICOBACTER PYLORI ON PERITONEAL MACROPHAGES

Neda Soleimani[1], Ashraf Mohabati Mobarez[2]
1.Dept. of Bacteriology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.
2.Associated of Bacteriology Dept. of Bacteriology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.

Background: The aim is effect of recombinant neutrophil-activating protein (HP-NAP) of *helicobacter pylori* on peritoneal macrophages (MQ).
Methods: Recombinant neutrophil-activating protein (HP-NAP) of *helicobacter pylori* were produced and in different concentration (0.01–100µg/ml) affected on macrophage lipopolysaccharide (LPS=10µg/ml) activated MQ; then proliferation assayed by MTT method. Nitrite levels were measured by using the diazotizationmethod based on the Griess reaction and MTT assay for evaluation of macrophages.
Results: Proliferation of Macrophages in all treated groups was activated. In LPS stimulated groups in dose dependent manner in high doses (10 and100?g/ml). Macrophages viability in all groups was the same and NO activation in unstimulated groups in all doses and in LPS stimulated groups in 3 higher doses (1,10 and100?g/ml) was significant. At least P value for significance consideration was $P<0.05$.
Conclusion: The results of this study indicate that recombinant neutrophil-activating protein (HP-NAP) of helicobacter pylori could active the immune system response and without cytotoxic effect on could active function and NO production of macrophages as main innate immune cells. These activities suggest HP-NAP may be a new tool for future therapeutic strategies aimed in cancer immunotherapy.

Keywords: *Helicobacter pylori*, LPS, Macrophage, Nitric Oxide

IN SILICO INVESTIGATION OF MECHANISM OF ACTION OF TPMP-1 - A PLATELET-DERIVED ANTI MICROBIAL PEPTIDE- IN STAPHYLOCOCCUS AUREUS

Elahe Mahdizade[1], Hassan Mohabatkar[1], Mandana Behbahani[1], Naghmeh PoorinMohammad[1]
1.Department of Biotechnology, Faculty of Advanced Sciences and Technologies, University of Isfahan, Isfahan, Iran.

Background: The aim of this study is to verify the mentioned suggestion using in silico techniques via studying of the interaction of tPMP-1 and β subunit of DNA-gyrase of *Staphylococcus aureus*.
Methods: After fetching the DNA-gyrase structure from the PDB, its pockets was analyzed via metapocket web server. On the other hand, the 3D structure of tPMP-1 was predicted using I-tasser protein modeling web server. Since the inhibition of central pocket in proteins may inhibit their biological activity with high possibility, using Cluspro docking web server the interaction of the mentioned peptide with the central pocket of beta-gyrase was studied.
Results: The results demonstrate that tPMP-1 can efficiently interact with the central pocket of beta-gyrase with a very low binding free energy.
Conclusion: In conclusion, it is bioinformaticaly validated that one of the anti microbial mechanisms of tPMP-1 is its ability to interact with beta-gyrase which finally results in inhibition of bacterial (here *Staphylococcus aureus*) replication.

Keywords: Anti Bacterial Peptide, Bioinformatics, DNA-Gyrase, Replication Inhibitor

DNA EXTRACTION OF MALARIA PARASITES FROM BLOOD SLIDES

sara moradi[1], Abbas Ali Eskandarian[2], Saeed Abedi[1]
1.Dept of Microbiology, Faculty of Biological Science, Islamic AzadUniversity, Falavarjan Branch, 84515/155, Isfahan, Iran
2.Dept of parasitology&mycology, Faculty of medicine, Esfahan university of medical sciences, Hezar garib street, Esfahan, Iran

Background: Different factors should be present for having good PCR results, among them the quality and quantity of template DNA has more importance. The blood is the commonest biological sample especially for malaria diagnosis. Extraction of DNA from fixed Giemsa – stained blood smears (Gsbs) is a considerable promising in several aspects of malaria molecular researches. A retrospective epidemiological study for diagnosis of malaria upon the parasites DNA which extracted from positive reported Gsbs was conducted in a hypo endemic area in Iran. Flowing up, present a simple and inexpensive DNA extraction method.
Methods: This study was carried out on the positive fixed Giemsa-stained slides were taken from patients in malarious regions of south and southeastern of Iran. DNA extraction was done with and scraped whole blood with sterile scalpel at the surface of blood smear, sentrifuging and at the end boiling. We useed Nested PCR for documentation of procedure efficiency in present experiment.
Results: Seven isolates from Isfahan, 26 isolates from Sistan and Baluchistan and 17 isolates from Hormozgan provinces of *Plasmodiumvivax* were analyzed. OD 260/280 that is about DNA, obtained from Nano Drop, the average of them was 1.12, the max was 1.67, the min was 0.9 and the mid of them was 1.1.
Conclusion: Although the microscopic examination of blood smears remains the method of choice for the diagnosis of malaria, in recent years, considerable attention has been attracted to molecular methods, including the PCR technique. In malaria researches, this method is considered to have a promising future, especially in areas where four *Plasmodium* species are present simultaneously.

Keywords: Malaria, *Plasmodium falciparum, Plasmodium vivax*, DNA Extraction, Fixed Giemsa- Stained

COMPARING CERUMEN BACTERIAL FLORA IN ACUTE OTITIS EXTERNA PATIENTS AND HEALTHY CONTROLS

Ramazan Rajabnia[1], Aynaz Khademian[2]
1.Department of microbiology, Babul University of Medical Sciences, Babul, Iran
2.Department of microbiology and, Babul University of Medical Sciences, Babul, Iran

Background: Cerumen is known as ear wax, produced regularly by cerumen and lipid secretary glands. Regarding the effect of Mazandaran province's humid weather on the prevalence of pathogenic microorganisms, this study was performed to determine the bacterial flora of the ear in patients with acute otitis externa and its comparison with healthy subjects.
Methods: In this case-control study,cerumen was collected and cultured from 40 patients with clinically diagnosed acute otitis externa and 80 healthy controls. The data were finally analyzed using SPSS.
Results: In the study group, *Staphylococcus aureus* (20.8%), *Bacillus* (18.9%) and *Pseudomonas* (11.3%) and in the control group *Staphylococcus epidermidis* (38.7%) and Diphtheroid (22.4%) were the most common bacteria, respectively.
Conclusion: The isolated bacteria from cerumen of healthy subjects were very different from those of acute otitis externa patients.

Keywords: Bacteria, Cerumen, Otitis Extern

PHENOTYPIC CHARACTERIZATION OF URO-PATHOGENIC ESCHERICHIA COLI STRAINS ISOLATED FROM PATIENTS WITH URINARY TRACT INFECTION IN TEHRAN, Iran

Mohsen Tabasi[1], Mohammad Reza Asadi Karam[1], Mehri Habibi[1], Saeid Bouzari[1]
1.Department of Molecular Biology, Pasteur Institute of Iran, Pasteur Ave., Tehran 13164, Iran

Background: In this study we assessed the biofilm formation, heamagglutination and hemolysis in UPEC strains isolated from patients with UTI in Tehran, Iran.
Methods: Biofilm production was assessed with microtiter plate method. In this method, bacteria were added to 96-well microtiter plate for 48 hours, and then the wells were washed and stained with crystal violet dye and biofilms were quantified at OD570 nm after solubilization in acetic acid. The heamolysin production was evaluated on blood agar medium. For Haemagglutination assay, cell pellets were mixed with 3% suspension of guinea pig erythrocytes in the presence or absence of mannose. Rapid clumping of the *E. coli* in the absence of mannose indicates the presence of type 1 fimbriae.
Results: Of the 103 UPEC isolated, 74 (71.8%) strains displayed a biofilm positive phenotype that 24 (32.4%) of the strains were classified as highly positive, 14 (19%) as moderate positive and 36 (48.6%) as weakly positive. Among the UPEC strains, 34 (33%) strains produced hemolysin that 27 (80%) of them showed biofilm production. All of the isolates tested agglutinated RBCs in the absence of mannose that indicated the presence of mannose-sensitive fimbriae.
Conclusion: UPEC are the second common cause of bacterial infections in the world. A hallmark of UPEC strains is their possession of specialized virulence factors such as fimbriae type 1and hemolysin. Biofilm production is a marker of virulence for clinically relevant UTI infections caused by UPEC. It has been shown that UPEC strains producing biofilms are more likely to be hemolysin producers. Our results showed that there is a relationship between the biofilm-formation and hemolysin production in UPEC strains.

Keywords: Biofilm, Uropathogenic *Escherichia coli*, Urinary Tract Infection, Hemolysin, Fimbriae

ANTIMICROBIAL PROPERTIES OF PLATELET-RICH PLASMA (PRP)

Elahe Mahdizade[1], Hassan Mohabatkar[1], Mandana Behbahani[1], Naghmeh PoorinMohammad[1]
1.Department of Biotechnology, Faculty of Advanced Sciences and Technologies, University of Isfahan, Isfahan, Iran.

Background: Platelet-rich plasma (PRP) is regarded as a portion of the plasma fraction having high platelet concentration. It has attracted great attention and has been increasingly used for a variety of clinical applications including orthopedic surgeries, periodontal and oral surgeries, maxillofacial surgeries, plastic surgeries, and sports medicine. More recently, the antibacterial properties of PRP against various bacteria have been assayed whether in vitro or in vivo and the results ranged from very active to completely inactive. Therefore, it suggests that PRP may contain different bioactive compartments using which there is a hope to overcome the current antibiotic resistances. The interest of identifying and purifying these agents is still increasing. The aim of this study is to review the antimicrobial effect of PRP.
Methods: By having a comprehensive literature survey and especial focus on the practicability of the study, the antimicrobial properties and applications of PRP is discussed here along with its mechanisms of actions and also the promising future trends of this issue.
Results: Studies have shown that anti microbial activity of PRP is dose and strain dependent. Although the mechanisms and extent by which PRP, exert antimicrobial activity, are still poorly understood, a vast range of mechanism of action have been reported; from generating oxygen metabolites to different antibacterial peptides and proteins such as RANTES, platelet factor-4 and many others.
Conclusion: Having such a wide variety in bioactive compounds, PRP is an appropriate candidate to identify novel antimicrobial compounds, which can be replaced with common problematic antibiotics.

Keywords: Antimicrobial Activity, Mechanism Of Action, Platelet-Rich Plasma, Platelet Factor-4.

MOLECULAR EVALUATION OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) BY PCR TO BACTERIOLOGICAL METHODS

Farnosh Gholamhoseinpor[1], Mohammad Navaderi[2], Mehdi Mirzaei[3]
1.Department of Chemistry, Faculty of science, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran
2.Department of Biology, Ahar Branch, Islamic Azad University, Ahar, Iran
3.Department of Plant Physiology, Islamic Azad University, Tonekabon Branch, Iran.

Background: The objective of the study was to evaluate the efficacy of cefoxitin disc diffusion method to detect MRSA and compare it with other phenotypic methods such as oxacillin disc diffusion test, oxacillin agar screening test and gold standard molecular method, which, as of now, is by the detection of mecA gene by polymerase chain reaction (PCR).
Methods: The study included75 *Staphylococcus aureus* (*S. aureus*) isolates obtained from clinical samples. All isolates were tested for MRSA using oxacillin screen agar plates with 4% NaCl and 6 µg/ml of oxacillin, cefoxitin disk diffusion method using 30 µg disc and zone sizes were measured and interpreted as per standard guidelines. Amplification of the mecA gene was performed by PCR.
Results: Out of 75 isolates, MRSA was identified in 45.4% isolates by oxacillin disc diffusion test, 50.9% by oxacillin screen agar method and 54.5% (50 isolates) by cefoxitin disc diffusion technique. For these 50 isolates mecA gene was also positive.
Conclusion: Cefoxitin disc diffusion test can be an alternative to technically demanding PCR for detection of MRSA isolates as the results of both techniques have shown 100% sensitivity and specificity.

Keywords: MRSA, Meca Gene, Cefoxitin, Oxacillin, PCR

CONSTRUCTION OF A RECOMBINANT VECTOR FOR SITE-DIRECTED MUTAGENESIS IN SALMONELLATYPHIMURIUM

Ania Ahani Azari[1], Taghi Zahraei Salehi[1], Bahar Nayeri Fasaei[1], Masoud Alebouyeh[2]
1.Department of Microbiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2.Bacis and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Background: There are varieties of techniques for Site-directed mutagenesis in bacteria to inactivate chromosomal genes for many purposes. In some techniques such as λ Red recombinase system, linear PCR products has been used to knock out chromosomal genes, so there is always the risk of digestion by host's Restriction enzymes that leads to the low frequency of recombination. To overcome this problem, we constructed a recombinant vector to disrupt phoP gene in *Salmonella typhimurium.*
Methods: To construct the vector, SOEing PCR method and restriction enzymes were used.
Results: The resulting plasmid, pTAAZ92, contains a Kanamycin cassette with two homologous arms flanking of the phoP gene.
Conclusion: After electrotransformation of the pTAAZ92 into the *Salmonella typhimurium*, the *phoP* gene is replaced by the Kanamycin cassette through homologous recombination. According to high homology of the phoP gene in many of *Salmonella* species the pTAAZ92 can be used to delete the *phoP* gene in most of them.

Keywords: *Salmonella enterica* Serovar *typhimurium*, Gene Disruption, Site-Directed Mutagenesis,

THE PREVALENCE OF ESCHERICHIA COLI STRAINS CARRYING VIRULENCE GENE IPAH ISOLATED FROM DIFFERENT WATER SOURCES IN ALBORZ PROVINCE

Sorayya Hosseini[1], Reza Ranjbar[2], Taghi zahraie salehi[3], Roohollah Kheiri[4], Fatemeh Pourali[5]
1.Department Of Microbiology, Faculty Of Science, Islamic Azad University, Saveh Branch, Saveh, Iran
2.Molecular Biology Research Center, Baqiyatallah University Of Medical Sciences, Tehran, Iran.
3.Department Of Microbiology, Faculty Of Science, Islamic Azad University, Saveh Branch, Saveh, Iran
4.Alborz Province Water And Wastewater Company. Karaj, Iran
5.Molecular Biology Research Center, Baqiyatallah University Of Medical Sciences, Tehran, Iran.

Background: *E. coli* is considered as one of the most important causes of bacterial infections transmitted through water and foods. Several factors are involved in the pathogenesis of *E. coli*. The aim of this study was to investigate the prevalence of *E.coli* strains carrying virulence gene ipaH isolated from different water sources in Alborz province.
Methods: This study was carried out in 2013. The research included all *E.coli* strains isolated from different surface water sources in Alborz province. *E.coli* isolates were detected and identified by standard microbiological and biochemical tests. The strains were evaluated for presence of virulence gene ipaH by PCR using specific primers. The PCR amplicons were visualized after electrophoresis and staining with ethidium bromide.
Results: Ninety nine *E. coli* strains were isolated and included in the study. The PCR results showed %88 of the strains harbored ipaH gene. There was any difference between detection rates of ipaH gene among the strains isolated from different water source. The most of bacterial strains harboring ipaH gene were multi drug resistance.
Conclusion: Our finding showed the prevalence rate of virulence gene ipaH is very high among *E.coli* strains isolated from different surface water sources in Alborz province. Considering its plasmid borne nature, the risk of transmission of this gene between other bacterial species could pose a high threat for public health.

Keywords: *E.coli*, Ipah, Pcr

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MOLECULAR CHARACTERIZATION OF 4 LYTIC BACTERIOPHAGES SPECIFIC FOR SALMONELLA ENTERITIDIS

Zahra Mirzaii[1], Peyman Zare[1]
1.Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran

Background: Progressing usage of various bacteriophages in genetic engineering, biotechnology and phage-therapy also using them as appropriate tools in order to determine type and rank of Barcia's focused attentions of most researches to introduce new types and vast application for them also. In researches, its required to introduce their new types and various usages and each invention opens a new vision in science expansion.
Methods: In order to separate bacteriophage, filtered suspension of bird samples added to fresh standard *salmonellaenteritidis* culture and bacteriophage pure with frequent passages. It was studied host domain and genetic ingredients of At least 4separated lytic bacteriophage. After genome extraction, genomic type and pattern was determined in agar gel through electrophoresis. Separated genome of each bacteriophage was studied through polymerase chain reaction PCR about STX gen existence (in order to study toxin production gen transfer capability) and INT gen (to study lytic bacteriophage potential probability conversion to lysogenic form.
Results: Findings showed that obtained bacteriophage host domain is specific and without any concern from dramatic changes in alimentary tract nature floor as the assistant tool of *Salmonella enteritidis* infection treatment. Specific pattern of genome and lack of studied genetic factors defined as assistant tool for detection and better evaluation with high biological safety were used.
Conclusion: Findings showed that obtained bacteriophage host domain is specific and without any concern from dramatic changes in alimentary tract nature floor as the assistant tool of *Salmonella enteritidis* infection treatment. Specific pattern of genome and lack of studied genetic factors defined as assistant tool for detection and better evaluation with high biological safety were used.

Keywords: *Salmonella enteritidis.*INT Gen.STX Gen. Bacteriophage

DETECTION OF LISTERIA MONOCYTOGENES IN VIABLE BUT NONCULTURABLE STATE BY 16S RRNA GENE EXPRESSION

Mehdi Zolfaghari[1], Masoud Rezaei,[2], Ashraf Mohebbati Mobarez[3], Mehdi Forozandeh Moghadam[3], Hedayat Hoseyni[4]
1.Department of Seafood Sciences and Technology, Faculty of Marine Sciences, Tarbiat Modares University, Tehran, Iran
1.Department of Seafood Sciences and Technology, Faculty of Marine Sciences, Tarbiat Modares University, Tehran, Iran
3. Dept. Medical Bacteriology, Faculty of Medical, Tarbiat Modares University, Iran
4.Dept. Food Sciences and Technology, Shahid Beheshti University, Iran

Background: This study aimed to investigate RT-PCR for 16S rRNA as a reliable method for precise and specific detection of VBNC state of *Listeria monocytogenes*.
Methods: For this purpose VBNC state of these bacteria was created in three environmental shocks: high osmotic shock, nutritional depauperation, cold temperature. The active and dead treatments were as positive and negative control groups. The culturability af treatments were checked on BHI agar containing yeast extract and sodium pirovate. Viability of treatment confirm by syto9 fluorescent dye by fluorescent microscope. Then the RNA extraction, Dnase treatment, cDNA synthesis and PCR for 16S rRNA were done for all samples.
Results: The results showed that the 16S rRNA gene in viable bacteria as a positive control, VBNC state from high osmotic shock, VBNC state from cold temperature and VBNC state from nutritional depauperation express.
Conclusion: Based on the results 16S rRNA is a reliable gene for RT-PCR for VBNC detection from various environments.

Keywords: *Listeria monocytogenes*, VBNC, Gene Expression, 16S Rrna

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EVALUATION OF THE ANALYTICAL SENSITIVITY OF LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) METHOD FOR RAPID DETECTION OF EXTENDED SPECTRUM BETA LACTAMASES, CTX-M-1 FAMILY

Shima Asadi[1], Keivan Majidzadeh Ardebili [2], Mohammad Soleimani[3]
1.Islamic Azad University, Qom Branch, Department of Microbiology, Qom, Iran
2.AJA University of Medical Sciences, Tasnim Biotechnology of Research Center (TBRC), Tehran, Iran,Cancer Genetics Research Group, Breast Cancer Research Center (BCRC), ACECR, Tehran, Iran
3.Islamic Azad University, Qom Branch, Department of Microbiology, Qom, Iran,AJA University of Medical Sciences, Tasnim Biotechnology of Research Center (TBRC), Tehran, Iran.

Background: In this study LAMP, a new molecular technique was evaluated for detection of CTX-M 1 gene.
Methods: Sequences of related gene of CTX-M-1 family were retrieved from Gene Bank and aligned. By using of the consensus sequence, LAMP specific primers by Eiken primer design online software were designed. Using the outer primers F3 and B3, the target gene in a strain producing CTX-M-1 was amplified and the product was cloned in E coli Top10 F' host. Confirming the recombinant plasmid obtained (pTZ-CTX-M1), its concentration determined by spectrophotometry. Tenfold serial dilution of the plasmid was prepared and subjected to evaluate by CTX-M1-LAMP method at standard conditions in presence of the designed primers (F3, B3, FIP, BIP). Amplification of the target sequence was detected by using Loopamp Real Time Turbidimeter LA-320C device and direct observation of generated turbidity.
Results: When using the turbidimeter, the last dilution of the plasmid that showed the proliferation, was 0.5395 fg or 161 copies of the CTX-M-1 gene. So the value was determined as the limit of detection of CTX-M1-LAMP method. The detection limit of the method when using direct observation of turbidity was calculated 5.395 fg or 1610 copies.
Conclusion: The CTX-M1-LAMP method detects CTX-M-1 type in 60 minutes and no need to temperature cycles and thermocycler device. This method with high analytical sensitivity has significant potential for routine using in clinical laboratories, particularly in laboratories with the low resources and economy.the LAMP method because of the simplicity, low cost and no need to complex equipment can be preferred to other molecular methods in the diagnosis of infection diseases. Also the LAMP assay for detection of PTZ-CTX-M1 was highly sensitive and specific.therefore this method could be a useful tool for rapid detection of CTX-M 1.

Keywords: Extended Spectrum Beta Lactamase (ESBL), *CTX-M-1*,Loop Mediated Isothermal Amplification (LAMP)

A ROAD TO CELL CYCLE CONTROLLING: CLONING OF A CDCH ANTISENSE CODING REGION IN A HALOBACTERIUM SALINARUM R1-E. COLI SHUTTLE VECTOR

Neda Mokari Bonabi[1], Ali Asghar Deldar[1], Ehsan Vafadar Nejad[2], Afruz Ali-Yari[1],, Baharak Mahyad[1]
1.*Department of Genetic Engineering, Biosciences and Biotechnology Institute, Malek Ashtar University of Technology, Tehran, Iran*
2.Department of microbiology, College of Science, University of Tehran, Tehran, Iran

Background: In the this research we designed and cloned an antisense coding fragment against cdcH gene in a *H. salinarum* – *E. coli* shuttle vector that seem involved in cell cycle.
Methods: First of all the cdcH gene sequence downloaded from NCBI Genbank. In order to amplification of the desired region of cdcH gene that included translation start codone and ribosome binding site، two primers designed. These primers were compliment to a fragment of cdcH gene which consists of 50 base upstream and 80 base downstream of the start codon. The primers have digestion sequences for Xba I and BamH I. After genomic DNA extraction and amplification, PCR product and cloning vector, pBluscript skII+, digested by the enzymes. Then digested fragment and vector ligated by T4 ligase and transformed into *E. coli* XLI Blue. Finally the fragment sub cloned into pVDSH1 shuttle vector in reverse orientation between promoter and terminator sequence of the vector.
Results: The results of sequencing and digestion showed gene fragment has been cloned in reverse orientation related to the promoter. This promoter is an inducible one, and decrease in KCl concentration lead to induction of antisense production.
Conclusion: The vector is ready to transform. Transformation of this vector in to *H.salinarum* and induction by decreasing of KCl concentrations may help us to investigate the role of cdcH gene in the *H. salinarum* R1 life cycle.

Keywords: *H. salinarum*,Cell Cycle, Antisense, Silencing

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CITRININ REDUCTION IN WHEAT FLOUR BY USING "YEAST SACCHAROMYCES CEREVISIAE"

Issa Gholampour Azizi[1], Moulod Gorgi[2], Samaneh Rouhi[3], Mahsa Azimi[4], Pegah Shakib[5], Babak Shahbazi[3], Noushin Abdolmaleki[5]

1.Department of Mycology sciences, Islamic Azad University Babol Branch, Babol, Iran
2.Department of Laboratory sciences, Islamic Azad University Babol Branch, Babol, Iran
3.Cellular&Molecular Research Center and Microbiology Department, Member of Student Research Committee, Kurdistan university of Medical Sciences, Sanandaj, Iran
4.Department of Microbiology sciences, Islamic Azad University, Ayatollah Amoli, Amol, Iran
5.Department of Microbiology, Member of Student Research Committee, Kurdistan university of Medical Sciences, Sanandaj, Iran

Background: Wheat is the main cereal that is used in Iran and in the world. Citrinin is a toxic metabolite produced by Penicillium, *Aspergillus* and Monascus into grains, foods, feedstuffs. Eating this contaminated product causes of acute and chronic diseases in humans and animals. The easiest and safest way to reduce mycotoxins in various contaminated foods with mycotoxins using certain strains of various microbes, such as *Saccharomyces cerevisiae.*
Methods: 15 samples of wheat flour were randomly collected from different bakeries in Babol city (Northern Iran). Citrinin level in samples was measured by ELISA method. Then Saccharomyces cerevisiae was added to wheat flour. After 48 h Citrinin contamination level in samples were measured by ELISA, again. Statistical analysis was performed by SPSS software and t-test.
Results: All samples were contaminated with Citrinin. The minimum and maximum levels of this mycotoxin in wheat flour before adding *S. cerevisiae* were 1.1 and 35.5 ppb, respectively. Minimum and maximum amount of Citrinin after adding *S. cerevisiae* was 0.8 and 30 ppb, respectively. According to the results, there is a significant relationship between the variables. Also minimum and maximum percent of Citrinin reduction was 28.6% and 11.54% in wheat flour containing yeast, S. cerevisiae.
Conclusion: *S.cerevisiae* reduced the citrinin level in wheat flour samples.

Keywords: Citrinin, Reduction, Wheat Flour, *Saccharomyces cerevisiae*

PECTINASE ENZYME DERIVED FROM *ASPERGILLUS FUMIGATUS* IS A BIOCATALYST THAT USED WIDELY IN FOOD INDUSTRY

Akram Songol[1], Mandana Behbahani[2], Mohammad Hosseini sade[3]

1.MSc of Biotechnology, Isfahan university, Isfahan, Iran
2.Associate professor of Biotechnology, Isfahan university, Isfahan, Iran
3.Department of agriculture Biotechnology, faculty of agriculture,Islamic Azad University sabzevar, Mashhad, Iran

Background: Pectinase is used in fruit juice industry, cleansing oil industry, paper-making, textile, fermented tea and coffee, water and waste treatment.The main strategies that are used to enhance production of this enzyme is mutagenesis or optimization By the experimental design. The purpose of this study is optimizing of pectinase enzyme production and rising of thermal stability is that.
Methods: In this study, optimization of enzyme production was performanced for the three fungi, *Aspergillus fumigatus, Penicillium chryzogenum* and *Rhizopus oryzea* by application of the factorial design which involves five factors, each at three levels. The five factors were carbon sources (whey, sugar, stevia) and ammonium sulfate, manganese sulfate, temperature, and pH). Pectinase concentration was measured by the Miller method.
Results: The results showed that the optimum of enzyme production was at 32 °C, PH = 6, 3g / L manganese sulfate, 2.75g / L of ammonium sulfate, 10g / L of each carbon source (whey, stevia, and glucose). A comparison was made between the three fungi *Aspergillus fumigatus* in addition to producing a higher amount of enzyme capable of producing the enzyme was 60 °.
Conclusion: The pectinase enzyme from *Aspergillus fumigatus* strains has considerable thermal stability, which is a great advantage for its use in the industry. This strain also could growth in a wide range of carbon sources, pH and temperature.

Keywords: Pectinase Enzyme, Fruit Juice Industry, Optimization, *Aspergillus fumigatus*

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OPTIMIZATION OF CELL GROWTH AND CAROTENOID PRODUCTION OF HALORUBRUM SP. TBZ112 THROUGH STATISTICAL EXPERIMENTAL METHODS

Masoud Hamidi [1], Hossein Nazemyieh [2], Mohammad Amin Hejazi [3], Mohammad Saeid Hejazi [4]
1.Young Researchers & Elite Club, Islamic Azad University, Hamedan Branch, Hamedan, Iran
2.Department of Pharmacognosy, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
3.West & Northwest Agricultural Biotechnology Research Institute of Iran (ABRII), Tabriz, Iran
4.Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

Background: The increasing demand for consumption of natural carotenoids has raised interest in their bioproduction. The objective of the present study was the analysis of environmental factors including temperature, pH and salinity through response surface methodology (RSM) on the cell growth and carotenoid production of Halorubrum sp. TBZ112. In addition the effect of light was evaluated.
Methods: Five levels of temperature, pH, and salinity were selected based on central composite design (CCD) and RSM to reach the optimum values for the cell growth and carotenoid production. Bio-production was carried out in an orbital shaker using a 10% (v/v) inoculum, and agitation at 120 rpm for 9 days in a non-illuminated environment. Dry cell weight was determined and total carotenoid was estimated by spectrophotometer.
Results: The production of biomass ranged from 0.04 to 1.6 g/L and the total carotenoid from 0.15 to 15.6 g/L. The optimum conditions for both cell growth and carotenoid production in Halorubrum sp. TBZ112 cultures determined by RSM, were temperature 31 ºC & 32 ºC, pH 7.51 & 7.94, and NaCl (w/v) 18.33% & 20%, respectively.
Conclusion: Optimization with RSM and light as an inducing factor, 170% increase in total carotenoid production by *Halorubrum* sp. TBZ112 was obtained as compared to the unoptimized medium.

Keywords: Carotenoid, *Halorubrum*, Light, Optimization, Salinity, Temperature

DOT BLOT SCREENING FOR RECOMBINANT PROTEIN PRODUCTION IN PICHIA PASTORIS

Leila Asadpour[1]
1.Department of Veterinary Science, Rasht Branch, Islamic Azad University, Rasht, Iran

Background: Recombinant protein production is an important means of obtaining large amounts of proteins to investigate their biological properties. The methylotrophic yeast Pichia pastoris is widely used for recombinant protein expression. Screening for protein expression on a small scale is an attractive way of identifying high yield expression constructs prior to large scale protein production
Methods: The S1 gene of infectious bronchitis virus was cloned in *P. pastoris* secretory expression vector pPICZαA and the recombinant plasmids were transformed into P.pastoris KM71H cells. To determine high yield expression constructs and the time-point of maximum yield of recombinant protein the supernatant of culture samples were harvested by centrifuging and subsequently analysed by Dot blotting.
Results: In the present study dot blotting was used for determining high yield expression constructs prior to SDS-PAGE and western blot analysis and good correlation was observed between the results of dot blot and western blotting.
Conclusion: The results confirmed that dot blot can be used as a screening method in order to reduce time consuming and cost in recombinant protein production.

Keywords: Dot Blot; Screening, Recombinant Protein*; P. pastoris*

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Optimization Of The Iron Bioleaching Parameters In Leptospirillum Ferriphilum UTMC 2299, Isolated From Gol-E-Gohar Iron Ore Mine

Mina Hamidpour[1], Javad Hamedi[2], Reyhaneh Papiran[2], Hamid Moghimi[2]
1.Advanced Science And Technology Faculty, Islamic Azad University Of Pharmaceutical Science Branch, Tehran, Iran
2.Department Of Microbial Biotechnology, School Of Biology And Center Of Excellence In Phylogeny Of Living Organisms, College Of Science, University Of Tehran, Tehran, Iran 2-Microbial Technology And Products Research Center, University Of Tehran, Tehran, Iran

Background: In this study, we evaluated some physical and chemical parameters to determine the optimum conditions for Biooxidizing of ferrus iron to the ferric form by Leptospirillum ferriphilum UTMC 2299.
Methods: Initially, the bacteria was cultured in 9K medium, pH 1.8 and incubated at 28 °C with shaking at 150 rpm in aerobic condition. In order to achieve the highest iron oxidation activity, the parameters including incubation time (3, 6. 9 and 15 day), particle size (45, 75, 106, 126 and 150 µm) initial pH (1.5, 1.75, 2, 2.5 and 3), initial inoculation volume (5, 7.5, 10 and 15% V/V), incubation temperature (28, 32, 37, 40 and 42 °C) and initial iron concentration (5, 7.5, 10, 15 and 20 g/l) were tested. Each test was performed in three repeats. Measurement of atomic absorption and total cell protein followed by Bradford test were used to evaluate the biooxidation and bacterial growth, respectively.
Results: The Gram-negative iron oxidizing bacteria, L. ferriphilum was isolated from Gol-Gohar iron mine in Kerman and preserved in University of Tehran Microorganisms Collection (UTMC), under the accession number, UTMC 2299 in the previous study. Based on atomic absorption and Bradford results, the optimum growth and iron oxidizing activity was observed in particle size of 45 µm, initial pH of 1.75, 10-15 g/l of iron in culture media with initial inoculums of 7.5% at 40 °C. Under optimized experimental conditions, the incubation period decreased from 10 to 3 days. Moreover, the capability of L. ferriphilum UTMC 2299 in oxidation of ferrus to ferric form, increased from 80% to 100%.
Conclusion: Optimizing the parameters affecting the bioleaching process is important for biomining industry. Since finding of a rapid growing and iron oxidizing chemolitotrophic bacteria is critical step in biomining projects, the obtained data in the current study can be useful for developing industrially remarkable bacteria with bioleaching property.

Keywords: Bioleaching, Optimization, Iron Biooxidation, Leptospirillum Ferriphilum

EXPRESSION OF SYNTHETIC PEPTIDE OF M2E-HA2 OF INFLUENZA VIRUS ON THE SURFACE OF BACTERIOPHAGE OF M13 IN ORDER TO INCREASE ITS ANTIGENICITY

Hadis Fathizadeh[1], Mehdi Golchin[1], Hadi Tavakkoli[1], Maryam Maazallahi[1]
1.Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran

Background: The small peptides can expressed in multiple copies on the filamentous phage M13 or fd as amino-terminal fusions. These peptides are usually expressed with the phage coat protein pIII or pVIII. Such fusion peptides are able to induce humoral as well as cell-mediated immune responses, which makes phage particles an attractive antigen delivery system. The aim of current study was to display a synthetic peptide containing two epitopes of Influenza virus (from M2e and HA2 regions) on the surface of pVIII bacteriophage of M13.
Methods: To express the peptide on the surface of bacteriophage M13, the Influenza peptide gene and gVIII gene were amplified and joined to each other by Assembly PCR technique. The constructed gene was then cloned into PIT2 phagemid vector and expressed in *E. coli* TG1 cells. The biological activities of hybrid phage was accessed after purification.
Results: The sequencing result revealed that peptide and pVIII genes have been cloned correctly in phagemid vector of PIT2. The expression of synthetic peptide on the surface of bacteriophage M13 was confirmed in ELISA and Western blotting using a polyclonal antibody.
Conclusion: This hybrid bacteriophage is a good candidate for displaying antigen and immunization.

Keywords: Bacteriophage M13, M2e-HA2, Influenza, Pviii

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ANALYTICAL SPECIFICITY AND SENSITIVITY OF LOOP MEDIATED ISOTHERMAL AMPLIFICATION ASSAY TARGETING COM1 GENE OF COXIELLA BURNETII

Razieh Zabihi[1], Mohammad Soleimani[2], Keivan Majidzadeh [3]
1. Islamic Azad University Qom Branch, Department of Microbiology, Qom, Iran
2.Tasnim Biotechnology Research Center (TBRC), AJA University of Mdical Sciences, Tehran, Iran.
3.Breast Cancer Research Center (BCRC), Iranian Center of Breast Cancer (ICBC), Academic Center for Education, Culture & Research (ACECR), Tehran, Iran.

Background: *C.burnetii*, the bacterium causing Q fever, is an obligate intracellular biosafety level 3 agent. Detection and quantification of these bacteria with conventional methods is time consuming and dangerous. Rapid detection of the agent improves the management of infection in natural or intentional epidemics. In the study, analytical properties of a com 1 gene based loop mediated isothermal amplification for detection of Q fever agent was evaluated.
Methods: LAMP primers were designed in accord with the com1 gene. Specificity of the assay was tested on genomic DNA of negative control bacteria. Sensitivity of the assay was calculated in serially diluted positive control plasmid containing PCR product of com1 gene from 100 billion to 10 particles. LAMP product was electrophoresis in 2% agaros gel (0.5X TBE) stained with Ethidium Bromide.
Results: The Electrophoresis of the LAMP products showed different size fragments in smear shape. No amplification signal observed in loop mediated isothermal amplification of negative control bacteria accompanied by specific *C.burnetii* primers. The last tube-showing signal of amplification in sensitivity testing, was related to 550 fg of the positive control plasmid. Converting the concentration to copy number value, the limit of detection of the assay was calculated 100000 copies of the com1 gene.
Conclusion: These results showed high specificity and sensitivity of the loop mediated isothermal amplification assay targeting com1 gene of the organism. Further evaluation of the assay using clinical specimens or artificially infected samples is essential to confirm the assay as a valid diagnostic test.

Keywords: LAMP, *Coxiella burnetii*, Analytical Specificity, Analytical Sensitivity.

DEVELOPMENT A TAQMAN REAL-TIME PCR ASSAY WITH AN INTERNAL POSITIVE CONTROL FOR QUANTITATIVE DETECTION OF NEISSERIA MENINGITIDES

Zakiyeh Ghadimi[1], Mohammad Soleimani[1], Keyvan Majidzadeh[1], Ali Mahmoudi[2]
1.Tasnim Biotechnology of Research Center (TBRC), Tehran, Iran
2.Genetic Department, Pastour, Tehran, Iran

Background: This study was development a TaqMan Real-time PCR assay for rapid and specific quantitative detection of *N. meningitidis*. Also, by designing and using an Internal Positive Control (IPC) to monitor the assay, we were able to distinguish the false negative results caused by PCR failure.
Methods: The specific primer pair and a CY5/BHQ2 dual labeled TaqMan probe were designed based on the *crgA* gene. The TaqMan reaction was set up on genomic DNA of *N. meningitides* (ATCC 13060). The *crgA* PCR product was cloned into pTZ57R/T plasmid and a standard positive control plasmid (pTZ-crgA) was resulted. Performing the assay on various bacterial genomes, its specificity was evaluated. The sensitivity was determined by performing assay on 10-fold diluting pTZ-crgA, which the first provided concentration was 220ng/µl. To quantify the crgA gene copy number in the assay, a standard curve was generated by plotting Cycle threshold (Ct) values relative to log crgA copy number for each reaction tube. Also, to design a competitive IPC, a 100 bp fragment of AOX1 gene of Pichia pastoris, was inserted into the crgA PCR product and replaced with some nucleotides between the primer pair annealing sequences.
Results: In the set up reaction the *crgA* gene was amplified by the specific primers and probe. In the specificity test, other bacterial genomes were not amplified. The detection limit of assay was shown 0.089fg or 240 copies of the gene. The standard curve showed R2 value, efficiency and slope of 0.999%, 93.4% and -3.48, respectively. The optimum concentration of IPC in multiplex reactions, which was amplified simultaneously with the lower detection limit of positive control plasmid, was 350fg.
Conclusion: The high sensitivity, speed, reproducibility and specificity of the novel crgA TaqMan assay using an IPC to monitor the false negative results, changed this method suitable for DNA extraction, PCR inhibitors control, diagnosis and bacterial load determination. It is considerably faster than conventional methods, too.

Keywords*: Neisseria meningitidis*, Rapid Diagnosis, Taqman Real-Time PCR, Internal Positive Control.

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KINETIC STUDY OF BACTERIOCIN PRODUCED BY ISOLATED LAB ON GRAM POSITIVE AND GRAM NEGATIVE PATHOGENIC BACTERIA

Paria Motahari[1], Saeed Mirdamadi[1]
1.Biotechnology Department,Iranian Research For Science And Technology,Tehran,Iran

Background: The objectives of this study are screening bacteriocinogenic LAB from traditional dairy products and kinetic study of bacteriocins on the selected spoilage and pathogenic microorganisms.
Methods: The agar diffusion bioassay was used to initiate screening of isolated lactic acid bacteria from traditional dairy products for bacteriocin against standard gram positive and gram negative bacteria (*Escherichia coli* (PTCC= 1330), *Micrococcus luteus*(PTCC= 1110), *Staphylococcus aureus*(PTCC= 1112), *Bacillus* cereus(PTCC= 1015), *Listeria* monocytogenes(PTCC=1306)). Out of seventy nine LAB isolates, thirty four were found to inhibit these bacteria and *Micrococcus luteus* was the most sensitive one, then Producers were selected to obtain the kinetic changes on growth curve of indicator organisms by turbidometric assay.
Results: The comparison of the bacteriocins inhibition effect on indicator growth curves showed the different effect based on the type of bacteriocin and the indicator. In this experiment change of lag phases, the slopes of growing curves and the shorter logarithmic period were obtained. The growth curves of Gram-negative bacteria were more changes in slope of log phase, and among gram positive bacteria lag phase elongated and shorter exponential phase was occurring.
Conclusion: Bacteriocins possess different mode of action, and based on inhibitory mechanisms,the dose and physiological state of the indicator cells can be bacteriostatic or bactericidal. In this regard, growth curve change is an effective screening tool to test the effect of bacteriocins on selected indicator. We can conclude that kinetic studies of bacteriocins on indicators could help us to improve our knowledge about the mechanisms of antimicrobial peptides.

Keywords: Bacteriocin, Lactic Acid Bacteria, Probiotic, Natural Preservation

CLONING AND EXPRESSION OF TNF ALPHA RECEPTOR IN E.COLI

Maryam Kaszadeh[1], Mojgan Bandehpour[2], Farzaneh Hosseini[1], Zeinab Soleimanifar[2], Bahram Kazemi[3]
1.Department of Microbiology, North Tehran Branch, Islamic Azad University, Tehran, Iran
2.Cellular & Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3.Biotechnology Department, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: TNF alpha is an adipokine involved in systemic inflammation and it is a member of a group of cytokines that stimulate the acute phase reaction. It is produced by macrophages and different cell types such as CD4+ lymphocytes, NK cells.Deregulation of TNF production has been implicated in a variety of human diseases such as arthritis rheumatoid. Recombinant TNF alpha receptor helps to control and treatment arthritis rheumatoid symptoms.
Methods: In this study, TNR sequence was synthesized based on NCBI genbank and cloned into PGH vector. The construct was transformed to *E.coli* strain BL21 and expressed in optimal conditions.
Results: The TNF receptor fragment in pGH vector was confirmed by PCR and restriction analysis. Expression and continue of procedure are carrying out.
Conclusion: Recombinant expressed protein approved by SDS-PAGE and confirmed by western blotting. This protein can be valuable for treatment of diseases such as arthritis rheumatoid.

Keywords: TNF Receptor, Arthritis, Recombinant Protein

INVESTIGATING OF PHYSALIN ANTI-LEISHMANIASIS EFFECT WITH MOLECULAR DOCKING

Fariba Sharifi Teshnizi[1], Mandana Behbahani[2], Akram Songol[1]
1.MSc of Microbial Biotechnology, Isfahan university, Isfahan, Iran.
2.Associate professor of Biotechnology, Isfahan university, Isfahan, Iran.

Background: This study is performed to explore how Physalin effect on *Leishmania* and compareing the binding ability of different Physalins with glycoprotein63.
Methods: In this study, molecular docking between glycoprotein 63 of *Leishmania major* and dozen Physalins is performed by using Molegro Virtual Docker for twelve times.
Results: The Results show that the binding ability between Physalin and glycoprotein63 of *Leishmania major* is appropriate. In addition, among Physalins there is the best connection between glycoprotein63 and PhysalinB, PhysalinA, PhysalinL and PhysalinO.
Conclusion: According to the results Physalins connection, especially PhysalinB, PhysalinA, PhysalinL and PhysalinO and the glycoprotein63 can creat the Physalin anti-*Leishmania* effect.

Keywords: *Leishmania*, Physalin, Molegro Virtual Docker, Glycoprotein63

CLONING OF THE CREATINASE GENE FROM PSEUDOMONAS PUTIDA IN E.COLI

Elnaz Afshari[1], Zahra Amini-Bayat[2], Setareh Haghighat[1], Sanaz Jafari[2]
1.Department of microbiology, Islamic Azad University of Pharmaceutical Sciences Branch, Tehran, Iran
2.Department of biotechnology, Iranian Research Organization for Science and Technology (IROST), Tehran, Iran

Background: The purpose of this study is production of recombinant creatinase.
Methods: To clone the creatinase gene in pET28a expression vector, the genome of *Pseudomonas putida* was extracted with genome extraction kit and then appropriate designed primers was used for gene amplification using PCR and gene was cloned into pET28a expression vector using NheI and XhoI restriction enzymes. Recombinant plasmid was transformed into DH5α and then will be transformed in to BL21 for expression. Cloning was confirmed by colony PCR, double restriction enzyme digestion and sequencing.
Results: The results of colony PCR, double restriction enzyme digestion and sequencing showed that creatinase gene was cloned into pET28a expression vector and was transformed into DH5α.
Conclusion: Production of recombinant protein is so important and has many applications for producing higher amount of recombinant enzyme. So The purpose of this study is production of recombinant creatinase.

Keywords: *Pseudomonas putida*, Creatinase, Creatine, Recombinant Protein

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EVALUATION OF ANTIOXIDANT ACTIVITY IN KOCURIA ASB107 EXTRACTION

Roya Ghaderpanah[1], Ozra Sabura [1], Ezat Asgarani[1], Vahid Yavarpoor[2]
1.Department of Biology, Alzahra University, Tehran, Iran
2.Department of Modern Science, Tehran University, Tehran, Iran

Background: Carotenoid pigment as extinguisher compounds of reactive oxygen species, are considered one advantage ecological bacterial species under oxidative conditions. Bacterial colonies of Kocuria ASB107 strains have orange carotenoid pigments that can be used in industries. The purpose of this study was measurement and comparison of the antioxidant power of the bacterial extracts under different culture conditions.
Methods: In this study, content of carotenoids and plenolic compounds were assayed, then antioxidant activity of Kocuria bacterial extracts were studied using three methods of antioxidant power assay (metal chelating ability, reduced power assay and deterrence of beta carotene whiteness method). The experiments were done in a statistical randomized complete block design and then data analyzed using one-way ANOVA test.
Results: In all three methods, there are a close correlation between antioxidant activity of the methanolic extracts and their carotenoids and phenolic compounds contents. Increasing the salt concentration, time of aeration and reduce of medium temperature led to increasing amount of carotenoid in deterrence of beta carotene whiteness method. While increasing of the phenolic compound contents in different extracts was correlated to their antioxidant activity in two other methods.
Conclusion: According to the our results, we can be concluded that culture of Kocuria ABS107 in present of Nacl 7%, content of carotenoids and phenolic compounds was highest and the extract of this treatments had more antioxidant activity relative to other treatments. Also, cultures of bacteria during 5-day and under temperature of 4℃ increased antioxidant activity of the methanolic extracts compared to the control (4-day culture at 30℃)

Keywords: Antioxidant, Carotenoids, Kocuria ASB 107, Phenolic Compounds

BIODEGRADATION OF RESORCINOL USING STREPTOMYCES SP. BACTERIA ISOLATED FROM THE NORTH WEST OF Iran

Ali Reza Dehnad[1], Faramarz Ahmadi[2], Ali Farzi[3], Nader Hajizadeh[4], Najibeh Shirzad[5]
1.Department of Microbial Biotechnology, AREEO, Tabriz, Iran.
2.Faculty of Basic Sciences, Islamic Azad university Uremia branch, Uremia, Iran.
3.Department of Chemical and Petroleum Engineering, University Of Tabriz, Tabriz, Iran.
4.Young Researcher and Elite Club, Ilkhchi Branch, Islamic Azad University, Ilkhchi, Iran.
5.Young Researcher and Elite Club, Ahar Branch, Islamic Azad University, Ahar, Iran.

Background: In this study, the ability of *Streptomyces* sp. bacterial strains isolated from the soil of the East Azerbaijan in biodegradation of resorcinol was investigated. These isolates are more compatible with the weather of northwest region because they are indigenous.
Methods: *Streptomyces* sp. bacteria were isolated from soil samples of East Azerbaijan. Initially, each of the bacterial isolates was cultured in Nutrient Agar and Muller-Hinton Agar. For performing biodegradation experiments Minimal Salts Medium (MSM) was used. Bacteria were also added to one of the mediums, in addition to the resorcinol, by comparison with mineral medium containing only resorcinol, the changes because of the effect of bacteria can be realized. To assess the amount of dye decomposition, an analysis was performed by spectrophotometer. Secondary metabolites and products were identified by gas chromatography.
Results: The results of spectrophotometry and gas chromatography showed that bacteria could degrade 56% resorcinol in industrial wastewater.
Conclusion: *Streptomyces* sp. bacteria could have an important role in the decomposition of dye and removing them from the environment. By improving growth conditions, proliferation of bacteria, analysis and identification of metabolites resulting from degradation, it can be applied in a semi-industrial pilot to attempt for clean-up the dyes and producing beneficial metabolites such as acids, alcohols and other harmless substances.

Keywords: Resorcinol, *Streptomyces*, Biodegradation, Dyes, Wastewater

CONSTRUCTION OF PVAX/IUTA CASSETTE AS A DNA VACCINE CANDIDATE AGAINST URINARY TRACT INFECTION AND EVALUATION OF IUTA TRANSCRIPTS IN COS7 CELL LINE

Ronak Bakhtiari[1], Shahin Ahmadian[1], Jalil Fallah Mehrabadi[2]
1.Department of Biochemistry,Institute of Biochemistry and Biphysics,University of Tehran,Tehran,Iran
2.The Lister Institute of Microbiology, Tehran, Iran

Background: Uropathogenic *Escherichia coli* are one of the major agents of urinary tract infection. Since it has intracellular propagation, cellular immune response is so important in this case. Accordingly, a genetic construct for inducing of cellular immune system was designed.
Methods: At first, chromosomal DNA extracted from *E.coli* 2510 and *iutA* gene amplified with this template by PCR. PCR product inserted to pVax.1 eukaryotic expression vector and confirmed the recombinant vector by sequencing. The COS7 cell line transfected with a complex of pVax/iutA and ExGen 500 poly cationic polymer.
Results: Expression of *iutA* gene in COS7 was confirmed by RT-PCR. Consequently, pVax/iutA cassette could express inserted iutA gene in eukaryotic cells and is a valuable DNA candidate cassette for urinary tract infection vaccination.
Conclusion: This is the first prompt to designing a DNA vaccine based *iutA* gene against urinary tract infection that caused by Uropathogenic *Escherichia coli.*

Keywords: Genetic Vaccination, Uropathogenic *Escherichia coli*, Iuta

STABLE TRANSFERRING OF MUTANT STREPTOKINASE GENE INTO E. COLI

Tokhanbigli Samaneh [1], Kazemi Bahram[2], Bandehpour Mojgan[2]
1.Cellular and Molecular Department, Islamic Azad University of Pharmaceutical Science Branch, Tehran, Iran
2.Biotechnology Department, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: In this research we used mutant Streptokinase lack 42 C-terminal amino acids that has shown to lower levels of antigenicity.
Methods: Two sequences from Arabinose operon was amplified by PCR using specific primers with appropriate restriction enzyme sites in their end. These genes were cloned in pBluescriptIISK(+/-). Then synthetic mut – C42 Streptokinase under PLP promoter was cloned. In future it will be transferred between two homologous arms in pBluescript. This construct will be transformed to *E. coli* (HB101) genome by homologous recombination, after that 420C heat will stimulate expression of Streptokinase.
Results: Cloning of two homologous arms were confirmed by colony PCR, cloning of PLP promoter was confirmed by enzymatic digestion. After transferring this construct into *E. coli* genome the expressed protein will be analyzed on SDS – PAGE and confirm on Western blotting using specific antibody.
Conclusion: Mutant Streptokinase is less antigenic than native one in thrombolytic therapy in clinical practice. By inserting mut – 42 C – terminal Streptokinase to bacterial genome, stable expression in large quantities of Streptokinase can be produced inexpensively via bacterial fermentation.

Keywords: Streptokinase, B – Hemolytic Streptococci, PLP Promoter

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PRODUCTION OF ACTINOMYCIN IN STREPTOMYCES ANTIBIOTICUS BY PCR TARGETING

Hossein Rassi[1], Behnam Nazari[2]
1.Department of Microbiology, Karaj Branch, Islamic Azad University, Karaj, Iran
2.National Institute for Agro-Environmental Sciences, Ibaraki, Japan

Background: The PCR targeting of the biosynthetic pathway in the *S. antibioticus* would provide an efficient and convenient method of generating new derivatives of this complex molecule in vivo.
Methods: Here we describe how promoters can be replaced or gene fusions can be generated using the existing disruption cassettes. we have used a simplified variation of PCR targeting to introduce point mutations into promoter of the *phs A* gene of S. *antibioticus*. In our version, Red recombination was not used to generate the point mutation itself, but instead it was used to introduce PCR fragments containing the point mutations into the corresponding cosmid by co-transformation as described for oligo-targeting.
Results: The resulting mutant selectively produced the actinomycin, at 6-fold higher titers than the wild type strain. This mutant and the biosynthetic gene cluster will facilitate engineered microbial production of actinomycin with improved properties.
Conclusion: In this mutant, Phenoxazinone synthase (PHS) is one enzyme that has been implicated in the biosynthesis of actinomycin. The expression of the enzyme in growing cultures is regulated at the transcriptional and posttranscriptional levels, and glucose repression of PHS synthesis also involves control at the level of mRNA synthesis. Interestingly, the transformation of S. antibioticus with a multicopy plasmid containing the cloned PHS gene leads to the premature cessation of mycelial growth and actinomycin production.

Keywords: Actinomycin, PCR Targeting,*Streptomyces antibioticus*

BIOSYNTHESIS OF SILVER NANOPARTICLES BY LACTOBACILLUS CASEI

Behin Omidi[1], Seyed Jamal Hashemi [2], Mansour Bayat[1], Kambiz Larijani[3]
1.Department of pathobiology, Science and Research Branch, Islamic Azad University, Tehran, Iran
2.Tehran University of Medical Sciences, School of Public Health Research
3.Department of chemistry, Science and Research Branch, Islamic Azad University, Tehran, Iran

Background: In this study we used *Lactobacillus casei* (PTCC1608) as a probiotic to biosynthesis of AgNp. AgNp biosynthesized by this safe microorganism can used in medicine and pharmacology.
Methods: *Lactobacillus casei* was cultured in MRS broth and incubated in 37 ˚c and 5% co2 for 24 h.Then its centrifuged in 4000 rpm for 20 min. Supernatant was filtered by 0.45 micron then supernatant was added to silver nitrat 0.001, 0.002M and incubated in 37, 28 ˚c for 1 week. Change of solution colour to brown was shown AgNp was made, to provide it was used of uv-vis spectrophotometer, XRD, XRF, AAS and TEM.
Results: Result was shown that *Lactobacillus casei* had potentiality biosynthesis of AgNp. Uv-Vis spectrophotometer, XRD and XRF diyagrams was provid AgNps in solution. Size of this AgNps was 50-60 nm and their concentration was 115 ppm.
Conclusion: In this present study we have reported a simple biological extracellular, easy, non toxic, economical and eco-friendly method for synthesizing silver nanoparticles by using one of the probiotics bacteria.

Keywords: Biosynthesis -Silver Nanoparticles -*Lactobacillus* Casei- Probiotic

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OPTIMIZATION OF BIOSURFACTANT PRODUCTION BY PSEUDOMONAS AERUGINOSA ISOLATED FROM ACTIVATED SLUDGE RESERVOIRS USING WASTE OIL

Fatemeh Saleh[1], Farnaz Kheirandish[2], Hossein Azizi[3], Mahmoud rezazad bari[4], Maryam Azizi[5]

1.Msc Of Microbiology, Razi Herbal Medicines Research Center, Lorestan University Of Medical Sciences,Khorramabad,Iran
2.Department Of Parasitology And Mycology, Razi Herbal Medicines Research Center, Lorestan University Of Medical Sciences, Khorram Abad, Iran
3.Razi Herbal Medicines Research Center, Lorestan University Of Medical Sciences,Khorramabad,Iran, Department Of Microbiology, Broujerd Branch, Islamic Azad University, Broujerd Iran
4.Department Of Food Science, Urmia University, Iran
5.Department Of Microbiology,Yazd Branch, Islamic Azad University, Yazd, Iran

Background: The aim of this study was to investigate the conditions of biosurfactant production by bacteria isolated from activated sludge reservoirs, using industrial waste as substrate.
Methods: Biosurfactant production by two different types of medium with various concentrations of carbon and nitrogen source was investigated. Samples were incubated at different temperatures. Optimum condition for biosurfactant production and activity was studied. Finally, the surface tension of the samples was measured.
Results: Results showed that different concentration of carbon and nitrogen source had significant role in the production of biosurfactants. In this study, the highest rate of biosurfactants production was observed at 30oC with 3ml oil concentration and 2 mg/ml nitrogen source.
Conclusion: Biosurfactants are potentially used for removing oil contamination. In addition, biosurfactants were involved in reducing hospital infections and medical systems rather than chemical drugs. The results of this study establish the optimal conditions for the production of biosurfactants effective and appropriate solution seems to be to increase production.

Keywords: Biosurfactant, *Pseudomonas aeruginosa*, Activated Sludge, Surface Tension

COMPARATIVE STUDY OF OF ANTIMICROBIAL ACTIVITIES OF CU AND ZNO NANOPARTICLES AGAINST THE PATHOGENIC STRAIN OF KLEBSIELLA PNEUMONIAE

Yasaman sadat Nabipour[1], Arman Rostamzad[2], Salman Ahmadi[3]

1. Department of Biology, Faculty of Science, Ilam University, Ilam, Iran
2.Assistant, Department of Biology, Faculty of Science, Ilam University, Ilam, Iran
3.Associate, Department of Cell and Molecular Science Faculty, Mazandaran University, Mazandaran, Iran

Background: The use of metal nanoparticles can be effective to eliminate the bacterial infections, as an alternative to antibiotics. The aim of this study was to detect the antibacterial properties of 0.01, 0.5 and 1% nano-Cu and nano-Zno against *Klebsiella pneumoniae* as a major and prevalent pathogenic bacterium.
Methods: *Klebsiella pneumoniae* was cultured in liquid and agar nutrient medium to evaluate the antibacterial effects of 0.01, 0.5and 1% of both nano-Cu and nano-Zno via the optical density (OD) and log CFU/ml measurements.
Results: Non-significant effect was seen for 0.01% of both nano-specimens.While, 0.5 and 1% of both nanoparticles showed considerably decreased bacterial number. A 4.1 and 1.7 times decrease in the OD value was found in the presence of 1 and 0.5% nano-Cu, respectively ($P< 0.01$). 1.2 and 3.5 times decreased OD was seen in the presence of 0.5 and 1% nano-Zno, respectively, as compared to control ($P<0.01$). In the second study, 6.3 log CFU/ml of *Klebsiella pneumoniae* were present in the cultures treated with 1% nano-Cu and Zno at 4 ºC in water. Control *Klebsiella pneumoniae* cells survived for 14 days while complete cell death was seen when 1% nano-Cu was applied for 14 hours as compared to 1% nano-Zno, which showed complete cell death after 17 hours. In the third study, *Klebsiella pneumoniae* was grown in the agar medium with and without both nanoparticles and suppressed growth (4.1 and 5.6 times; $P<0.01$) was seen in the presence of 1% nano-Zno and -Cu, respectively.
Conclusion: In spite of the fact that both nanoparticles showed bactericidal activity, nano-Cu has proven to be more efficient antibacterial agent as compared to nano-Zno.

Keywords: Nano- Particles, Antibacterial, *Klebsiella pneumoniae*

ISOLATION AND IDENTIFICATION OF L-ASPARAGINASE PRODUCING BACTERIA FROM FRESHWATER SHELL (CORBICUAL SP) AND OPTIMIZATION OF CONDITIONS FOR ENZYME PRODUCTION

Mohammad Amin Abedi[1], Ghilamhossein Ebrahimipour[1], Abdolrazagh Marzban[2]
1.Department of Microbiology, Faculty of Biological Sciences, Shahid Beheshti University, Tehran, Iran
2.Biotechnology Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

Background: The aim of this study was to extract LAse from marine bacteria screened from freshwater shell (Corbicual sp) and assessment of enzymatic efficiency against a tumor cell line in vitro.
Methods: To produce Lase, bacteria isolated were purely cultured on nutrient broth for 24 h at 30̊ C. The partial purification of Lase is performed using ammonium sulfate precipitation and then Gel filtration. Assessment of LAse activity is done by measuring ammonia released using Nessler reagent and protein assay by Lowry method.
Results: Out of 5 colonies, only selected 2 colonies were Lase. These colonies seem to produce potent enzyme due to color change from yellow to red was observed very sharp and severe.
Conclusion: As now reported in many literatures, only bacterial L-asparaginase isolated from *E.coli* and Erwinia caratovora possess antitumor activity. However, we hope to our bacterial isolate to be have desirable activity, although needs to further studies about its immunogenicity and affinity in vitro and in vivo that has to be approved.

Keywords: Bacteria, L-Asparaginase, Antitumor, *Corbicual* Sp

STUDY OF PHAGE INFECTION IN LACTIC ACID BACTERIA ISOLATED FROM HOME MADE

Mona Basmechi[1], Saied Mirdamady[2], Fatemeh Bagheri[1], Farzaneh Azizmohseni[2]
1.Islamic Azad University of pharmaceutical sciences Branch, Tehran, Iran
2.Biotechnology department Iranian Research Organization for Science and Technology (IROST), TEHRAN, Iran

Background: The aim of this study is assessment the contamination of important lacto*Bacillus* in Iranian traditional dairy products.
Methods: Sixty five lacto*Bacillus* strains isolated from dairy products tested with double- layer plaque assay and Heap and Lawrence test for phage screening. Strains were grown in MRS (de Mann Rogosa and Sharp) broth containing 10 mM CaCl2. 6 H2O (MRS-Ca2). Baetriophage were detected in cell lysates induced by mitomycin C, UV irradiation and temperature
Results: Baetriophage were detected in cell lysates induced by mitomycin C, UV irradiation and temperature. The sensitivity of detection in different mentioned methods were varied and estimated up to 50 percent by some methods.
Conclusion: In conclusion, LAB cultures of Iranian traditional dairy products may not be safe for use in industries without any modification.

Keywords: Lactic Acid Bacteria, Phage, Dairy Products

MOLECULAR STUDY OF EXTENDED-SPECTRUM BETA-LACTAMASE (TEM-1) GENE IN ISOLATES COLLECTED, FROM OSTAD ALINASAB HOSPITAL IN TABRIZ

Azizeh Shadi Dizaji[1], Mohammadreza Nahaei[1], Hayedeh Mobayyen[1]
1.Faculty of Sciences, Department of Microbiology, Urmia Branch, Islamic Azad University, Urmia, Iran

Background: Emergence of antibiotic resistance is one of problems in microbial infection control. These types of enzymes can lead to resistances against the third generation cephalosporin's and hydrolysis of monobactams. This study was conducted to evaluate antibiotic sensitivity profiles and the presence of blaTEM gene in *E. coli* isolates collected from clinical specimens of Ostad Alinasab hospital in Tabriz.
Methods*: Escherichia coli* (100 isolates) were detected by using conventional bacteriologic tests and then antibniotic sensitivity tests were performed according to Kirby-Bauer method. Confirmatory test was also performed by Combind disk test method. Finally, blaTEM gene was detected by using PCR technique. Of 100 *E. coli* 23% of isolates contained blaTEM gene.
Results: 18% of isolates were resistant to ceftazidime, while 30% of isolates were resistant to cefotaxime and the remaining's were sensitive. 46% of isolates were ESBL producers from which 23% of them contained blaTEM gene.
Conclusion: High resistance of *E. coli* isolates to the third generation cephalosporin's underlines need for accurate sensitivity tests, also avoidance from inappropriate use of antibiotics.

Keywords: Extended-Spectrum Beta-Lactamases (Esbls), Blatem Gene, *Escherichia coli*, Polymerase Chain Reaction (PCR)

USING 2X CONCENTRATION (200µG/ML) OF AMPICILIN IS THE MOST OPTIMUM CONCENTRATION FOR GROWING BACTERIA REGARD TO PLASMID EXTRACTION

Hossein Khanahmad[1], Faezeh Sabzehei[1], Shirin Kohpayeh[2], SADEGH FEIZOLLAHZADEH[2], ZAHRA HEJAZI[1], ILNAZ RAHIMMANESH[1]
1.Department Of Genetics And Molecular Biology School of Medicine Isfahan University of Medical Sciences Isfahan, Iran
2.Department of Immunology School of Medicine Isfahan University of Medical Sciences Isfahan, Iran

Background: The aim of this study is to determine the best concentration of medium antibiotics for the best yield of plasmid extraction.
Methods: *Escherichia coli* (*E.coli*) strain Top 10 F' was used as host for Green Fluorescent Protein (GFP) expression vector encoding ampicillin resistance gene. *E.coli* Top 10 was grown in Luria–Bertani (LB) broth medium containing 50 µg/ml tetracycline under aerobic condition and then has subjected to transformation with the vector of interest. The transformed *E.coli* was then cultured in LB broth containing 100 µg/ml Ampicillin for 4 hours. Then 150 µl of this medium were added to 4.5 ml of LB broth medium containing different concentrations (0,100, 200 and 300 µg/ml) of ampicillin and cultured at 37°C with shaking at 250 rpm for 16 hours. The medium containing bacteria were washed with sterile PBS and analyzed with flowcytometry.
Results: The flow cytometric results showed that the population of GFP positive bacteria was grown in 200 µg/ml of ampicillin was higher than other bacteria grown in 0, 100 or 300 µg/ml of ampicillin.
Conclusion: Our results suggest that the best medium for plasmid extraction is 2X ampicilin (200µg/ml ampicillin containing medium). Perhaps the beta lactamase, which is expressed by bacteria containing plasmid, cleaves ampicillin molecules and during the cultivation the concentration of ampicillin decreased and some bacteria which is lost their plasmids can grow but in higher concentration this will not occur.

Keywords: Plasmids, Green Fluorescent Protein, *Escherichia coli*,Ampicillin

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PROTECTIVE EFFECT OF CAROTENOID EXTRACTION FROM « KOCURIA ASB107 » BACTERIA ON DNA PLASMID

Roya Ghaderpanah[1], Ezat Asgarani[1], Ozra Sabura[1]
1.Department of Biology, Alzahra University, Tehran, Iran

Background: In this study, the role of carotenoids extracted from this bacterium in free radicals scavenging and protection of plasmid DNA was assayed.
Methods: In this study, oxidative damage on DNA induced by treatment of pUC18 with, hydrogen peroxide (H2O2) and tin chloride (SnCl2).The induced damage in plasmid DNA was carried out in the absence of extracted carotenoid from Kocuria ASB107, and also in the presence of various concentrations of extraction in Dimethyl sulfoxide (DMSO) solvent. A double-strand plasmid DNA break was determined on the rate of conversion supercoiling double-strand DNA to nicked (open) circular and linear forms and create smear on gel agarose.
Results: Agarose gel analysis of treated plasmid DNA with SnCl2 showed that in the absence of carotenoid extract, one smear was formed, but with increasing concentration of carotenoid extract from Kocuria, the rate of smear dropped and the bands related to Open Circular (OP) and Supercoil (SC) forms were determined. In the presence of 10% carotenoide concentration. No smear was observed and two specified bands of the OP and SC were appeared. Also, in plasmid that treated with hydrogen peroxide, in the absence of carotenoid extract, one smear was formed without any specified OC and SC bands. With increasing concentration of carotenoid extract, mentioned two forms of plasmid intensified.
Conclusion: The results of treatments showed that with increasing concentrations of carotenoid extracts, the extent of damage to DNA was reduced. Therefore we suggested that caotenoides in Kocuria ASB107 prevent of oxidation and destroye of important biomoleculs such as DNA by protecting them from free radicals and play effective role in the resistance of this bacterium against environmental mutagenic agents and ionizing radiation.

Keywords: Carotenoid, DNA Damage, Kocuria ASB 170 Bacteria

IN VIVO BIOACTIVITY OF TRUNCATED STREPTOKINASE LACKING 42 AMINO ACIDS IN C-TERMINAL REGION

Marzieh Sameni[1], Mazaher Gholipourmalekabadi[2], Mojgan Bandehpour[2], Farzin Sahebjam[3], Mehrdad Hashemi[4], Bahram Kazemi[2]
1.Department of Biology, Faculty of Basic Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran
2.Biotechnology Department, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3.Cellular & Molecular Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
4.Department of Genetics, Tehran Medical Branch, Islamic Azad University, Tehran, Iran

Background: In the present study, the bioactivity of the recombinant streptokinase (rSK) was evaluated in vivo.
Methods: Streptokinase mutant gene, lacking 42 amino acids, was cloned in pET32a. Expression of the rSK was confirmed by western blotting and then purified by affinity chromatography. For in vivo investigation, blood clot was created in the jugular vein of rabbit after a general anesthesia. Clot formation was confirmed by Doppler ultrasound The rSK (4000 IU) was administered in marginal vein of rabbits' ear. The animals were kept under standard conditions. After 1 h, level of the D-dimer, in plasma of the animals was measured and compared with those of the animals treated with a commercial whole length streptokinase (Heberkinase). Distribution of data was assessed by Kolmogorov–Smirnov test and analyzed by independent-sample t-test. A P value of < 0.05 was defined as the level of significance.
Results: Doppler ultrasound showed clot formation in jugular vein of the rabbits. In addition, there was a significant difference between d-dimer levels in plasma of the animals before and after the SK administrations, indicating the in vivo bioactivity of both truncated and commercial SKs ($p < 0.05$). On the other hand, a decrease in bioactivity of the rSK was seen in comparison with the commercial SK.
Conclusion: According to the results, the truncated SK had in vivo bioactivity, although its' activity was less than commercial SK. With confirming the stability and non-immunogenicity of the rSK in vivo, this product could be a promising choice for clinical applications.

Keywords: B Hemolytic Streptococci; Thrombolysis; Recombinant Streptokinase

PREDICTION OF ANTIGENIC SITES ON ALS1 AND HWP1 PROTEIN SEQUENCES IN ISOLATED C. ALBICANS OF VAGINAL INFECTIONS USING BIOINFORMATICS ANALYSIS

Mona Pakdel[1], Parisa Mohammadi[1], Mahbobeh Zarrabi[1], Asgarani Ezat[1]
1.Department of biology, Faculty of sciences, Alzahra University, Tehran, Iran.

Background: The ability to predict antigenic sites on proteins is of major importance for medication. The aim of this study was to predict the antigenic sites on Agglutinin Like Sequence (ALS1) and Hyphal Wall Protein sequences (HWP1) in *Candida albicans* isolated of vaginal infections using Physico-Chemical Profiles server.
Methods: Seven isolates were obtained from women with vaginal infection which were collected from various medical centers of Tehran in 2011 and 2012. At the First, DNA was extracted and Multiplex PCR was performed using specific primers. In order to do bioinformatic studies, the genes were sequenced and then translated. Antigenic sites of protein sequences were identified by Physico-Chemical Profiles program.
Results: The results showed the presence of two genes als1 and hwp1 in isolates. In ALS1 and HWP1, respectively 2 and 1 antigenic site with the most antigenicity were identified using Physico-Chemical Profiles program.
Conclusion: According to previous studies, Serine and Threonine phosphorylation is an important mechanism in pathogenesis of ALS1 and HWP1 proteins. Results in this study showed that serine and threonine are the most amino acids in the antigenic sites with high antigenicity property.

Keywords: ALS1 Protein; HWP1 Protein; *Candida albicans*; Antigenic Sites; Bioinformatics

THE EXTENT APPLICATION OF RNAI FOR TREATMENT OF ANIMAL VIRUSES

Azam Mokhtari[1]
1.*(DVM, PhD) Assistant Professor in field of Virology Pathobiology Department, Shahrekord University, Shahrekord, Iran Tel: +98 381 4424401 EXT. 2172 Fax: +98 381 4424427*

Background: RNA silencing or RNA interference (RNAi) is a conserved biological phenomenon that the small RNA-guided gene silencing ocuurs in a wide range of eukaryotic and Prokaryotic organisms and viruses. In recent years, progress is being made in the development of RNA interference-based (RNAi-based) strategies for the control of gene expression and RNAi has been widely exploited to silence gene expression. Both chemically synthesized siRNAs and promoter-dependent expressed shRNAs show potent gene-silencing activity in vitro and in vivo.
Methods: As part of this special issue on the biology, mechanisms and applications of RNAi, we reviewed the recent advances on applying of RNAi in the development of nucleic acid-based technologies for therapeutic gene suppression against animal viral infections.
Results: This review focuses on therapeutic use of in vitro and in vivo RNAi-based therapeutics as well as the challenges involved in developing RNAi for clinical use. Although not complete, the list of potential therapeutic applications of RNAi in our study illustrate that the advantages inherent in RNAi technology such as specificity, potency, and versatility has led to numerous progresses in animal and human antiviral therapy in recent years.
Conclusion: So it can be hoped that RNAi be widely used for clinical treatment of animal viruses in the near future.

Keywords: Rnai, Shrna, Sirna, Antiviral Therapy, Animal Virus.

THE STUDY OF ANTI-MICROBIAL CYCLOTIDE ENCODING GENES FROM IranIAN VIOLA TRI-COLOR

Zahra Khoshkamz[1], Mahboubeh Zarrabi[1], Zargham Sepehrizadeh[2]
1.Faculty of basic science, Biotechnology department, Alzahra University, Tehran, Iran
2.Faculty of pharmacy, pharmaceutical biotechnology department, Tehran University, Tehran, Iran

Background: The cyclotides are the largest known family of cyclic proteins which have been found in plants from the Rubiaceae, Violaceace and Cucurbitaceae families. The complex structure of these small proteins consists of approximately 30 amino acid residues and the cyclic cystine knot motif.
Methods: In this article the main content and genetic structure of cyclotides from Iranian Viola tricolor, have been studied by cDNA screening, using a degenerate primer against a conserved (AAFALPA) motif in the cyclotide precursor ER signal sequence. For this purpose, from the fresh plant tissue, total RNA extraction and cDNA synthesis, and then in a good condition PCR was performed by using the conserved primer and oligo dT20. The resulted bands were cloned via pUC19 and the plasmids of white colonies were sent for sequencing studies.
Results: Good results of sequencing of cyclotide encoding precursor genes from flowers and leaves of Viola tricolor were achieved and analyzed through BLAST and alignment in NCBI.
Conclusion: This study resulted in relatively new gene sequences encoding antimicrobial cyclotides.

Keywords: Viola Tricolor, Cyclotides, Gene Structure, Cdna Screening

USING PCR TO SPECIFY THE GENUS OF NO-CARDIA BACTERIA IN PATIENTS SUFFERING FROM MS (MULTIPLE SCLEROSIS)

Behrooz Taherijavan[1], Mhamad Hassan Shahhosseiny[2]
1.Department of Biology, East Tehran Branch, Islamic Azad University,Tehran, Iran
2.Iranian Gene Fanavar(IGF),Tehran, Iran

Background: The aim of this research is molecular detection of *Nocardia* spp. DNA in MS patients and healthy people.
Methods: 50 samples from M.S patients and 50 samples from healthy people were collected for this research. DNA extraction was performed using DNG-PLUS kit. NG1 and NG2 as primers and 16s-rRNA as a target gene, were chosen. PCR method optimized and limit of detection, specificity evaluated by standard method. Amplicon was cloned by T/A cloning method.
Results: Amplicon with size of 596 bp, amplified and observed in Agars gel electrophoresis. Sequencing confirmed the correct PCR product. DNA of MS patients and healthy people serum extracted by DNG PLUS successfully and were used in PCR test.3(6%) patient serum was positive in PCR test healthy people serum was positive by only 1(2%) sample from PCR method.
Conclusion: Positive PCR *Nocardia* test in MS patients was more than healthy people. Apparently *Nocardia* species have a role in Multiple sclerosis and PCR test is a accurate, sensitive and specific method for detection of *Nocardia* DNA

Keywords: Ms, Nocardia, Pcr

OPTMIZIATION OF CARBONE AND NITROGEN SOURCE AND PH FOR PRODUCTION OF PHYTASE FROM THE ASPERGILLUS FUNGUS

Farideh Hayati[1], Jamshid Fouladi[1], Sara Gharavi[1]
1.Department of biology, Faculty of basic sciences, Azzahra University, Tehran, Iran

Background: Phytase (myo-inositol hexakisphosphate phosphohydrolase) is a phosphatase, that catalyzes the dephosphorylation of phytate (myo-inositol- hexakisphosphate) to inositol and inorganic phosphate.Phytate is the major storage from of phosphorus in the,legumes,grainsand oil plant seeds.
Methods: To optimiziation of carbone and nitrogen source on phytase production medium(phytase –screening medium(PSM)) was supplemented with different sugars(at 1%w/v concentration) and nitrogen(0.3%) whit 0.1% sodium phytate.Effect of different PH on phytase activity was studied in the pH range(3-10).After incubation for 72 h the culture filtrate was analysed for phytase activity.
Results: The maximum phytase activity observed at pH 6.5 whit sucrose as carbone source and ammonium solphate as nitrogen source
Conclusion: Phytase production by this strain is influenced by various physical and nutritional parameters. Enzyme production was find maximum in presence of sucrose as carbon source, ammonium sulphate as nitrogen source, PH 6.5.

Keywords: Optimiziation, *Aspergillus*, PSM, Phytase, Phytate

MICROBIAL SYNTHESIS OF SILVER NANOPARTICLE BY CULTURE SUPERNATANTS OF ALCALIGENES SPECIES STRAIN CK-CR6

Ramin Rostami-Taghi Dizaj[1], Nasrin Mollania[1], Farangis Gharib-Bibalan[1]
1.Department of Biology, Faculty of Basic Sciences, Hakim Sabzevari University, Sabzevar, Iran

Background: Nanotechnology has dynamically developed as an important field of modern research with potential effects in electronic, chemistry and medicine.
Methods: After culturing the bacteria in the culture medium, supernatant phase was separated by centrifugation. Production of silver nanoparticles using bacterial supernatant phase was carried out by adding an aqueous concentrations of AgNo3(1mM to 5mM) solution. The prepared mixtures were incubated at different temperatures (25 °C-50 °C) after 24 hour was absorbed. UV-Vis spectra of the mixtures of nanoparticles were recorded in the range of 300-700 nm. Other characteristics of produced nanoparticles such as average particle size.XRD,DLS,zeta potential and FTIR were also determined.
Results: 16SRNA molecular techniques were used to characterization the strain type,also phylogenetic analysis showed that the most similar to Alcaligenes species.Biosynthesis of silver nanoparticles occurred at 50 and 70°C in various range concentrations of Agno3.optimization of Silver nanoparticle biosynthesis at different condition was also carried out. UV-Vis spectra of the mixtures of nanoparticles were recorded in the range of 300-700 nm. The sharp band ofAgNPs was observed at 412 nm. The intensity ofabsorption band increases with increasing time period.Analysis of XRD spectra obtained showed that silver nanoparticles graph are similar to the standard graph.
Conclusion: In the present study, silver nanoparticles biosynthesis showed that with the increases of temperature and concentration of AgNo3, causes change at mixture.that showed change at shape and size.

Keywords: Bio-Nanoparticle,,Biosynthesis,Silver Nanoparticle,Supernatant

PHYSICOCHEMICAL METHODS IN ENDOTOXIN REMOVAL FROM RECOMBINANT ACTIVE PHARMACEUTICAL INGREDIENT (API)

Elhamosadat Ghorbi[1], Seyed Nezamedin Hosseini[2], Alireza Harifi Mood[3]
1. Department of Microbiology, Tehran Medical Science Branch, Islamic Azad University, Tehran, Iran.
2.Production & Research Complex, Pasteur Institute of Iran, Iran
3. Department of Chemistry, Tarbiat Moallem University, Karaj, Tehran, Iran.

Background: In this research, physical and chemical methods were used and the efficiency of each of those evaluated.
Methods: In this research we have selected ultrafiltration (UF) 10KD as a physical method and incubation with sodium deoxicolate as a chemical method.The above methods are some example of effective processes in endotoxin removal. TAGUCHI experimental designe software was used by 2 factors: time and sodium deoxicolate concentration were examined at three levels. Time was examined at three levels of 0, 12, and 24 h and sodium deoxicolate Concentration at three levels 0.5%, 1% and 1.5%.
Results: By using of TAGUCHI designing method, 9 experiments were designed. Endotoxin levels were measured by a Limulus Amebocyte Lysate gel-clot assay and showed that incubation with 1.5% sodium deoxicolate at 24 h was the most effective method in reducing the amount of endotoxin present in API from >1 EU/ml level to <0.5 EU/ml.
Conclusion: Physical and chemical method was used to reduce the endotoxin level from API and result showed that combination of two methods had efficient effection in reducing the amount of endotoxin

Keywords: Active Pharmaceutical Ingredient (API); Endotoxin; Sodium Deoxicolate; Ultrafiltration

AMPLIFICATION AND CLONING OF A GEN ENCODING A 41 KDA OUTER MEMBRANE PROTEIN (LIPL41) OF LEPTOSPIRA INTERROGANS SEROVAR CANICOLA

Maryam sadat Soltani[1], Pejvak Khaki[2], Soheila Moradi bidhendi[2], Sama Rezasoltani[3]
1.Department of Biology, Science and Research Branch,Islamic Azad university,Tehran,Iran
2.National Reference Laboratory for Leptospira, Department of Microbiology, Razi Vaccine & Serum Research Institute, Karaj, Iran,
3.Department of Biology, Science and Research Branch,Islamic Azad university,Tehran,Iran,

Background: Leptospirosis has been recognized as an important emerging infectious disease caused by pathogenic *Leptospira* spp. A major challenge of this disease is the application of basic research to improve diagnostic method. Outer membrane proteins of *Leptospira* are potential candidate that could be useful in diagnostic method. In order to evaluate genetic conservation of the lipL41 gene, we cloned and sequenced this gen from *Leptospira* interrogans serovar Canicola.
Methods: Following DNA extraction from this serovar. The lipL41 gene were amplified and cloned in the pTZ57R/T vector. Recombinant clones were confirmed by colony PCR and DNA sequencing. The related sequences were analysed and compared with sequences in the Genbank database.
Results: It revealed >95.9% homology with other serovars that indicates genetic conservation of this gene.
Conclusion: Hence the cloned gene in this study may be a suitable candidate in developing diagnostic method.

Keywords: Amplification, Cloning, Leptospira, Outer Membrane Proteins

DESIGN, SYNTHESIS, MOLECULAR CLONING AND EXPRESSION EVALUATION OF WHEAT (TRITICUMAESTIVUM) DEFENSIN PROTEIN IN E.COLI HOST

Maryam Mortazavi[1], Behnaz Saffar[2], Neda Mirakhorli[3], Mohsen Mobini Dehkordi[4]

1. Department of Genetics, Faculty of Basic Sciences, Shahrekord University, Shahrekord. Iran
2. Department of Genetics, Faculty of Basic Sciences, Shahrekord University, Shahrekord. Iran
3. Department of Plant Breeding and Biotechnology, Faculty of Agricultur, Shahrekord University, Shahrekord. Iran
4. Department of Genetics, Faculty of Basic Sciences, Shahrekord University, Shahrekord. Iran

Background: This study was done to design, synthesis, cloning and expression evaluation of wheat defensin protein.
Methods: In this study, defensin gene optimized and fused to sumo gene for more expression in *E. coli* Origami. Sumo gene encodes a protein with chaperon property that facilitates protein folding and increases its solubility. Then sumo_defensin sequence cloned into the pET-32a(+) vector and the resulting construct was used to transform bacterial cells (*E.coli* origami), bacteria grown in medium Luria-Bertani under 1 mM IPTG induction in 30 degrees and fusion protein was expressed in *E. coli* Origami. The fusion protein with a molecular weight of approximately 35.52 kDa was analyzed on SDS-PAGE gel and confirmed by western blotting and dot blotting.
Results: Most expression observed in 6 hour after IPTG induction on SDS-PAGE gel and Western blot and dot bot results showed that the recombinant protein specific antibody mouse anti-(His) 6 peroxidase is connected.
Conclusion: Expression of recombinant defensin protein in microbial host offers the potential development of biological productions such as antibiotics and fungicides.

Keywords: Antimicrobial Peptides, Cloning And Expression, SDS-PAGE Gel, Wheat Defensin Gene (K9M2T4)

ANTIBIOTIC RESISTANCE OF LACTIC ACID BACTERIA ISOLATED FROM HOME-MADE IranIAN DAIRY PRODUCTS

Mona Basmechi[1], Saeed Mirdamadi[2], Farzaneh Azizmohseni[3], Fatemeh Bagheri[4]

1.Islamic Azad University of pharmaceutical sciences Branch, Tehran, Iran
2.Biotechnology department Iranian Research Organization for Science and Technology (IROST), TEHRAN, Iran
3.Biotechnology department Iranian Research Organization for Science and Technology (IROST), TEHRAN, Iran
4.Islamic Azad University of pharmaceutical sciences Branch, Tehran, Iran

Background: Probiotics and dairy starter cultures are microorganisms which administered in adequate amount in fermented food. The main starter bacteria are strains belonging to the genera lacto*Bacillus* which is inhabitants of the human intestinal microbiota. The antibiotic susceptibility patterns of some species of lactic acid bacteria isolated from traditional dairy products made in different geographic regions of Iran have been evaluated by analyzing their isolated strains used for production of probiotics and starters.
Methods: Some strains were found to harbor plasmids caring resistance genes and some other strains have intrinsic resistance genes. In this study sixty three strains isolated from dairy products tested for their susceptibility to 22 antibiotics, in particular: penicillin,ampicillin, ofloxacin, oxacillin, erithromycin, tetracyclin, with disc diffusion test
Results: It was found that antibiotic resistance lactic acid bacteria are widespread among iranian traditional dairy products and resistance incidences depended on manufacturing area of the food. Distribution of resistance was found in different species. The aim of this study is assessment strains with plasmid resistance genes which are unacceptable for use in dairy product.
Conclusion: Antibiotic resistance was found in different species of probiotic strains isolated from traditional dairy products made in different geographic regions of Iran which can poses a threat to food safety.

Keywords: Probiotic, *Lactobacillus*, Antibiotic Resistance

OVERPRODUCTION OF DOXORUBICIN AND DAUNORUBICIN IN STREPTOMYCES PEUCETIUS BY PCR TARGETING

Hossein Rassi[1], Behnam Nazari[2]
1.Department of biology, Collage of Basic Science, Karaj Branch, Islamic Azad University, Alborz, Iran
2.National Institute for Agro-Environmental Sciences, Ibaraki, Japan

Background: *Streptomyces peucetius*, a microorganism that produces the anticancer drugs doxorubicin and daunorubicin, is itself resistant to the action of these drugs. Biosynthesis of doxorubicin (DXR) is tightly regulated, limiting its production in S. peucetius cultures. The genes conferring resistance to doxorubicin and daunorubicin in S. peucetius have been sequenced. Two open reading frames, drrA and drrB, were proposed to encode for an ABC (ATP-binding cassette) type of permease that carries out export of the antibiotics in an ATP-dependent manner.
Methods: This article reports a simplified variation of PCR targeting to introduce point mutations into promoter of the *drrA and drrB* genes of *S. peucetius* ATCC 27952. The resistance genes drrAB from S. peucetius were cloned into the pIBR25 expression vector under a strong ermE* promoter to enhance doxorubicin (DXR) production.
Results: The recombinant expression plasmids, pDrrAB30 and pDrrAB45, were constructed to overexpress drrAB in S. peucetius ATCC 27952. The recombinant strains produced more DXR than the parental strain: a 8-fold increase with pDrrAB30 and a 12-fold increase with pDrrAB45.
Conclusion: This study showed that DXR production was significantly enhanced by overexpression of resistance genes drrAB by PCR targeting.

Keywords: PCR Targeting, *Streptomyces peucetius*,Doxorubicin And Daunorubicin, Drra And Drrb

CLONING OF PSEUDOMONAS AERUGINOSA LIPASE GENE INTO PET28A EXPRESSION VECTOR

Maryam Roohi[1], Mojgan Bandehpour[2], Behzad Lamerad[3], Ameneh Koochaki[4], Bahram Kazemi[5]
1.Biochemistry Department, Payame Noor University, Tehran Branch, Tehran, Iran
2.Cellular and Molecular Biology Research Center, Shahid Beheshti university of medical Sciences, Tehran, Iran
3.Biochemistry Department, Payame Noor University, Tehran Branch, Tehran, Iran
4.Biotechnology Department, School of Medicine, Shahid Beheshti University of medical Sciences, Tehran, Iran
5.Biotechnology Department, School of Medicine, Shahid Beheshti University of medical Sciences, Tehran, Iran

Background: Lipase are enzymes which catalyze the hydrolysis of triglyceride to give di and mono glyceride, glycerol and free fatty acids. Our research is about cloning of *Pseudomonas aeruginosa* lipase gene in pET28a in order to cause random mutagenesis in future
Methods: After DNA extraction from culture of *Pseudomonas aeruginosa*. The lipase gene was amplified by PCR reaction and digested using BamHI restriction enzyme. In next step this fragment was cloned into pET28a by T4 DNA ligase.
Results: Cloning of lipase gene was confirmed by PCR and restriction analysis.
Conclusion: Our purpose is to increase efficiency of recombinant lipase enzyme of *Pseudomonas*.

Keywords: Recombinant Protein, Lipase, Cloning

IN SILICO STUDIES FOR CONSTRUCTION OF NOVEL FUSION PROTEIN MRPH.FIMH AGAINST URINARY TRACT INFECTION

Mehri Habibi[1], Mohammad Reza Asadi Karam[1], Mana Oloomi[1], Anis Jafari[1], Saeid Bouzari[1]
1.Department of Molecular Biology, Pasteur Institute of Iran, Pasteur Ave., Tehran 13164, Iran

Background: In this regard, with in silico studies, different forms of fusion protein were assessed and expressed in *E. coli*.
Methods: Different forms of MrpH and FimH fusion protein were modeled using I-Tasser server. The validity of structures was investigated using RAMPAGE and ProSA web. Modeled structures were docked to TLR-4 using Hex docking server. Depending on the energy value and pose of interaction, the best fusion form was selected and subjected to cloning into pET28a vector and expressed in BL21 (DE3) host.
Results: Fusion protein consisting of MrpH.FimH showed the best interaction affinity to the receptor (TLR-4). Cloning of this fusion was confirmed by DNA sequencing and their expression was observed on SDS-PAGE and confirmed by western blotting.
Conclusion: According to our in silico results, MrpH.FimH fusion protein showed the best interaction tendency to the receptor. This fusion protein was successfully cloned and expressed in *E. coli*. Further in vitro and in vivo studies are still necessary to evaluate the ability of this construct in activating immune responses.

Keywords: In Silico, Mrph, Fimh, Uropathogenic *Escherichia coli*

DESIGNING AND IN SILICO ANALYSIS OF A CHIMERIC PROTEIN CONTAINING FIMH AND FLIC AS A CANDIDATE VACCINE AGAINST UROPATHOGENIC E.COLI (UPEC)

Seyed Mohammad Gheibi hayat[1], Hekmat allah Moradi mogarmon[2], Babak Elyasifar[3], Sajjad Yazdansetad[4]
1.Department of Biology, School of science, Shahed University, Tehran, Iran
2.Department of EDU, School of Medical, Medical University of Shiraz, Tehran, Iran
3.Department of Microbiology, Faculty of Basic science,Damghan Branch, Islamic Azad University, Damghan, Iran
4.Department of Molecular Biology, Faculty of Basic science,Ahar Branch, Islamic Azad University, Ahar, Iran

Background: Then in this study chimeric proten cotaining FimH and FliC, was designed and insilico analysys was performed.
Methods: Chimeric protein involving FimH of UPEC, and FliC of EAEC was designed. These proteins were fused together by (EAAAK)4 linker. The phisico-chemical parameters of the chimeric protein were obtained. In silico Linear and conformational epitopes for fliC were predicted by appropriate softwares. Then the protein was reverse-transcribed to DNA. The multigenes DNA was then codon optimized for expression in *E. coli*Bl 21 host. At the end, the RNA secondary structure and RNA stability was determined.
Results: The results showed a good 3D structure of the protein in which many of epitopes were exposed and the domains were separated completely. The mRNA structure was in good condition and it was stable invivo.
Conclusion: The designed protein structurally and immunologically has almost all factors of an efficient candidate vaccine and can be tested in experimental tests.

Keywords: Flic, Fimh, In Silico Analysis, Urinary Tract Infection

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THREE DIMENTIONAL STRUCTURE PREDICTION OF CHIMERIC PROTEIN COMPRISING FIMH AND FLIC

Seyed Mohammad Gheibi hayat[1], Hekmat allah Moradi mogarmon[2], Babak Elyasifar[3], Sajjad Yazdansetad[4], Tahereh Azimi[5]

1.Department of Biology, School of science, Shahed University, Tehran, Iran
2.Department of EDU, School of Medical, Medical University of Shiraz, Tehran, Iran
3.Department of Microbiology, Faculty of Basic science,Damghan Branch, Islamic Azad University, Damghan, Iran
4.Department of Molecular Biology, Faculty of Basic science,Ahar Branch, Islamic Azad University, Ahar, Iran
5.Department of Biology, Faculty of Basic science, Kharazmi University, Tehran, Iran

Background: In this in silico study 3D structure of Chimeric protein including FimH and FliC, an appropriate vaccine candidate, is predicted.
Methods: The Protein sequence of FimH and FliC were collected from UniProt. These proteins were fused together by (EAAAK)4 linker. The secondary structure of hypothetical protein was predicted by GORIV secondary structure prediction method. For 3D structure prediction, I-TASSER ab initio online software,was used. The predicted structure was validated by Ramachandran plot in PROCHECK software.
Results: The top three structures were selected and refined with kobamin software that reduces the structures errors. The refined structures were evaluated with Qmean score and the quality of structures were observed to have improved.The Ramachandran plot analysis revealed that 73% of amino acid residues were incorporated in the favored regions.
Conclusion: The present study demonstrated the best refined 3D structures of Chimeric protein including FimH and FliC with the highest score.

Keywords: 3D Structure, Fimh, Flic

PROTECTION AGAINST P. AERUGINOSA WITH A SYNTHETIC PEPTIDE DERIVED FROM OMP F CONJUGATED TO OUTER MEMBRANE VESICLE (OMV) OF NEISSERIA MENINGITIDIS

Ali Farjah[1], Parviz Owlia [2], Seyed Fazlollah Mousavi [3], Seyed Davar Siadat [4], Mehdi Shafiee Ardestani [5]

1.Department of Biology, Shahed University, Tehran, Iran
2.Molecular Microbiology Research Center, Faculty of Medicine, Shahed University
3.Department of Microbiology, Microbial research center, Pasteur Institute of Iran
4.Department of Microbiology, Microbial research center, Pasteur Institute of Iran
5.Department of Radiopharmacy, faculty of pharmacy, Tehran University of Medical Science, Tehran, Iran;

Background: *Pseudomonas aeruginosa* is an opportunistic pathogen that causes serious infections, is usually resistant to antimicrobial agents and is the leading cause of morbidity and premature mortality in hospitalized and burned patients.
Methods: In this work, the bioinformatics approach was used to design and synthesis a peptide derived from OMPF (ERRANAVRDVLVNEY).This peptide contained both B- and T-cell epitopes based on prediction models. We conjugated this peptide to the outer membrane vesicle (OMV) of *Neisseria* meningitidis serogroup B, which is a safe carrier protein, and evaluated its efficacy in mice.
Results: Results of this study on mice showed that the conjugate elicited anti-alginate-IgG that were not detected after immunization with naive peptide. IgG1 was also dominant among IgG subclasses. Immunization of mice with the peptide-OMV conjugate raised the levels of opsonic antibodies, and the vaccinated mice were protected when challenged intranasally with P. aeruginosa. Further studies showed that the conjugated vaccine could eliminate *P. aeruginosa* from the lungs of infected mice.
Conclusion: Thus, tests confirmed ability of this conjugate to elicit protective and opsonophagocytic antibodies that candidate our vaccine for further studies.

Keywords*:* *Pseudomonas aeruginosa*, Conjugate Vaccine,Synthetic Peptide; Bioinformatics

IN SILICO PREDICTION AND FUSION OF TWO HIGH IMMUNOGENIC EPITOPIC FRAGMENTS IN CLOSTRIDIUM PERFRINGENS EPSILON TOXIN AND CLOSTRIDIUM NOVYI ALPHA TOXIN

Mohsen Fathi Najafi [1], Mohsen Mehrvarz[1], Behjat Majidi.[1]
1.Razi Vaccine and Serum Researches Institute,I.R.Iran,Mashhad.

Background: The goal of present study is to design a fusion protein composed of two in silico selected epitopes of alpha and epsilon toxin.
Methods: Materials and methods: Mouse constructs as a human model was designed based predicted B and T cells epitopes. Primary sequence analysis was accomplished in NCBI (national center for biotechnological information) and CLC-Main work bench 5. PEST searches were searched based by EPESTFIND. Molecular properties were estimated in expasy online softwares. Immunoinformatic analysis was performed in RANKPEP, CED, SEPPA and CEP tools and CLC-Main work bench 5. The linker was designed based on LINKER online algorithm.
Results: Based on all received data from used tools, the highest scored fragments as the best epitopes were selected and to produce a perfect immunogenic multi-epitope vaccine a good linker was designed.
Conclusion: Compared in two native protein with selected and designed fusion protein the immunogenicity was not lower and the novel designed subunit vaccine after production can be used to make immunization faster, cheaper and even more effective.

Keywords: *Clostridium novyi, Clostridium perfringens*, Multi-Epitope Vaccine, Epsilon Toxin, Alpha Toxin.

ELIMINATION OF BACTERIAL AND FUNGAL CONTAMINATIONS FROM MICROALGAL CULTURE

Leila Aliasgharzade Rumiani [1], Hasan Jalili[1], Morteza Pahlavan Yali[2]
1.Department Of New Science And Technology, University Of Tehran,Tehran,Iran
2.Phd Student Of Seafood Processing In Iran Fisheries Research Organization, Iran

Background: The use of antibiotics is one of the means of dealing with contamination, but it should be noted that Chlorella is eukaryotic and anti-fungal compound might have negative effects on it.
Methods: The alga strain used in this experiment was Chlorella volgaris which was isolated from fresh water.It was pure but contaminated by bacteria and fungi. 1.5% Microbiological agar (Merck, Germany) was added to modified Bristol medium to prepare a solid medium. To eliminate bacterial contamination, the filtered-pharmaceutical tetracycline, ampicillin, and penicillin G have been used at 0.01 g/ml concentration in the medium. Pharmaceutical nystatin in 40 to 100 µg / ml Concentrations has been used and added to the medium in petri dishes in order to eliminate the fungal contamination. The control culture did not have any antibiotics.This experiment carried out in three repetitions. Cultures were incubated at room temperature under a 4000 lux light intensity with a 14: 10 h light photoperiod for 10 days in order to obtain free bacterial and fungal Chlorella colonies.
Results: The bacteria were sensitive to antibiotics and none of cultures shows any colonies of bacteria.In the cultures containing lower concentrations than 70µg / ml, colonies of fungi have been observed. But in the cultures that containing 70 to 90 µg / ml haven't been observed colonies of fungi.Although in the cultures with 100 µg / ml nystatin, Chlorella couldn't grow.
Conclusion: The experimental results showed that the increased levels of nystatin up to 90µg / ml had negative effect on Chlorella volgaris survival.And 70 to 90 µg / ml nystatin is more efficient for elimination of fungal contamination from algal culture.

Keywords: Microalge Culture, Purification, Contamination

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SECRETORY EXPRESSION OF HEMAGGLUTININ GLOBULAR DOMAIN (HA1) OF THE INFLUENZA A (H5N1) VIRUS IN BACILLUS SUBTILIS

Moein Aliakbari[1], Farida Behzadian[1], Ali Asghar Deldar[1], Baharak Mahyad[1], Maryam Bidram[1], Maedeh Bahreinian[1]
1.Molecular Genetics department, Research Centre for Sciences and Biotechnology, Malek Ashtar University, Tehran, Iran

Background: Here we produced secretory recombinant HA1 protein in *B. subtilis*.
Methods: The PCR-amplified HA1 sequence was cloned into pGEM® 5Zf(–)vector and subjected for sequencing. It was then subcloned into *E.coli-B.subtilis* shuttle vector PHT43 and transferred to *E.coli* for replication. The recombinant plasmid PHT43-HA1 was extracted from *E.coli* and used to transform of *B.subtilis* by electroporation. Following the verification of the new recombinant *B.subtilis*, it was subjected for HA1 production by IPTG induction. Total cell protein and the protein secreted into media were analysed through a time course using SDS-PAGE.
Results: The accuracy of PHT43-HA1 construct was confirmed by Sequencing and enzymatic digestion analysis. SDS-PAGE results showed that the recombinant HA1 protein was successfully expressed and secreted into medium. It reaches to maximum level 4 hours after induction.
Conclusion: *B.subtilis* is a free endotoxin host which could be a favorite prokaryotic platform for production of the recombinant HA1 protein.The HA1 protein produced here could be considered and evaluated as a candidate vaccine which its immunogenicity potential needs to be assessed in animal models along with proper control groups.

Keywords: *Bacillus subtilis*, Secretory Expression, Influenza, H5N1, PHT43

TAKEN THE SOLUBLE MUTANT FORM OF RICE-NSLTP2 IN E.COLI, STRATEGIES AND CHALLENGES

Fatemeh Zahra Darvishi[1], Mehran Miroliaei[2], Mahboobeh Nazarai3[3]
1.Msc Student In Cellular & Molecular Biology, Department Of Biology, Faculty Of Science, University Of Isfahan, Iran
2.Associated Professor In Biochemistry, Department Of Biology, Faculty Of Science, University Of Isfahan, Iran
3.Assistant Professor In Biochemistry, Department Of Recombinant Technology Research, Nanobiotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran

Background: In this paper, a soluble form of mutant type from Rice-nsLTP2 is expressed using fusion protein technology.
Methods: The designed mutant construct was subjected to expression and optimization in *E.coli*. *E.coli* BL21 (DE3) pLysS was the host bacterial strain for the pET32a vector expression system. The protein expression was optimized by changing in temperature, time and IPTG concentration. The production of mutant nsLTP2 was analyzed by SDS-PAGE.
Results: The new construct was transformed by heat shock procedure and the soluble recombinant nsLTP2 was expressed. Therefore, the fusion protein technology was mentioned to overcome difficulties in protein solubility. The new construct has thioredoxin tag that improves the yield of active soluble protein.
Conclusion: In the present study, we have obtained manipulated ns-LTPs. Using PCR approaches, phenylalanine 39 was mutated to alanine in Iranian ns-LTP2. The final sequence was expressed flanked by thioredoxin tag. A significant amount of soluble protein was obtained after optimized expression.

Keywords: Nsltp2, Mutant, Soluble, Drug Delivery

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DETERMINING THE EPIDEMIOLOGICAL CORRELATION OF COAGULASE POSITIVE STAPHYLOCOCCI ISOLATED FROM SWEETMEATS AND STAFF BY PCR- RFLP-BASED METHOD

Mohamad Hassanvand[1], Gholamreza Godarzi[2]
1.MA In Microbiology From The Azad University Of Tunkabon, Iran
2.Assistant Professor, Parasitology And Mycology Department, Medical Collage, University Of Luristan Medical Sciences, Iran;

Background: Microbial confectionary contamination in the world is widespread. Confectionary products form an important part of foodstuffs in this country. The aim of this study is to isolate the epidemiological correlation of coagulase positive staphylococci isolated from sweetmeats and staff by PCR- RFLP-based method.
Methods: We randomly selected 300 samples in some confectionary workshops in Khoramabad city and tested them for possible contamination using the related standards of Organization of Standards and Industrial Research. We also gathered 91 swabs of the nose fluid samples of the staff. Considering the existence of coagulase gene in the samples, we used a PCR- RFLP test to identify *Staphylococcus aureus*. We also used AluI digesting enzyme to determine the epidemiological correlation of the isolates. We used IBM SPSS Statistics 19 to analyze the data.
Results: By PCR amplification of coagulase gene, *S. aureus* was detected in 21 fragments (7%) of the sweetmeat samples. Furthermore, 13 fragments (14.28%) were detected from the swaps of the nose fluid samples of the staff. Three different band sizes of 420bp (26.47%), 580bp (64.7%), and 780bp (5.53%) were observed in amplicons of coagulase gene. The amplified isolated genes of Coagulase were further discriminated by digestion with AluI restriction enzyme. Heterogeneous bands of different sizes were observed. The findings indicated that the there was no significant epidemiological correlation among the coagulase positive staphylococci isolated from the samples of the sweetmeats and staff.
Conclusion: Therefore, it is required to control and monitor this product and its basic material through all relevant processes proactively

Keywords: Coagulase Gene, Alui Enzyme, PCR-RFLP, Food Poisoning

STUDY OF MICROSPORIDIOSIS IN CORNEAL SCRAPING OF KERATITIS PATIENTS

Zarrintaj Valadkhani[1], Mir Mostafa Ghamiloui[1], Firozeh Rahimi[2], Nayyereh Hassan[1], Zohreh Aghighi[1]
1.Pasteur Institute of Iran, Tehran
2.Farabi research eye center, Professor of ophthalmology,Tehran University of Medical Science,

Background: The current study aimed to investigate the microsporidial keratitis in patients referred to the corneal clinic of Farabi hospital, Iran, and compare with other countries.
Methods: Two scraping samples from each 91 keratitis patient referred to the Farabi hospital were collected by an ophthalmologist. In order to culture, the microorganisms in a Vero cell culture, one sample was stored in microtubes containing PBS while the other sample used for the preparation of Giemsa and/or Gram staining slides. The samples cultured in Vero cell culture, and after 30 days, the cells scrapped, centrifuged and used for DNA extraction and the Nested PCR-based detection method. The Nested primer pairs designed by Using CLC Genomics workbench V.3.6.1 software to amplify all major microsporidian pathogens reported to infect cornea; such as E. cuniculi, E. hellem, E. intestinalis and Vittaforma corneae, as well as E. bieneusi that isolated from stool to use as positive control.
Results: Results from the nested PCR showed a negative presence of microsporidium in all the samples and the Gram and Giemsa staining also all were negative in the detection of any spores.
Conclusion: The rate of human microsporidiosis reported worldwide is in the range of 0%-50%. This study, with all the proved negative samples, indicates that the rate of this infection amongst Iranian eye patients lies in the bottom quartile. Few reports are available on this issue thus the parameters are poorly understood. By gathering several lines of evidence, studies focused in this field can move a step forward and can open new insights for researchers to investigate in immunosuppressive drugs users and AIDS patients as well.

Keywords: Microsporidiosis, Cornea, Cell Culture, Nested Pcr, Iran

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PREVALENCE OF STAPHYLOCOCCUS SPP. AND MECA GENE FROM SPECIMENS IN KURDISTAN MEDICAL UNIVERSITY HOSPITALS, Iran

Hanar Narenji[1], Himen Salimizand[2], Rashid Ramazanzadeh[3], Babak Shahbazi[4], Masomeh Khonshah[4]
1.Msc Of Microbiology,Faculty Of Medicine, Kurdistan University Of Medical Sciences, Sanandaj, Iran
2.Kurdistan University Of Medical Sciences,School Of Medicin,Department Of Microbiology, Iran
3.Cellular & Molecular Research Center, Faculty Of Medicine, Kurdistan University Of Medical Sciences, Sanandaj, Iran
4.Students' Research Committee, Faculty Of Medicine, Kurdistan University Of Medical Sciences, Sanandaj, Iran

Background: *Staphylococcus aureus* is a major human pathogen. Methicillin-resistant *S. aureus* (MRSA) have greater virulence than methicillin-susceptible strains. MRSA is a major cause of nosocomial and community infections,The aim of this study was a survey on prevalence of isolated MRSA from nosocomial infections and environmental specimens in Kurdistan University Hospitals.
Methods: In this study, 264 samples were collected. Samples confirmed with biochemical tests for S. aureus. 156 staphylococci bacteria were related to clinical samples, 46 environmental samples and 59 samples were related to staff in hospitals wards. Sensitivity to antibiotics was determined by using Kirby-Bauer methods with gentamicin (10µg), vancomycin (30µg), ciprofloxacin (5µg), and erythromycin (15µg). Also agar screening with 6 µg/ml oxacillin and PCR were performed for predicting mecA gene
Results: Frequency of isolated Staphylococcus was following: In staff, *S. aureus* was18(36.73), S. epidermidis was 31 (63.27%) and S. saprophyticus was not found. In environmental samples, *S. aureus* 20 (33.9%), *S. epidermidis 38 (64.41%) and S. saprophyticus* 1 (1.69%) were found. In Clinical samples, *S. aureus* was 50 (32.05%), S. epidermidis was 101 (64.74%) and S. saprophyticus was 5 (3.21%). Results showed that vancomycin (69.7%), ciprofloxacin (43.94%), gentamicin (28.3%) and erythromycin (9.09%) were most effective antibiotic against staphylococci that were isolated from samples. Also 6 (6.82%) of isolates were Methicillin-Susceptible *S. aureus* (MSSA), 82 (93.18%) of the isolates were (MRSA), 151(85.79%) were Methicillin-resistant coagulase-negative staphylococci (MRCNS) and 25(14.21%) were Methicillin-Susceptible coagulase-negative staphylococci. *mecA* gene was isolated from 66 (75%) of samples.
Conclusion: Prevalence of MRSA was highest among isolated *S. aureus*. This result indicates a potential risk of staph infections resistant. So appropriate method for prevention of this problem should be considered

Keywords: MRSA,Nosocomial Infections, *Staphylococcus aureus*

MOLECULAR TYPING OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS STRAINS BY PCR-RFLP OF SPA GENE

Masume Aslanimehr[1], Amir Peymani[1], Rezvan Yaghoobfar[1], Mahnaz Tavakoli[2]
1.Department of Microbiology, Faculty of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran
2.Department of Microbiology, Faculty of Medicine, Shahid beheshti University of Medical Sciences, Tehran, Iran

Background: Protein A of *Staphylococcus aureus* is a pathogenic factor whose encoding gene, spa, shows a variation in length in different strains.In this study to characterize methicillin-resistant *Staphylococcus aureus* (MRSA) strains we applied molecular typing based on polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of spa gene.
Methods: Studies were undertaken on 116 MRSA isolates of *Staphylococcus aureus* which we were isolated from Qazvin and Tehran hospitals, Iran. Methicillin resistance first determined by disc diffusion and agar screen tests and then confirmed by PCR using specific primers of mecA gene. Isolates were typed by PCR-RFLP of spa gene using Hin6I restriction enzyme. Analysis of the amplified spa gene fragment of the representative RFLP patterns was performed with standard protocols.
Results: All the isolates resistant to methicillin were found to contain mecA gene. In 6 (5.2%) strains of them no spa gene was detected, and 1(0.86%) had a dual band (1200 and 1500 bp). The most prevalent length was 1200 bp (45%). 17 different PCR-RFLP patterns were observed among 116 MRSA strains.
Conclusion: The study demonstrates the importance of spa genotyping in the discrimination of MRSA strains. The study provides valuable information on the epidemiological characterization of MRSA strains.

Keywords: *Staphylococcus aureus*, Spa, PCR-RFLP

MOLECULAR IDENTIFICATION OF NOCARDIA SPP. COLLECTED FROM PATIENTS WITH SYMPTOM TUBERCULOSIS

Saeed Zaker Bostanabad [1], Abdolrazagh Hashemi-Shahraki, [2], Parvin Heidarieh[3]
1.Islamic Azad University, Parand branch, Science Faculty, Biology Group, Parand -Theran, Iran
2.Pasteur institute of Iran, Epidemiology Department
3.Alborz Medical University, Pathobiology Department

Background: The improved identification of isolates using molecular techniques have shown that the genus exhibits considerable taxonomic complexity and phenotypic base identification can be ambiguous. The aim of this study was to assess the species distribution of *Nocardia* strains mostly recovered from patients suspected of having tuberculosis.
Methods: The clinical isolates were identified to species level using conventional tests and genotypic methods using single and multi locus sequence analysis (MLSA) of 16S rRNA, gyrB and secA1 genes.
Results: Nocardiosis was diagnosed in 46 patients. The most frequent underlying condition were organ transplantation (6 patients; 13%), cancer (6 patients; 13%), human immunodeficiency virus (HIV) (6 patients; 13%), non-infectious chronic lung disease (5 patients; 10.8%) and tuberculosis (4 patients; 8.7%). *Nocardia* species was recovered from 46 different clinical specimens, the most common of which was bronchoalveolar lavage (BAL) (43.5%). In conclusion, infection caused by *Nocardia* species appears to be more common than is generally appreciated.
Conclusion: The current study provides further evidence that *Nocardia* species are capable of causing a wide range of human diseases in healthy and immunocompromised patients. MLSA is a reliable method for accurate species identification of *Nocardia* isolates and would be more feasible for routine use in clinical laboratories.

Keywords: Molecular Identification - *Nocardia*

THE SEROEPIDEMIOLOGICAL SURVEY OF HTLV1, 2 INFECTIONS IN NEYSHABOUR CITY (NORTHEAST OF Iran)

Mohammad Salehi[1], Masoud Mobini[2], Maryam Asghar Heydari[2]
1. Master Of Science Of Microbiology,Iranian Academic Center For Education, Culture And Research Of Mashhad (ACECR), Neyshabor Branch. Neyshabour. Iran.
2.Master Of Science Of Microbiology,Young Researchers Club, Tonekabon Branch, Islamic Azad University, Tonekanbon, Iran

Background: HTLV was the first human retrovirus to be discovered. this virus is endemic in several regions around the World such as the Razavi Khorasan Province, Northeast of Iran. The aim of this study is investigate HTLV-1, 2 infections prevalence rate in patients who referred to Iranian Academic Center for Education, Culture and Research of Mashhad (ACECR), Neyshabor branch.
Methods: All patients referred to ACECR were tested for evaluation of HTLV-1, 2 using ELISA method (Dia.pro diagnostic bioprobes, Italy) from March 2013 to February 2014 in this Center. The variables were age and gender.
Results: According to the results of enzyme linked immunosorbent assay (ELISA), HTLV1, 2 infections were positive in 7.2% (153/2118) of the participants. Infection was significantly associated with age and gender of participants.
Conclusion: It seems that HTLV infection is highly endemic in Neyshabour and it is seems that more effective prevention strategies of infections prevalence are needed, especially in women population (positive infectious rate in female = 6.7%).

Keywords: Seroepidemiology, Htlv-1, 2

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EVALUATION OF HUMAN PAPILLOMAVIRUS PREVALENCE AMONG SYMPTOMATIC PATIENTS USING MOLECULAR REVERSE HYBRIDIZATION METHOD IN TEHRAN

Nasrin Sheykhi[1], Shahin Pourazar Diziji[1], Ensiyeh Ketabchi[2], Mehdi Shekarabi[3]
1.Molecular Unit, Masoud Laboratory, Tehran, Iran
2.Institute Pasteur of Iran, Mycobacteriology Department, Tehran, Iran
3.Department of Immunology, School of Medicine, Tehran University of Medical Science, Tehran, Iran

Background: Human papillomavirus (HPV) infection has been identified as a major risk factor for invasive cervical cancer. Based on their presence in cervical carcinoma specimens these genotypes are classified as high-risk or low-risk. In this study We evaluated different specimens genotyping to identify HPV genotypes that are more common in Iran.
Methods: This cross sectional study was conducted in Massoud laboratory in Tehran during 2011. Seventy two symptomatic patients attending the laboratory entered to this study and their samples were evaluated using PCR. Twenty five samples were positive for HPV DNA. Positive samples for HPV DNA were evaluated using the INNO-LiPA HPV Genotyping method.
Results: Out of 72 samples that suspected to have HPV attending Massoud laboratory during 2011, HPV DNA was found in 25 cases. Genotyping was performed on positive cases which determined 8 different genotypes in samples. HPV 16, 31 and 18 were the most common genotypes respectively.
Conclusion: Persistence of high-risk HPV infections is associated with an increased risk of cervical cancer. Molecular typing methods to determine patients infected with high risk HPV genotypes are critical and are necessary to set best protocols for treatment and prophylaxis of HPV infections.

Keywords: HPV, Genotyping, Cervical Cancer

THE GENETIC DIVERSITY OF ESCHERICHIA COLI STRAINS ISOLATED FROM DIFFERENT WATER SOURCES IN ALBORZ PROVINCE

Parichehr Pezeshknejad[1], Reza Ranjbar[2], Kumars Amini[3], Roohollah Kheiri[4], Fatemeh Pourali[5]
1.Department of Microbiology, Faculty of Science, Islamic Azad University, Saveh Branch, Saveh, Iran
2.Molecular Biology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
3.Department of Microbiology, Faculty of Science, Islamic Azad University, Saveh Branch, Saveh, Iran
4. Alborz province Water and wastewater Company. Karaj, Iran
5.Molecular Biology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

Background: *E. coli* is one of the most common causes of water borne infectious disease. The study of genetic relationships is an important issue between bacterial strains particularly those are spreading via water. The aim of this study was to apply ERIC-PCR method for molecular typing of *E. coli* strains isolated from different surface water sources in Alborz province.
Methods: This study was performed in the year 2013 on the *E. coli* isolated from surface waters such as springs, refinery inputs, and a number of wells. Bacterial isolation and identification were performed using standard microbiological methods. The genetic relationship between the strains was investigated by ERIC-PCR using specific primers.
Results: The results showed that the ERIC-PCR was able to generate 10-17 amplified DNA bands. All strains were typable when subjected to ERIC PCR. The technique differentiated all strains to more than 9 ERIC clusters.
Conclusion: The results showed that *E. coli* strains circulating in different water sources such as springs, refinery inputs, and wells are belonging to the different genotypic clusters. We also found that ERIC-PCR had a good discriminatory power for molecular typing of *E. coli* strains isolated from water sources.

Keywords*: E. coli*, ERIC PCR, Water

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ERIC FINGERPRINTING AND GENOTYPING OF E. COLI STRAINS ISOLATED FROM 5 SURFACE WATER SOURCES OF TEHRAN

Leili Akhtari[1], Roohollah Kheiri[2]
1.Department of Microbiology, Faculty of Science, Islamic Azad University Science And Research Branch Of Kordestan
2.Alborz province water and wastewater company.Karaj.Iran

Background: The aim of the current study was to apply ERIC-PCR method for molecular typing of *E. coli* strains isolated from different surface water sources in Tehran province.
Methods: Current study included *E. coli* strains all isolated from 5 different surface water sources of Tehran province in 2013 including Bilaghan, kan, sohanak, Jalalieh and 4&5. Bacterial isolates were all detected and identified by standard microbiological and biochemical tests. Genomic DNA was extracted applying AccuPrep® Genomic DNA Extraction Kit and genomic fingerprints and genetic relationship between the strains was evaluated by ERIC-PCR using specific primers. The PCR amplicons were visualized after electrophoresis and staining with SYBR green and dendogram was constructed based on Dice Comparison method and UPGMA Clustering method applying online in silico tool.
Results: One hundred and six *E. coli* isolates were collected from Tehran surface water sources. Applying ERIC-PCR, all strains were typeable, since 18 different bands ranging from 200 to 4000 bp were amplified in different profiles. Following dendogram analysis, ERIC-PCR could categorize the strains within 11 ERIC clusters.
Conclusion: Among different typing techniques, ERIC-PCR is a rapid, simple, and reproducible method to identify the genetical variety of bacterial strains as well as their evolutionary changes. The results of current study confirmed that *E. coli* strains isolated from different water sources of Tehran province belong to diverse clones and different genotypes while ERIC -PCR is a powerful molecular tool with high performance and good discriminatory power.

Keywords: *E. coli*, ERIC, UPGMA, Water

DIFFERENT PHENOTYPIC ASPECTS WITH NO GENOTYPIC HETEROGENEITY IN LEISHMANIA MAJOR ISOLATES OF SUSPECTED PATIENTS IN NORTHERN KHUZESTAN PROVINCE

Adel Spotin[1], Soheila Rouhani[1], Parviz Parvizi[2], Parnazsadat Ghaemmaghami[2], Ali Haghighi[1], Aref Amirkhani[3], Ali Bordbar[2], Amir Yaghoubinezhad[2]
1.Department of Parasitology and Mycology, Medical Faculty, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2.Molecular Systematics Laboratory, Parasitology Department, Pasteur Institute of Iran, 69 Pasteur Ave., Tehran, Iran
3.Department of Epidemiology, Medical Sciences of Tehran branch, Islamic Azad University, Tehran, Iran

Background: In this inquiry, behavioral aspects of *L.major* isolates are evaluated in two forms of genotypic and phenotypic characters among suspected patients of some exceptional zoonotic cutaneous leishmaniasis (ZCL) locations in northern Khuzestan, Iran.
Methods: A total, 200 suspected patients were collected in ZCL foci over two successive years in 2012-14. Prepared slides were stained, scaled and measured precisely by ocular micrometer and Dino-captureu under a light microscope. Whole DNA was extracted directly from smears; ITS-rDNA gene amplified, subsequently amplicons digested with HaeIII restriction enzyme. In order to discriminating of *Leishmania* species, PCR-RFLP, sequencing and phylogenetic analyses were established by CLC DNA Workbench 5.05, Sequencher Tmv 4.0.4 and PAUP software respectively.
Results: Only L. major was identified in suspected patients with different regular (oval or round) and irregular (spindle, pear or cigarette) morphometric amastigotes' shapes which had the average size of 3- 4.5 m into each of the dry and mixed, classical and non-classical wet lesions, All sequenced ITS-rDNA gene of L. major (27 sequences) did not have any variation (? 2 test: P> 0.05) with only one common haplotype (GenBank access No. EF413075). Shush district had higher infection rates than other locations. Interestingly, sequence analyses indicated that unlike previous studies, there is not a meaningful correlation between phenotypic and genotypic features of L. major isolates (P > 0.05).
Conclusion: This study is considered as a first comprehensive report to incriminating morphometric shapes of L. major amastigotes which remarkably enhances our knowledge concerning their relevance with various clinical lesions and genotypic traits in southwestern Iran.

Keywords: *Leishmania major*, Amastigote Morphometrics, Molecular Variation,

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EVALUATION OF THE GENETIC SIMILARITY OF MYCOBACTERIUM TUBERCULOSIS STRAINS ISOLATED FROM IranIAN AND AFGHAN TB PATIENTS USING RFLP-BASED METHODS

Behnam Rafiee[1], Sepideh Ghani[2], Davoud Sadeghi [3], Nader Mosavari [4], Keyvan Tadayon [4], Ali Rezaei [5], Ali Asghar Farazi [6]

1.Department of Biology, Payam-e-Noor University, Arak, Iran
2.Young Researchers Club and Elite, Arak Branch, Islamic Azad University, Arak, Iran
3.Department of Biology, faculty of basic science, Imam hosein University, Tehran, Iran
4.Razi Vaccine and Serum Research Institute, Karaj, Iran
5.Department of Biology, Arak University, Arak, Iran
6.Department of Infectious Diseases, Arak University of Medical Sciences, Arak, Iran

Background: Tuberculosis is an old problem that is currently considered a great challenge. Noticing Iran's borders with Afghanistan and Pakistan, which are among the 22 high burden countries around the world. this study awareness of possible genetic similarities between *Mycobacterium tuberculosis* isolated from Iranian and Afghan patient living in Markazi province, also examines the role of migration based on the epidemiology of tuberculosis using RFLP (Restriction fragment length polymorphism) was performed.
Methods: In this study, 57 sputum specimens from smear positive patients admitted to health centers in Markazi province were cultured on specific mycobacterial culture media. Genomic DNA was extracted by standard protocols of WHO and digested separately by PvuII and AluI. Electrophoresis was performed and DNA fragments were transferred to positively charged nylon membrane by southern blotting method and hybridization by PGRS, DR and IS6110 probes. The hybridized strains were subsequently detected by enzymatic reaction and analyzed by Gel pro analyzer software.
Results: In this study a sample of mycobacteria isolated from Afghan patients were similar to isolates from Iranian patients and this similarity using all methods used in the study was confirmed
Conclusion: The results of this research based on genetic similarity, suggesting activation of circulating *Mycobacterium tuberculosis* strains among Iranian and Afghan nationals. However, it seems due to the limited number of samples effect of transmission through migration is very low.

Keywords: Epidemiology, Genetic Similarity, *Mycobacterium tuberculosis*, RFLP

ANTI-TUBERCULOSIS DRUG RESISTANCE IN WEST OF Iran

Parviz Mohajeri[1], Baharak Norozi[2], Abbas Farahani[3]

1.Department of Microbiology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran
2.Student Research Committee, Kermanshah University of Medical Sciences, Kermanshah, Iran.
3.PhD student of Medical Bacteriology, Dept. of Microbiology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Background: *Mycobacterium tuberculosis* has developed resistance to anti-tuberculosis drugs and becoming a major and alarming public health problem in worldwide. This study aimed to determine anti-tuberculosis drug resistance rate and to identify multidrug resistant tuberculosis (MDR-TB) in west of Iran.
Methods: Total of 130 samples were included Between Decembers 2011 and July 2012 in the study from that 112 cases were *Mycobacterium* tuberculosis. The proportional method was carried out according to the CLSI on Lowenstein-Jensen against isoniazid, rifampcin, streptomycin, ethambutol, pyrazinamide, para aminosalsilic acid, etionamid, cyclocerin. The microdillution method was carried out using 7H9 broth with 96 well-plates.
Results: From 112 isolates, resistance was observed to isoniazid 18 (16.07%), rifampicin 16 (14.28%), streptomycin 25 (22.32%), ethambutol 15 (13.39%), pyrazinamide 27 (24.10%), para aminosalicylic acid 19 (16.96%), cyclocerin 4 (3.57%) and ethionamid 14 (12.5%) cases. 16 isolates were multidrug-resistant.
Conclusion: The high prevalence of MDR-TB in our study is assumed to be due to recent transmission of drug resistant strains. Overall, the rate of drug resistance in our study was high, which is in line with findings of some high-burden countries. Therefore, that early case detection, rapid drug susceptibility testing, effective anti-TB treatment is necessary.

Keywords: *Mycobacterium tuberculosis*, Drug Resistant

CIPROFLOXACIN SUSCEPTIBILITY OF CLINICAL AND ENVIRONMENTAL NONTUBERCULOUS MYCOBACTERIA ISOLATED FROM ISFAHAN, Iran

Fatemeh Sadat Zarkesh Isfahani[1], Bahram Nasr-Esfahani[2], Nima Bahador[1], Tooba Radaei[2]
1. Department of Microbiology, Science and Research Branch, Islamic Azad University, Fars, Iran.
2. Department of Microbiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.

Background: In this study, the susceptibility of 20 environmental and 21 clinical isolates of Non- tuberculous Mycobacteria to ciprofloxacin was determined by agar dilution method.
Methods: Total of 41environmental and clinical isolates of NTM from microbial collections of Isfahan Microbiology Department and Tuberculosis center were obtained. The isolates were identified by conventional including pigmentation, growth temperature, rate of growth, Ziehl-Neelsen staining and multiplex PCR as molecular methods. The susceptibility of isolates to different concentrations of ciprofloxacin (1, 2 and 4μg/ml) was determined by agar dilution method according to the CLSI guideline
Results: From 41 isolates identified by phenotypic and molecular methods, the frequency of isolates were as follow: *M. fortuitum*; 27 cases, *M. gordonae*; 10 cases, M. smegmatis; 1 case, *M. conceptionense*; 1 case and *M. abscessus*; 2 cases. All isolates except *Mycobacterium* abscessus were sensitive to all three concentrations of 1, 2 and 4 μg/ml ciprofloxacin (MIC< 1 μg/ml).
Conclusion: Due to the sensitivity of NTM isolates (except for *M. abscessus*) to ciprofloxacin, this antibiotic should be regarded as a primary choice drug in treatment of these groups of infections

Keywords: Nontuberculous Mycobacteria, Agar Dilution Method, Ciprofloxacin

OPTIMIZATION OF METHODS FOR ISOLATING ENVIRONMENTAL MYCOBACTERIA FROM WATER BY THE PARALLEL APPLICATION OF 3 ISOLATION METHODS

Sarvenaz Falsafi[1], Saeed Zaker Bostanabad[2], Mohammad Mehdi Feizabadi[3], Ramezan Ali Khavari-Nejad[4], Abdolrazagh Hashemi Shahraki[5], Mostafa Ghalami[6], Nasrin Sheykhi[6]
1.Department Of Biology, Science And Research Branch, Islamic Azad University,Tehran, Iran
2.Department Of Microbiology And Biology, Islamic Azad University, Parand Branch, Tehran, Iran
3.Department Of Microbiology, School Of Medicine, Tehran University Of Medical Science, Tehran, Iran
4.Department Of Biology, Science And Research Branch, Islamic Azad University,Tehran, Iran
5.Institute Pasteur Of Iran, Epidemiology Department, Tehran, Iran
6.Mycobacteriology Unit, Masoud Laboratory, Tehran, Iran

Background: Cultivation of mycobacteria requires long-term incubation in rich media and inactivation of rapidly growing microorganisms whose growth impedes observation of mycobacterial colonies.
Methods: 38 samples were collected from surface waters in Tehran. Water sampling was carried out in a volume of 200-100 ml. water sample was transferred immediately to the laboratory and examined. Three methods (Cetylpyridinium chlori, Petroff, Tacquet-Tison) for the isolation of mycobacteria were compared by applying them in parallel to 38 samples of surface water. Each method was defined by a particular combination of decontamination method. The efficacy of each method was determined by calculating the positivity rate, negativity rate, contamination rate, mean number of mycobacterial colonies grown and mean number of different mycobacterial strains isolated. The last value was determined by subjecting the isolates to PCR.
Results: Decontamination with CPC appeared to be the best decontamination method, on the one hand, it significantly decreased the level of non-target microorganisms and, on the other hand, it was significantly less lethal for the NTM strains studied.
Conclusion: Our goal was to measure the effects of various methods known to inhibit the growth of non-target microorganisms, while we also took into consideration the inhibitory effects of these methods on the growth of NTM. We propose that CPC procedure could be used for detection of NTM in aquatic samples.

Keywords: Aquatic Ecosystems, CPC, Nontuberculous Mycobacteria

SYNCHRONOUS COMPARISON OF HUNTER – GASTON DISCRIMINATORY INDEX FOR MYCOBACTERIUM TUBERCULOSIS EPIDEMIOLOGY STRAINS BY15 LOCUS AND 12 LOCUS MIRU-VNTR TECHNIQUE

Samin Zamani[1], Mohammad Javad Nasiri[2], Mohammad Mehdi Feizabadi[3]
1.Department of Microbiology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Background: Tuberculosis (TB) is considered one of the most important pathogens in the world. Identifying the source of transmission infection is necessary to decrease tuberculosis. In this study we compared 15 locus and 12 locus mycobacterial interspersed repetitive unit variable-number tandem repeat MIRU-VNTR methods to determine discriminatory power of them.
Methods: Standard 15-locus and 12- locus (VNTR)typing was applied to genotype 121 MTB clinical isolates
Results: The Hunter – Gaston discriminatory index (HGDI) for 15 VNTR loci and 12 VNTR loci were 0.97 and 0.94 indicating a high power of discrimination for MIRU-VNTR typing. A highly discriminatory subset of 15 loci was selected for first-line epidemiological investigations.
Conclusion: For 15 locus MIRU VNTR typing HGDI was 0.97, indicating a high power of discrimination for MIRU-VNTR typing compared to 12 locus. Sets of 15 MIRU VNTR loci have been suggested to further improve the discrimination of isolates, as compared to that provided by 12-loci system. This method could be considered a suitable tool for studying the transmission routes of TB and leading to more appropriate measures for tuberculosis control.

Keywords: *Mycobacterium tuberculosis*, MIRU-VNTR, Iran

EFFECT OF ZINC SUPPLEMENTATION ON BIOCHEMICAL MARKERS SERUM CONCENTRATION OF UREA, URIC ACID, CREATININE, ALP, AST, ALT, ALBUMIN, TOTAL PROTEIN AND ZN IN THE TREATMENT OF PULMONARY TUBERCULOSIS.

MUCORMYCOSIS; CLINICAL MANIFESTATION, DIAGNOSIS AND MANAGEMENT

Farhad Abbasi[1], Masoud Mardani [2], Davood Yadegarynia [2], Morteza Aghahasani [2]
1.Bushehr University of Medical Sciences, Bushehr, Iran
2.Shaheed Beheshti Medical University, Tehran, Iran

Background: Mucormycosis is an aggressive fungal disease that involves the paranasal sinuses, orbit, central nervous system and other organs. This infection usually occurs secondary to immune suppression, diabetic ketoacidosis, and prolonged use of antibiotics, steroids, and cytotoxic drugs. Management of the condition consists of treatment of the underlying disease and surgical debridement combined with intravenous antifungal agents.
Methods: In a retrospective study we evaluated nine cases of mucormycosis in several tertiary care hospitals in Tehran, Iran during several years. Clinical manifestations, diagnosis including laboratory and radiologic study, management including surgical and medical interventions were evaluated.
Results: In this study there were six cases of rhinocerebral mucormycosis, one case of pulmonary mucormycosis, one case of pelvic mucormycosis and one case of isolated cerebral mucormycosis. Risk factor for most of our patients was diabetes mellitus. We use amphotericin B and posaconazole as medical therapy. Surgical intervention and radical debridement was performed for all of patients. Five cases (55.5%) of our patients survived. The patients that expired were those who developed cerebritis, meningitis or brain abscess.
Conclusion: High index of suspicion, early diagnosis, treatment with antifungal agents and surgical debridement may improve the prognosis of this infection. Resistant to Amphotericin-B is increasing in mucoral agents. Mocurmycosis has a good response to posaconazole.

Keywords: Mucormycosis, Amphitericin-B, Posaconazole

MYCOBACTERIUM TUBERCULOSIS AND CRYPTOCOCCUS NEOFORMANS COINFECTION MENINGITIS IN A YOUNG IMMUNCOMPETENT WOMAN

Farhad Abbasi[1], Bahram Nasri Razin [2], Simin-Dokht Shoaei [2], Alireza Family [2], Mahmood Nabavi[2]
1.Bushehr University of Medical Sciences, Bushehr, Iran
2.Shaheed Beheshti Medical University, Tehran, Iran

Background: Meningitis is a severe and potentially fatal form of tuberculosis. The diagnostic workup involves detection of acid-fast bacilli (AFB) in the cerebrospinal fluid (CSF) by microscopy or culture, however, the difficulty in detecting the organism poses a challenge to diagnosis Cryptococcosis is an opportunistic fungal infection caused by Cryptococcus neoformans. Generally, the disease affects the central nervous system
Methods: The patient was a 35 year-old woman who was admitted in hospital due to fever, headache and changes of mental status. Physical examination reviled neck stiffness. Kernig's and Brudsinsky signs were positive.
Results: Cerebrospinal fluid analysis showed lymphocytic pleocytosis. Culture of cerebrospinal fluid reviled *Mycobacterium tuberculosis* and *cryptococcus neoformans.*
Conclusion: Tuberculosis meningitis should be considered in patients with chronic meningitis especially in endemic area. Cryptococcus neoformans meningitis may occur in immuncompetent patient and confection with tuberculosis meningitis is possible

Keywords: Chronic Meningitis, *Cryptococcus Neoformans*, *Mycobacterium Tuberculosis*

SUSCEPTIBILITY OF CANDIDA SPECIES ISOLATED FROM ORAL CANDIDIASIS AND DIAPER DERMATITIS INFECTIONS IN NEONATES TO COMMON ANTIFUNGALS

Mohamad Reza Havasian[1], Jafar Panahi[1], Iraj Pakzad[2]
1.Student research of committee, Ilam University of Medical Sciences, Ilam/ Iran
2.Clinical Microbiology Research Center, Ilam University of Medical Sciences, Ilam/ Iran

Background: Oral candidiasia and diaper dermatitis are common infections in children which can be treated with antifungals. However, extensive use of such medications would lead to the creation of resistant candida species. We aimed to evaluate the sensitivity of candida species isolated from oral candidiasis and diaper dermatitis infections in children.
Methods: In this study, 248 oral candidiasis and diaper dermatitis samples were collected from children referring to private and public clinics in Ilam, Iran. Candida species were identified using standard culture methods. Resistance and sensitivity to amphotericin B, nystatin, ketoconazole,fluconazole, itraconazole, clotrimazole, and posaconazole were determined using the CLSI M44-A standard disk diffusion method.
Results: Of the 248 studied samples, 149 were positive for candida, among which the most *Candida albicans* was the most prevalent (64.4%). The resistance of different candida species to nystatin, itraconazole, fluconazole, ketoconazole, clotrimazole, voriconazole, and posaconazole were 4, 43, 34.2, 34.9, 21.5, 6, and 6.7%, respectively. No resistance to amphotericin B was observed.
Conclusion: Considering that a relatively low resistance was found to nystatin, this drug is the best treatment choice for oral candidiasis and diaper dermatitis.

Keywords: Drug Resistance And Sensitivity, *Candida*, Oral Candidiasis, Diaper Dermatitis, Antifungals

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EVALUATION OF FUNGAL KERATITIS IN ADENOVIRUS SUSPECTED SAMPLES BY MOLECULAR METHOD

Fahimeh Tehranchi[1], Mohammad Hassan Shahhoseiny[1]

1.Iranian Gene Fanavar Institute (IGF), Tehran/ Iran

Background: In this study, PCR techniques and fungal specific primers for samples taken from patients with suspected Adenovirus keratitis is used.
Methods: In this research 72 adenoviruses keratitis suspected sample collected from f Farabi hospital. Samples prepared by scraping of corneal surface with special needle. DNA was extract by DNG plus kit and fungal PCR test with Mitochondrial B cytochrome target Gene were optimized. Limit of detection and specificity carry out by standard methods.. Finally Amplicons were cloned in pPZ57R vector by T/A cloning method.
Results: Two PCR tests optimized and products 430 bp for fungi and 301 bp for Adenoviruses amplified and observed in agarose gel electrophoresis. Limit of detection determined 10 particle for Adenovirus and 50 CFU for fungi. Of 72 investigated samples, 22(30.5%) was positive for Adenovirus, and no any positive for fungi in positive and negative Adenoviruses.
Conclusion: Because of that keratitis is dangerous, timely and exact detection is important to cure. Molecular technique, especially PCR can be reliable,exact and rapid for detection. According to result, fugi keratitis in Adeno viruses keratitis suspected samples is scarce and apparently fungal along with viruses does not cause keratitis.

Keywords: Keratitis,Fungi,PCR,Adenoviruse

EFFECT OF LONG-TERM EXPOSURE OF CANDIDA ALBICANS TO MOBILE PHONE RADIATION ON ITS AINT1 GENE SEQUENCE

Ariyo Shahin Jafari[1], Mohammad Hasan Shahhosseiny[2], Mohammad Hasan Shohhosseiny[3], Mansour Bayat[4], Parviz Tajik[4], Shahla Roudbar Mohammadi[5]

1.Department of Pathobiology, Science and research Branch, Islamic Azad University, Tehran, Iran
2.Department of Microbiology, Islamic Azad University, Qods branch, Tehran, Iran
3.Iranian Gene Fanavar institute (IGF), Tehran, Iran
4.Faculty of veterinary Specialized Sciences, Science and research Branch, Islamic Azad University, Tehran, Iran
5.Faculty of Medical Mycology, Tarbiat Modares University, Tehran, Iran

Background: In this study, for the first time, the mutagenic impact of long-term exposure to 900-MHz radiation on *Candida albicans* alpha-Int1 gene sequences were evaluated.
Methods: A standard EMF generator with output ranging from 860 to 940 MHz was used for radiation. 10 ml volumes from a stock suspension of *Candida albicans* (2x10^6 cell/ml, in log phase of growth, prepared in PBS) were transferred into ten 15 ml volume polystyrene tubes. 5 tubes were incubated at 4°C and exposed (distance=0~15 cm) to fixed magnitude of radiation with different time periods of 10, 70, 210,350 and 490 hours. The other 5 tubes were kept at the same conditions but far enough from radiation generator. Exposed and unexposed samples went under genomic DNA extraction using a standard method. PCR amplification of αInt1 gene sequence was done using a set of primers (forward and reverse) with optimized conditions. PCR products were resolved using 1.5% agarose gel electrophoresis and the nucleotide sequences were determined using Sanger method.
Results: All exposed and unexposed samples showed a clear electrophoretic band around 441 bp and further sequencing of the products revealed the amplified DNA segments are exactly related to alpha-Int1 gene of *Candida albicans*. No any type of mutations in the gene was seen in radiation exposed samples.
Conclusion: The findings indicate that long-term exposure of *Candida albicans* to mobile phone radiation under above mentioned conditions had no mutagenic effect on αInt1 gene sequence.

Keywords: *Candida albicans*, Aint1 Gene, EMF, Mutation

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DETECTION OF ASPERGILLOSIS IN IMMUNO-COMPROMISED PATIENTS

Parisa Badiee [1], Hadis Jafarian[1]
1.Professor Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Immunocompromised patients have been increasing in recent decades and *Aspergillus* species are the most frequent moulds have been isolated from immunocompromised patients. As the mortality rate is very high in these patients early detection and treatment is very crucial. In this study the incidence of aspergillosis was investigated in transplant patients by conventional and molecular methods.
Methods: In total 490 immunocompromised patients (liver and bone marrow transplant) were screened for aspergillosis by conventional and molecular methods. Clinical samples including: urine, sputum, fluids and tissue were examined by KOH smear, cultured on Sabouraud Dextrose Agar with Chloramphenicol, and incubated in room temperature for 14 days. Five ml blood of each patient was cultured using the BACTEC system and 1 ml serum of each patient was used for real-time *Aspergillus* PCR.
Results: The mean age of patients was 27 and 62% of patients were male. Blood cultures were negative in all patients. *Aspergillus* PCR and culture were positive in 32(6.5%) and 27(5.5%) of patients, respectively. All the patients had clinical and radiological signs and symptoms of fungal infection. Isolated etiologic agents were: 13 *Aspergillus fumigates*, 12 *Aspergillus flavus* and 2 *Aspergillus niger*. Twenty one of infected patients (65.6%) were died.
Conclusion: Despite using antifungal prophylaxis, invasive aspergillosis is a major problem with high mortality rate in immunocompromised patients so early detection and suitable treatment is very important. *Aspergillus* PCR is a sensitive, specific and rapid method for *Aspergillus* detection.

Keywords: Immunocompromised, Aspergillosis, Mortality Rate

FUNGAL INFECTION IN PEDIATRIC WARDS, NEMAZEE HOSPITAL, SHIRAZ, Iran

Parisa Badiee[1], Hadis Jafarian[1]
1.Professor Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Fungal infections have increased over recent years because of growing number of immunocompromised patients and potentially fatal fungal strains resistant to many commonly antifungal agents. This study focused on the incidence of fungal infections in the pediatric wards.
Methods: A prospective study was performed in pediatric wards in Nemazee hospital during 7 months. A physician followed each patient daily from admission until hospital discharge. Clinical samples collected from the patients with suspected fungal infections and examined by culture and KOH smear. Patient's blood samples were cultured by BACTEC system. Susceptibility of the isolates to antifungal agents was also determined by E-Test method.
Results: Totally, 697 patients were admitted to pediatric wards during the study. Mean time of hospitalization was 7.9 days. Twenty eight patients (4%) with mean age of 6 years had proven fungal infection, with 60% being male. The etiologic agents were 14 *Candida albicans*, 2 Candida dubliniensis, 1 Candida glabrata, 1 Candida famata, 2 Candida spp, 3 *Aspergillus* fumigatus, 2 *Aspergillus* flavus, 1 Fusarium spp., 1 Entomophthora spp and 1 chrysosporium spp. Antifungal resistance to different drugs was detected.
Conclusion: As clinical signs and symptoms of fungal infections are the same as the other infectious diseases. Reports on the incidence of fungal infections can be helpful to the respective clinicians in the more effective management of patients in hospital wards.

Keywords: Fungal Infection, Paediatric, *Aspergillus*, *Candida*

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RHINO- ORBITAL MUCORMYCOSIS

Ali Naseri[1], Abdolmajid Fata[1], Mohammad Naseri[1], Mahmoud Parian[1]
1.Department of Medical Parasitology and Mycology, Imam Reza Hospital, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Background: Mucormycosis is the acute and rapidly progressive diseases and the most dramatic of all fungal infections. The patients have uncontrolled diabetes and other underlying diseases. The usual presenting symptoms involved first the nose and then the eye and brain. We report a case of rhino- orbital mucormycosis in Mashhad (Iran).
Methods: A -31- year old woman with lesions in right eye and suspected to have mucormycosis was referred to the Medical Mycology laboratory, Imam Reza Hospital, Mashhad University of Medical sciences. She was diabetic. Physical examination of eye, nose and mouth revealed chemosis, ptosis and severe edema of eye lids. The other findings in the eye include orbital pain, proptosis and sensitivity to light. There were reddish black necrosis areas in the nasal turbinate and hard palate. Samples were taken from nose and hard palate. All collected specimens were analyzed by direct microscopy and culture.
Results: In direct mount from nasal and hard palate scraping board, irregular branched and non septate hyphae were observed. Rapidly growing colony yielded after 2 days on culture media. The colonies were light- gray and wooly in surface and hyaline to cream on the reverse. After 5 days petri dish completely filled by the fungus. The colonies became dark with age. Slide culture revealed board hyaline hyphae. Sporangiophores were irregularly branched with spherical sporangia. spores and columellae were ovoid to globose. Stolon and rhizoids were absent. The fungus was identified as mucor sp. and the patient was successfully treated with posaconazole.
Conclusion: Because mucormycosis is one of the most rapidly progressing mycoses, control of predisposing factors and prompt diagnosis is crucial for successful management and therapy.

Keywords: Mucormycosis, Mucor, Uncontrolled Diabetes, Posaconazole

ANTI-CANDIDA ACTIVITY OF TRACHYSPERMUM AMMI ESSENTIAL OIL ON AZOLES RESISTANT CANDIDA ALBICANS ISOLATES FROM ORAL CAVITY OF HIV+ PATIENTS

Iradj Ashrafi Tamaei[1], Aghil Sharifzadeh[2], Alireza Khosravi[2]
1. Department Of Microbiology, Faculty of Veterinary Medicine, Tehran University, Tehran, Iran.,1. Department of Pathobiology, Faculty of Paraveterinary Science, Bu-Ali Sina University, Hamedan, Iran. 2.Mycology Research Center, Faculty of Veterinary Medicine, University of Tehran,Tehran, Iran

Background: Oral candidiasis, caused by *Candida albicans*, is one of the most common infections in immunocompromised patients, especially in HIV+ individuals.The aims of this study were to evaluate the susceptibility of C. albicans isolates to azole drugs and Trachyspermum ammi essential oil.
Methods: Oral swabs were cultured from 70 HIV+ patients and In order to identify and confirm of C. albicans isolates, Chrom agar, Corn meal agar, germ tube production, carbohydrate assimilation, growth at 45°C and PCR were performed. Sensivity to fluconazol, ketoconazole and clotrimazol were assessed by disc diffusion and also the effect of T.ammi essential oil was determined by disc diffusion and microdilution broth methods.
Results: The causative agent, in 50 patients with oral candidiasis, was C. albicans (71.4%). In sensivity determination survey to antifungal drugs, the resistance of isolates to fluconazole, ketoconazole and clotrimazole were determined 32%, 28% and 14%, respectively. In disc diffusion, all isolates have an acceptable sensivity at 10 - 20 µL of the oil and 30 µL inhibit the growth completely in plate. Minimum Inhibitory Concentrsation(MIC) by microdilution broth method was 500ppm and 750ppm in 72% and 28% of isolates, respectively, and Minimum Fungicidal Concentration(MFC) in 70% of isolates were 750ppm and for the rest of the isolates (30%) were 1000ppm.
Conclusion: We conclude that use of this native plant, as an antifungal compound, could act as a treatment of the patients with mucosal candidiasis, beside of other drugs in to the future.

Keywords: Azole, *Candida albicans, Trachyspermum ammi*

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THE EFFECT OF CARVACROL AND EUGENOL AGAINST FOOD SPOILAGE ASPERGILLI

Aghil Sharifzadeh[1], Sepideh Abbaszadeh[2], Saeid Razzaghi[1]
1.Mycology research center, faculty of veterinary medicine, university of Tehran, Tehran, Iran
2.Baqiyatallah university of medical sciences, Tehran, Iran

Background: Moulds from the genus *Aspergillus* often spoil food products and produce mycotoxins. Over the last few years herbal compounds have attracted significant interest due to their antimicrobial activity, including activity against a wide range of fungi.This study aimed to evaluate the effectiveness of carvacrol and eugenol against four food-decaying *Aspergillus* species (A.niger – PTCC 5154, A.fumigatus – PTCC 5009, *A. flavus* - PTCC 5004 and A. ochraceus – PTCC 5017).
Methods: The susceptibility test for compounds was carried out in terms of the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) using microdilution method in 96 multiwell microtiter plates. Various solutions (25,50,75,100,125,150,175,200,250,300,400,450 and 500 µg/ml) of carvacrol (Sigma-Aldrich Corp.,St. Louis, MO, USA W224502) and (100,200,300,350,400,450,500,550 and 600 µg/ml) of eugenol (Sigma-Aldrich Corp.,St. Louis, MO, USA E51791) was prepared in dimethyl sulfoxide diluent (DMSO 5%). MIC and MFC were determined according to the CLSI M38-A standard method.
Results: In the current study, the MICs of carvacrol and eugenol ranged from 50 to 100 µg/mL (mean value: 87.5 µg/mL) and 350 to 450 µg/mL (mean value: 387.5 µg/mL) for different *Aspergillus* species, respectively. In addition, carvacrol had better fungicidal activity (mean value of MFC = 112.5 µg/ml) than eugenol (mean value of MFC = 450 µg/ml).
Conclusion: Our results suggest that application of these herbal components could be considered as a good alternatives to inhibit fungal growth and to reduce the use of synthetic fungicides.

Keywords: Carvacrol, Eugenol, *Aspergillus*

COMPARISON OF AFLATOXIN B1 LEVELS IN IraniAN AND INDIAN SPICES BY ELISA METHOD

Amir Sasan Mozaffari Nejad[1], Masoud Sabouri Ghannad[1]
1.Department of Microbiology, Research Center for Molecular Medicine, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

Background: This study was carried out to detect the presence of aflatoxin B1 (AFB1) in 36 samples of spices from Iran and India that inclued of chilli powder (n=12), black pepper powder (n=12) and whole black pepper (n=12).
Methods: Enzyme-linked immunosorbent assay (ELISA) was applied to analyse AFB1 in the samples. All the analyses were done twice.
Results: Aflatoxin B1 was found in all the spices samples, the concentration of AFB1 in Iranian samples was ranged from 63.16 to 626.81 ng/kg and in Indian samples was ranged from 31.15 to 245.94 ng/kg. The mean of AFB1 concentration in the chilli powder was significantly higher ($P < 0.05$) than the whole and powdered black pepper. However, none of the samples exceeded the maximum prescribed limit i.e. 5000 ng/kg (5 µg/kg) of European Union regulations for aflatoxin B1.
Conclusion: Although, the present study was not wide, it provides valuable information on aflatoxin B1 levels in Iranian and Indian spices.

Keywords: Aflatoxin B1, Spices, Elisa, Iran, India.

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IN VITRO INHIBITORY EFFECT OF ALCOHOLIC EXTRACT OF INNER STRATUM OF OAK FRUIT (JAFT) ON CANDIDA ALBICANS

Mohamadreza Feili[1], Jafar Panahi[1], Mohamad Reza Havasian[1]
1.Student Research Of Committee, Ilam University Of Medical Sciences, Ilam, Iran

Background: Candidiasis is one of the most common opportunistic fungal diseases in humans. This disease is caused by a fungus, yeast called candidial. Candida vaginitis is the most important fungal disease in women. Traditionally, in western parts of Iran different plant extracts are used for treatment of vaginal infections. This study aims to evaluate alcoholic extract of inner stratum of oak fruit (Jaft) on *Candida albicans* isolated from vaginitis.
Methods: the fruits of this Plant were collected from Ilam Mountain then they were dried in shadow, the alcoholic extraction was carried out. Inhibitory effect was studied by disk diffusion and agar well diffusion assay and the statistical analysis was done with SPSS 16 with used of repeat measure examination.
Results: in disk diffusion method, high inhibition zone was in 80 µg/disk and in agar well diffusion method 80 mg/ml has the highest inhibition zone.
Conclusion: alcoholic extract of jaft contains some metabolites that have inhibitory effect on *Candida albicans*. We suggest determining effective components of this extract.

Keywords: Inhibitory, *Candida albicans*, Disk Diffusion, Phenolic Extract

COMPARISON OF THE ENZYMATIC ACTIVITIES OF CANDIDA SPECIES IN WOMEN WITH VAGINITIS

Mahnaz Fatahinia [1], Marzie Halvaeezadeh[1]
1.Department of Medical Mycology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Background: The aim of this study was to assess the enzymatic activity of Candida species isolated from the vagina of women with vaginal candidiasis.
Methods: The research was performed on 250 vaginal samples from patients referred to health centers in Khuzestan province. After isolation and identification of Candida species, enzyme activities hemolysin α, hemolysin β, esterase and proteinase were measured in the laboratory. To calculate Index of each enzyme, the colony diameter was divided on the clear zone diameter or Sediment created, and the number 1 shows no enzymatic activity (Index=(colony diameter)/(clear zone diameter or Sediment diameter)).
Results:: Of the 250 samples, 80 (32%) Candida isolates were recovered. respectively, *Candida albicans*, 52 (65%), *C. glabrata* 12 (15%), C. dubliniensis, 10 (5/12%), C. Crusei 4 (5%) and C. tropicalis and C. Parapsilosis each case (3/1%) was isolated. The mean of enzymatic activity containing hemolysin A, hemolysin B, proteinase, esterase and Phospholypase are 0.5420, 0.2895, 0.7413, 0.5753 and 0.7446 respectively. The amounts of enzymatic activities of different Candida species were evaluated separately.
Conclusion: The percentage of Candida isolates in our study was higher than the average of other investigators. The minimum and maximum enzymatic activity was related to *Candida albicans*. Nearly 100% of *Candida albicans* produced all of mentioned enzyms. 86% of C.glabrata did not produce proteinase and phospholipase.

Keywords: Enzymatic Activities, Vaginities, *Candida albicans*

METACASPASES ACTIVITY IN ASPERGILLUS PARASITICUS TREATED WITH VARIOUS CONCENTRATIONS OF AMPHOTERICIN B

Seyyed Amin Ayatollahi Mousavi[1]
1.Dept. of Medical Mycology, Faculty of Medicine,Kerman University of Medical Sciences, Kerman, Iran

Background: In this study, cell death following treatment with amphotericin B (amB) was investigated using a number of well-characterised biochemical markers of apoptosis
Methods: DNA fragmentation using the TUNEL assay, translocation of phosphatidylserine (PS) from the inner to the outer leaflet of the plasmalemma using annexin V-FITC and caspase activity. Propidium iodide (PI) staining was used as a marker of cell viability.
Results: When treated with low but toxic concentrations of amphotericin B, an apoptotic phenotype also developed within 2 h and appeared prior to cell death as followed by PI staining. Higher concentrations induced immediate cell death with no apoptotic phenotype. However, whilst inhibiting protein synthesis blocked the development of an apoptotic phenotype at low concentrations of amB, the caspase inhibitor had no effect nor was any caspase activity toward substrates for caspase-1, -3 or -8 detected,
Conclusion: Suggesting either a different metacaspase was involved or that a metacaspase independent pathway was operating.

Keywords: Metacaspase, Amphotericin B, *Aspergillus parasiticus*

EVALUTION OF ANTIFUNGAL ACTIVITY THE BACILLUS SUBTILIS AGAINST DERMATOPHYTES FUNGI

Hamideh Babaei[1], Masoomeh Shams Ghahfarokhi[2], Mahboobeh Madani[3], Razzaghi Abyaneh [4]
1.M.Sc. Microbiology, School of Basic Sciences, Islamic Azad University, Flavarjan Branch, Isfahan, Iran
2.Department of Mycology Faculty of Medical Sciences,Tarbiat Modarres University, Tehran, Iran
3.Department of Microbiology, School of Basic Sciences, Islamic Azad University, Flavarjan Branch, Isfahan, Iran
4.Department of Mycology, Pastor Institiute of Iran, Tehran, Iran

Background: The purpose of this study was to evaluate the antifungal activity of *B.subtilis* culture filtration against dermatophytes fungi.
Methods: Fifty soil samples were collected from so different part of Tehran and cultured on glucose yeast extract agar for isolate of B.subtilis. All isolated *B.subtilis* were screened for their ability to inhibit all fungi growth using the dual culture on medium GYA on base visual agar plate assay, then of *B.subtilis* culture filtration showed most remarkable antagonistic activites against fungi was prepared serial dilution and antagonistic activity amount was evaluated. The results were analyzed using the SPSS software program.
Results: Totally 38B.subtiliswere obtained from soil samples that showed antagonistic activites against fungi. Bacteria cultured filtrated was able to inhibition more than 50% related to the evaluated fungi. This inhibition amount reported related to the current fungi in comparision to control group using the SPSS software, statistically, less than 0/05 in significant level.
Conclusion: The results show that the *B.subtilis* with produce antifungal metabolites can be an appropriate tool for biological control fungi in nature.

Keywords: *Bacillus subtilis*, Fungal Diseases, Antagonistic Activity, Filtration Medium

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ANTI-BACTERIAL EFFECT OF MENTHA SPICATA L. ESSENTIAL OIL ON EIGHT STANDARD SPECIES OF GASTRO INTESTINAL PATHOGENS

Majid Zare Bidaki[1], Mina Arab[2], Mohtarame Khazaei[2], Ehsan Afkar[3]
1.Assistant professor in medical microbiology, paramedical faculty, Birjand University of Medical sciences, Birjand, Iran
2.Student of laboratory sciences, paramedical college, Birjand University of Medical sciences, Birjand, Iran
3.Statistical consultant, deputy of research & technology, Birjand University of Medical sciences, Birjand, Iran

Background: Nowadays resistance to antibiotics and their side effects has encountered the human with a big problem. As a result, tend to use anti-bacterial compounds of plant origin has been increased. Mint plant scientifically called Mentha spicata L. has been a matter of research and debate for its pharmaceutical and antibacterial effects by a number of researchers. We aimed to study antibacterial effects of Mentha spicata L on 8 standard bacterial strains.
Methods: In this experimental study, the essential oil was extracted by steam distillation using clevenger apparatus. Using broth micro-dilution testing, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of mint essential oil were determined against 8 standard bacterial strains. This exam was repeated for three times. The results were analyzed by SPSS, v. 16 using one-way ANOVA, LSD and T statistical tests.
Results: The MIC tested for Mentha spicata L essential oil showed a significant difference ($p < 0.05$) among the different bacterial species. The highest growth inhibitory effect was found against *Bacillus* cereus and the least was recorded against *Klebsiella pneumoniae* and *Staphylococcus aureus*. No significant difference was found between the MBCs ($p > 0.05$).
Conclusion: This study showed that the essential oil of this plant can be used as an antiseptic agent in pharmaceutical and food industries.

Keywords: Menth Aspicata L, Essential Oil, Anti-Bacterial Effect, MIC, MBC

ANTIBACTERIAL EFFECT OF AQUEOUS EXTRACTS MEDICINAL PLANTS ON STANDARD STRAINS

Fariba Asgharpour[1], Zahra Moulana[1], Mehrasa Rezaie[2], Yasaman Alizade[3]
1.Department of Laboratory Sciences, Facultyof Para-Medicine; Babol University of Medical Sciences, Babol, Iran.
2.Faculty of Veterinary Medicine University of Urmia, Urmia, Iran
3.Islamic Azad University North Tehran, Iran

Background: In recent years, it is recommended to use natural materials, instead of chemically synthesized drugs with undesirable side effects, in the control and treatment of infections. Increasing usage of medical plants for medical treatment made for this branch of complementary medicine a unique place.
Methods: The present research was done for studying of anti-microbial effect of aqueous extracts of some medicinal plants such as Securigera securidaca, Coriandrum satirum, Allium satirum, Salvia officinal, Eucalyptus globules, Juglans regia. Vitis vinifera, Viscum Album, Pyrus biosseriana on bacteria P. aeruginosa, *E. coli*, *Staphylococcus aureus*, *Klebsiella* pneumonia, *Bacillus* subtillis in invitro conditions by using disc diffusion and micro broth dilution methods and Minimum inhibitory concentrations (MIC) and minimum bactericidalconcentration (MBC) of aqueous extracts plant have been evaluatead.
Results: Aqueous extracts medicinal plants had no effect on gram-negative bacteria but aqueous extracts Allium satirum and Eucalyptus globules inhibits the growth of gram-positive bacteria. Allium satirum to inhibition zone diameter 24 mm and MIC 12.5 µg/mL on *Staphylococcus aureus* had the greatest effect.
Conclusion: Based on the research finding, mentioned herbal extract can be a good candidates for laboratory studies to separate active compounds in this plant to achieve effective antimicrobial drugs.

Keywords: Antimicrobial Effects, Aqueous Extracts, Medicinal Plants

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ANTIMICROBIAL ACTIVITY OF ETHANOL AND ACETONE EXTRACTS OF OREGANO

Ali Heshmati[1], Iraj Khodadadi[2], Ali Asghar vahidinia[1]
1.Department ofNutrition, Faculty of Medicine, Hamadan University of Medical Sciences and Health Services, Hamadan, IR Iran
2.Department of Biochemistry and Nutrition, Faculty of Medicine, Hamadan University of Medical Sciences and Health Services, Hamadan, IR Iran

Background: This investigation was carried out to assess antimicrobial activity of ethanol and acetone extracts of oregano against two gram negative food spoilage bacteria Pseudomonas sp., *Escherichia coli* and two gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*.
Methods: In this research, ethanol and acetone extracts of oregano obtained by maceration. Antibacterial activity was performed by disc diffusion method. Minimum inhibitory concentration (MIC) value of the extracts of the oregano was determined the serial dilution technique
Results: *Pseudomonas* sp., *E. coli*, *Bacillus subtilis* and *Staphylococcus aureus* were sensitive to ethanol extract. Acetone extract of spices exhibited highest activity against *Escherichia coli* whereas it showed no activity against *Staphylococcus aureus* and *Bacillus subtilis*. According to the results obtained, minimum inhibitory concentrations (MIC) of ethanol extract were found to be 64µg/ml against *Bacillus subtilis*, *Staphylococcus aureus* and *E. coli*. Against *Pseudomonas* Sp., the MIC values were found 64µg/m. The MIC values of acetone extract were found to be 128µg/ml against both Pseudomonas sp., and *Bacillus subtilis*. Against *E. coli*, the MIC values were found 16µg/ml.
Conclusion: Based on our findings, oregano ethanol extract has a significant antibacterial effect on food borne pathogens and can be applied as a preservative in food.

Keywords: Oregano, Antibacterial, Extract

ANTI-BACTERIAL EFFECT OF ZATARIA MULTI-FLORA BOISS ESSENTIAL OIL ON EIGHT STANDARD SPECIES OF GASTRO INTESTINAL PATHOGENS

Majid Zare Bidaki1[1], Mohtarame Khazaei[2], Mina Arab[2], Ehsan Afkar[3]
1.Assistant professor in medical microbiology, paramedical faculty, Birjand University of Medical sciences, Birjand, Iran
2.Student of laboratory sciences, paramedical college, Birjand University of Medical sciences, Birjand, Iran
3.Statistical consultant, deputy of research & technology, Birjand University of Medical sciences, Birjand, Iran

Background: Due to worldwide development of antibiotic resistance, the tendency to herbal medicine has grown in recent years. Antibacterial substances derived from plants have many applications in controlling microorganisms. Thyme plant scientifically called Zataria multiflora Boiss is a matter of research and debate for its pharmaceutical and antibacterial effects. We aimed to study antibacterial effects of Zataria multiflora Boiss on 8 standard bacterial strains.
Methods: In this experimental study, the essential oil was extracted by steam distillation by means of clevenger apparatus. Using broth micro-dilution testing, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of thyme essential oil were determined against 8 standard bacterial strains. This exam was repeated for three times. The results were analyzed by SPSS, v. 16 using one-way ANOVA, Tukey and T statistical tests.
Results: The MIC found for Zataria multiflora Boiss essential oil showed a significant difference ($p<0.001$) among 8 different bacterial species. The highest growth inhibitory effect was found against *Bacillus* cereus and the least one was against *Pseudomonas aeruginosa*. There was no significant difference between the MBCs ($p> 0.05$).
Conclusion: The present study showed that Zataria multiflora Boiss essential oil has significant antibacterial effect. Therefore, it can be used as an antiseptic agent in pharmaceutical and food industries.

Keywords: Zataria Multiflora Boiss, Essential Oil, Anti-Bacterial Effect, MIC, MBC

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ANTI-TRICHOMONAS VAGINALIS ACTIVITY OF THE FOENICULUM VULGARE FROM IranIAN SEMI-ARIED REGION

Zaynab Joneydi[1], Maryam Niyyati[1], Ali Haghighi[1], Mohammad KamaliNejad[1], Fatemeh Memari[1], Mahmood Reza Behravan[1]
1.Department of Medical Parasitology and Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: *Trichomonas vaginalis* is a parasitic protozoan with a predilection for human urogenital tract and causative agent for vaginitis, cervicitis and urethritis in females. The drug of choice for therapy is metronidazole however the main drawback to metronidazole is wide adverse effects and also there are evidence of emerging resistance. This study was aimed to determine the effect of Foeniculum vulgare on *Trichomonas vaginalis* isolated from a female patient with urogenital complication.
Methods: *Trichomonas vaginalis* were cultured axenically in the TYM medium for 24-48 h. An initial inoculum of 1x 106 trophozoites/ml was achieved. The ethanolic extract of Foeniculum vulgare was obtained and different concentrations of Foeniculum vulgare were performed (75 µg/ml, 150 µg /ml, 300 µg /ml, 600 µg /ml, 800 µg /ml). T. vaginalis were incubated with the mentioned concentrations of extracts for 24-48 h along with positive and negative control. Positive and negative control was metronidazole (0.1 µg/ml) and culture medium, respectively. Experiments were performed in triplicate.
Results: The extract of Foeniculum vulgare showed a remarkable trichomonocidal effect with minimal inhibitory concentration (MIC) at 800 µg/ml in 48 hour incubation periods. At this concentration 79.4% of parasites were killed. It should be mention that the concentrations 75-800 µg/ml failed to completely destroy the parasites.
Conclusion: Due to adverse effect of chemical drugs such as metronidazole, use of plant based compounds could be an alternative approach for treatment of Trichomonasiasis. The present Results revealed that Rheum Nobile could be considered as an appropriate drug for treatment of T. vaginalis. In vivo study regarding Foeniculum vulgare is recommended in future.

Keywords: *Trichomonas vaginalis*, Foeniculum Vulgare, Invitro Assay

THE SURVEY OF ANTIBACTERIAL ACTIVITY OF ETHANOLIC AND METHANOLIC EXTRACT OF EUCALYPTUS MICROTHECA

Parinaz Lordifard[1], Seyyed Mansour Seyyednejhad[1], Hosein Motamedi[1], Seyed Soheil Rahmatabadi[1], Mostafa Amopour Bahnamiry[1]
1.Department of Biology, Faculty of science, Shahid Chamran University, Ahvaz, Iran

Background: The tendency to replace synthetic drugs with plants products has been increased in recent decades. Increase in Bacterial antibiotic resistance to antibiotics is a major public health challenges. Plant matter can be a good option in the management and treatment of bacterial antibiotic resisatance. The aim of this study was to investigate the antibacterial activity of the alcoholic extract of Eucalyptus microtheca.
Methods: 10 gr dried powder of E. microtheca leaf were mixed in 100 cc of %96 ethanol stayed in refrigerator overnight; then was pure with purification paper and extractions were obtained after alcohol evaporation. Preparation of methanolic extract was done due to the same method. Different concentrations of this methanolic and ethanolic extracts from 0.05 to 0.4 g/ml were tested by disc diffusion method against *Staphylococcus aureus* ATCC: 25923, *Bacillus subtilis* ATCC: 6633, *Escherichia coli* ATCC: 25922 and *Pseudomonas aeruginosa* ATCC: 2785.
Results: The results showed that the most antibacterial effects of both ethanolic and methanolic extractions was against *S. aureus* and it has reduced in order to *B. subtilis*, P. aeruginosa, and *E. coli*. Besides, the effect of methanolic extractions was more than ethanolic extractions.
Conclusion: Based on the above results, this extract can use as a broad-spectrum antibacterial agent that can use as a good choice against antibiotic-resistant bacteria. Better effect of methanolic extract than ethanol extract could be attributed to the higher polarity that leads to differences in the types of compounds which have been isolated from plants. Survey of various kinds of Eucalyptus compands can explore different combinations to find alternatives to antibiotic.

Keywords: Antibacterial Agent, Plant Matter, Eucalyptus Microtheca

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THE SURVEY OF ANTIBACTERIAL ACTIVITY OF ETHANOLIC AND METHANOLIC EXTRACT OF OLEA EUROPAEA

Parinaz Lordifard[1], Seyyed Mansour Seyyednejhad[1], Hosein Motamedi[1], Seyed Soheil Rahmatabadi[1], Fatemeh Ghazbanzadeh[1], Mostafa Amopour Bahnamiry[1]
1.Department of Biology, Faculty of science, Shahid Chamran University, Ahvaz, Iran

Background: Plants are important resources for searching new drugs. Todays, as the spread of antibiotic resistance in bacteria, tendency of replacing synthetic materials with plants materials have been increased. Plant materials because of lower toxicity and production cost can be introduced as new drugs. The purpose of this study was to investigate the antibacterial activity of the alcoholic extract of Olea europaea (olive).
Methods: 10 gr dried powder of O. europaea leaf were mixed in 100 cc of %96 ethanol stayed in refrigerator overnight; then was pure with purification paper and extractions were obtained after alcohol evaporation. Preparation of methanolic extract was done due to the same method, too. Different concentrations of this methanolic and ethanolic extracts from 0.05 to 0.4 g/ml were tested by disc diffusion method against *Staphylococcus aureus* ATCC: 25923, *Bacillus subtilis* ATCC: 6633, *Escherichia coli* ATCC: 25922 and *Pseudomonas aeruginosa* ATCC: 2785.
Results: The given results showed that O. europaea leaf's just has effect on *S. aureus* and the effect of methanolic extractions was more than ethanolic ones.
Conclusion: Based on the results obtained from the above results, Olive leaf extract could be a good candidate for using as antibacterial matter against S. aureus, and can be used as a substitute matter or concurrent with antibiotics. More Reviews for combination with other antibiotics, as well as the toxicity of the extract is required.

Keywords: Antibacterial Agent, Plant Matter, Olea Europaea

ANTIBACTERIAL EFFECT OF RHEUM RIBES ON ESCHERICHIA COLI

Fatemeh Ebrahimi[1], Zahra Fatahi[2], Fatemeh Jabbari[1], Leila Aboali[1]
1.Shahid Ahmadi Roushan Student Research Center, Education office, Baharestan 1, Golestan, Tehran, Iran
2.Neuroscience Research Center, Shahid Beheshti University of Medical Science, Tehran, Iran

Background: In the current study, the antibacterial effect of Rheum ribes on *Escherichia coli* was investigated
Methods: After preparation of the extract, the minimum inhibitory concentration and minimal bactericidal concentration (MIC / MBC) were determined. In disc diffusion method, the mean diameter of growth inhibition zones on agar media were determined by preparing discs from different concentrations (400, 600 and 800 ppm). In order to compare the antibacterial effect of Rheum ribes extract with antibiotics, antibiogram kits for gentamicin, ampicillin, ciprofloxacin, and cefotaxime was used as a positive control groups.
Results: The results revealed that certain concentrations of the extracts showed significant antibacterial effect on the strains. Extracts with 400, 600 and 800 ppm concentration showed defined growth inhibitory effect and 600 and 800 ppm concentration showed both inhibitory and bacteriocidal effects on the bacteria. The results of used antibiogram kits indicated that the effect of extract on *Escherichia coli* was similar to the effects of gentamicin and ciprofloxacin antibiotics.
Conclusion: Findings from this study showed that Rheum ribes extract inhibits the growth of Gram-negative bacteria *Escherichia coli*. This plant can be considered as a medicinal plant used for treating infections caused by *Escherichia coli*.

Keywords: *Escherichia coli*, Rheum Ribes

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THE ROLE OF NISIN, MONOLAURIN AND EDTA IN STRENGTHENING ANTIBACTERIAL EFFECT OF ROSEMARY AND CINNAMON ESSENTIAL OILS AGAINST ESCHERICHIA COLI, SALMONELLA TYPHIMURIUM, STAPHYLOCOCCUS AUREUS AND LISTERI

Mojtaba Raeisi[1], Mojtaba Raeisi[1]
1.Department of Public Health, Health Faculty, Golestan University of Medical Sciences, Gorgan, Iran

Background: Essential oils have great value in food industry due to their pharmaceutical, antimicrobial, and antioxidant properties. However, they are frequently used as flavoring agents and are also important in perfumery.Nowadays, numerous essential oils such as Thyme, Clove, Oregano, Cinnamon and Rosemary are used in the preparation and/or protection of foods.
Methods: In this study, the role of nisin, monolaurin and EDTA in strengthening antibacterial effect of the essential oils of Rosemarinus officinalis L. and Cinnamon zeylanicum Boiss. were tested against foodborne pathogens including *Escherichia coli* (ATCC43894), *Salmonella* typhimurium (ATCC1730), *Listeria monocytogenes* (ATCC19118) and *Staphylococcus aureus* (ATCC6538).
Results: According to GC/MS analyses, 14 and 15 components were identified representing 95.1% and 94.25% of the total oils, respectively. In the case of R. officinalis oil, 1,8-cineol (24.3%), ?-pinene (22.8%), and camphor (12.1%) were determined as the main volatiles. On the other hand, cinnamaldehyde (79.74%) was determined as the major compound for C. zeylanicum. Essential oils and individual components tested in this study were found effective against all of the bacteria tested. According to antimicrobial activity tests, *L. monocytogenes* was found as the most sensitive microorganism again. It is followed by S. aureus, *E. coli*, and S. typhimurium, respectively.
Conclusion: In general, EDTA made the weakest contribution to the MIC and MBC values of the essential oils. However, in some cases, the presence of EDTA enhanced the activity of oils two-fold. On the other hand, nisin made the most promising contribution to the MIC and MBC values of the oils. In some cases, nisin and monolaurin decreased the MIC and/or MBC values of the oils four-fold against microorganisms.

Keywords: Rosemarinus Officinalis L Essential Oil, Cinnamon Zeylanicum Boiss

EVALUATION OF ANTIBACTERIAL ACTIVITY OF THE ESSENTIAL OIL FROM PEPPERMINT (MENTHA PIPERITA L.) IN VITRO AND IN VIVO SYSTEMS

Shirin Ghanooni[1], Abolfazl Dadkhah[2], Mohammad Reza Zolfaghari[1]
1.Department of Microbiology, Qom Branch, Islamic Azad University, Qom, Iran
2.Department of Medicine, Qom Branch, Islamic Azad University, Qom, Iran

Background: The antibacterial activities of peppermint (Mentha piperita L.) essential oils in vitro and in vivo systems were evaluated in the present research.
Methods: To evaluate the antibacterial activities of this aromatic extracts; its in vitro antibacterial activities were determined by disk diffusion testing and minimum inhibitory concentration (MIC) against *Escherichia coli* and *Staphylococcus aureus*. Polymicrobial sepsis induced by cecal ligation and puncture (CLP) is the most frequently used animal model for investigation in vivo system. For this purpose, the blood was taken from hearts of rats for colony forming units (CFU) measurements.
Results: The results indicated that the oil was effective in reducing CFU caused by sepsis-induced CLP operation. Also, in vitro system, the test organisms were found to be inhibited by peppermint oil at lower concentration in broth dilution method as compared with disk diffusion method.
Conclusion: This study indicated the antibacterial activities of peppermint oils both in vivo and in vitro systems.

Keywords: Antibacterial Activity; *Mentha piperita* L.; Essential Oils; Sepsis; CLP

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A SURVEY ON THE ANTIMICROBIAL EFFECT OF METHANOLIC EXTRACT AND ALKALOID FRACTIONS OF AERIAL PARTS OF GLAUCIUM VITELLINUM AGAINST DIFFERENT BACTERIAL STRAINS AND CANDIDA SPP WITH DISK DIFFUSION AND MIC

Mina Mehrara[1], Nahid Rahimifard[2], Mojdeh Hakemi-Vala[3], Jinus Asgarpanah[1], Mohsen Heidary[3], Saeed Khoshnood[3]

1.Pharmaceutical sciences branch, Islamic Azad University of medical sciences, Tehran-Iran
2.Food and Drug Control Laboratories and Research Centre, Ministry of Health and Medical Education, Tehran-Iran
3.Department of microbiology, Medical school, Shahid Beheshti University of medical sciences, (SBMU), Tehran-Iran

Background: In this study the antibacterial effect of Glaucium vitellinum against some bacterial strains and C. albicans were evaluated by disk diffusion and microdilution methods.
Methods: Aerial parts of the plant were collected in April-May 2012 from Khansar and Golpayegan heights, Isfahan province, Iran and dried by natural drying method. Extraction of aerial parts was done by perculator apparatus and using methanol 80%.Different alkaloids solvents such as petroleum and chloroform were added based on standard methods.The antimicrobial effect of methanolic extract and alkaloid extracts were evaluated against *Staphylococcus aureus* (PTCC 1431), *Escherichia coli* (PTCC 1399), *Pseudomonas aeruginosa* (PTCC 1430), *Klebsiella pneumonia* (PTCC 1053), *Salmonella* typhimurium (PTCC 1639), *Candida albicans* (PTCC 5027) based on CLSI 2012 protocol by disk diffusion method.Further microdilution was done to determine Minimal inhibitory concentration(MIC).
Results: based on the results, only by microdilution method not disk diffusion, alkaloid extracts in 1000 mg/ml concentration inhibit the growth of *E. coli*, *K. pneumonia* and *P. aeruginosa* in comparison to control antibiotics with 0.0481±0.0183 mg/ml (p=0.0001). Also, alkaloid extract inhibit the growth of *S.aureus* in comparison to control as0.03052±0.09155mg/ml.
Conclusion: No detection of antimicrobial effect in disk diffusion method and detection the antimicrobial effect in microdilution test in some cases like this study, is related to no penetration and distribution of alkaloid extracts in agar. So, using all methods are needed in negative results before any report.

Keywords: Glaucium Vitellinum, Bacterial Strains, *Candida albicans*, Antibacterial Effect

IN-VITRO ANTIFUNGAL ACTIVITY OF ECHINOPS CEPHALOTES ON CANDIDA TROPICALIS CBS 94 AND USING GC-MS METHOD

Maryam Hadian [1], Mahboobeh Madani [2]

1.MS Student, Department of Microbiology, science and research Branch, Islamic Azad University, Arak, Iran
2. Assistant Professor of Medical Mycology, Department Of Microbiology, Falavarjan branch, Islamic Azad University, Isfahan, Iran.

Background: *Candida tropicalis* is a diploid ascomycetes yeast commonly found on the skin and in digestive tracts healthy human hosts worldwide in recent years
Methods: The objective of the current study was to evaluate the inhibitory effect of Echinops cephalotes against C.tropicalis CBS 94 in laboratory conditions. To this end, in April 2013, Echinops cephalotes were collected from Komshecheh, Ardestan. The leaves and stems of these plant were dried in shadow and the acetonic extract were prepared by the Soxhlet apparatus. The antifungal activity were investigated in concentrations of 7.8, 15.8, 31.2, 62.5, 125, and 250 mg/ml through well diffusion method.Minimum Inhibitory Concentration (MIC) and Minimum fingicidal Concentration(MFC) were determined.To identify the components of the plant, the GC-MS technique was used. The results were evaluated by Kruskal Wallis and non-parametric Mann-Whitney statistical tests
Results: The acetonic extract in different concentrations had a remarkable effect on C.tropicalis CBS 94.However, the difference was not significant in the concentrations of 62.5, 125, and 250. Six investigated concentrations did not show any significant difference in the diameter of non-growth halo. The results of the MIC and MFC were reported to be 7.8, 7.8 and 15.6, 15.6 respectively. Based on the results, the extract had fungicidal properties on C.tropicalis CBS 94. The available components in Echinops cephalotes were not identified via the GC-MS technique
Conclusion: The results concluded that the acetonic extract of Echinops cephalotes is a potential natural antifungal agent, however its effect is dependent on the source and extraction method

Keywords: Echinops Cephalotes, Antifungal Activity, *Candida tropicalis*, GC-MS

Iranian J Publ Health, Vol. 43, Supplementary Issue, No. 2, 2014
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IN-VITRO ANTIFUNGAL ACTIVITY OF ECHINOPS CEPHALOTES ON CANDIDA TROPICALIS CBS 94 AND USING GC-MS METHOD

Maryam Hadian [1], Maryam Hadian [1]
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Conclusion: The results concluded that the acetonic extract of Echinops cephalotes is a potential natural antifungal agent, however its effect is dependent on the source and extraction method

Keywords: Echinops Cephalotes, The Acetonic Extract, Antifungal Activity, Candida Tropicalis, GC-MS

INVESTIGATION OF ANTIMICROBIAL ACTIVITY OF TOTAL EXTRACTION AND FRACTIONS OF TWIGS OF GAILONIA AUCHERI AGAINST DIFFERENT BACTERIAL STRAINS AND CANDIDA SPP.

Mojdeh Hakemi-Vala[1], SAEED KHOSHNOOD[1], Mojgan por ramezan[2], mohammad hossein salehie[2], Mohsen heidary[3]
1.Department of microbiology, Medical school, Shahid Beheshti University of medical sciences, (SBMU), Tehran-Iran
2.Pharmaceutical sciences branch, Islamic Azad University of medical sciences, Tehran-Iran
3.Medical school, Shahid Beheshti University of medical sciences, (SBMU), Tehran-Iran

Background: The aim of this research was to study of the antimicrobial activity of total extract and petroleum ether, chloroform, ethyl acetate, methanol and aqueous fractions of twigs of Gailonia aucheri.
Methods: Aerial top branches of plant G. aucheri were gathered from Bandarabas in June 2012. The preliminary phytochemical screening was carried out with different standard tests. Primary extracting was performed in percolation by methanol. Further aquatic and organic extracts were yielded after a week. The (MIC) of resulted extracts were evaluated against *S. aureus* ATCC: 9026, *E. coli* ATCC: 8739, *P. aeruginosa* ATCC: 9027, S. typhi ATCC: 1622, and C.albicans ATCC: 10231 by micro dilution method.
Results: Alkaloids, tannins, anthraquinone, flavonoids were absent in G. aucheri. MIC of metanolic extract against C.albicans and *S. aureus* were 0.7324±0.3453 mg/ml and 23.33±11.05 mg/ml, MIC of petroleum ether fraction against *S. aureus* was 125±0.0 mg/ml, MIC of ethyl acetate fraction against *S. aureus* and C. albicanse were 0.092±0.031 mg/ml and 0.0481±0.0183 mg/ml, MIC of methanolic fraction against *S. aureus* and C. albicans were 0.092±0.031 mg/ml and 2.9297±1.38 mg/ml and the MIC of aquatic fraction against *S. aureus* and C. albicans were 23.33±11.05 mg/ml and 0.03662±0.17 mg/ml, respectively. Total extract and other fractions of this plant against other tested bacterial strains had not significant effect.
Conclusion: With respect to significant effectiveness of total methanolic extract and other fractions of G. aucheri against *S. aureus* and C. albicans, it is suggested to perform the other complementary tests in vivo and in vitro in future studies.

Keywords: Gailonia Aucheri, Antimicrobial Activity, Candida,Bacterial Strains

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STUDY ROLE OF TEMPERATURE FACTOR FOR DETERMINE THE SENSITIVITY OF COMMON YEAST IN THE MOUTH TO MOUTHWASHES

Somayeh Talebi[1], Azar Sabokbar[1], Majid Riazipour[2], Mohsen Saffari[3]
1.Department of Microbiology, Karaj Branch, Islamic Azad University, Karaj, Iran
2.Department of Parasitology and Mycology, School of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran
3.Health Education Department, School of Health, Baqiyatallah University of Medical Sciences, Tehran, Iran

Background: The aim of the present study, investigation role of temperature factor for determine the sensitivity of common yeast in the mouth to mouthwashes
Methods: In the present study, a standard strain of *Candida albicans* PTCC 5027 was used as a common fungal yeast in the mouth. The effect of mouthwashes against the common yeast of mouth, C.albicans Evaluated by agar diffusion methods. At first the suspension was provided by physiological saline and fresh culture of C.albicans (24 hours) and the OD was read at 530 nm wavelength by spectrophotometer. 10 µl of providing suspension was transferred to SDA medium. In the next step, two wells were made with suitable distance in culture medium, filling with. For decreasing the error, the test was repeated 3 times. Plates were placed in the incubator at 25 ºC,30 ºC, 37 ºC for 24 hours.Inhibition zone was measured and recorded by using a Collis. The data were analyzed by SPSS software (18th edition), using one sided variance analysis (ANOVA-one way) with P ‹0.05.
Results: Mean of inhibition zone diameter due to effect of the mouthwashes on C.albicans in 37 ºC greater than 30 ºC and 25 ºC. In 30 ºCApproximately equal to 25 ºC.
Conclusion: The temperature was effective for the effect of mouthwashes on C. albicans. Mouthwashes were used in this study, showed the best effect on candidate was incubated at 37 compared to 30 ºC and 25 ºC, but no significant differences observed between them (p›0.05).

Keywords: Temperature Factor, Sensitivity,Common Yeast, Mouthwash

ANALYSIS OF A HEAVY METAL EXTRACTION BY NATIVE BACTERIA

Abbas Rashidi[1], Faezeh Fatemi[2], Samaneh Jahani[2]
1.Department of Chemical Engineering, Faculty of Engineering, University of Mazandaran, Babolsar, Iran
2.Nuclear Fuel Cycle Research School, Nuclear Science and Technology Research Institute, Tehran, I.R. Iran

Background: Acidithio*Bacillus* thiooxidans is a sulfur-oxidizing bacterium that used in industrial process of bioleaching. Bioleaching is the extraction of metals from their ores through the use of living organisms. This processes are commonly more eco-friendly than physico-chemical processes. So, in the present study, native Acidithio*Bacillus* thiooxidans used for uranium extraction.
Methods: Bioleaching experiment carried out in 250 ml flask containing 90 ml APH medium, 5% uranium ore powder and 10% native Acidithio*Bacillus* thiooxidans. APH medium containing 2 g (NH4)2SO4, 0.5 g K2HPO4, 0.5 g MgSO4.7H2O, 0.1 g KCl, 0.01 g Ca(NO3)2.4H2O, 10 g S and 1000 ml of distilled water. pH was adjusted to 4 with H2SO4 (10N). Then, the flask was maintained in a shaker incubator at 150rpm and 35◦C. In following, the soluble extracted uranium, variation of pH and Eh measured daily.
Results: The result of bioleaching experiment showed that the bacterium was proficient in total uranium extraction (100%) during 5 days at 5% pulp density of the ore. The pH and Eh curve variations showed that the pH of the medium decreased in 4 to 1.46 and Eh increased from 142 mV to 425 mV.
Conclusion: In the present study, we found that the native Acidithio*Bacillus* thiooxidans is capable of uranium extraction from low grade uranium mine.

Keywords: Bioleaching, Uranium, Acidithio*Bacillus* Thiooxidans

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CHARACTERIZATION OF LEISHMANIA PARASITES IN SUSPECTED PATIENTS OF CUTANEOUS LEISHMANIASIS BY TARGETING ITS-RDNA, ITS-MICROSATELLITE IN SHUSH CITY, NORTHERN KHUZESTAN (2012-2013S).

Parnazsadat Ghaemmaghami[1], Parviz Parvizi[1], Adel Spotin[2]

1.Molecular Systematics Laboratory, Parasitology Department, Pasteur Institute of Iran
2.Department of Medical Parasitology & Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: This study was done to identify the *Leishmania* parasites using microscopic and molecular methods in suspected patients of cutaneous leishmaniasis by targeting ITS-rDNA and ITS-microsatellite genes in Shush city, Northern Khuzestan.
Methods: 65 smears from confirmed patients' lesions of ZCL, in Shush city during 2012-13s, were taken, stained and examined under a light microscope. Whole DNA of parasites was extracted directly from prepared slides based on Phenol-Chloroform method and examined by polymerase chain reaction (PCR) of ribosomal DNA (rDNA) internal transcribed spacer (ITS) and ITS-microsatellite markers. Some positive amplicons were digested with BsuRI restriction enzyme according to RFLP method. In order to re-confirm, suspected PCR products were sequenced, edited and aligned by Sequencher TM v. 4.1.4 software and exported to MEGA5.05 for phylogenetic analysis.
Results: 50 out of 65 and 57 out of 65 samples were detected as *Leishmania* positive using microscopic and molecular methods respectively. All 57 positive samples digested with BsuRI, were identified as *Leishmania* major. Seven positive slides, which were negative by targeting ITS's-rDNA gene, were then identified positive by ITS-microsatellite.
Conclusion: Findings of this investigation proved that applying molecular method with appropriate markers had more sensitivity than conventional methods in identifying *Leishmania* parasites. Also, it seems that ITS- microsatellite gene because of having a short tandem repeat, to be good marker in detection and evolutionary studies of *Leishmania* parasites.

Keywords: *Leishmania major*, Microscopic And Molecular Methods, ITS-Rdna

IDENTIFYING OF CAUSATIVE AGENTS OF CUTANEOUS LEISHMANIASES BY AMPLIFYING CYT B GENE IN INDIGENOUS FOCI OF Iran

Neda Samei[1], Parviz Parvizi [2], Adel Spotin[3], Mohammad Reza Khatami Nezhad[4], Narmin Najafzadeh [3], Amir Yaghoubinezhad[5]

1.Department of Cellular and Molecular Biology, Islamic Azad University, Tonekabon Branch, Tonekabon, Iran
2.Molecular Systematics Laboratory, Parasitology Department, Pasteur Institute of Iran, Tehran, Iran
3.Parasitology Department, Medical Faculty, Shahid Beheshti University of Medical Science, Tehran, Iran
4.Department of Cellular and Molecular Biology, Islamic Azad University, Tonekabon Branch, Tonekabon, Iran
5.Department of Cellular and Molecular Biology, Rab-Rashid Higher Education Institute, Tabriz, Iran

Background: In this study, simultaneously identifying of three causative agents species of CL is followed by targeting Cyt b gene in three regions of Turkmen Sahara, Ahvaz and Abarkouh.
Methods: Smears of suspected patients infected with zoonotic cutaneous leishmaniasis (ZCL) were stained and examined under a light microscopic observation. DNA of parasites within human ulcers was extracted directly from their smears. PCR standard was used to amplify the Cyt b gene of *Leishmania* parasites in human from Turkmen Sahara, Ahvaz and Abarkouh. Based on RFLP method by digesting Ssp1 restriction enzyme and more precisely sequencing of Cyt b was shown to be species-specific.
Results: In this investigation 290 of 310 confirmed patients were infected with L. major by having high density and low genetic diversity. Unlikely, 18 out of 310 cases were determined to be L. tropica with low density and high diversity. Besides, for the first time L. turanica was isolated from two patients in Turkmen Sahara with low density rate and low molecular variation.
Conclusion: Results showed that at least three species of genus *Leishmania* are the causative agents of CL that are unequivocally circulating in endemic foci of Iran. Molecular analysis demonstrated that Cyt b is an appropriate evolutionary marker for identifying *Leishmania* spp., to survey variation range and phylogenetic annotations.

Keywords: Key Words: *Leishmania turanica*, Cyt B, Molecular Variation, ZCL.

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USE OF LYOPHILIZED RABBIT SERUM AS NUTRITIONAL REPLACEMENT FOR THE FOETAL CALF SERUM IN CULTIVATION OF LEISHMANIA MAJOR AND LEISHMANIA INFANTUM PROMASTIGOTES

Vahid Nasiri[1], Abdolhossein Dalimi asl[2], Gholamreza karimi[1], Habibollah Paykari[1], Fatemeh Ghaffarifar[3]
1.Parasitology Department, Razi Vaccine and Serum Research Institute, Karaj, Alborz, Iran.
2.Department of Parasitology, Faculty of Medicine, Tarbiat Modares University, Tehran, Iran
3.Department of Parasitology, Faculty of Medicine, Tarbiat Modares University, Tehran, Iran

Background: Genus *Leishmania* is a protozoan parasite that causes different severe diseases. Fetal Calf Serum (FCS) is the major part of the *Leishmania* culture media and the most expensive ingredient in these media. In the present work, the nutritional efficacy of the lyophilized Rabbit serum was evaluated in *Leishmania* culture media.
Methods: The efficacy of lyophilized Rabbit serum was evaluated by assassing the parasite growth and replication pattern after adding 5%-10% lyophilized Rabbit serum to the RPMI-1640 medium. The growth ability and behavior of promastigotes of Leishmanian parasites assessed at in vitro condition.
Results: According to our finding, 5% lyophilized Rabbit serum can be used for cultivation of *Leishmania major* and can support the growth of them.
Conclusion: The ability of the parasites to survive and proliferating in the presence of lyophilized Rabbit serum indicating that this serum is a good nutritional source. This study open a new way to make low- cost replacements for FCS that could be used in cultivation of Leishmanian parasites.

Keywords: *Leishmania major*, Rabbit Serum, Fetal Calf Serum, Promastigote

COMPARISON OF ANTIMICROBIAL ACTIVITY OF FOUR HERBAL EXTRACT AGAINST SOME STRAINS OF BACTERIA

Ebrahim Hossein Abadi[1], Nahid Tavakoli[2]
1.Faculty of veterinary Medicine, University of Zabol, Iran
2.faculty of pharmacy,Tehtan university of medical siences,Iran

Background: The aim of the present study was to compare Antimicrobial activities of the crude ethanolic extracts of four plants against *E.coli* and S.aureus.
Methods: Fresh leaves bark and root of four different plants (Acacia nilotica, Terminalia arjuna, Cynamomum zeylanicum, Eucalyptus globulus) were collected and washed thoroughly 2-3 times with running water and once with sterile distilled water, leaf, bark and root material was then air-dried on sterile blotter under shade. Maceration method was used for extraction and methanol is used as solvent. After filtration and evaporation of ethanol, the extracts were oven dried. For experiments, each extract was redissolved in ethanol to the desired concentration.
Results: All the plant extracts showed antimicrobial activity against microorganisms tested. Extracts of A. nilotica, C. zeylanicum showed the most potent activity against microorganisms studied. Eucalyptus globulus and *Terminalia arjuna* showed antimicrobial effects only on S.aureus.
Conclusion: The ethanolic extracts of A. nilotica, C. zeylanicum could be a possible source to obtain new and effective herbal medicines to treat infections caused by microorganisms from community as well as hospital settings.

Keywords: Antimicrobial,Ethanolic Extract,Herbal Medicine

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EVALUATION OF THE EFFECT OF COPPER OXIDE AND ALUMINUM OXIDE NANOPARTICLES ON THE BRUCELLA MELITENSIS 16 M IN VITRO

Ghasem Rahimi[1], Masoud Negahdari[1], Marzieh Ajdary[2], Ali Roostaei[3]
1.Young Researchers and Elite Club, Marvdasht Branch, Islamic Azad University, Marvdasht, Iran
2.Yang researchers club, Khorasgan branch, Islamic azad university, Isfahan, Iran
3.Department of Microbiology, Science and Research Branch, Islamic Azad University, Fars, Iran

Background: Recently inorganic nanomaterials characterized with high level thermal stability and new physical and chemical properties were considered for antimicrobial therapy. In the present study, the antibacterial activity of copper oxide and aluminum oxide nanoparticles was evaluated against *Brucella melitensis* 16M.
Methods: For the first stage, physical and chemical properties of nanoparticles used in this study were checked and confirmed by means of double-beam ultraviolet-visible spectrometer, X-Ray diffractometer underCuKα beam emission, and transmission electron microscope. Subsequently the minimum inhibitory concentrations value (MICs) of nanoparticles was calculated using broth microdilution test. Time kill study was also performed to show the effect of antibacterial to inhibit the growth of *Brucella* by time. Eventually NCCLS disc diffusion method was carried out to investigate the effect of nanoparticles tested against *Brucella melitensis* 16 M.
Results: Our results showed that nanoparticles tested could be able to inhibit the growth of sessile *Brucella* cell. Indeed MICs was ranged from 0.24 μgml-1 and 0.42 μgml-1 for copper oxide and aluminum oxide nanoparticles, respectively. Disc diffusion method also showed the significant reduction of growth Brusella after treatment by nanoparticles tested at level ($p < 0.05$). Furthermore, it is also demonstrated that the copper oxide nanoparticles showed more antibacterial activity than aluminum oxide against *Brucella melitensis* 16 M.
Conclusion: These favorable results need to be supported by animal modeling.

Keywords: Antibacterial Activity, *Brucella melitensis* 16 M, Copper Oxide Nanoparticle, Aluminum Oxide Nanoparticle

ANTIMICROBIAL PROPERTIES OF SODIUM CASEINATE FILM CONTAINING POMEGRANATE PEEL EXTRACT ON THE GROUND MEET

Arezoo Karamimoghadam[1], Zahra Emam-Djomeh[2]
1.Department of food science,college of Agriculture,Tehran science and Research branch,Islamic Azad university,Tehran,Iran
2.Faculty of Agricultural Engineering and Technology, University of Tehran, Iran

Background: Microbial growth is considered as the main cause of food spoilage that can be controlled by the use of films containing antimicrobial compounds. In recent years numerous studies have been conducted to evaluate, the impact of the antimicrobial activity of various extracts and spices in edible films. Phenolic compounds of pomegranate peel have been demonstrated to have high antimicrobial activities.
Methods: Therefore in the present study, casein-based edible films containing 1, 1.5 or 2 times of minimum inhibitory concentration (MIC) of pomegranate peel extract were prepared and their antimicrobial impacts against two Gram-positive and Gram-negative bacterial strains were investigated using diffusion disk method. Antimicrobial impact of edible films was also studied on ground meat.
Results: According to the results, pomegranate peel extract-free films had no antimicrobial activity. However, the antimicrobial activity of films was enhanced by increase for extract. Films containing peel extract were more effective on *S. aureus* (24.90 mm average halo diameter at 2× MIC) rather than *E. coli* (19.2 mm average halo diameter at 2× MIC). It was also observed that antimicrobial activity of films was mostly depended on the types of microorganisms presented in meat.
Conclusion: Although they were not a suitable replacement for common films such as cellophane, they could substantially extend the shelf life of ground meat antimicrobial effectiveness.

Keywords: Antimicrobial Packaging, Sodium Caseinate Film, Pomegranate Peel Extract, Ground Meat

DESIGN AND CLONING OF AN ANTISENSE AGAINST BACTERIORHODOPSIN-LINKED PROTEIN CODING GENE OF HALOBACTERIUM SALINARUM R1.

Afrouz Ali- yari[1], Ali Asghar Deldar[1], Neda Mokari Bonabi[1], Ehsan Vafadar Nejad[2], Ayman Bazzaz[1]
1.Department of Genetic Engineering, Biosciences and Biotechnology Institute, Malek Ashtar University of Technology, Tehran, Iran
2.Department of microbiology, College of Science, University of Tehran, Tehran, Iran

Background: Our goal in this study is designing antisense against *blp*. To identify function of genes there are multiple ways. One of these approaches is designing of antisense that prevent function of desired gene.
Methods: At the first stage we designed a primer for a 180 bp area that contains ribosome binding site and initial part of gene. Then restriction sites of *BamH1* and *Xba1* inserted at the 5' of them with 5 random nucleotides at the end of each primers. The primer was analyzed be oligoanalyzer software. We amplify 180 bp fragment by PCR and cloned it in pBluscript SKII cloning vector. Then 180 bp subcloned into shuttle vector pVDSH1, that is a vector contains promoter and terminator sequences and our 180 bp fragment between these essential elements.
Results: The results of digestion and sequencing showed that this 180 bp fragment was inserted into vector in inverse direction and placed downstream of an inducible promoter and with manipulating of KCL concentration, we can control the rate of final target protein production and consequently evaluate the rate of silencing of the gene.
Conclusion: Transforming of this vector into *Hallobacterium sallinarom* and induction of antisense production through KCL removal may results in evaluating of rate of silencing of this gene and consequently we could analyze effect of silencing of this gene in production of bacteriorhodopsin.

Keywords: *Halobacterium salinarum* R1-Antisense-Blp

SURVEY THE QUALITATIVE AND QUANTITATIVE GROWTH OF SCIENTIFIC PRODUCTION OF IranIAN RESEARCHERS IN THE FIELD OF MICROBIOLOGY BY ISI CITATION DATABASES

Aboozar Ramezani[1], Iraj Nikokar[2], Mohammad Esmaeilpour Bandboni [3], Behrad Rohi[4]
1.Knowledge & Information Science (Medical), Guilan University of Medical Sciences, Rasht, Iran
2.Laboratory of Microbiology and Immunology of Infectious Diseases, Paramedicine Faculty, Guilan University of Medical Sciences, Rasht, Iran
3.Assistant Professor, PhD in Nursing, Guilan University of Medical Sciences, Rasht, Iran.
4.Guilan University of Medical Sciences, Rasht, Iran.

Background: Today, researchers have published papers in academic life, the total number of citations to articles, the average number of citations per paper and journal of productive that articles have been published. Among the criteria considered in the evaluation of scientific output of researchers and research centers worldwide. In this research, the scientific productivity of Iranian researchers in the field of microbiology has been studied in 2014.
Methods: This study is a survey of scientometrics. Using quantitative and qualitative indicators and citation database of scientific papers indexed in Web of Science. All scientific documents indexed using the formula (SU=MICROBIOLOGY AND CU=Iran) were collected and analyzed.
Results: Iranian researchers have 4954 papers in the field of microbiology in scientific journals indexed in the ISI. These articles have already received 21,666 citations. However H-index was calculated as 47 for scientific field microbiology Iran. Iranian scientists in their research by 1268 scientists worked in other countries.
Conclusion: Published articles in the field of microbiology in Iran interdisciplinary research most interaction and communication areas of biotechnology and microbiology as well as biochemistry and molecular biology are applied. According to the results of the fastest growing research field of microbiology Iran is about twelve years. Indexing of scientific productivity shows the quality of the articles published in this area is at an acceptable level.

Keywords: Science Production; Microbiology; Iran; Scientometrics; H-Index

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OPTIMIZATION OF BACILLUS CEREUS DETECTION BASED ON HEMOLYSIN BL AND NON HEMOLYTIC ENTEROTOXIN C GENES BY MULTIPLEX PCR

Kasra Sadeghi[1], Sina Mirza Ahmadi[1]
1.Department of Microbiology, Faculty of Basic and Medical Sciences, Zanjan Branch, Islamic Azad University, Zanjan, Iran.

Background: *Bacillus cereus* is a Gram-positive, facultative anaerobic, spore forming bacterium, which are widely spread in nature. *B.cereus* as a food-poisoning organism can cause two types of illness, the diarrhoeal form and the emetic form. *B. cereus* produces several toxins, including emetic toxin and at least four other enterotoxins: hemolysin BL or Hbl, nonhemolytic enterotoxin or Nhe and two single proteins, cytotoxin K or CytK and enterotoxin FM or EntFM. This study investigates the molecular detection of *Bacillus cereus* by Multiplex PCR and using *hblA* and *nheC* genes.
Methods: Isolateswere grown overnight at 37 °C on BHI broth, and purification of DNA was performed. The PCR reactions were performed for genes *hblA* and *nheC*, the PCR of those genes were optimized.
Results: Molecular detection of *B. cereus* was performed by using specific primers. Multiplex PCR were done for both genes and we were able to see the bands.
Conclusion: Multiplex PCR method has high accuracy and is significant in identifying the pathogen. The results of this study suggest that by using this method we can simultaneously, in less time and with much higher precision than the traditional method and other methods, attempt to review and identify pathogens in food.

Keywords: *Bacillus cereus*; Enterotoxin Genes; Foodborne Disease; Multiplex PCR

VANA AND VANB POSITIVE VANCOMYCIN-RESISTANT STAPHYLOCOCCUS AUREUS AMONG CLINICAL ISOLATES FROM CATS AND DOGS

Ali Ghorbani Ranjbary[1], Nazanin Ghorbani Ranjbary[1], Zahra Ghorbani Ranjbary[1], Ali Bahmani[1]
1. Young Researchers and Elite Club, Kazerun Branch, Islamic Azad University, Kazerun, Iran.

Background: *Staphylococcus aureus* is one of the most common nosocomial and community-acquired infections worldwide. Vancomycin is still preferred for treatment of methicillin-resistant *S. aureus* (MRSA) infections. The purpose of this study was to determine the prevalence of vancomycin-resistant *Staphylococcus aureus* isolated from among clinical Isolates from Cats and Dogs.
Methods: From March to December 2012, 100 *S. aureus* isolates (mainly from wound and Skin) were collected from two veterinary clinics in southern Iran. After identification of *Staphylococcus aureus* by biochemical, microbiological and molecular methods, antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion test for 13 different antibiotics. Vancomycin-resistant *Staphylococcus aureus* isolates were determined by vancomycin agar screening test and PCR for vancomycin resistant genes (i.e. *vanA* and *vanB*).
Results: The lowest and highest resistance was seen for quinupristin-dalfopristin (n = 1) and ampicillin (n = 95), respectively. Vancomycin agar screening test showed that 48 isolates can grow on these media. Further study by PCR also was detected vanA and/or vanB genes in all of these strains. Also, 15 isolates showed either vanA or vanB but were susceptible according to vancomycin agar screening test. Totally, vanA and vanB resistant genes were detected in 43% and 51% of clinical isolates, respectively.
Conclusion: The results showed that the frequency of vancomycin resistance genes (vanA, vanB) is very high in *Staphylococcus aureus* strains isolated from two veterinary clinics in southern Iran.

Keywords: *Staphylococcus aureus*, Zoonosis, Vancomycin Resistance, Vana And Vanb

COMPARING CULTURE AND PCR FOR DETECTION OF ORNITHOBACTERIUM RHINOTRACHEALE INFECTION

Aidin Azizpour[1], Yashar Azizpour[2]
1.Meshginshahr Faculty of Agriculture, University of mohaghegh Ardabili, Ardabil, Iran
2.Tehran University of Medical Sciences,Tehran, Iran

Background: Ornithobacteriosis is caused by Ornithobacterium rhinotracheale (ORT), a pathogenic bacterial agent in fowl and turkey flocks that can cause economic losses to the poultry industry annually. No doubt the definitive diagnosis of this emerging respiratory disease is important. The purpose of this study was to diagnose the ORT using polymerase chain reaction (PCR) in comparison with culture.
Methods: The PCR was carried out using the primer combination OR16S-F1 and OR16S-R1. All samples were prepared for DNA extraction using phenol-chloroform method. In this study, 22 lung and tracheal swabs from 11 broiler flocks with respiratory disease were examined for the presence of ORT using culture and PCR assay.
Results: A fragment of 784-bp was observed in 15 out of 22 samples tested. Eight out of 11 flocks studied were positive in PCR assay and only 1 flock was negative in culture. 7 infected flocks were positive in both culture and PCR.
Conclusion: The results of this study showed that polymerase chain reaction is a rapid and reliable method for diagnosis of *Ornithobacterium rhinotracheale.*

Keywords: *Ornithobacterium rhinotracheale*, PCR, Broiler Flocks.

GENOMIC DETECTION OF COXIELLA BURNETII (Q FEVER AGENT) IN CATTLES MILK SAMPLES IN ANIMAL FARMS BONAB TOWNSHIP. Iran

Peyman Khademi[1], Mohammadreza Mahzounieh[2], Mahmoud Esmaeili Koutamehr[3]
1.Master of Science Bacteriology, Pathobiology Department, Faculty of Veterinary Medicine, Shahrekord University, Iran
2.Associate Professor, Pathobiology Department, Faculty of Veterinary Medicine and Research Institute for Zoonotic Diseases, ShahreKord University, Iran
3.Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran

Background: This study was conducted to determine the seasonal prevalence rate of *Coxiella burentii* in raw samples obtained from different cattles in Bonab.
Methods: In this cross-sectional study (at Spring 2014), 100 cattles milk samples were collected randomly. These samples were tested for the presence of *Coxiella burnetii* by the Nested PCR method.
Results: In this study, 100 samples (26%) were found to be positive for the presence of *Coxiella burnetii.*
Conclusion: The analysis of the collected data in different seasons and areas revealed that, more than 74 percent of the samples were negative and about 26 percent were positive in terms of *Coxiella burnetii* presence. It can be concluded from this study the season and the region of sample collecting affects the amount of bacteria excreted, and that cattle milk can be one of the potential sources of Coxiella burnetii in Iran.

Keywords: Q Fever, *Coxiella burnetii*, Cattle Milk, Bonab, Nested PCR

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ANTIBIOTIC SUSCEPTIBILITY PATTERN OF PSEUDOMONAS AERUGINOSA ISOLATED FROM PATIENTS WITH URINARY TRACT INFECTION ADMITTED TO DIFFERENT HOSPITALS OF TEHRAN,Iran

Mohsen Tabasi[1], Mohammad Reza Asadi Karam[2]
1.MSc student of Medical Microbiology, Molecular Biology Department, Pasteur Institute of Iran, Iran
2.Molecular Biology Department, Pasteur Institute of Iran-Tehran, Iran

Background: Antibiotic resistance pattern of *P. aeruginosa* isolates varied with geographical location and hospitals environments.The present study was conducted to determine the antibiotic susceptibility pattern of *Pseudomonas aeruginosa* from urine samples of urinary tract infection patients admitted to different hospitals of Tehran.
Methods: The urinary samples from 100 patients with urinary tract infection were collected consecutively between January 2013 to January 2014 and were cultured and identified. According to CLSI (Clinical Laboratory Standards Institute) guidelines antimicrobial susceptibility testing was performed by disc diffusion method.
Results: A higher resistance to *Pseudomonas aeruginosa* isolates was observed with piperacillin/tazobactam and cefipime i.e. 42% and 40% respectively. Imipenem was found to be most effective antibiotic against *Pseudomonas aeruginosa* (76% sensitivity) but amikacin resistance was continuously increasing. In conclusion the frequency of *Pseudomonas aeruginosa* was also higher among urinary tract infection patients with alarmingly high rate of resistance among widely used antibiotics.
Conclusion: These findings focused on careful consideration for monitoring and optimization of antimicrobial use in order to reduce occurrence and spread of antimicrobial resistant pathogen.

Keywords: Antimicrobial Susceptibility, *Pseudomonas aeruginosa*

NESTED-PCR AS GENE-MAPPING TOOL TO RECOGNIZE LENGTH OF INTEGRON CLASS I AMONG P.AERUGINOSA

Reza Azizian[1], Azar Hasanvand[2], Saeed Hemati[2], Mostafa Akbari Qomi[3], Mohammad Masbi[3], Lida Bimannad[2], Nourkhoda Sadeghifard[3], Mahnaz Shafiee[3]
1.Research and Developing Center, Mahan Gene Poyesh, Tehran, Iran
2.Dep. of Medical Microbiology, Ilam University of Medical Sciences, Ilam, Iran
3.Clinical Microbiology Research Center, Ilam University of Medical Sciences, Ilam, Iran

Background: Integron as mobile elements could be help to induce resistance among species of bacteria. Three class of Integron is known and among them Integron class I by high frequency among *P.aeruginosa* play mainly role to resistant to antibiotics. Molecular detection of the resistance genes carried by *Pseudomonas aeruginosa* is important in assessing spread and colonization and in treatment of infections. This study designed to detect whole consequence of Integron class I among isolated P.aerugionsa from urine samples.
Methods: *P.aeruginosa* strains isolated form Urine samples and screened for Integron class I presence by PCR for intl1 gene. Length of integron class I were recognized by Nested-PCR for intl1-f (CAGTGGACATAAGCCTGT), qacEΔ1-(TGAGCCCCATACCTACAAAGC) and sul1-R (GTTTCCGAGAAGGTGATTGCG). First PCR done by intl1 F and sul 1 R and its product used for next PCR (intl1 F and qacEΔ1 R).
Results: Among 61 isolated P.aeruginosa, 46 detected as Integron class I positive and 6 isolates were blaVIM positive. All of Integron class I positive strains had 3500 bp band in first PCR that indicated all of them ended to sul 1 gene. And also, all of isolates had 2500 bp band in second PCR that shown qacE?1 located on Integron class I before sul 1 gene and sul 1 gene has near to 1000 bp length.
Conclusion: Regards to these results, Integron class I with various length ended to sul gene as a conservative consequence and in majority of them, qacEΔ1 gene located before sul 1gene. This study is in progress to determine whole genes on Integron class I in P.aeruginosa.

Keywords: *P.aeruginosa*, Integron Class I, Nested-PCR, Gene-Mapping.

RECOMBINANT PCRV INDUCED POLY-ISOTYPIC HUMORAL IMMUNE RESPONSES AGAINST PSEUDOMONAS AERUGINOSA IN BALB/C MICE

Isar Dejban Golpasha[1], Parviz Owlia[2], Seyed. Fazlollah Mousavi[3], Seyed Davar Siadat[3], Shiva Irani[1]
1.Department of Biology, Faculty of Basic Sciences, Science and Research branch, Islamic Azad University, Tehran, Iran
2. Department. of Microbiology, Faculty of Medicine, Shahed University, Tehran, Iran
3.Department of Microbiology, Pasteur Institute of Iran, Tehran, Iran

Background: The purpose of this study was to evaluate the feasibility of using PcrV as a candidate vaccine against *Pseudomonas aeruginosa* and also to examine whether PcrV would promote the immune responses and induce poly-isotypic humoral immune response in burned mouse models.
Methods: Following the expression and purification of recombinant PcrV (r-PcrV), immunization process was done, it must be reminded that, after each immunization course specific total antibodies responses were measured to declare the efficacy of immunization. At the end different isotype-specific antibodies (IgG1, IgG2a, IgG2b and IgM) against r-PcrV measured with ELISA method. Four week after last immunization, Control (PBS injected group) and immunized (r-PcrV injected group)group of mice were burned and challenged (subeschar) with approximately 5-3×102CFU (3 times LD50) of *Pseudomonas aeruginosa* PAO1.Survival rate and bacterial quantity in skin and internal organs (Liver and Spleen) were evaluated 25 hour post infection to study systemic infection and reveal the pattern of immune responses.
Results: All immunized animals (rPcrV injected group) were able to produce specific antibodies against PcrV and highest antibody titer was detected after third immunization (second booster), Immunization with candidate vaccine (r-PcrV) significantly increased specific IgG1, IgG2a, IgG2b and IgM isotype antibodies compared with control group, ($P<0.001$). Also immunization with r-PcrV protein resulted in a significant improvement in survival rate of infected mouse against *Pseudomonas aeruginosa* PAO1 infections in immune group (r-PcrV injected group) in contrast to non-immune group (PBS injected group) (65%).
Conclusion: Our result declares that r-PcrV can promote the humoral and cellular immune responses. Moreover r-PcrV induced poly isotypic humoral responses.

Keywords: Recombinant-Pcrv, Poly Isotypic Antibodies, Immunization, *Pseudomonas aeruginosa*

EFFECT OF CULTURE SUPERNATANTS FROM PSEUDOMONAS AERUGINOSA ON GROWH AND BIOFILM FORMATION OF STAPHYLOCOCCUS EPIDERMIDIS

Maryam Naserinejad[1], Fereshteh Eftekhar[1]
1.Department of Microbiology, Faculty of Biological Sciences, Shahid Beheshti University, Tehran, Iran

Background: The goal of this study was to investigate the in vitro effect of *P. aeruginosa* culture supernatant on S. epidermidis growth and biofilm formation.
Methods: A *P. aeruginosa* CF isolate (PAO1) was grown in trypticase soy broth at 37 °C for 24 h and the culture supernatant was centrifuged at 7000×g for 10 minutes and filter sterilized using a 0.22 µ Millipore filter. The effect of *P. aeruginosa* supernatant was determined on growth and biofilm formation of 17 biofilm producing isolates of S. epidermidis. For biofilm experiments, overnight cultures of S. epidermidis in trypticase soy broth (TSB) were diluted (1: 200) in TSB and 200 µl of each culture with or without 40 µl of *P. aeruginosa* culture supernatant was added to 4 wells of 96 well flat-bottomed microtiter plates. The plates were incubated at 37 ºC for 24 h. Bacterial growth was measured by measuring the optical density at 630 nm and biofilm formation was evaluated by determining the optical density of the attached cells at 492 nm using an ELISA plate reader. Finally the optical densities of the supernatant treated biofilms were compared with the untreated controls.
Results: The *P. aeruginosa* culture supernatant displayed no inhibition on the growth of S. epidermidis strains. On the other hand, biofilm formation by S. epidermidis was significantly reduced in 11 out of 17 (%64) of the strains ($P<0.05$).
Conclusion: In conclusion, culture supernatant of *P. aeruginosa* decreased the amount of biofilm produced by some strains of S. epidermidis but did not affect bacterial growth.

Keywords: Biofilm Formation; *Staphylococcus epidermidis*; *Pseudomonas aeruginosa*; Culture Supernatant.

EVALUATION OF THE EFFECT OF CHITOSAN ON IMPROVING THE EFFICACY OF PHOTODYNAMIC INACTIVATION OF PSEUDOMONAS AERUGINOSA BIOFILMS

Masoumeh Shahbazi[1], Nasim Kashef[2]
1.Msc student, Department of Microbiology, School of Biology, College of Science, University of Tehran, Tehran, Iran
2.Assistant Professor of Medical Bacteriology, Department of Microbiology, School of Biology, College of Science, University of Tehran, Tehran, Iran

Background: In this study, we aimed to use chitosan, a polycationic biopolymer, to improve the efficiency of PDI using methylene blue (MB) on *P. aeruginosa* growing as biofilms.
Methods: Effect of MB concentration (200 μM) and light dose (47 J/cm2) on PDI of five drug-resistant *P. aeruginosa* isolates in biofilm forms was investigated. In vitro bactericidal effect of MB-PDI on *P. aeruginosa* biofilms treated with chitosan (1 mg/ml) was also studied.
Results: For this set of PDI parameters, *P. aeruginosa* isolates showed 0.58-1.5log10 killing. However, MB-PDI applied on biofilms treated with chitosan was significantly able to disrupt pre-formed biofilms (viable count reduction ranging from 4.14-7.4log10-unit in comparison to controls in all tested isolates).
Conclusion: Chitosan/PDI combination had significant ability to eradicate the pre-formed mature biofilms of P. aeruginosa. These results indicate that chitosan may improve the uptake of MB, so it can potentiate the PDI efficacy against biofilm cells.

Keywords: *Pseudomonas aeruginosa*, Photodynamic Inactivation (PDI),Chitosan

THE PREVALENCE OF RESISTANCE TO 15 ANTIMICROBIAL DRUGS IN PSEUDOMONAS AERUGINOSA RECOVERED FROM PATIENTS IN TEHRAN, Iran

Horieh Saderi[1], Batoul Basatiyan [2], Shahram Boroumandi [3],Roxana Sahebnasagh[4]
1.PhD, Molecular Microbiology Research Center, Shahed University, Tehran, Iran
2.M. Sc. Student in Microbiology Department, School of Medicine, Shahed University, Tehran, Iran
3.PhD, Microbiology Laboratory, Pars General Hospital, Tehran, Iran

Background: *Pseudomonas aeruginosa* is an opportunist pathogen which causes different infections especially in patients with impaired immunity.
Methods: In the three-month period in spring of 2013, all the clinical isolates of *P. aeruginosa* were studied from the aspect of resistance to 15 antimicrobial drugs by the means of disk diffusion method using Rosco company disks (Denmark) in the laboratory of Pars Hospital in Tehran, Iran.
Results: A total number of 55 *P. aeruginosa* isolates were recovered from different clinical samples of hospitalized patients (27.3%) and outpatients (72.7%). Totally 54.5% of these patients were male and most of them were at the age of 45 or above (81.5%). Most of the isolates were collected from sputum (40%), urine (38.2%) and wound and abscess (12.7%). Among the studied antibiotics, the lowest level of resistance was documented to colistin (3.7%). Resistance to meropenem and piperacillin-tazobactam were also low (22.6% and 24.1%, respectively). Resistance to ceftriaxone, amikacin, ceftazidime, ciprofloxacin, gentamicin and tobramycin were detected in 38.6% to 50.9% of isolates. Moreover, resistance to cefotaxime and trimethoprime-sulfamethoxazole were observed in 69% and 73.6% of isolates, respectively, while resistance to ampicillin, amoxicillin-clavulanic acid, cefuroxime and nitrofurantoin were observed in 94.4%, 87.9%, 93.5% and 95.2% of isolates, respectively. Most of the isolates were exhibited multidrug resistant.
Conclusion: The prevalence of resistance to the wide range of antimicrobial agents in *P. aeruginosa* isolates in Tehran emphasizes that it is vital to prevent from their dissemination especially in patients with impaired immunity. In addition, use of appropriate drug especially in empirical treatment is very important.

Keywords: *Pseudomonas aeruginosa*, Antimicrobial Susceptibility, Multidrug Resistant

USE OF MULTILOCUS SEQUENCE TYPING (MLST) IN MOLECULAR EPIDEMIOLOGY STUDY OF ANTIBIOTIC RESISTANCE PSEUDOMONAS AERUGINOSA ISOLATED IN Iran

Hossein Fazeli[1], Hooman Sadighian[1], Bahram Nasr Esfahani[1], Mohammad Reza Pourmand[1]
1.Microbiology Department, Medical School, Isfahan Universityb of Medical Science, Iran

Background: Nosocomial infections produced by multi-drug-resistant (MDR) *Pseudomonas aeruginosa* strains in Iran severely increased. Also nosocomial outbreaks of *P. aeruginosa* infection, particularly by MDR strains, have become problematic in hospitals in various countries. The object of this study was to determine ST type of antibiotic resistance *Pseudomonas aeruginosa* isolated in Iran
Methods: The real-time RT-PCR was performed to determine the expression level of mexY, ampC and oprD for isolates. The isolates were typed by multilocus sequence typing (MLST) a strain-typing system that focuses strictly on conserved housekeeping genes. Also Antimicrobial Susceptibility testing was performed.
Results: 75% of clinical isolates were multidrug-resistant. The blaOXA group-I and blaPER alleles were identifiedin 28 and 10 *P. aeruginosa* isolates respectively. The majority of blaPER positive isolates belonged to the same MLST clone and was identified as ST235. The types of remaining isolates were ST360 and ST861. Among 10 blaPER positive isolates, eight isolates demonstrated reduced oprD expression and mexY overexpression.
Conclusion: Our data further highlight the epidemic potential of the international clone ST235. According to the results different resistant mechanisms identified among ST235 isolates which were resistance to ceftazidime, imipenem, ciprofloxacin and amikacin.

Keywords: MLST, PER, *Pseudomonas aeruginosa*, ST235

DETERMINATION OF METALLO BETA LACTAMAS BLA-IMP-1 AND BLA-VIM-1 GENES AMONG CARBAPENEM RESISTANT PSEUDOMONAS AEROGINOSA ISOLATED FROM CLINICAL PATIENTS IN KERMANSHAH,Iran.

Farzaneh Nazari[1], Mohsen Mirzaie[1], Hossien Fazeli[2]
1.Microbiology departman,medical school,Boroujerd University,Boroujerd,Iran
2.Microbiology departman,medical school,Isfahan University,Isfahan,Iran

Background: Effective treatment multi- drug-resistant (MDR) *P. aeroginosa* infection is challenging, because of narrowed therapeutic options. Increasingly carbapenem resistant among these isolated is significant attention. *Pseudomonas aeroginosa*, frequently cause hospitalization- associated infections participating to raise hospital morbidity and mortality.
Methods: The total of 100 isolates of *P. aeroginosa* were recovered from patients. Identification of imipenem resistant and their antimicrobial susceptibility pattern was performed by disc diffusion. metallo beta lactamases (MBLs) enzyme detection was carried out by E-test. The presence of genes encoding MBLs bla-IMP-1 and bla-VIM-1 was determined by PCR.
Results: A total of 100 investigated *P. aeroginosa*, 99 (99%) were MDR, 45 (45%) of MDR were resistant to imipenem and 28 (28%)were MBL producers by MBL E-test.The outbreak bla-IMP-1 and bla-VIM-1 were 3(3%), 19(19%).
Conclusion: This is the first report of metallo beta lactamas bla-IMP-1 and bla-VIM-1 genes producing *P.aeroginosa* from Kermanshah. Early recognized of the emergence of carbapenem-resistant *P. aeroginosa* is an effect for preventing their spread.

Keywords: *P. aeroginosa*, Multi Drug Resistant, Metallo Beta Lactamases

DISTRIBUTION OF CTX-M B-LACTAMASE GENE AMONG PSEUDOMONAS AERUGINOSA STRAINS

Delsuz Rezaee[1], Mohammad Ahangar Zade Rezaee[2], Gholamreza Zarrini[1]
1.Department of Microbiology, Department of Biology, Faculty of Natural Sciences, University of Tabriz. Tabriz-Iran.
2.Department of Microbiology, Faculty of Medicine, University of Medical Sciences Tabriz, Tabriz-Iran.

Background: The production of Extended Spectrum Beta Lactamases (ESBLs) by *P. aeruginosa* is the main cause of resistance to Cephalosporins. In the past decade, CTX-M Enzymes have become the most prevalent ESBLs in Europe, Canada, and Asia.. In this study, the frequency of ESBL producing *P. aeruginosa* and molecular detection of the CTX-M gene was investigated.
Methods: In this study, a total of 33 clinical isolates *P. aeruginosa* were collected from patients hospitalized in Tehran. The isolates were identified using standard Biochemical tests. The resistance to ceftazidim was assessed by Kirby-Bauer disk diffusion method. The confirmatorytest for detection of resistant isolates wascarried out by double disk method at the presence of ceftazidim, cefepime and clavulanic acid. The presence of β-lactamase gene of blaCTX-M in ESBL was assessed by PCR.
Results: Among.of.33.isolates, 30 isolates (90.9%) are resistant to ceftazidim. four (13.3%) of them are confirmed as ESBL producing P. aeruginosa. The results showed all isolates were blaCTX-Mnegative.
Conclusion: Many isolates of *P. aeruginosa* exhibited high resistance to beta-lactams, but they did not produce any CTX-M gene.

Keywords: *Pseudomonas aeruginosa*, Extended Spectrum Beta Lactamase, CTX-M, Double Disk Method.

ROLE OF MICROBE-DERIVED COMPOUNDS ON MALARIA CONTROL

Reza Soltani[1], Saba Belgheisi[2]
1.Faculty Of Food Industry And Agriculture, Department Of Biology, Standard Research Institute (SRI), Karaj, Iran.
2.Faculty Of Food Industry And Agriculture, Department Of Food Science & Technology., Standard Research Institute (SRI), Karaj,Iran.

Background: Microbial applications in malaria transmission control have drawn global attention. Malaria transmission control relies mainly on vector control. However, the current vector control measures, based on insecticide-treated nets (ITNs) and residual sprays, cannot interrupt the transmission of malaria in the most intensely endemic parts of Africa and the Pacific.
Methods: Cycloprodigiosin hydrochloride,a stable fluorescent red pigment obtained from the marine bacterium Pseudoalteromonas denitrificans Then incorporated into P. falciparum cells upon incubation.
Results: Results shown a potent antimalarial activity.It has been shown that the concentration required for 50% of the activity is stronger than that of chloroquine. The compound did not affect growth rate of mammalian cells and it's in vivo antimalarial activity was observed indicating its antimalarial potency.
Conclusion: The encouraging results of many microbes and/or compounds derived from them could be employed in the biological warfare against malaria parasite and/or its mosquito vectors.

Keywords: Microbe-Derived Compounds, Malaria, Control

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ANTIBACTERIAL ACTIVITY OF OLEA EUROPAEA AND CHAMAEMELUM NOBILE EXTRACTS ON IMP-TYPE METALLO-BETA-LACTAMASE-PRODUCING PSEUDOMONAS AERUGINOSA

Masoud Dadashi[1], Gita Eslami[1], Hossein Goudarzi[1], Fatemeh Fallah[2], Ali Hashemi[1], Soudabeh Taheri[1], Zohreh Ghlavand[1]

1.Department of Microbiology,school of medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2.Pediatric Infections Research Center, Mofid Children's Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: The aim of this study was to evaluate the antibacterial activity of Chamaemelum nobile and Olea europaea extracts on IMP-producing *P.aeruginosa* strains.
Methods: This descriptive study was carried out on hospitalized burnt patients during 2013 year. Antibiotics and extracts susceptibility tests were performed by disc diffusion and broth microdilution methods based on Clinical Laboratory Standards Institute (CLSI) guidelines. MBL detection was performed by Combination Disk Diffusion Test (CDDT).The bla(VIM) and bla(IMP) genes were detected by PCR and sequencing methods.
Results: Using Combination Disk Diffusion test method, it was found that among 83 imipenem non-susceptible *P.aeruginosa* strains, 48 (57.9%) were MBL producers. PCR and Sequencing methods proved that these isolates were positive for blaIMP-1 genes, whereas none were positive for bla(VIM) genes. The nucleotide sequence data reported in this paper have been submitted to the GenBank sequence database and assigned accession no.JX648311. The mortality rate due to MBL-producing Pseudomonas infection was 4(8.3%) among the hospitalized patients. It was shown that Chamaemelum nobile extracts had the highest antibacterial effect on standard and IMP-producing *P.aeruginosa* strains in 3.12 µ.g/ml concentration and Olea europaea extracts in 100 µ.g/ml.
Conclusion: The prevalence of beta-lactamase-producing *Pseudomonas aeruginosa* detected in this study is of great concern and highlights the need of infection control measures including antibacterial management and prompt identification of beta-lactamase-producing isolates. In this study, it was shown that extracts of Chamaemelum nobile has higher antibacterial effects on β-lactamase producer *P.aeruginosa* strains than other extracts and antibiotics.

Keywords: *P. aeruginosa*, Metallo-B-Lactamases, Olea Europaea,

EFFICACY OF METHANOLIC EXTRACT OF GREEN AND BLACK TEAS AGAINST EXTENDED-SPECTRUM B-LACTAMASE-PRODUCING PSEUDOMONAS AERUGINOSA

Arezou Taherpour [1], Ali Hashemi[2], Soroor Erfani-manesh[2], Elahe Taki [2]

1.Microbiology Department, Kurdistan University of Medical Sciences, Sanandaj, Iran
2.Department of Microbiology, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: *Pseudomonas aeruginosa* is one of the major bacteria causing acute infections. β-Lactamase production is the principal defense mechanism in gram negative bacteria. The aim of our study was to evaluate the antibacterial activity of Methanolic Extracts of Green and Black Teas on *P. aeruginosa* Extended Spectrum-β-Lactamases (ESBLs) production.
Methods: This research was carried out on burn wounds of 245 hospitalized patients in Kerman, Iran. *P. aeruginosa* ESBLs and MBL producing strains were detected by Combination Disk Diffusion Test (CDDT) and Epsilometer test (E-test) strips, respectively. Minimum inhibitory concentration (MIC) was measured for Ceftazidime, Meropenem, Imipenem, Aztreonam, Cefotaxime and methanollic extracts of Camellia Sinensis (Green Tea).
Results: From 245 patients in the burn ward, 120 cases were infected with P.aeruginosa. 41 isolates contained ESBL while MBL was not detected. *P.aeruginosa* were resistant to Cefotaxime, Aztreonam, Ceftazidime, Meropenem and Imipenem, 72 (60%), 50(41.66%), 79(65.83%), 33 (27.5%) and 24(20%), respectively. Green tea extract had the highest anti-bacterial effect on standard and *P.aeruginosa* strains in 1.25 mg/ml concentration.
Conclusion: This study determined that the methanolic extract of green tea has a higher effect against ESBL producing *P.aeruginosa* than Cefotaxime, Aztreonam and Ceftazidime.

Keywords: *Pseudomonas aeruginosa*, Beta-Lactamases,Antibiotic Resistance, Camellia Sinensis

IDENTIFICATION THE PRESENCE OF PSLA GENE IN PSEUDOMONAS AERUGINOSA ISOLATES AND IT'S EFFECT ON BIOFILM FORMATION

Shiva Emami[1], Iraj Nikokar[1], Yusuf Ghasemi[1], Afshin Araghian[1]
1.Laboratory of Microbiology and Immunology of Infectious Diseases, Para medicine Faculty, Langeroud, Iran

Background: In this study we investigated the Presence of pslA genes in strains that we had already measured their biofilm formation by Phenotypic methods (microplate assay).
Methods: fifty isolates of *P. aeruginosa* were employed of wastewater of burn infection center. Biofilm formation was measured by the microtiter plate assay. The genome of all isolates was extracted by boiling method to be used in PCR reaction. The specific primers were used to check the presence of the pslA gene.
Results: We found that the pslA gene presented in 44% of biofilm high-producer strains, 42% of strains, which produce biofilm weakly, and it was not seen in biofilm non-producer strains. So it means the pslA gene is important to forming biofilm. Since this gene was not found in all of biofilm producers, it seems there are other genes or factors to forming biofilm too.
Conclusion: We found that biofilm formation in pseudomonas aeroginosa depends to different factors. One of the most important factors is pslA gene that presents widly in biofilm producer isolated.

Keywords: Biofilm Formation, Psla Gene, Microtiterplate, PCR

DETECTION OF TOXOPLASMA GONDII OOCYSTS IN SOIL OF URBAN PARKS, BASED ON MOLECULAR AND STAINING TECHNIQUES (ARAK, Iran)

Hadis Solyemane[1], Zahra Eslamirad[2], Mansour Bayat[3]
1.Department of Microbiology, Islamic Azad University, Science and Research Branch, Tehran, Iran
2.Departments of Parasitology, School of Medicine, Arak University of Medical Sciences, Arak, Markazi, Iran
3.Department of Medical and Veterinary Mycology, Faculty of Specialized Veterinary Sciences, Islamic Azad University, Science and Research Branch, Tehran, Iran

Background: In this study, soil contamination of public parks in the Arak city with Toxoplasma gondii oocysts was examined.
Methods: Sixty soil samples were collected from 15 main parks of Arak city. Samples were taken from 4 places at each public parks. The site includes a potting area, around toilets, children's play area and the place of rest. The samples were transported to the Laboratory of Parasitology, Medical Sciences, and were dried at laboratory temperature. Floatation in saturated sucrose was performed for isolation of oocysts from the soil samples. Floating debris was tested by two methods: staining by the modified Ziehl-Neelsen technique and polymerase chain reaction.
Results: From 60 soil samples of public parks of Arak city, 8 samples (13%) were suspected to be contaminated with Toxoplasma oocyst by staining method. But, only 3 samples (5%) of 60 samples were positive in PCR. The results showed that the Ziehl-Neelsen staining was not good method to differentiate of Toxoplasma oocysts in the soil. Molecular method for the detection of parasites in the soil was more suitable.
Conclusion: This study showed soil of public parks in the Arak city were contaminated to oocyst of Toxoplasma gondii and may play a role in the epidemiology of toxoplasmosis in Arak city.

Keywords: Oocyst, PCR, Soil, *Toxoplasma gondii*

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ISOLATION AND MOLECULAR IDENTIFICATION FREE LIVING NITROGEN FIXING BACTERIA FROM VERMICOMPOST

Nooshinkaramatvash[1], Morteza Abasi[1]
1.Islamic Azad University of Urmia, Iran

Background: many bacteria are able to fixing nitrogen, which is one of the magor nutrients essential for all living beings. The bacteria isolated from the soil can be used for various purposes.
Methods: In this research the samples were collected from 10 different cities of Iran and the preliminary screening tests such as gram staining, oxidase test,catalase test,nitrate test and some other tests were perforformed. The selected strains were tested by complementary screening tests such as culturing in N-free malate agar media and those which had the highest ability to fixate nitrogen were selected.PCR and 16SrRNA Gene seguence analysis were used for molecular identificationn of superior strains.
Results: After enrichment and growth in Burk's N-free medium plates, 70 bacterial strains were isolated and by performing nitrate test 40 of them were recognized as nitrogen fixing strains. These 40 strains were subjected to complementary screening tests such as culturing them in N-free malate agar media and 10 strains were selected as the highest nitrogen fixing strains, considering diameter of colored regions and the halo formed by these bacteria. Finally, based on 16S rRNA Gene Sequence Analysis, molecular identification of the 10 superior strains was performed and revealed that all strains belonged to pseudomonas genus.
Conclusion: Considering high potential of the isolated strains such as significant increase in the soil nitrogen content and by further investigation of characteristics associated with these strains, their crucial role in agriculture can be expected.

Keywords: Malate Agar,Pcr,Gene Seguence, Burk's N-Free

ISOLATION AND MOLECULAR IDENTIFICATION OF NATIVE RHODOCOCCUS STRAINS FROM MANGANESE MINE SOIL IN QOM CITY

Seyyed Soheil Aghaei[1], Nima Bahador[1], Majid Baserisalehi[2]
1. *Department of Microbiology, Science and Research Branch, Islamic Azad University, Fars, Iran.*
2. Department of Microbiology, Kazeroun Branch, Islamic Azad University, Kazeroun, Iran.

Background: The aim of this study was isolation and molecular identification of *Rhodococcus* native strains from soil samples of manganese mine in Qom city.
Methods: Totally 125 samples have been collected from soil around manganese mine in Qom city. The samples were serially diluted (10 -1- 10-7) and cultivated on different selective media. Then the pure coloies were physiologically and biochemically determined and characterized at 30°C.Genomic DNA extraction and PCR amplification of the 16 SrRNA genes were done and aligned with the 16rsRNA gene sequences of other *Rhodococcus* species obtained from GeneBank/EMBL/DDBJ.
Results: Overall, 125 bacterial strains were achieved after culturing on different selective enriched media such as ISP5 and Bennet agar and 87 strains were similar to the coryne form among them two strains (85, 87) which is isolated from ISP5 agar, were identified and confirmed by biochemical tests and 16srRNA PCR amplification method as *Rhodococcus rhodnii* and *Rhodococcus rhodocorrhous*.
Conclusion: 16S rRNA phylogenic analysis was carried out to confirm isolated strains which can use for further studies.

Keywords: Key Words: Manganese Mine, *Rhodococcus*, Soil, 16srrna

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PARASPORIN, A CYTOTOXIC PROTEIN AGAINST HUMAN BREAST CANCER CELL OF BACILLUS THURINGIENSIS

Marjan Estakhr[1], Negar Azarpira [2], Elham Moazamian[1]
1.Department of Microbiology, Science and Research Branch, Islamic Azad University, Fars,Iran
2.Organ Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Parasporin, a *Bacillus thuringiensis* parasporal protein, is unique in having a strong cytocidal activity preferential for human cancer cells. In this study, we characterized parasporin activities against human breast cancer cell line.
Methods: Soil samples were collected and then isolates were identified using phenotypic and Cry toxin staining. In next step, toxin was separated, purified, and activated using proteinase-K enzyme. The toxin molecular weight was evaluated using SDS-PAGE method. Breast cancer cell line was cultured and then treated with activated toxin.
Results: *B. thuringiensis* strain Kla4 produced one parasporal inclusion proteins with a molecular mass of 37 kDa that was converted to 30 kDa toxin when activated by proteinase K digestion. This toxin exhibited strong cytocidal activity against human breast cancer cell, while the isolate lacked lymphocyte toxicity and hemolytic activity against sheep erythrocytes.
Conclusion: Our results provide evidence that the parasporin producing organism is a common member in *B. thuringiensis* populations occurring in natural environments of Iran. Parasporin acts as a cytolysin that permeabilizes the plasma membrane with target cell specificity and subsequently induces cell decay and death.

Keywords: Parasporin, *Bacillus thuringiensis*, Cancer Cell Lines, Cytotoxicity

ISOLATION AND SCREENING OF NATIVE STREPTOMYCES STRAINS ABLE TO PRODUCTION OF BIOSURFACTANT FROM SALINE SOIL

Farzaneh Fakharian[1], Soheil Aghaee[1], Mohammad Reza Zolfaghari[1], Mohammad Soleimani[1], Ali Javadi[1]
1.Department of Microbiology, Qom Branch, Islamic Azad University, Qom, Iran

Background: Objective of the present study is isolation and screening of biosurfactant producing *Streptomyces* from saline soil in Qom city.
Methods: Totally 40 soil samples were collected from different regions of saline lake in Qom city and were dilluted (10 -1- 10-7) and cultivated on different media including: starch casein agar and ISP4 medium. The plates were incubated at 30°C for 3-7 days and then the pure isolates were identified due to biochemical and morphological tests. Biosurfactant activity was determined for the pure culture of *Streptomyces* isolates by different methods namely: 1) hemolysis 2) drop collapsing test 3) oil spreading 4) lipase production 5) surface tension measurement.
Results: In the serial dilution and plating on starch casein agar about 95 Actinobacterial strains were isolated. Among the isolated bacteria 25 strains belonged to *Streptomyces* genus. In general, six isolates were capable of biosurfactant production. Morphologically distinct colonies were selected for further analysis.The selected strains were screened for production of surface active molecules.Inoculation of isolates on blood agar plate produced a clear zone around the colonies indicates the biosurfactant activity.In the drop collapsing test, a flat drop and in oil spreading method, a clear zone was observed around the colonies of the isolates which indicates a biosurfactant activity.
Conclusion: Overall, the results obtained from this study indicated that isolated *Streptomyces* strains from saline soil samples showed biosurfactant activities.Biosurfactant productions of these isolates were confirmed with the oil spreading and drop collaosing methods(Youssef et al.,2004).

Keywords: Screening, Streptomyces, Biosurfactant, Saline Soil

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ENRICHING VERMICOMPOST BY NITROGEN FIXING BACTERIA ISOLATED FROM VERMICOMPOST

Nooshinkaramatvash[1], Mahmod Rezazade Bari[2]
1.MA degree in Microbiology, Department of Microbiology, Islamic Azad University, Urmia Branch, Urmia, Iran
2.Assistant Professor, Department of Microbiology, University of Urmia, Urmia Branch, Urmia, Iran

Background: Addition of nitrogen fixing bacteria improves the quality of vermicompost,nitrogen is one of the major nutrients essential for all living beings,also any change in population of microorganisms in vermicompost has a significant influence on its quality. Present study was performed with aimed to possibility of changing in the content of nitrogen in the vermicompost.
Methods: In this research vermicompost was inoculated by 10 strains free-living nitrogen-fixing bacteria of the genus Pseudomonas,which were previously isolated and identified from vermicompost. This research was conducted to increase the nitrogen content in vermicompost. In order to accomplish this goal, inoculums from nitrogen fixing bacteria genera were prepared,then bacteria inoculated to vermicompost beds, for 60 days at a temperature of 28°C storage, and biological and chemical factors were measured on days 0, 20, 40 and 60 in vermicompost.
Results: Results showed that with increasing incubation time, the population of bacteria,nitrogen increased, According to results of this study, 20 to 40 day incubation period could have very good results on the chemical and biological properties in vermicompost.These bacteria can cause a significant increase in vermicompost nitrogen.
Conclusion: Considering high potential of the isolated strains such as significant increase in the soil nitrogen content and by further investigation of characteristics associated with these strains, their crucial role in agriculture can be expected.

Keywords: Vermicompost

ISOLATION AND IDENTIFICATION OF BACILLUS SPP. FROM SOIL OF BAHMANSHIR RIVERSIDE IN ABADAN CITY-Iran AND EVALUATION OF THEIR ANTIBACTERIAL PROPERTIES

Zeinab Rakhisi[1], Nabi Jomehzadeh[2], Mansour Amin[3]
1.Islamic Azad University Fars Science and Research Branch, Fars, Iran
2.Abadan School of Medical Sciences, Abadan, Iran
3.Department of Microbiology, School of Medicine, Health Research Institute, Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Background: *Bacillus* species being the predominant soil bacteria because of their resistant endospore formation and production of essential antibiotic like bacitracin. The aim of this study was to isolation of *Bacillus* Spp. From riverside soil and investigation of antimicrobial characteristics against some pathogenic bacteria
Methods: fifty soil samples were collected from different sites of Bahmanshir riverside in Abadan city, and analyzed for the presence of *Bacillus* species. The media used in this research was nutrient broth and agar medium. The *Bacillus* species were differentiated by their biochemical characteristics. The inhibitory effect of *Bacillus* strains against the target bacteria including: *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* typhi, *Shigella* Spp. and Corynebacterium diphtheria was examined.
Results: The identified *Bacillus* species included *Bacillus* cereus (93.3%), *Bacillus subtilis* (6.6%). The evaluation of the antimicrobial activity of the extracted compounds from isolated bacteria was carried out against 5 target bacteria. Antibiotic production tests indicated that two *Bacillus* cereus isolated in this study showed antimicrobial properties.
Conclusion: This study suggests that some *Bacillus* species have potential to produce high quality antibiotics that can be use to control microbial growth.

Keywords: Soil Bacteria, Antibiotic Production, *Bacillus* Species

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THE EFFECT OF SILVER NONOPARTICLES ON STAPHYLOCOCCUS EPIDERMIDIS BIOFILM

Hanieh Mortazavi[1], Mahboobeh Nakhaei Moghaddam[2], Khadijeh Nejad Shahrokh Abadi[2]
1.Department of Biology, Damghan Branch, Islamic Azad University
2.Department of Biology, Mashhad Branch, Islamic Azad University, Mashhad, Iran

Background: Background and aims: Biofilm is a functional consortium of microorganisms attached to the surface and is embedded in the extracellular polymeric substances (EPS) produced by the microorganisms. Biofilm, due to the special structure and EPS are more resistant to antimicrobial agents. The purpose of this study was to investigate the effect of silver nonoparticles on *Staphylococcus epidermidis*.
Methods: Methods: Bacteria were isolated from different patients' samples referred to Mashhad hospitals and identified by biochemical tests. Nanoparticles with a diameter of 20 nm were purchased from Nanosany Company (USA). Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of nonoparticles were determined by using agar dilution method in 24- well micro titer plate. 96- Well micro titer plate and ELISA reader were used for evaluating the effect of silver nanoparticles on the biofilm of *Staphylococcus epidermidis*. Biofilms were stained with safranin.
Results: Results: Silver nanoparticles showed antibacterial activity on 15 clinical isolates and the reference strain of S. epidermidis (ATCC12226). The MIC and MBC of silver nanoparticles were 175 and 400 for reference strain. The MIC was 150-350 ppm and MBC was 400-800 ppm for clinical isolates of S. epidermidis bacteria. Silver nanoparticles showed an anti- biofilm activity at ≥ 0/5 ppm concentration.
Conclusion: Conclusions: The results showed that silver nonoparticles with the size of 20 nm had an effective activity on *Staphylococcus epidermidis* biofilm. Thus due to the staphylococcal biofilm formation on medical devices, silver nanoparticles can be used as an inhibitory factor for the development of these biofilms.

Keywords: Biofilm *Staphylococcus epidermidis* Silver Nanoparticles

A COMPARISON BETWEEN PCR, OXACILLIN AGAR DILUTION AND CEFOXITIN DISK DIFFUSION METHODS IN DETECTION OF METHICILLIN RESISTANCE IN S. AUREUS ISOLATES COLLECTED FROM CLINICAL

Asghar Havaei[1], Mojtaba Shahin[2], Sharareh Moghim[1], Abbas Farahani[2], Fahimeh Ghanbari[3], Maryam Afzali[2]
1.Department of Microbiology, Faculty of medicine, Isfahan University of Medical sciences, Isfahan, Iran
2.Ph.d student of medical bacteriology, Department of Microbiology, School of Medicine, Infectious & Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
3.Department of Microbiology, Islamic Azad University, Falavarjan Branch, Isfahan, Iran

Background: It is crucial to identify the methicillin resistant *S. aureus* (MRSA) strains and implement the necessary treatment in order to control the spread of hospital infections.
Methods: A total of 150 *S. aureus* isolates were collected from samples of patients admitted to alzahra and Shariati hospitals. PCR for mecA gene was performed in all strains. There after MIC for oxacillin was carried out using agar dilution method, which was determined 8 micrograms according to CLSI regulations. Then antibiogram using disk diffusion for cephoxitin was performed according to CLSI regulations on isolates containing mec A gene.The three methods were then compared.
Results: Of the 150 samples tested, 62samples (41%) were found to carry mecA gene using PCR however, agar dilution for oxacillin found 56 samples (90.3%) to harbour mec A gene.Disk diffusion method revealed that 53 samples (85.4%) contain mecA gene.
Conclusion: Agar dilution method was found to have a higher sensitivity in determining antibiotic sensitivity compared to disk diffusion method. However PCR was identified as the ideal method for detecting MRSA strains. Since a number of strains were found to be sensitive to oxacillin in phenotypic test due to lack of mec A expression while still containing the gene.

Keywords: *Staphylococcus aureus*, Methicillin, Meca, MRSA

COMMUNITY-ACQUIRED METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS NASAL COLONIZATION PREVALENCE; A PROSPECTIVE STUDY IN Iran

Shervin Shokoohi[1], Sasan Sazgar[1], Farhad Abbasi[2], Bahareh Hajikhani[1], Mohtaram sadat Kashi[1], Shahram Sabeti[1], Parvin Sarbakhshi[1]
1.Shaheed Beheshti Medical University, Tehran, Iran
2.Bushehr University of Medical Sciences, Bushehr, Iran

Background: Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) is a serious pathogen and its nasal carriage is a risk factor for subsequent infections. This study aims at determining the prevalence of and risk factors for CA-MRSA colonization at the time of hospital admission in our community.
Methods: Anterior nares cultures were obtained from patients coming to the emergency department of Loghman Hakim hospital in Tehran within 24 hours of admission. Antibiotic susceptibility tests (E-Test) were performed. A positive culture of MRSA within 24 hours of admission was considered as CA-MRSA. Data analysis was performed for assessment of associations between culture results and risk factors.
Results: 56 (14%) and 11(2.7%) of 400 patients had a nares culture positive for *S.aureus* and MRSA respectively. HIV infection (P value=.001), nursing homes residence (P value=.033) and nasal anatomic abnormalities (P value=.033) had significant association with CA-MRSA cultures. However in logistic regression, no statistically significant association was found. 45% of MRSA cultures showed induced resistance to clindamycin on D-test. Based on a 25µg/ml cutoff for susceptibility to Tigacyline On E-test, 18.1% showed resistance.
Conclusion: Our study showed CA-MRSA prevalence to be 2.7% and didn't demonstrate any association between recent hospitalization, antibiotic use and IV drug use with CA-MRSA carriage.

Keywords: *Staphylococcus Aureus*, Methicillin-Resistant, Nasal Colonization

THE EFFECT OF GARLIC JUICE ON THE GROWTH AND EXTRA CELLULAR PROTEIN PROFILE OF METHICILLIN- RESISTANT STRAINS OF S.AUREUS

Leila Asadpour[1], Sima Abdollahi[2], Najmeh Ranji[3]
1.Department of Veterinary science, Islamic Azad University, Rasht Branch, Rasht, Iran
2.Department of Microbiology, Guilan Science and Research Branch, Islamic Azad University, Guilan,Iran
3.Department of Biology, Islamic Azad University, Rasht Branch, Rasht, Iran

Background:: *Staphylococcus aureus* is an important etiologic agent of food-borne diseases due to its ability to produce heat-resistant staphylococcal enterotoxins (SEs) when it grows in foods and also the rise in antibiotic resistance has further amplified public health concern. Because of importance of staphylococcal exotoxines and exoenzymes in Staphylocoous aureus pathogenesis, in the present study, we examined the effect of garlic juice on growth and extracellular proteins production of methicillin-resistant *Staphylococcus aureus*.
Methods: A total of 20 methicillin resistant *S.aureus* isolated from different hospitals in Rasht was determined by using Kirby bauer disc diffusion technique. Antimicrobial activity of fresh garlic juice against test bacteria was evaluated by using agar well diffusion. Minimum inhibitory and minimum bactericidal dilution of garlic juice were determined using the microdilution method. To verify the inhibitory effect of garlic juice on extracellular proteins production, the staphylococcal cells from an BHI overnight culture, containing different concentrations of garlic juice, were harvested by centrifugation and separated by polyacrylamide gel electrophoresis.
Results: All of the MRSA strains were sensitive to garlic juice with minimum bactericidal dilution of 12.5%-50% v/v. Changes in protein profile between control and garlic juice containing cultures, showed that garlic juice can inhibit some *S.aureus* exoproteins production.
Conclusion: Several methods are used for identification of exotoxin production of *S.aureus* isolates in food. In the present study electrophoretic method could show the inhibitory effect of garlic juice on *S.aureus* growth and exoprotein production so garlic can be used as a natural therapeutic agent and natural antimicrobial preservative in food industry.

Keywords: *S.aureus,* Extra Cellular Protein, Garlic Juice,SDS-PAGE

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SCCMEC TYPING OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN HOSPITAL-RELATED HEALTHY CARRIERS IN MOFID CHILDREN HOSPITAL

Masoumeh Navidinia[1], Fatemeh Fallah[2], Shadi Alimehr[3], Behnaz Lajevardi[4], Ashraf Karimi Rahjerdi[4], Mehdi Shirdoost [4]

1.Assistant Professor Of Paramedical Science Faculty Of Shahid Beheshti Medical Science University,Tehran,Iran
2.Professor, Pediatric Infection Research Center, Mofid Children Hospital, Tehran, Iran
3.Pediatric Infections Research Center, Mofid Children Hospital, Shahid Beheshti University Of Medical Sciences,Tehran,Iran
4.Pediatric Infections Research Center, Mofid Children Hospital, Shahid Beheshti University Of Medical Sciences,Tehran,Iran

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the main causes of nosocomial diseases in hospitalized children. Then, screening healthy carriers of these bacteria who are in direct contact with patients in hospital is nesceccery.
Methods: Two hundreds tweny nine medical staff were examined and nasal samples for *S. aureus* culture and sociodemographic data were obtained from them during one year, 2012–2013. After screening for oxacillin and cefoxitin susceptibility, all isolates were examined for antimicrobial susceptibility pattern and staphylococcal cassette chromosome mec (SCCmec) type I–V genes.
Results: From 229 tweny nine nasal samples examination, 27 Staphylococci were isolated and 21 of them were Methicillin-resistant *Staphylococcus aureus*(MRSA). The highest prevalence resistant to antibiotics were: penicillin (90.3%), ceftazidime(77.4%), aztreonam (79%). Resistant to trimetoprime-sulfametoxazol (19%), doxycyclin(29%), minocyclin (12%), rifampicine(16%) were not high. All isolates sensitive to linezolid and vancomycin. Of those tested, 4 isolates was nontypable by using the published primers, perhaps indicating the existence of a novel SCCmec class. Two isolates(9%) had SCCmec I, 5 (23%) had SCCmec II, 9 (42%) had SCCmec III, and 1 (4%) had SCCmec IV.
Conclusion: Incidence of MRSA in hospital-related colonized healthy subjects is very dangerous. The diagnosis of a MRSA infection requires laboratory testing to determine which antibiotics might be useful in treating it. Since methicillin resistant Staphylococci is very problematic, this may propose the significance of detecting the carriers and decolonizing them to reduce transmission of *S. aureus* in the hospital.

Keywords: Scc Mec Typing, Methicillin-Resistant *Staphylococcus aureus* (MRSA), Healthy Carriers

STAPHYLOCOCCAL ENTD GENE DETECTION IN BLOOD OF RHEUMATOID ARTHRITIS PATIENTS

Reyhane Kashefi[1], Ramezan Ali Ataee[2]

1.*Microbial Biotechnology, Faculty of Science, Payame Noor University, Unite of Tehran Center, Tehran, Iran*
2.Department of Medical Microbiology, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran

Background: However, Rheumatoid arthritis is an inflammatory disease with unknown etiology. The super antigens may have a role triggering the disease. Since *Staphylococcus aureus* produce and elaborated several of the classical super antigens (enterotoxins) in the body. The aim of this investigation was seeks to identify Staphylococcal entD gene in the blood of Rheumatoid arthritis patients
Methods: In this study based on ref Gene the specific primer pairs was designed and bioinformaticly were evaluated. Then, set up the PCR protocol and 70 blood Samples of patients with Rheumatoid arthritis were assayed. The finding data were descriptively analyzed.
Results: The result of primer designed was amplification of a 294bp amplicon. The results of the PCR assay indicated that in several patients' blood sample the Staphylococcal entD gene were detected
Conclusion: The result of this study showed that a high percentage of Rheumatoid arthritis patients have entD gene in blood. This finding may be indicating in addition of intoxication, other role of this superantigen is involved

Keywords: Rheumatoid Arthritis, Staphylococcal Entd Gene, Blood, PCR

PREVALENCE OF AAP, FBE, BHP IN CLINICAL ISOLATES OF STAPHYLOCOCCUS EPIDERMIDIS.

Shahin Najar Peerayeh[1], Ali Jazayeri Moghadas[2], Mehrdad Behmanesh[3]
1.Tarbiat Modares University, Faculty of Medical Science, Department of Microbiology, Iran
2.Semnan University of Medical Sciences, Faculty of Medicine, Department of Microbiology
3.Tarbiat Modares University, Faculty of biological Science, Department of genetic

Background: *Staphylococcus epidermidis* surface components are able to establish bacteria on the host surface, biofilm formation and cause infection. The frequency of aap, fbe, bhp in clinical isolates of *Staphylococcus epidermidis* were investigated in this study.
Methods: 59 *S. epidermidis* isolates were collected, from blood (50), wound (1), urine (4), and tracheal (4) samples between September 2010 and September 2010 from three different hospitals, Tehran, Iran. *S. epidermidis* isolates were identified with conventional bacteriological tests. Virulence-associated genes were detected by specific PCRs.
Results: Of the 59 *S. epidermidis*, fbe was found in 89.8%, while aap and bhp were observed in 64.4% and 15.3% respectively. Coexistence of aap and fbe was found in 32 isolates, while coexistence of bhp and fbe was observed in 5 isolates. Two isolates were negative for investigated gens.
Conclusion: Prevalence of fbe and also aap are significantly different with similar studies, but frequency of bhp is in accordance with other studies.

Keywords: *Staphylococcus epidermidis*, Aap, Fbe, Bhp

CORRELATION OF MACROSCOPIC BIOFILM PRODUCTION AND PRESENCE OF VIRULENCE DETERMINANTS IN CLINICAL ISOLATES OF STAPHYLOCOCCUS EPIDERMIDIS.

Shahin Najar Peerayeh[1], Ali Jazayeri Moghadas [2], Mehrdad Behmanesh[3]
1.Tarbiat Modares University, Faculty of Medical Science, Department of Microbiology
2.Semnan University of Medical Sciences, Faculty of Medicine, Department of Microbiology
3.Tarbiat Modares University, Faculty of biological Science, Department of genetic

Background: Ability of biofilm production is one of the molecular mechanisms of S. epidermidis pathogenesis. Virulence related gens play role in the bacterial attachment to artificial surface and host surfaces which covered by proteins. This study was investigated the correlation of icaA, aap, fbe, bhp and IS256 presence and ability of macroscopic biofilm production in S. epidermidis clinical isolates.
Methods: Macroscopic biofilm production assay has been done by microtiter plate method. Bacteria were cultured in TSB medium included NaCl and glucose, pour in polystyrene well, incubated in 370C, washed with P.B.S, stained by safranin, and then the OD measured in 490 nm. S. epidermidis virulence-associated genes were detected by specific PCRs.
Results: Ability of macroscopic biofilm production was observed in 61% of isolates by microtiter plate method. The most frequent genotype icaA+IS256+aap+ fbe+ was seen in 41.67%.
Conclusion: Presence of icaA and IS256 has significant affect on macroscopic biofilm formation than other virulence determinants. One isolate with icaA- IS 256 - aap- bhp+ genotype was able to produce macroscopic biofilm which need more investigation

Keywords: *Staphylococcus epidermidis*, Virulence Determinants, Biofilm

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STUDY OF BIOFILM FORMATION AND DETECTION OF THE ICAADBC GENE CLUSTER AMONG CLINICAL ISOLATES OF STAPHYLOCOCCUS AUREUS AND STAPHYLOCOCCUS EPIDERMIDIS IN SHIRAZ TEACHING HOSPITALS, 2013

Reza Khashei[1], Mehrdad Zalipour[1], Jamal Sarvari[1], Marzieh Taheripour[1]
1.Department of Medical Bacteriology and Virology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: This study aimed to determine biofilm producing ability and presence of icaAD gene in clinical staphylococcal isolates as well as to assess the reliability of two phenotypic methods used for detection of biofilm.
Methods: This study was performed on 151 staphylococcal isolates (79 and 72 isolates of *Staphylococcus aureus* and *Staphylococcus epidermidis*, respectively) obtained from different clinical specimens during a 5 months period in 2013 from four Shiraz Teaching Hospitals. Slime production assay was performed by cultivation on Congo Red Agar (CRA). Quantitative biofilm formation was assessed by microtiter plate assay (MPA) and PCR was used for detection of icaAD gene. In addition, antibiotic susceptibility profiles were determined by the disc diffusion method according to CLSI guidelines.
Results: Of the S. epidermidis isolates, 71% were CRA positive, 76% adherence positive using the MPA and 75% carried the icaAD gene. Among *S. aureus* isolates, 54%, 58% and 63% were CRA, adherence and icaAD positive, respectively. Resistance to linezolid was never observed. Interestingly, biofilm forming strains exhibited a significantly higher prevalence in the resistance to the gentamicin, sulfamethoxazole and to ciprofloxacin in comparison to non-producing isolates. Moreover, multiple drug resistance was more frequent among exopolysaccharide forming strains.
Conclusion: Despite the presence of icaAD gene, it is not always correlate with in vitro biofilm formation. The biofilm-forming ability of some isolates in absence of icaAD gene highlights the importance of further genetic investigations of ica locus independent biofilm formation mechanisms. Compared to phenotypic methods, MTP remains a better tool for biofilm screening.

Keywords: *Staphylococcus*, Biofilm, Congo Red Agar, Microtiter Plate, Antibiotic Susceptibility

MOLECULAR INVESTIGATION OF GENES ENCODING CAPSULAR POLYSACCHARIDES TYPE 5 AND TYPE 8 IN STAPHYLOCOCCUS AUREUS ISOLATES COLLECTED FROM SHOHADA HOSPITAL, TABRIZ

Fahimeh Rahimi Dijvijin[1], Haedeh Mobaiyen [2], Mohammad Reza Nahaeii [2]
1.Department of Basic Science, Ahar Branch, Islamic Azad University, Ahar, Iran.
2.Department of Microbiology, College of Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran.

Background: *Staphylococcus aureus* is a major cause of hospital acquired and community-acquired infections. More than 90% of *S. aureus* strains produce capsular polysaccharides (CPs). Although 11 serotypes of CP have been identified in *S. aureus* strains, more than 80% of the clinical isolates produce either type 5 CP (CP5) or type 8 CP (CP8).This study was aimed to investigate the CP5 and CP8 genes in *S.aureus* strains, isolated from surgical wounds of impatients in Shohada hospital , Tabriz from 2010 to 2012.
Methods: The CP5 and CP8 genes was evaluated in 110 clinical specimen of *S. aureus* strains, isolated from surgical wounds of impatients in Shohada hospital , Tabriz from 2010 to 2012. The CP5 and CP8 genes were detected by PCR method.
Results: The CP5 and CP8 genes were detected in 43 (39/1%) and 64 (52/8%) isolates, respectively, whereas 3 (8/6%) were non-type 5 or non-type 8. There was a significant difference between the ability to producing CPs in these isolates ($P<0.05$).
Conclusion: This study confirmed that the prevalence of the CP5 and CP8 genes in *S.aureus* were predominant, and the prevalence of *S.aureus* type 8 was greater compared to type 5.

Keywords: Capsular Polysaccharides Types 5 And 8، *Staphylococcus aureus* ،PCR.

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ASSOCIATION BETWEEN PRODUCTION OF THE ENTEROTOXIN A AND THE PRESENCE OF COA AND NUC GENES AMONG S. AUREUS ISOLATED FROM VARIOUS SOURCES, IN SOUTHERN Iran

Reyhaneh Hamidi[1], Saeid Hosseinzadeh[2], Seyyed Shahram Shekarforosh[2], Maryam Poormontaseri[2], Abdollah Derakhshandeh[3]

1.Food Hygiene department of vetrinary medicine of Shiraz University, Iran
2.Department of Food Hygiene, Veterinary School, Shiraz University, Iran
3.Department of microbiology, Veterinary School, Shiraz University, Iran

Background: *Staphylococcus aureus* is considered as a common cause of food-borne disease, worldwide. The present study was aimed to identify the frequency of coagulase (Coa) and thermonuclease (Nuc) genes and Staphylococcal enterotoxin A (Sea) production among *S. aureus* isolated from various sources in Shiraz. Moreover, the correlation between the Sea gene and coagulase and thermonuclease enzymes is also considered.
Methods: A total of 100 *S. aureus* isolated from various sources including 40 humans, 30 animals and 30 food samples by the routine biochemical tests. The frequency of Coa, Nuc and Sea genes was evaluated by PCR assay. The enterotoxin production was confirmed using SDS-PAGE method. Correlation among those genes was finally evaluated by statistical analysis.
Results: The PCR results showed that the prevalence of Coa, Nuc and Sea genes were 91%, 100% and 14%, respectively. The evaluation of the enterotoxin production indicated that 78% of the Sea gene was expressed. No correlation was shown among the presence of enterotoxin A gene and production of the coagulase and thermonuclease enzymes meaning the presence of gene was not necessarily associated with the production of toxin.
Conclusion: As a conclusion to detect the enterotoxigenic strains, both genotypic and phenotypic methods are highly recommended.
Keywords: *Staphylococcus aureus, PCR, Iran*

EVALUATION OF ANTIMICROBIAL ACTIVITY OF GREEN TEA EXTRACT ON BACTERIA ISOLATED FROM DENTAL PLAQUE

Masoud Dadashi[1], Gita Eslami[1], Faezeh Ranjbar[1], Hossein Goudarzi[1], Fatemeh Fallah[1], Soudabeh Taheri[1], Majid Ayatollahi[2]

1.Department of Microbiology,school of medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2.Phytochemistry Research Center,Shahid Beheshti University of Medical Sciences,Tehran,Iran

Background: Green tea is one important factors to inhibition of growth of oral bacteria like *Streptococcus mutans* and *Enterococcus* faecalis.This study investigates the antibacterial effect of green tea extract,on *Enterococcus* faecalis and *Streptococcus mutans* of dental plaques.
Methods: After obtained of samples,entered in transport medium(TSB) and were transported to the microbiology laboratory of Shahid Beheshti University of Medical Sciences.The samples were cultured in blood agar medium,Mitis salivarius agar,bile esculin agar and were incubated in proximity of Co2(5-10%) at 37 ° C for 48 hours.After observing the colonies,the slides were prepared and then they stained with Gram's method.Then to ensure the authentication and identification of microorganisms, common microbial and chemical tests were used.After determining the type of bacteria,antibiogram test was done to determine bacterial sensitivity and resistance to green tea,sodium hypochlorite and chlorhexidine by Disc Diffiusion Agar according to CLSI guidelines.
Results: In Streptococcus mutans,diameter of growth inhabitation of green tea was not so different from chlorhexidine($p=0.305$)but significantly was less than sodium hypochlorite($p=0.0001$) and also inhibitory effect of sodium hypochlorite was significantly higher than chlorhexidine ($p=0.038$).in *Enterococcus* faecalis,sodium hypochlorite was more effective than green tea and chlorhexidine ($p=0.0001$) and chlorhrxidine effect was significantly more than Green Tea($p=0.0001$).
Conclusion: Sodium hypochlorite had the highest inhibitory effect on growth of *Streptococcus mutans* and *Enterococcus* faecalis.Since there was no significant difference between inhibitory effect of green tea in *Streptococcus mutans* and chlorhexidine,using it as a mouth wash,can be a good way to prevent tooth decay by the other ways.

Keywords: *Enterococcus faecalis*,*Streptococcus mutans*,Green Tea Extract,Dental Plaque

DISTRIBUTION OF THE CYANIDE-RESISTANT ALTERNATIVE OXIDASE IN THE FUNGAL CLASS OF EUROTIOMYCETES (ASCOMYCOTA, PEZIZOMYCOTINA)

Mojtaba Asadollahi[1], Ákos P. Molnár[1], Erzsébet Fekete[1], Levente Karaffa[1], Michel Flipphi[1], Erzsébet Sandor[2]
1.Department of Biochemical Engineering, Faculty of Science and Technology, University of Debrecen, Hungary
2.Institute of Food Processing, Quality Assurance and Microbiology, University of Debrecen, Hungary

Background: Here we investigated the distribution of this enzyme within the fungal class of Eurotiomycetes.
Methods: 111 AOX protein sequences were mined from various nucleotide databases available at the National Center for Biotechnology Information (NCBI) and the Joint Genome Institute (JGI). Gene models and products were deduced manually. The resultant peptidic sequences were aligned with MAFFT version 7 using the E-INS-i algorithm and a BLOSUM 30 similarity matrix. Curation was carried out with Block Mapping and Gathering using Entropy (BMGE) with a BLOSUM 45 similarity matrix. A maximum likelihood tree was then obtained with PhyML and drawn with FigTree.
Results: Half of the sequenced *Aspergillus* and all Pencillium species specify a second alternative oxidase, which is likely to have evolved within the Aspergillaceae family (Eurotiales order) by duplication before the divergence of the two genera. Two species (A. sydowii and P. brevicompactum) even have three isoenzymes. The second enzymes in *Aspergillus clavatus* and *monascus* are however, related to Dothideomycetes enzymes possibly suggesting gene transfer events. Another horizontal gene transfer involves the second AOX in Byssochlamys spectabilis (Thermoascaceae, Eurotiales) positioned at the basis of the Sordariomycetes clade. A sole alternative oxidase gene is found in species of Onygenales, *Aspergillus* section fumigati, *A. nidulans*, and all species of the section nigri, except A. niger. Finally, the enzyme found in the species within the orders of Chaetothyriales and Verrucariales is not closely related to those in Eurotiales and Onygenales – this latter cluster with Dothideomycetes, the sister class of Eurotiomycetes – but instead cluster together with the earlier diverged classes of Sordariomycetes and Leotiomycetes.
Conclusion: The laboratory model organism *Aspergillus* nidulans and its relative *Aspergillus niger* appear suitable to study alternative oxidase function and regulation at the molecular level and the fermentation performance of the two AOX isozymes, respectively.

Keywords: Eurotiomycetes, Alternative Oxidase, Alternative Respiration, Aspergillus, Pencillium

MOLECULAR TYPING OF WATERBORNE ESCHERICHIA COLI BY REP-PCR

Maryam Makhmalzadeh[1], Reza Ranjbar[2], Naser Harzandi[1], Roohollah Kheiri[4]
1.Department of Microbiology, College of basic Science, Karaj Branch, Islamic Azad University, Alborz, Iran
2.Molecular Biology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.
3.Alborz province Water and wastewater Company. Karaj, Iran

Background: Molecular typing is a valuable tool for investigation of genetic relationship between the microbial strains. The aim of the current study was to determine the molecular types of waterborne *E. coli* strains using Rep-PCR.
Methods: Current study included one hundred waterborne *E. coli* strains isolated from different water sources in Karaj in 2013. Bacterial isolates were detected and identified by standard microbiological and biochemical tests. Genomic DNA was extracted by AccuPrep® Genomic DNA Extraction Kit and genetic relationship between the strains was evaluated by Rep-PCR using specific primers. PCR amplicons were visualized after electrophoresis and staining with ethidium bromide and dendogram was constructed based on Dice Comparison method and UPGMA Clustering.
Results: Using Rep-PCR, all strains were typeable. More than fifteen different bands ranging from 130 to 2300 bp were amplified in different profiles. Following dendogram analysis, Rep-PCR could categorize the strains within 9 Rep clusters.
Conclusion: The results of current study indicated that *E. coli* strains isolated from different water sources in Karaj belong to diverse clones and different genotypes. Our finding also showed that Rep-PCR is a powerful molecular tool with high performance and good discriminatory power for molecular typing of waterborne *E. coli.*

Keywords: *E. Coli*, Rep PCR, Water

DETERMINATION OF UROPATHOGENIC AND FECAL E.COLI O SEROTYPES ISOLATED FROM ZABOL BY MULTIPLEX PCR

Hussein Ali Abdi[1], Ahmad Rashki[2], Zahra Rashki Ghalhnoo[2]
1.Department of Biology - Faculty of Science - National University of Zabol - Zabol – Iran
2.Assistant professor in Molecular Genetics, Department of physio-pathology, faculty of Vet-medicine, University of Zabol-Iran

Background: *Escherichia coli*(*E.coli*) can be serotyped by determination of O antigens, and some relationship exists between specific O types and pathogenic behavior. There are rare information about the prevalence of O serogroups of uropathogenic and fecal *E.coli* isolates in Iran. This study was performed to determine the distribution of the 12 principal O-Serotypes among uropathogenic and fecal *E.coli*.
Methods: In total, 194 *E. coli* isolates collected from hospital patients of the Zabol hospitals (Zabol, Iran) were studied: 100 uropathogenic and 94 fecal *E. coli* isolates. Confirmation of *E.coli* samples were done by biochemical tests. The identification of O-serogroups(O1, O2, O4, O6, O7, O12, O15, O16, O18, O25, O75 and O157) were performed by Multiplex PCR method.
Results: Among uropathogenic and fecal *E.coli*, 73 (73%) and 67(71.27%) were serotyped successfully by multiplex PCR, respectively. In this study was determined that O2, O6 and O18 Serogroups in uropathogenic *E.coli* and O6, O16 and O18 Serogroups in fecal *E.coli* had the highest presence rates of O antigens. O15 type not found in none of the uropathogenic, whereas was observed in fecal *E.coli* isolates. Other antigens including O1, O4,O7, O12, O25, O75 and O157 were observed in both groups of uropathogenic and fecal *E.coli* isolates.
Conclusion: The identification of O-serogroups by Multiplex PCR could be employed in microbial laboratories in the *E. coli* isolates screening, including the possibility of vaccine strain selection and epidemiological searches.

Keywords: O Serotypes, Uropathogenic *E. coli*, Fecal *E.coli*, Multiplex PCR

MOLECULAR SURVEY OF ANAPLASMA OVIS AND ANAPLASMA MARGINALE IN SHEEP AND CATTLE FROM WEST AZERBAIJAN PROVINCE

Davood Bastani[1], Vahid Noaman[2], Masoud Soltani Alvar[3]
1.Graduated from Boroujerd Science and Research Branch, Islamic Azad University, Boroujerd, Iran
2.Department of Veterinary Research, Isfahan Research Center for Agriculture and Natural resources, Isfahan, Iran
3.Department of Parasitology, Boroujerd Science and Research Branch, Islamic Azad University, Boroujerd, Iran

Background: This study was carried out to determine the presence and frequency of Anaplasma ovis and Anaplasma marginale in sheep and cattle from Azerbaijan province, Iran.
Methods: A total of 200 blood samples were collected via the jugular vein from apparently healthy cattle (100) and sheep (100), randomly. The extracted DNA from blood cells were first screened by Anaplasma spp., genus-specific nested-PCR using 16S rRNA gene primer sets. Species-specific PCR were set up using msp4 gene primer sets. No cattle blood sample was positive for Anaplasma spp. by first nested PCR.
Results: In 100 sheep blood samples, 7 samples were Anaplasma spp. positive in first nested PCR. The extracted DNA from positive Anaplasma spp. samples were amplified by Anaplasma ovis -specific PCR, based on msp4 gene. The percentages of positive animals for A. ovis were 5%.
Conclusion: This study is the first molecular detection of A. ovis in sheep from west Azerbaijan province.

Keywords: *Anaplasma ovis*, *Anaplasma marginale*, Sheep, Cattle, West Azerbaijan, Iran

ORNITHOBACTERIUM RHINOTRACHEALE: DEVELOPMENT AND STANDARDIZATION OF AN INDIRECT ELISA KIT

Davoud Nikoo[1], Mohsen Manavian[1], Mohammad Javad Mehrabanpour[1], Ali Shirazinezhad[1], Mohammad Hoseyn Hoseyni[1]
1.Razi vaccine and serum research institute, Shiraz, Iran

Background: Ornithobacterium rhinotracheale is a pleomorphic Gram-negative rod shaped bacterium that is associated with respiratory disease in poultry. This study was conducted to design and standardize of a diagnostic ELISA kit for detection and quantitation of antibodies against ornithobacterium rhinotracheale serotype A in chicken sera.
Methods: An indirect ELISA assay was standardized for detection of antibodies. For that, plates were coated with different quantities of inactivated ornithobacterium rhinotracheale serotype A bacterium. Using chekerbord method plate was charged wit
h different dilutions of positive and negative antiserum as well as conjugated antibodies. Colour developing time was also standardized.
Results: After assessment of 4 factors for kit standardization in this investigation the best results was achived with: the number of coated bacteria 5×?10?^6 bacteria per well; serum dilution 1: 50; conjugate dilution 1: 4000 and 15 min colour developing time.
Conclusion: Our study showed that this designed ELISA kit is able to detect specific antibodies against *Ornithobacterium rhinotracheale* serotype A in chickens.

Keywords: *Ornithobacterium rhinotracheale*, Indirect ELISA, Chicken.