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Original Article

Molecular Epidemiology of Aminoglycosides Resistance in *Acinetobacter* Spp. with Emergence of Multidrug-Resistant Strains

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Abstract

Background: *Acinetobacter* spp. is characterized as an important nosocomial pathogen and increasing antimicrobial resistance. Our aim was to evaluate antimicrobial susceptibility and aminoglycosides resistance genes of *Acinetobacter* spp. isolated from hospitalized patients.

Methods: Sixty isolates were identified as *Acinetobacter* species. The isolates were tested for antibiotic resistance by disc diffusion method for 12 antimicrobials. The presence of *aphA6, aacC1 aadA1,* and *aadB* genes were detected using PCR.

Results: From the isolated *Acinetobacter* spp. the highest resistance rate showed against amikacin, tobramycin, and ceftazidim, respectively; while isolated bacteria were more sensitive to ampicillic/subactam. More than 66% of the isolates were resistant to at least three classes of antibiotics, and 27.5% of MDR strains were resistant to all seven tested classes of antimicrobials. The higher MDR rate presented in bacteria isolated from the ICU and blood samples. More than 60% of the MDR bacteria were resistance to amikacin, ceftazidim, ciprofloxacin, piperacillin/tazobactam, doxycycline, tobramycin and levofloxacin. Also, more than 60% of the isolates contained phosphotransferase *aphA6, and* acetyltransferase genes *aacC1,* but adenylyltransferase genes *aadA1* (41.7%), and *aadB* (3.3%) were less prominent. 21.7% of the strains contain three aminoglycoside resistance genes (*aphA6*, *aacC1* and *aadA1)*.

Conclusion: The rising trend of resistance to aminoglycosides poses an alarming threat to treatment of such infections. The findings showed that clinical isolates of *Acinetobacter* spp. in our hospital carrying various kinds of aminoglycoside resistance genes.

Keywords: Acinetobacter *spp*., *Antibiotic resistance, PCR gene detection, Aminoglycoside resistance genes, Iran*

Introduction

Acinetobacter spp. are ubiquitous, non-fermentative, gram-negative bacilli which play a significant role in colonization and infection in hospitalized patients. *A. baumannii* is the predominant species associated with outbreaks of nosocomial infections (1). Extensive use of antimicrobial chemotherapy in clinical cases has contributed to emergence and dissemination of nosocomial *A. baumannii* infections. These infections are difficult to treat due to the presence of multidrug-resistant (MDR) organisms, which includes resistance to β-lactams, aminoglycosides, fluoroquinolones and more recently, carbapenems. Administration of combination therapy is usually required for effective treatment (2). Resistance of *Acinetobacter* spp. to aminoglycosides primarily results from inactivation of the antibiotic by specific modifying enzymes. Multiple aminoglycoside-modifying enzymes including acetylases, adenylases, and phosphorylases and these classes have been identified in *Acinetobacter* spp. (3-6) The genes encoding aminoglycoside-modifying enzymes may be located on plasmids and transposons, and some of these genes have been found on class 1 integrons in MDR *A. baumannii* strains in Europe (7-9).

The aim of the present study was to determine the genetic basis of aminoglycoside resistance in *A. baumannii* strains isolated from hospitalized

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patients in Kashan, Iran. To this aim, the occurrence of different genes encoding aminoglycoside-modifying enzymes and aminoglycoside resistance phenotypes was investigated.

Materials and Methods

This descriptive study was carried out in Beheshti Hospital (Kashan City, Isfahan Province, Iran) in 2008. It is a tertiary-care 500-bed general teaching hospital. Sixty isolates of *Acinetobacter* spp. from patients were enrolled in this study. Conventional biochemical tests were used for identification at the species level in 60 gram negative, short rods showing both a negative reaction on oxidase testing and the lack of lactose fermentation. The strains were isolated from blood (58.3%), urine (13.3%), cerebrospinal fluids (8.3%), the trachea (8.3%), sputum (8.3%), and pleural fluid (3.3%) samples. *A. baumannii* ATCC 19606 (11B) and *A. lwoffli* ATCC type (13A) strains were used as quality controls in each susceptibility determination. Antimicrobial susceptibility testing was performed on all 60 isolates according to the standard method established by the CLSIs (10). Imipenem (10μg), ciprofloxacin (5μg), levofloxacin (5μg), ceftazidime (30μg), sulbactam/ampicillin (10/10 μg), tazobactam/piperacillin (100/10μg), amikacin (30μg), gentamicin (10μg), tobramicin (10μg), SXT/TMP (1.25/23.75μg), doxycycline (30μg), and minocycline (30μg) disks (Becton Dickinson Microbiology Systems) were used. Multidrug resistance was defined in this analysis as resistance to three or more representatives of the following classes of antibiotics: quinolones (ciprofloxacin, levofloxacin), broad-spectrum cephalosporins (ceftazidime), beta-lactamase inhibitor/ beta-lactams (sulbactam/ ampicillin, tazobactam/ piperacillin), aminoglycosides (amikacin, gentamicin and tobramycin), tetracyclines (doxycycline, minocycline), trimetprim-sulfamethoxazole and carbapenems (imipenem).

All target genes and corresponding primers used for PCR amplification are listed in Table 1. For PCR, a 1:10 dilution of an overnight culture was boiled for 10 min. Then amplification was performed with 1:10 of this dilution as the DNA template. PCR conditions included 30 cycles of amplification under the following conditions: denaturation at 95° C for 30 sec, annealing at 50° C for 30 sec and then at 40° C for 40 sec, and cycling was followed by a final extension at 72°C for 30 min. PCR products were resolved on 2.0% agarose gels, stained with ethidium bromide, and photo-graphed by UV illumination. Either the 1-kb DNA ladder or the 100-bp DNA ladder (Bio NEER, Korea) was used to assess PCR product size. We investigated genes encoding aminoglycoside resistance genes including *aacC1* that confers gentamicin resistance, *aadA1* that confers streptomycin and spectinomycin resistance, *aadB* confers tobramycin, gentamicin, and kanamycin resistance, and *aphA6* that confer amikacin, gentamicin, kanamycin, and neomycin resistance.

Results

Acinetobacter spp. isolates were recovered from 60 patients including 35 men (58.3%) and 25 women (41.7%). The descriptive statistics of the study population are summarized in Table 2. The mean age of the population was 39.3 (±19.2) yr, with a range of 4 to 85 yr old. Table 3 summarizes the isolation sites and antibiotic resistance patterns found in this study. From the point of view of the hospital departments and type of specimen 86.7% (13/15) of isolates from ICU and 54.3% (19/35) of blood isolates were MDR. The Susceptibility test of *Acinetobacter* spp. isolates to different antibiotics in our hospital showed the wonderful high resistance rate to amikacin (80%) and tobramicin (68.3%). We found that *Acinetobacter* spp. was more sensitive to ampicillin/sulbactam than other tested antibiotics. From the 60 isolates, 40 (66.7%) were resistant to at least three classes of antibiotics and classified as multi-drug resistance (MDR). Surprisingly, among the MDR isolates 97.5% were resistant to amikacin, 80% to ceftazidime, 77.5% to ciprofloxacin, 75% to piperacillin/tazobactam, 70% to doxycycline, 65% to tobramicin and levofloxacin, 62.5% to trimthoprim/sulphamethoxazole, 37.5% to imi-

penem, and 30% to ampicillin-sulbactam and minocycline. Sixty-five percent of the isolates contained the phosphotransferase gene *aphA6* which confers to amikacin, gentamicin, kanamycin, and neomycin resistance, 63.3% of isolates contaied acetyltransferase genes *aacC1* that confers to gentamicin resistance, and 41.7% contained adenylyltransferase genes *aadA1* as streptomycin and spectinomycin resistance, and 3.3% of the isolates contained *aadB* that confers resistance to tobramycin, gentamicin, and kanamycin. 76.9% of isolates contained the *aphA6*, and 19 of 25 strains (76%) contained the *aadA1,* 28 out of 38 (73.7%) isolates with *aacC1,* and both of two isolates, which contained the aadB, were MDR. Table 4 shows the percentage of aminoglycosides resistance genes detected in *Acinetobacter* spp. strains according to the site of isolation. The strains carrying *aphA6* showed remarkably high level of resistance to amikacin 82.1% (32/39), ceftazidime 76.9% (30/39), ciprofloxacin 66.7% (26/39), and piperacillin/tazobactam 64.1% (25/39). In addition, 11 out of 40 MDR strains (27.5%) were resistant to all of the seven tested classes of antimicrobial agents. Table 5 shows the frequency of aminoglycosides resistance encoding genes detected in *Acinetobacter* spp. isolates in relation with antibiotics sensitivity patterns.

Table 1: The various Primers used for amplification of genes from *Acinetobacter* spp. Isolates

Primer name	Primer sequence (5′ to 3′)	Target gene(s)	Bp	Reference
aacC1-5	ATGGGCATCATTCGCACATGTAGG	aacC1	465bp	11
aacC1-3	TTAGGTGGCGGTACTTGGGTC			
aadA1-5	ATGAG GGAAGCGGTGATCG	aadA1	792bp	11
aadA1-3	TTATTTGCCGACTACCTTGGTG			
aadB-5	ATGGACACAACGCAGGTCGC	aadB	534bp	11
aadB-3	TTAGGCCGCATATCGCGACC			
aphA6 FOR	ATGGAATTGCCCAATATTATTC	aphA6	797bp	11
aphA6 REV	TCAATTCAATTCATCAAGTTTTA			

Table 2: The demographic characteristics of the study population

Parameter	No. (%) of patients	Mean±SD (range[minimum, Maximum])
Age (yr)	≤40 29(48.3) > 40 31(51.7)	39.27±19.20(81[4,85])
Sex (male/female)	35/25 (58.3/41.7)	
Hospital sampling ward		
Emergency room	24 (40)	
Internal medicine	15(25)	
ICU	15(25)	
Pediatrics	6(10)	

Table 3: Antibiotic resistance patterns of *Acinetobacter* spp. isolates according to the various specimen from the hospitalized patients in Kashan, Iran

Specimens	No. of isolates	CIP	LVX	CAZ	TOB	AMK	GEN	SXT	IPM	TZP	SAM	MIN	DOX
Blood stream	35	15	9	16	25	27	8	12	8	12	5	14	17
Cerebrospinal fluid	5	3	4	3	4	5	3	4	1	3	3	3	3
Urine	8	5	4	5	6	5	4	6	4	5	2	5	6
Trachea	5	5	5	5	2	5	5	2	1	5	1	3	1
Sputum	5	3	3	5	3	4	3	4	1	4	1	3	2
Pleural fluid	2	2	1	2	1	2	1	1	0	2	0	1	1
All	60	33	26	36	41	48	24	29	15	31	12	29	30

ciprofloxacin, CIP; levofloxacin, LVX; ceftazidime, CAZ; tobramycin, TOB; Amikacin, AMK; gentamicin, GEN; trimethoprim-sulfamethoxazole, SXT; imipenem, IPM; piperacillin-tazobactam, TZP; ampicillin-sulbactam, SAM; minocycline, MIN; doxycycline, DOX

Table 4: The percentage of aminoglycosides resistance gene detected in *Acinetobacter* spp. strains according to the site of isolation

Genes	Blood stream	Cerebrospi-nal fluid	urine	Trachea	Sputum	Pleural fluid	Gene detection in isolates No. (%)
aphA6	19	4	7	5	3	1	39(65)
aacC1	21	3	6	3	4	1	38(63.3)
aadA1	11	2	5	3	3	1	25(41.7)
aadB	0	0	1	1	0	0	2(3.3)

Table 5: Frequency of aminoglycosides resistance encoding genes detected in *Acinetobacter* spp. isolates in relation with antibiotics sensitivity patterns

Antibiotics	*aphA6* (No. 39)			*aacC1* (No. 38)			*aadA1* (No. 25)		
	S	I	R	S	I	R	S	I	R
Amikacin(30µg)	3	4	32	3	4	31	1	2	22
Gentamicin(10µg)	16	-	23	18	-	20	12	1	12
Tobramicin(10µg)	5	12	22	5	10	23	1	6	18
Tazobactam/ Piperacillin (100/10µg)	10	4	25	12	5	21	5	5	15
Sulbactam/Ampicillin(10/10µg)	18	10	11	23	8	7	14	5	6
Ceftazidime(30µg)	3	6	30	4	9	25	2	6	17
Ciprofloxacin(5µg)	4	9	26	3	9	26	1	10	14
Levofloxacin(5µg)	15	-	24	17	-	21	12	-	13
Imipenem(10µg)	26	1	12	28	1	9	17	-	8
Doxycycline(30µg)	3	13	23	4	12	22	1	10	14
Minocycline(30µg)	3	12	24	1	11	13	3	14	21
SXT/TMP(1.25/23.75µg)	2	13	24	3	16	19	-	11	14

S: sensitive, I: intermediate, R: resistance

Discussion

From the total of the 60 *Acinetobacter* spp. isolates in the present study, 48 (80%) were identified as *A. baumannii*, 10% *A. lwoffli* and 10% as other genomic species of the *Acinetobacter*. In general, the investigated isolates showed some resistance or decreased susceptibility phenotype mostly to all of the tested antimicrobial agents. Aminoglycoside resistance is common in *Acinetobacter* and primarily results from inactivation of the antibiotic by specific modifying enzymes such as acetyltransferases, phosphotransferases, and adenylyltransferases (3). Many reports have documented the high rates of antibiotic resistance found in *Acinetobacter* spp. These organisms are frequently resistant to multiple antimicrobial agents and recently, there are several reports on strains resistant to virtually all clinically relevant drugs (12, 13). Differences in antibiotic susceptibility have been observed between countries, probably as a result of environmental factors and different patterns of antimicrobial usage. Gaur et al, report more than 80% of isolates to be insusceptible to second and third-generation cephalosporins, aminoglycosides, and quinolones (14).

The presence of high frequent *aphA6*, *aacC1* and *aadA1* genes are in agreement with the previously published data on clinical isolates of *Acinetobacter* spp. (15). The presence of at least one of the following aminoglycosides resistance gene was detected in 40 (66.7%) MDR strains: *aphA6* (n = 30), *aacC1* (n= 28), *aadA1* (n= 19) and *aadB* (n= 2). In this study, 21.7% of the strains have been observed to contain three aminoglycoside resistance genes (*aphA6*, *aacC1* and *aadA1)*. In the present study, bloodstream infections were the most common clinical specimen of *Acinetobacter spp.* The frequency of isolation and variety of clones isolated bacteria found in clinical specimens in different countries widely varies (16, 17). Potential

risk factors for colonization or infection of hospitalized patients with multidrug-resistant *Acinetobacter* strains include length of ICU stay, underlying diseases, or conditions, exposure to carbapenems or third-generation cephalosporins, hospital size and using urinary catheterization (18, 19).
In conclusion, the present study showed that the emergence of *Acinetobacter* spp. resistance to antimicrobial agents in our hospital is associated with the spread of MDR strains. In summary, more than 60% of MDR *Acinetobacter* spp. isolated from patients in our hospital were resistant to aminoglycosides, broad-spectrum cephalosporins, piperacillin/tazobactam, doxycycline and trimthoprim/sulphamethoxazole. Aminoglycosides resistance in *Acinetobacter* spp. has emerged as a significant health problem, leaving limited options for antimicrobial therapy. The findings showed that clinical isolates of *Acinetobacter* spp. in our hospital carrying various kinds of aminoglycoside resistance genes.

Ethical Consideration

All Ethical issues (such as informed consent, conflict of interest, plagiarism, misconduct, co-authorship, double submission, etc) have been considered carefully.

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