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Study of *Mycoplasma* and *Ureaplasma* Species as Contaminats of Cell Cultures

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Abstract

Mycoplasma and *Ureaplasma* species are the most serious contaminants of cell cultures and this remains one of the major problem encountered in biological research using cell cultures. The aim of this study was to investigate the rate of *Mycoplasma* and *Ureaplasma* species as contaminats of cell cultures.

A total of 100 different cell culture specimens including, R228, Hela, Vero, MDCK, L20, RD and Hep2 were collected. Then the specimens were investigated by culture method and biochemical tests.

The rate of contaminant cell culture specimens was 32%. The highest and lowest rate of contamination was observed among Hela cell culture (25%) and R238 cell culture (0%), respectively. Moreover, the results of $\chi 2$ statistical analysis test, showed significant difference between contamination rate and kind of cell culture (p = 0/001).

It is concluded that *Mycoplasma* and *Ureaplasma* species are the most common source of further contamination of cell cultures.

Keywords: *Mycoplasma, Ureaplasma,* Contamination, Cell cultures

Introduction

Contamination is now observed frequently in many continuous cell lines of human or animal origin, in primary humans, animals, or insect cell lines and in such other cultured cells as hybridomas. The occurrence, detection and control of such infections have been reviewed extensively (5, 9). The extent of the contamination is variable, depending upon geographical location and quality control endeavours, but is estimated to occur in about 10-25% of all cell lines examined. Six Mycoplasmas currently are responsible for almost all the reported infections, 3 species of animal origin (*M. arginini, M. hyorhinis* and *A. laidlawii*) and 3 species found in humans (*M. orale, M. salivarium* and *M. fermentans*). Cell infections with animal Mycoplasmas or acholeplasmas can usually be traced to contaminated serum (usually fetal bovine) employed in cell culture media. Contamination with Mycoplasmas of human origin usually comes from breaks in sterility within the cell culture laboratory that allow normal human respiratory tract organisms (*M. orale and M. salivarium*) into cell lines, or to an infection of human primary lymphocyte or macrophage cells with *M. fermentans* (14).

The purpose of this study was to investigate the rate of *Mycoplasma* and *Ureaplasma* as contaminats of cell cultures.

Materials and Methods

In the present study 100 different cell culture specimens including Hep2, L20, RD, MDCK, Vero, Hela and R288 were collected from the department of pathobiology, School of Public Health and Institute of Public Health Research, Tehran University of Medical Sciences during 2000-2001. In the bacteriology laboratory, PPLO broth media containing specimens filtered by 0.5 Mm pore size filter paper (Millipore) and inoculated in to the special PPLO broth and PPLO agar media, that comprises a beef heart infusion broth supplement with fresh yeast extract (10% V/V; 25% Wt /V), horse serum (20% V/V), phenol red (0.002% and 1% urea, arginine and or glucose (Difco). Then these cultured media were incubated at 37°C, under 5% Co2 (3, 12, 15). After sufficient incubation, on PPLO agar the colonies of *Mycoplasma* and *Ureaplasma* species and in PPLO broth metabolizing urea (*Ureaplasma urealyticum*), arginine (*M. hominis, M. primatum, M. orale, M. salivarium* and *M. spermatophilum*), glucose (*M. pneumonia* and *M. genitalium*) and or both of arginine, and glucose (*M. fermentans* and M. *denetrans*) were investigated. (4, 8, 13).

Results

Table 1 shows the rate of different cell cultures contamination. The highest rate of contamination was among Hell and vero cell cultures (8%) and the lowest, found in R288 cell culture (0%). The rate of contaminants of cell cultures on the basis of metabolizing urea, arginine and glucose, are shown in table 2. The results of $\chi 2$ statistical analysis test, showed significant difference between the contamination rate and kind of cell culture ($\chi 2$ = 24.15, df = 6, P = 0.001).

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Table 1 : The rate of different cell cultures contamination

Cell cultures	Contamination				Total (n = 100)	
	Positive (n = 32)		Negative (n = 68)			
	N	%	N	%	N	%
Hela	8	25	22	32.4	30	30
Vero	8	25	0	0	8	8
Hep2	2	6.25	16	23.5	18	18
R 288	0	0	2	2.9	2	2
MDCK	6	18.8	6	8.8	12	12
L 20	4	12.5	12	17.6	16	16
RD	4	12.5	10	14.7	14	14

Table 2 : The rate of contaminants of cell cultures on the basis of their reaction in 4 key differential tests

Cell cultures	Biochemical Tests (positive)									
	Arginine (n = 18)		Glucose (n = 6)		Urea (n = 6)		Arginine+Glucose (n = 2)		Total (n = 32)	
	No	%	No	%	No	%	No	%	No	%
Hela	6	33.3	0	0	2	33.3	0	0	8	8
Vero	4	22.2	2	33.3	2	33.3	0	0	8	8
Hep2	2	11.1	0	0	0	0	0	0	2	2
R 288	0	0	0	0	0	0	0	0	0	0
MDCK	4	22.2	0	0	0	0	2	100	6	6
L 20	0	0	4	66.6	0	0	0	0	4	4
RD	2	11.1	0	0	2	33.3	0	0	4	4

Discussion

Mycoplasma and *Ureaplasma* species are the most serious contaminants of cell cultures. Its detection is as important as its eradication in virology, (6). Acquisition of mycoplasmas is generally through direct host to host contact, or through a number of secondary transmissions, including aerosols or fomites, food or water, insect vectors or carriers, and nosocomial acquisition (organ or tissue transplants) (14).

Several reviews in recent years have emphasized the problems created by *Mycoplasma* contamination of cultured cells. Because of the hazards of interpreting data derived from Mycoplasmas contaminated cells, most cell biologists

routinely screen their cultures for the presence of these organisms (11). It is reported that 20-90% of cell cultures worldwide are contaminated with one or more species of *Mycoplasma* (1, 2, 6, 7, 10). Our finding showed that 32 cell cultures (32%) were contaminant and these results are nearly in accordance with some above mentioned reports.

The high rate of contamination found calls for the adoption of measures for the prevention of this hazard: The elimination of mouth pipetting, the use of aseptic techniques and rigid control of trypsin, serum and other components of cell culture media. The substitution of Mycoplasmas-free cultures for all contaminated ones and the performance of periodical tests for Mycoplasmas detection must also be carried out to prevent and avoid the dissemination of these organisms.

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