MOLECULAR CHARACTERIZATION OF *MYCOPLASMA* CONTAMINATING TISSUE CULTURES

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ABSTRACT

One of the main problems in cell culture unit is mycoplasma contamination. It can extensively affect cell physiology and metabolism. As the applications of cell culture increase in research, industrial production, cell therapy and its importance for diagnostic routine work in selected labs, so more concerns about mycoplasma contamination will arise. In our study, a total of 50 cell cultures from Animal Health Research Institute, Dokki, Giza., VACSERA and Veterinary Serum & Vaccine institute Abbasia were monitored for mycoplasma using culture and PCR methodology. The contamination was detected in the cell culture collected from all laboratories. Mycoplasmas were detected by culture in 29/50 (58%) of the cell culture samples which subsequently identified with PCR using Mycoplasma group specific primer detecting all species of mycoplasma which gave bands at 280 bp in all positive culture samples, the most frequent species was *M. arginini* (30%), followed by *M. orale* (28%). MDBK were positive only for *M. arginini* which gave band at 326 bp, while VERO and BHK cells were infected only with *M. orale* which gave bands at 87bp. Awareness about the sources of mycoplasma and pursuing aseptic techniques in cell culture unit along with reliable detection methods of mycoplasma contamination can provide an appropriate situation to prevent mycoplasma contamination in cell culture.

Keywords: *Mycoplasma, Tissue Culture, contamination, PCR.*

INTRODUCTION

Mycoplasma is the smallest free-living organisms that, unlike other bacteria, lack a cell wall. The outer layer is instead, a three layered membrane containing sterols. Diameters of these organisms may range from 0.2-0.3 µm and, due to their plasticity, are able to pass through the pores of a 0.2 micron filter with applied pressure. Because the morphology of Mycoplasma is pleomorphic, they occur as two different structural forms during a life cycle: coccidian, a spherical or spheroidal shape, and filamentous, resembling rods. (Davis, 1994).

Each batch of live viral vaccine, each lot of master seed virus (MSV), each lot of primary and master cell stock (MCS), and all ingredients of animal origin not steam sterilized should be tested for the absence of mycoplasmas. Solid and liquid media that will support the growth of small numbers of tested organisms such as typical contaminating organisms *Acholeplasma laidlawii*, *Mycoplasma arginini*, *M. fermentans*, *M. hyorhinis*, *M. orale*, and *M. synoviae* should be used. The nutritive properties of the solid medium should be such that no fewer than 100 CFU should occur with each tested organism when approximately 100–200 CFU are inoculated per plate. An appropriate colour change should occur in the liquid media when approximately 20–40 CFU of each tested organism are inoculated. The ability of the culture media to support growth in the presence of product should be validated for each product to be tested, and for each new batch or lot of culture media. (OIE Terrestrial Manual, 2012).

The field of tissue culture has developed very rapidly during recent years and tissue culture methodology has become a useful research tool in many branches of science. Animal tissue culture is concerned with the study of cells, tissues and organs explanted from animals and maintained or grown in vitro for more than 24 hours. Dependent upon whether cells, tissues or organs are to be maintained or grown, two methodological approaches have been developed in the field of tissue culture, cell culture and tissue or organ Culture.

The application of cells in research laboratories (Nikfarjam *et al.,* 1999; Isaian *et al.,* 2003), regenerative medicine and biotechnological productions is growing extensively. Cells are used in wide-ranging activities from studies on cell proliferation to the production of biologically active substances. Due to restrictions on the use of laboratory animals by animal protection laws, the use of cell cultures will continue to increase in the future.

The importance of viral isolation as a diagnostic method still remains necessary because it is the only technique capable of providing a viable isolate that can be used for further characterization, such as with phenotypic antiviral susceptibility testing. Because no one cell culture type can support the growth of all medically relevant viruses, virology laboratories must maintain several different cell culture types. Barth and Melvin (2000).

In order to achieve reproducible results from cells, good cell culture conditions are vital. Despite the importance of bacterial and fungal contaminations in cell culture, they are not such a serious problem because they are usually obvious and easily detected. The most serious problem is mycoplasma contamination since these microorganisms are subtle (Hay,1986 and Harlin and Gajewski, 2008).

Mycoplasma contamination of cell cultures is a serious problem across the world because of the infection of cell cultures with mycoplasma can have different cytogenetic effects (Rottem and Barile, 1993). Mycoplasmas usually adhere to cells but, depending on the species, may fuse with the host cell or even invade it (Balish *et al.,* 2002; Dimitrov, 1993 and Lo *et al.,* 1993). These bacteria deplete the nutrients of cell cultures and interfere with the response of these cells when challenged experimentally (Miyazaki *et al.,* 1990). Interruption of cell metabolism (Pollack *et al.,* 1997), modulation of the immune response (Chambaud *et al.,* 1999 and D'Orazio *et al.,* 1996), modification of cellular morphology, interference with viral replication, chromosome modifications, or cell transformation may occur (Razin *et al.,* 1998). The identification of these phenomena in accidental or experimental infections may contribute to the understanding of the relationship between mycoplasmas and the host cell (Rottem, 2003).

This distress contamination effects on eukaryotic cells represented in two significant problems to the culture of mammalian cells used for research as they can alter every cellular parameter leading to unreliable experiment results and potentially unsafe biological products (vaccines), first is Mycoplasma contaminate cell lines will produce much poorer yield of final cell numbers as they do not grow as high cell density as normal cells. Freshney (1994).

Second Mycoplasma also cannot detect by visual inspection using a normal light microscope and thus can remain unnoticed in cellular cultures for long period of time because mycoplasma are the smallest organism their genome size range from 0.6-1.3 mega base size characterized by lacking cell wall that are capable of self-replication and cause various disease in human and animals and plants. Razin (1996).

There are Different sources for the spreading of mycoplasma in the laboratory such as, a laminar flow hood during a routine subculturing procedure, Media, sera or reagents contaminated with mycoplasma, Nonsterile supplies, media and solutions, laboratory personnel, incubators, improper sealing of culture dishes. (Barile, 1973; McGarrity, 1976; Polak-Vogelzang *et al.,* 1990 and Hay, 1991).

Robinson and Wichelhausen (1956) reported the first isolation of mycoplasma from a cell culture. Since then, mycoplasma had become recognized as a major culture contaminant. Published mycoplasma test results for cell lines during this time demonstrated rates of mycoplasma contamination between 57% to 92% (Barile, 1973).

There are currently more than 183 species in 8 genera, many of which are pathogenic (Manual of Clinical Microbiology, 2003). The vast majority of cell culture contaminants belong to only 6 species primarily of human, bovine or porcine origin. Of these six species, *M. orale* and *M. hyorhinis* are the most common historically, accounting for over 50% of all mycoplasma contaminated cultures (McGarrity *et al.,* 1979 and Barile *et al*., 1973).

As mycoplasma species grow extremely slow in tissue culture medium and do not destroy the cell lines, they can persist in cell cultures for an indefinite time without being noticed. Therefore, fast and reliable methods are required to detect mycoplasma contamination. Out of a vast array of techniques toward the detection of mycoplasma contamination of cell cultures, polymerase chain reaction (PCR)- based detection methods have become more popular, as they are not only highly sensitive and specific, but also simple, rapid, efficient, and cost effective (Drexler and Uphoff, 2002 and Kalhor *et al.*, 2014).

The purpose of the present study was to introduce molecular characterization for mycoplasma species contaminated tissue culture as well as awareness about the sources of mycoplasma and pursuing aseptic techniques in cell culture unit along with reliable detection methods of mycoplasma contamination to provide an appropriate situation to prevent mycoplasma contamination in cell culture.

MATERIAL and METHODS

1- Samples: A total of 50 cell culture of animal origin samples were studied, including 23 Madin Darby Bovine Kidney (MDBK) Cells samples, 13 Baby Hamster Kidney (BHK) Cells samples, 13

Green Monkey Kidney Cells (VERO) and one primary cell culture sample, these cell cultures were tested for potential mycoplasma contamination These cell lines were supplied from three sources:-

1- Tissue Culture Unit, Virology Department, Animal Health Research Institute, Dokki, Giza.

2- The Holding Company for Biological Products and Vaccines (VACSERA) Dokki, Giza.

3- Veterinary Serum and Vaccine institute Abbasia–Cairo.

These cell monolayer's were propagated using Eagle's MEM with Earl's salts or with Hanks salts and 10% sterile newborn calf serum or 10% fetal calf serum. They distributed in 75ml sterile tissue culture flasks.

2- Media used for propagation of tissue cultures:

2.1-Newborn Calf Serum: Supplied by GIBCO-Newzeland (STERILE.A), Invitrogen Corporation, (500 ml / Bottle), Stored at -20 °C.

2.2- Fetal Calf Serum: Supplied by Biowest- South America, (Vol.: 100 ML), Stored at -20°C.
Fetal and newborn calf sera were essential for preparation of all types of media, as well as considered one of the potential source for mycoplasma contamination of T.C.

2.3- Minimum Essential Medium (Eagle's):
Supplied by Sigma, USA with Hank's salt's and L.glutamine and without Sod. Bicarbonates, kept at 2 - 8 °C.

2.4- Trypsin versine:

2.4.1. Trypsin:-
Supplied by Sigma, (1: 250).

2.4.2. Versine:
Supplied by WINLAB as power form.

2.5. Stock antibiotic solution: It was prepared as 1,000,000 IU Penicillin G Sodium, 1 g Streptomycin sulphate and 1g mycostatin dissolved in 100 ml HBSS. 1% of this solution was added to cell culture media and solutions to yield a final concentration of 100 IU of penicillin G. Sodium, 100 µg of streptomycin sulphate and 100 µg mycostatin /1 ml.

2.6. Sodium bicarbonate solution (4.4%): 4.4 g $NaHCO_3$ dissolved in100 ml double distilled water and sterilized by autoclaving. It was used to adjust the required pH of the cell culture media and solutions.

3- Media used for cultivation and isolation of Mycoplasma:

3.1- Liquid and solid media for the isolation and propagation of mycoplasma were prepared as described by Sabry and Ahmed (1975).

3.2- Digitonin sensitivity test was done for the obtained isolates according to Erno and Stipkovits (1973).

3.3- Biochemical characterization was carried out by glucose fermentation and arginine deamination tests as described by Erno and Stipkovits (1973). Film and spot formation medium (Fabricant and Freundt, 1967).

4- Serological identification was conducted by growth inhibition test as described by (Clyde (1964). Standard antisera - locally prepared.

5) Polymerase chain reaction:

a) Preparation of samples for DNA extraction (Yleana *et al.*, 1995)*:* 5ml of a 24 hour broth cultures of isolates were centrifuged for 10 minutes at 12000 r.p.m. The pellet was washed twice in 1 ml of phosphate buffered saline pH 7.2 (PBS) and suspended in 50 µl PBS. The cell suspension was heated directly at 100°C for 10 min. in a heat block to break the cell membranes, and then cooled on ice for 5 min. Finally, the cell suspension was centrifuged for 5 min. and the supernate containing chromosomal DNA was collected and stored at -20°C until used.

b) Oligonucleotide primers: The oligonucleotide primers used, including previously published primers, primer specificities, and expected amplicon lengths are shown in Tables 1.

c) Procedure for DNA amplification: PCR amplification was performed in 50 µl reaction mixture consisting of 5 µl of 50 ng M. agalactiae genomic DNA, 10 µl of 10 x Taq buffer (10mM tris-HCl [pH 8.8], 50 mM KCl), 1 µl of 50 pM of each primer, 1.5 mM MgCl2, 1 µl of 2U of Taq thermos Table DNA polymerase, 1 µl of 50 uM of each dNTP, and 31µl of DNase- RNase- free, deionized water. DNA amplification was carried out in PTC-100 programmable thermal controller (MJ, Research Inc.). The thermal profiles were as follows: Denaturation at 94 ^{o}C for 45 seconds, primer annealing at 60°C for 1 min., and extension at 72°C for 2 min. The amplifications were performed for 30 or 35 cycles with a final extension step at 72^{o}C for 3 min. After the reaction, the amplified DNA was electrophoresed on 1.5% agarose gel for 90 min. at 100 volts, DNA Ladders: 100 bp (Pharmacia), Cat. No. 27-4001-01 and 50 bp (Promega), Cat. no. G316A, USA were added then stained with ethidium bromide.

The resulting PCR product was separated by electrophoresis in 1.3% agarose gel, stained with ethidium bromide, visualized by UV transillumination and documented by photography. Image analysis was made by ImageQuantTL-V2003.03 (Amersham Biosciences).The length of the amplification products were 87 to 1000 bp depending on mycoplasma species (Table 1, Photo 8-11).

Table 1: The used oligonucleotide primers, primer specificities, thermal cycle, expected amplicon lengths and the method reference.

	Mycoplasma Species	Forwarded primer	Reverse primer	product size (bp)	Thermal cycle	Reference
1	**The mycoplasma group-specific primer set**	GPO-3 (5'-GGG AGC AAA CAG GAT TAG ATA CCC T-3')	MGSO (5'-TGC ACC ATC TGT CAC TCT GTT AAC CTC-3')	280-bp	The thermal profile consisted of 40 cycles of denaturation at 94°C for 1 min, primer annealing at 55°C for 1 min, and primer extension at 72°C for 2 min.	**Van Kuppeveld *et al.* (1994)**
2	**Sequence of 16S common gene for Mycoplasma**	MunivF 5'- AGA CTC CTA CGG GAG GCA GCA -3'	MunivR 5' ACT AGC GAT TCC GAC TTC ATG 3'	1000-bp	an intial denaturation step at 94 ºC for 5 min., followed by35 cycles of denaturation at 94 ºC for 1 min, annealing at 55 ºC for 1 min., and extension at 72 ºC for 1.5 min. A final extension step at 72 ºC for 10 min	**Alberto *et al.* (2006**)
3	***M. arginini***	TGA TCA TTA GTC GGT GGA GAG TTC	TAT CTC TAG AGT CCT CGA CAT GAC TC	326-bp	initial denaturation at 94 _C for 3 min, 32 cycles consisting of denaturation at 94 ºC for 60 s, annealing at 60 ºC for 30 s and extension at 72 ºC for 60 s, followed by a final extension at 72 ºC for 5 min.	**Vahid *et al.*** (**2009**)
4	***M. orale***	GCA AAG CTA TAG AGA TAT AGT A	GTC CTG CAA CGA GCG CAA C	87-bp	10 min initial denaturation at 95 °C, followed by 40 cycles with 15 second at 95 °C C and 60 second at 60°C.	**Karin *et al.* (2014)**
5	***M. bovis***	MboF 5'-CCT TTT AGA TTG GGA TAGCGGATG-3'	MboR 5-CCGTCAAGGTAG CGT CAT TTCCTAC-3	360-bp	Denaturation at 94 ºC for 45 seconds, primer annealing at 60 ºC for 1 min., and extension at 72 ºC for 2 min. the amplifications were performed for 30 or 35 cycles with a final extension step at 72 ºC for 3 min.	**Chávez González *et al.* (1995)**

RESULTS

Mycoplasma contamination of cell cultures is a serious problem across the world because of the infection of cell cultures with mycoplasma can have different cytogenetic effects. The cell culture were examined for any changes or effects before uses, due to mycoplasma contamination many cytogenetic effects were detected, such as decrement of viability, detachment of adherent cells from the cell culture vessel surface (Photos 1&2), inhibition of proliferation, cell growth interference, morphological changes (Photos 3&4). As well as severe cytopathic effects characterized by stunted, abnormal growth and rounded, degenerated cells (Photos 5&6) were also detected.

A total of 50 cell culture samples were monitored for potential mycoplasma contamination using culture and PCR methodology. Mycoplasmas were detected by culture in 29/50 (58%) of the cell culture samples. The cultured mycoplasmas presented "fried egg" colonies (Photo7), shifted the pH of the broth without turbidity and were subcultured after filtration. The mycoplasma reference strains grew on solid and liquid medium and were confirmed at the species level by PCR. Table 2, Photo 8-11 shows the general results obtained for the samples evaluated by culture and PCR.

Infected cultures were initially identified using common primers detecting all species of mycoplasma "MGSP" Mycoplasma group specific primer which provided by GPO and MGSO primers, as described in the Materials and Methods. Which gave bands at 280 bp (Photo 8) in 29/50 (58%) of the cell culture samples.

As MDBK of bovine origin, so we make PCR for it with a common primers of bovine (16S rRNA) which give positive band at 1000 bp (Photo 9), so it may be *M. bovis* or *M. arginini*. Making PCR using *M. bovis* and *M. arginini* primers as described in the Materials and Methods gave a positive band only for *M. arginini* at 326 bp (Photo 10), while negative for *M. bovis* with a frequenting of 15/50 (30%) of the cell culture samples, which subsequently indicate that MDBK infected with only one *Mycoplasma* species *M. arginini*.

On the other hands, the infected VERO and BHK cells gave positive with a common primers "MGSP" but were negative with 16*S* r*RNA* primers of ruminant origin and positive for *M. orale* which gave bands at 87bp (Photo 11) in 14/50 (28%) of the cell culture samples, indicating that VERO and BHK also infected only by *M. orale*. The most frequent mycoplasma species was *M. arginini* (30%), followed by *M. orale* (28%).

Table 2: Results of isolation and biochemical of tissue culture samples.

Type	Samples		Positive samples by isolation		Digitonin test	Glucose fermentation
	No	%	No	%		
MDBK	23	46	15	30	+VE	-VE
BHK	13	26	8	16	+VE	+VE
VERO	13	26	6	12	+VE	+VE
Original media (primary)	1	2	0	0	- VE	- VE
Total	50		29	58		

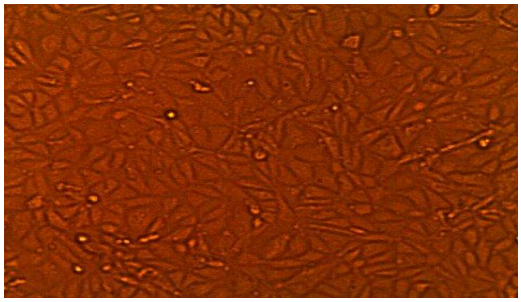

Photo 1: Normal MDBK Cell Line (Negative)

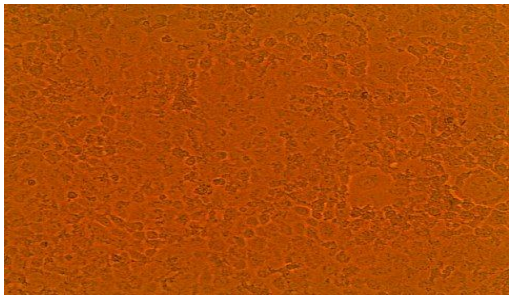

Photo 2: Vacculations and Detachment of MDBK cell layer

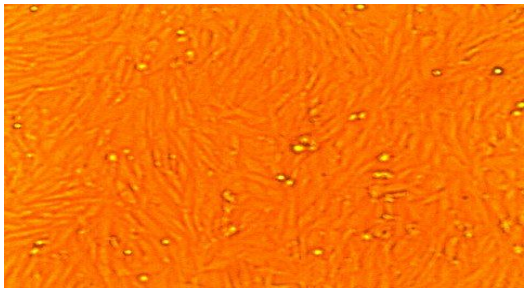

Photo 3: Normal BHK Cell Line (Negative)

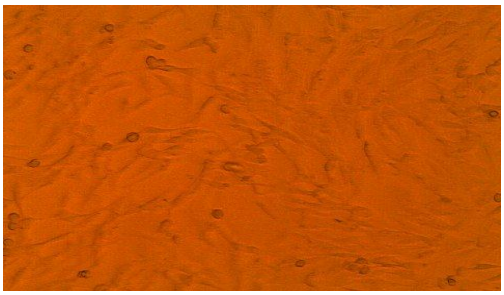

Photo 4: Deviation from normal fibroblastic morphology (24 hours).

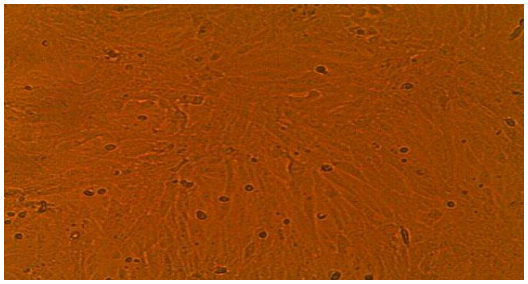

Photo 5: Normal Vero Cell Line (Negative)

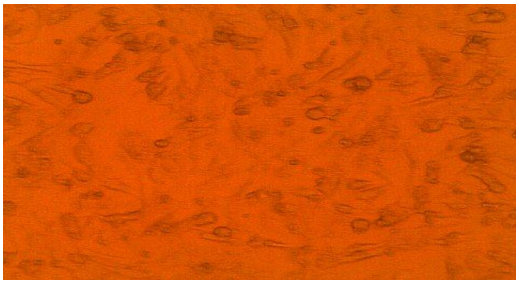

Photo 6: Loss of characteristic spindle shape of normal Vero cells, some rounding and finally, detachment (48 hours)

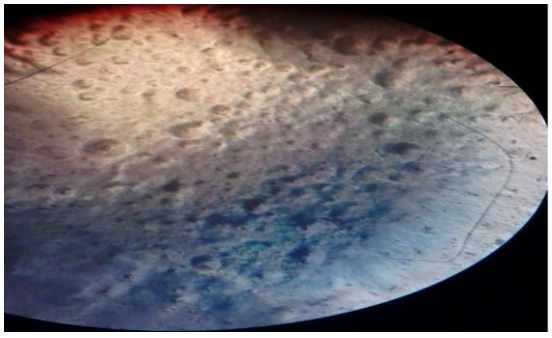

Photo 7: production of "fried egg" colonies on agar plates

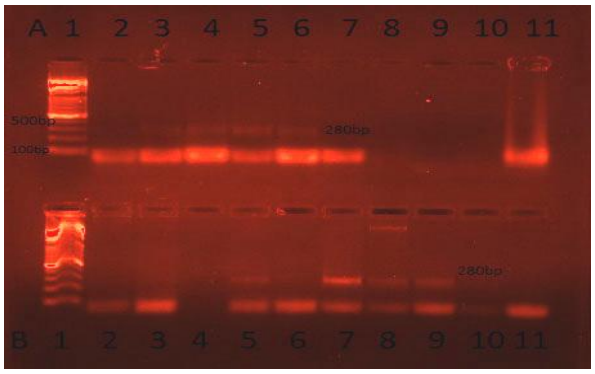

Photo 8: Above **(A):** lane 1: DNA 100bp marker, lane 2: negative control, lanes 3: positive control, lanes 4-7: positive samples at 280bp band, lanes 8, 9&11: negative samples. Down **(B):** lane 1: DNA 100bp Marker, lanes 5, 7, 8&9: positive samples, lanes 2, 3, 4, 6, 10&11: negative samples using the *mycoplasma* group-specific primer set, "MGSP".

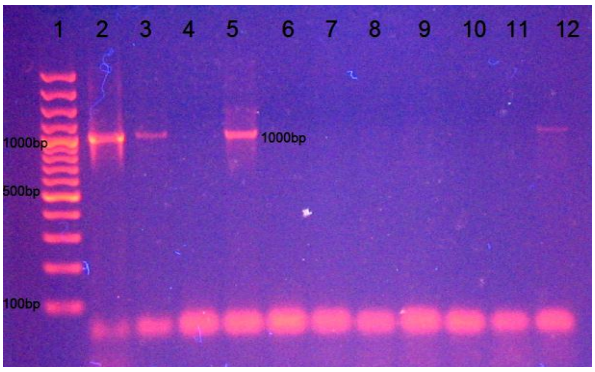

Photo 9: lane 1 DNA 100bp Marker, lane 2: positive control, lane 4: negative control, lane 3,5&12: positive samples 1000bp band, lanes 6-11: negative samples by 16S rRNA common gene for Ruminant *Mycoplasma.*

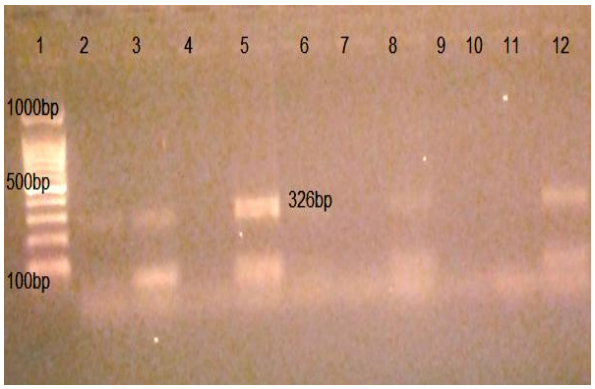

Photo 10: Lane 1: DNA 100bp Marker, lane4: negative control, lane 5: positive control *M. arginine*, lane 2,3,8&12: positive sample, lanes 6,7,9,10&11: negative samples using specific primer for *M. arginine*.

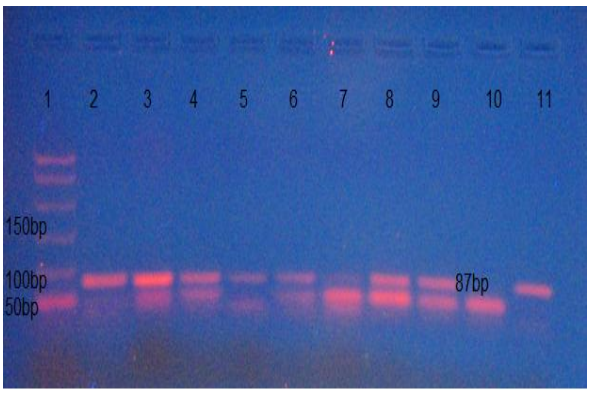

Photo 11: Lane 1: DNA 50bp Marker, lanes 2-9&11: positive samples, lane 10: negative sample using specific primer for *M. orale.*

DISCUSSION

Mycoplasma is the most important and common source of cell culture infection, worldwide. Unlike bacterial and fungal infections which are readily identified by microscopic and visual inspections, mycoplasma infected cultures are hardly, if ever, detected by these routine inspections (Hay, 1986 and Harlin and Gajewski, 2008).

Accidental infection of cell cultures with mycoplasmas, especially when unnoticed, usually invalidates the results of biomedical researches. The cell cultures may not die, but remain altered and inadequate for experimentation for a long period of time (Fleckenstein and Drexler, 1996 and Drexler *et al.*, 2002).

Mycoplasma-infected cell lines are themselves the single most important source for further spreading of the contamination. This is due to the high concentration of mycoplasmas in infected cultures, and the prolonged survival of dried mycoplasmas (Drexler and Uphoff, 2002). Operator-induced contamination is also a potential issue. Mycoplasmas spread by using laboratory equipment, media or reagents that have been contaminated (Drexler and Uphoff, 2002).

The behavior of mycoplasmas in cell culture is different; therefore, no consistent effects have ever been reported. The activity of arginine deiminase as well as uptake and depletion of the growth medium by mycoplasmas can inhibit the cell proliferation and induce apoptosis in cell lines.

Reduction of arginine will result in abnormality of growth rate, decrement of viability, detachment of adherent cells from the cell culture vessel surface and granulation of cells. Moreover, chromosomal aberration will happen due to the lack of arginine as a major component of the histone in the nucleus (Ben-Menachem *et al*., 2001). Chromosome breakage, multiple translocation events, and numerical chromosome changes are other effects of different species of mycoplasma on cell cultures (McGarrity *et al.,* 1984).

Generally, the side effects of mycoplasma contamination on cell cultures are:
1- Inhibition of proliferation, cell growth interference, morphological changes.

2- Severe cytopathic effects (CPE) characterized by stunted, abnormal growth and rounded, degenerated cells.

3- Amino and nucleic acid metabolism alterations, Increment in cell death.

4- Fragmentation of DNA, Chromosomal aberrations.

5- Change of gene expression patterns.

6- Morphological features of apoptosis (Sokolova *et al*., 1998).

The presence of mycoplasmas in cell cultures has also different side effects including loss of time, money, valuable cells and misleading publications, besides personal embarrassment and biosafety concerns (Nikfarjam and Farzaneh, 2011).

Several methods for the detection of mycoplasmas in cell cultures have been reported. The use of two methods has been the most recommended strategy to minimize false results. PCR in combination with culture is the most widely recommended procedure (Uphoff and Drexler, 2002).

The isolation of more than one mycoplasma species is time consuming even for a specialized laboratory. In this respect, PCR permits not only the diagnosis of mycoplasmas in cell cultures but also allows the determination of the distribution of species (Smith and Mowles, 1996). PCR permits the detection of femtogram amounts of mycoplasma DNA, corresponding to one bacterial cell. However, the sensitivity of specific PCR varies from species to species.

In a study by Timenetsky *et al.* (2006), *M. orale, M. hyorhinis* and *A. laidlawii* were found to be the most frequent species in cell cultures. While Uphoff and Drexler (2002) observed most of their cell lines were contaminated with *M. fermentans*, in our study, however, the most frequent species was *M. arginini* (30%), followed by *M. orale* (28%).

M. arginini has a wide host range as they have been isolated from mammals, birds and insects (Drexler and Uphoff, 2002). *M. fermentans* was believed to be associated with the development of AIDS in HIV-positive individuals in 1986. However, the species is now considered to be a normal inhabitant of the human urogenital tract (Blanchard and Montagnier, 1994). Therefore, the increase in the frequency of *M. fermentans* in cell cultures can be explained by the growing use of human blood cells or tissues for primary culture (Timenetsky *et al.,* 2006). The high incidence of the species *M. arginini* seems to be in direct correlation with the use of fetal or newborn bovine serum (Barile and Rottem, 1993). *M. orale* inhabits the human oropharynx and in addition to cell line cross-contamination may infect cell cultures through the aerosol generated by mouth pipetting (Razin *et al.,* 1998), although this practice is now prohibited in most cell culture laboratories.

In summary, mycoplasmal contamination status of 50 cell lines including different mammalian cell types deposited in the different institutes in Egypt was obtained using PCR-based assay that allowed detection and identification of mycoplasmal contaminants in different types of cell lines with high sensitivity showed that *M. arginin* and *M. orale* were the most Mycoplasmal contaminant of cell culture. Due to the high Mycoplasmal contamination recovery 29/50 (58%) in this study, which had personal embarrassment and biosafety concerns, regular microbiological examination for mycoplasma contaminating cell culture should be necessary methods for determination of quality and safety of tissue culture.

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التصنيف الجزيئي للميكوبلازما الملوثة لخلايا الزرع النسيجي

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التلوث بالميكوبلازما واحدة من اهم المشاكل الرئيسية في وحدة زراعة الخلايا. التي يمكن أن تؤثر على نطاق واسع فى فسيولوجيا الخلية والتمثيل الغذائي. ولان تطبيقات خلايا الزرع النسيجي زادت فى المجال البحثي، الإنتاج الصناعي، العلاج بالخلايا والعمل التشخيصي الروتيني في قسم علم الفيروسات ، ادى ذلك الي المزيد من المخاوف بشأن التلوث الذي قد ينشأ بالميكوبلازما. وخلال دراستنا تم رصد مجموع ٥٠عينه مزارع خلايا من معهد بحوث صحة الحيوان، الدقي، الجيزة، فاكسيرا ومعهد المصل واللقاحات البيطرية بالعباسية لكشف وجود الميكوبلازما وذلك بطريقه الزرع واختبار تفاعل البلمره المتسلسل (PCR). وقد تم رصد التلوث في خلايا الزرع النسيجي من كل المختبرات. وتم اكتشاف الميكوبلازما عن طريق الزرع (٢٩ عينه من٥٠) بنسبه ٥٨٪ و بالتبعيه تم فحصها بأختبار تفاعل البلمره المتسلسل بأستخدام البادي الخاص بمجموعه الميكوبلازما والذي يقوم بالكشف عن جميع أنواع الميكوبلازما وذلك بوجود حزمه حامض نووى عند ٢٨٠ زوج من القواعد بكل عينات الخلايا الايجابيه وكانت الأنواع الأكثر شيوعا هي ميكوبلازما ارجينينى بنسبه (٣٠٪) وتليها ميكوبلازما اورال بنسبه (٢٨٪). كانت MDBK إيجابي فقط لميكوبلازما ارجينينى والتي اعطت حزمه حامض نووى عند ٣٢٦ زوج من القواعد ، فى حين اصيب كل من خلايا VERO ، BHK بميكوبلازما اورال فقط والذان اعطو حزمة حامض نووى عند ٨٧. التعرف علي مصادر الميكوبلازما ومتابعة تقنيات التعقيم في وحدة خلايا الزرع النسيجي بجانب الكشف عن التلوث بالميكوبلازما بطرق موثوقه يمكن أن توفر الوضع المناسب لمنع التلوث بالميكوبلازما في خلايا الزرع النسيجي.