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Culturing and PCR Methods for Detection of *Mycoplasma hominis* and *Ureaplasma urealyticum* in Women with Genitourinary Tract Infections

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Abstract

A total of Seven hundred and thirty five samples included; urine, endocervical, high vaginal and urethral swabs were collected from married 530/735 (72.1%) and unmarried women 205/735 (27.9%) who admitted to Al-Diwaniya Maternity and Pediatric Teaching Hospital, Al-Diwaniya Teaching Hospital and private clinics in Al-Qadisyia province, Iraq through a period of twelve months (from April 2011 to March 2012) in an attempt to detect the role of Mycoplasma and Ureaplasma in genitourinary tract infections in women. Culturing and PCR techniques were used in this study. Culturing method basing on diagnostic characteristics revealed positive results of Ureaplasma urealyticum in 352 cases while 91 cases were positive for Mycoplasma hominis in same method. According to the cases, the sources of isolates, 90 isolates of U. Urealyticum and 60 isolates of M. hominis were selected for PCR investigation. The results of this method indicated that 65/90 (72.2%) isolates of U. urealyticum were positive with PCR method versus 25/90(27.8%) were negative while among the 60 isolates of M. hominis, only 37/60 (61.7%) were positive with PCR technique versus 23/60 (38.3%) isolates exhibited negative results, accordingly, one can conclude that not all isolates being positive for culture can be positive for PCR too. These results evidently indicate that culturing methods for diagnosis of bacteria are still the confidential gold methods.

Keywords: Culturing, PCR, *Mycoplasma*, *Ureaplasma*, women, genitourinary tract infection.

Introduction

Mycoplasmas and *ureaplasma* are the smallest and simplest self-replicating bacteria. These organisms are so fastidious and completely depend on host biosynthetic precursors[1]. Their cells contain the minimum set of organelles essential for growth and replication; a plasma membrane, ribosome and a genome consisting of a double-stranded circular DNA molecule. They have no cell wall, consequently, they are fully resistant to penicillins and cephalosporins[2]. Growing evidence suggests that *Ureaplasma urealyticum* causes nongonococcal urethritis in human. Ureaplasmas have also been associated with chorioamnionitis, abortion, infertility and low-weight infants. *Mycoplasma hominis*, a common inhabitant of the vagina of healthy women, becomes pathogenic once it invades the internal genital organs, where it may cause pelvic inflammatory diseases such as tubo-ovarian abscess or salpingitis and postpartum fever[3].

Mycoplasma spp. have very little DNA of its own, but are capable of using DNA from the host cell leading to malfunction cell or can cause DNA mutation of host cell[4].

Molecular assays have developed for detection and specific identification of *M. hominis* and *Ureaplasma spp.* in different specimens such as genital tract specimens, amniotic fluids, and respiratory tract specimens from newborns. PCR assays for *M. hominis* have used specific 16S rRNA sequences as the target for amplification and detection, where as similar assays for *U. urealyticum* have used specific 16S rRNA sequence, or urease gene sequence for detection and identification of this organism[5,6].

PCR technique has been described as more sensitive than culture in specimen analyzed soon after collection. If neonates acquired ureaplasmas during birth, the organism would be at a low density at the first day and increase over time. Thus, rapid PCR assays in which early diagnosis is important and can be of tremendous benefit in designing antibiotic treatment protocols[7].

Different target sequences within specific genes have been used for detection of *M. pneumonia* by PCR. These include: P1 adhesin gene and 16S rRNA. The P1 adhesin gene is an appropriate target for PCR, because of its repetitive nature within the genome and similar P1 adhesin genes have been found in other *Mycoplasma* species[8].

The main goal of this study is to detect the role of *M*ycoplasma spp. and *Ureaplasma spp.* in clinical problems occurring in genitourinary tract of women by using both, culturing and PCR methods.

Material and Methods

Specimens collection: A total of 735 specimens were aseptically collected from women who Admitted to the Maternity and Pediatric Teaching Hospital and General

Teaching Hospital, in Al-Qadisyia Province, Iraq during a period of 12 months (from April 2011 to April 2012). These specimens obtained from married women 530/735 (72.1%), either pregnant or non pregnant. The specimens taken from this group included; high vaginal swabs (264), urethral swabs (216), endocervical swabs (42) and urine sample (8) samples. The remain unmarried women 205/735 (27.9%) were tested by investigating their urine only, 205 urine samples, one sample from each.

Swabs were inserted in tubes containing special transport medium to maintain the swabs wet[9]. Each specimen was coated in ice bag until be taken to the laboratory for bacteriological analysis. Specimens were first incubated at 37^0C for 30 minutes, then a loop-full from each sample was transferred to Arginine broth and A7 broth for *Mycoplasma hominis* and *Ureaplasma urealyticum* respectively[10,11]. The tubes were incubated aerobically at 37^0C for 24-72 hours. When the broth became an alkaline (Arginine and urea changes) a small inoculum have spread on Arginine agar medium for *M. hominis* and on A7 agar for *U. urealyticum* and incubated at 37^0C for 3-12 days in candle jar with small wet cotton to provide a little moisture. Colonies were investigated directly by microscope, since the colonies of *M. hominis* appear as fried-egg appearance, while the colony of *U. urealyticum* appear granular and dark brownish color due to accumulation of manganese oxide[12].

Molecular assay: Molecular experiments included the extraction and amplification of *Mycoplasma* and *Ureaplasma* DNA.

DNA extraction: The DNA was extracted according to extraction kit recommended by Intron co., Korea. Preparation of primers suspension: The DNA primers were resuspended by dissolving the lyophilized primers provided by (Integrated DNA Technologyies -USA) after spinning down with TE buffer depending on manufacturer instruction as stock suspension. Working primer tube was prepared by diluted with TE buffer. The final picomoles depended on the procedure of each primer (10 pco/ mol) as recommended by Kong et al[13] and Boesen T. et al[14].

The reaction mixture: Amplification of DNA was carried out in a final volume of 25 µl containing the contents shown in table-2.

Detection *M. hominis* and *U .urealyticum* by PCR: Amplification of specific gene 16S rRNA for *M. hominis* nd UreB-UreC gene spacer region for *U. urealyticum* made according to method recommended by Biernat-Sudolska M. et al[15] and Sambrook J. et al[16].

Detection of amplified products by agarose gel electrophoresis: Successful PCR amplification was confirmed by agarose gel electrophoresis as mentioned by Luki N. et al.[17].

Results and Discussion

According to the origin of isolates (abortion cases, infertility cases and urinary tract infections), 90 isolates out of 352 of *U. urealyticum* isolates revealed positive culturing results and 60 isolates from 91 *M. hominis* isolates were selected for investigation by PCR method. The results of this experiment are shown in table 3. Not all these isolates revealed positive results with PCR as in culture method, since only 65 *U. urealyticum* isolates out of 90 (72.2%) were PCR positive versus 25/90 (27.8%) were negative, while 37 isolates among 60 isolates of *M. homonis* (61.7%) exhibited positive test by PCR method versus 23/60 (38.3%) were negative as shown in table 3.

To show the results of This experiment in this paper, only five isolates of *M. homonis* (figure 1) and eight isolates of *U. urealyticum* (figure 2) were selected. All five isolates (1 to 5) of *M. hominis* revealed positive results by PCR method (figure 1), while 6 isolates (1, 2, 3, 5, 7 and 8) of *U. urealyticum* among eight isolates were positive by PCR method whereas two of these isolates (4 and 6) were negative (figure 2).

Table-1
PCR primers employed in the detection *M. hominis* and *U. urealyticum*.

Organism	Primer (F) (R)	Sequence (5'-3')	Size of amplified product(bp)	Target gene
M. hominis	HOM+ UNI-	TGAAAGGCGCTGTAAGGCGC TAATCCTGTTTGCTCCCCAC	589	16SrRNA
U.urealyticum	UUS2c UUA2c	CAGGATCATCAAATCAATTCAC CATAATGTTCCCCTTCGTCTA	420	Ure-B Ure-C

Table-2
Contents of the reaction mixture

No.	Contents of reaction mixture	Volume
1.	2X PCR ImaxIImaster mix	12.5 µl
2.	Upstream primer	2.5µl
3.	Downstream primer	2.5 µl
4.	DNA template	5 µl
5.	Nuclease free water	2.5 µl
Total volume		**25 µl**

Table-3
Results of PCR method versus culturing method in detecting *Ureaplasma* and *mycoplasma*

Organisms	Positive cases by culture	Tested cases by PCR	Positive cases by PCR	Negative cases by PCR
U. urealyticum	352	90	65(72.2%)	25(27.8%)
M. hominis	91	60	37(61.7%)	23(38.3%)

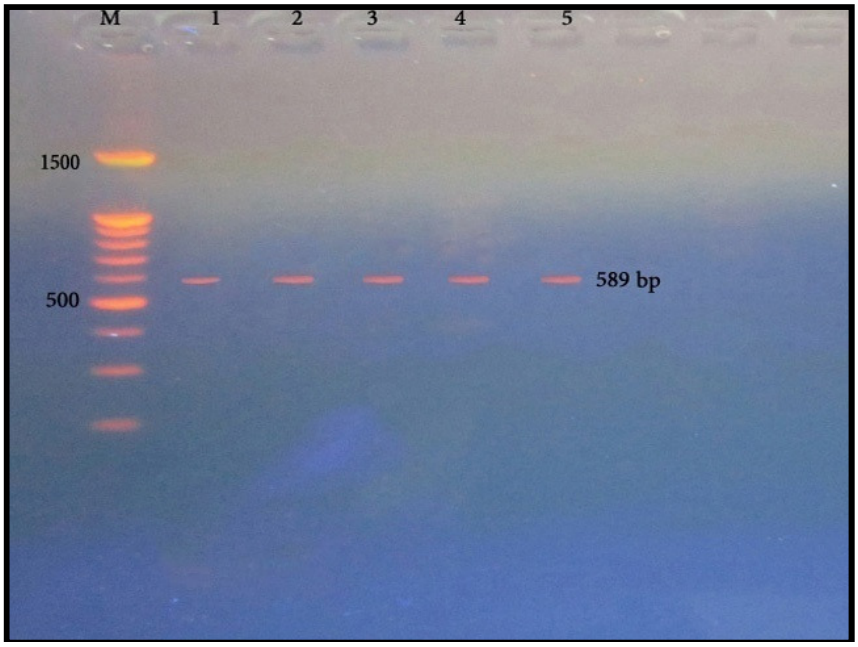

Figure-1
Ethidium bromide stained 2% agarose gel shows the PCR amplification products with 16S rRNA gene (589 bp) primer for *M. hominis*. M: 100 bp. DNA marker, lanes from 1 to 5 positive results with 589 bp. product

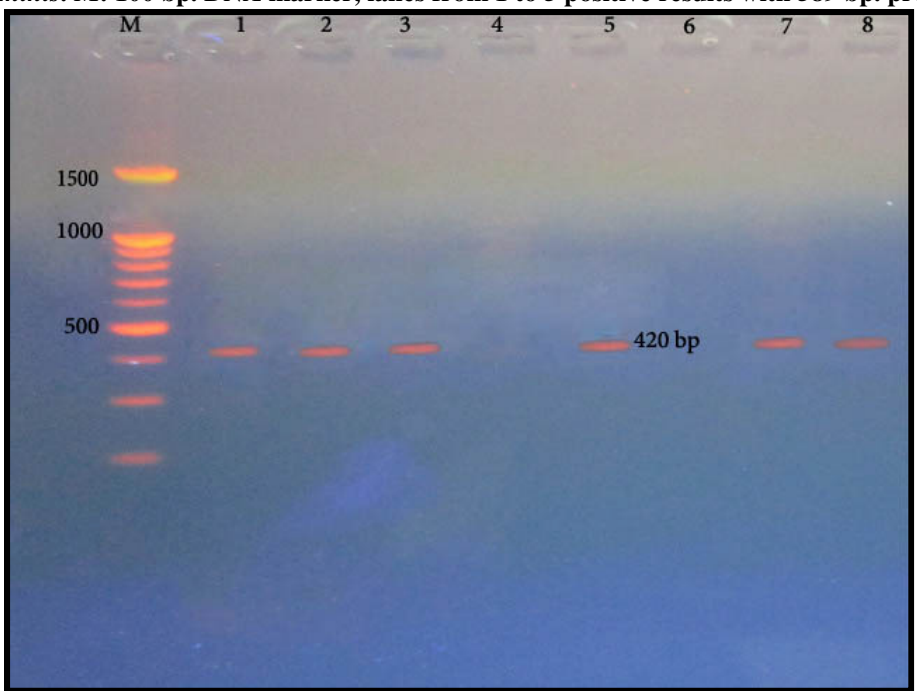

Figure-2
Ethidium bromide stained 2% agarose gel showing PCR amplification products with (420 bp) primer for *U. urealyticum*. M: 100 bp. DNA marker. Lanes; 1, 2, 3, 5, 7 and 8 are positive with 420bp. Product, Lanes; 4 and 6 are negative

We used in this study numerous and various clinical specimens from genitourinary tract of women who suspected to be infected with *Mycoplasma* and/or *ureaplasma* compared with other related studies. The results indicated that not all specimens revealed culture positive are necessary to be PCR positive, since among 90 culture positive isolates of *U. realyticum* , 65 (72.2%) isolates were positive by PCR versus 25 (27.8%) isolates were negative. Regarding *M. hominis*, only 37 (61.7%) isolates were PCR positive out of 60 culture positive isolates while 23 (38.3%) of those were negative. Variation results in detection of *Mycoplasma* and *Ureaplasma* by culturing and PCR assay have been also reported elsewhere 18, 19, 20, 21. The lower frequency of these organisms detection by PCR may due to in part to degradation in bacterial DNA through processing of DNA, heterogeneity of the intergenic spacer regions 21, presence of some inhibitors of the PCR reaction in the specimen under test or chromosomes rearrangements, since the mycoplasmal chromosome is a genetically dynamic structure that undergoes frequent rearrangements, insertions, deletions, and inversions of genes 22, 23. Another possibility is that the *Mycoplasma* and/or *Ureaplasma* DNA loading may below the detectable level by PCR.

Mycoplasma and ureaplasma are well known as unusual bacteria among all prokaryotes. Attempts have made worldwide to obtain valuable method for isolation and identification of these organisms but still there is an argumentation about consistent method. However, at the present, there are two main methods used for diagnosis *mycoplasma* and *ureaplasma*, culturing and PCR, even though, there are some advantages and disadvantages for each method. The positive results obtained by each method are not quite satisfactory with these bacteria in comparison with other bacteria, may because they are a unique group. It is agreed that culturing method of *Mycoplasma* and *Ureaplasmas* is difficult, expensive and time consuming, since special rich media and special growth conditions are required. Even though, isolation by culture is still considered as the gold standard technique for detection of these organisms. However, this reliability is limited by PCR technique which has the advantages of being easy and rapid in detection these organisms, but the matter is, there are no commercially available molecular tests, therefore, efforts for new techniques to detect *Mycoplasma* and *Ureaplasma* are ongoing at present time since various procedures based on DNA amplification have been developed for detection of *M. pneumoniae,* such as capillary PCR 24, nucleic acid sequence-based amplification and enzyme-linked gel assay 25, real time PCR 26, multiplex PCR and loop-mediated isothermal amplification 27. In conclusion, culture method remains preferential method in isolation and identification of *Mycoplasma* spp. and *Ureaplasma* spp., since they form colonies with unconfused detectable diagnostic characteristics but this method like PCR method, needs more attention to modify and develop new qualified medium to support the growth of *Mycoplasma hominis* and *ureaplasma urealyticum* and to minimize the incubation period to 1 – 2 days. We believe that it is possible to modify and develop new culture medium to enhance the growth Mucoplasmas/Ureaplasmas, accordingly, we are focusing at the present time on this project since some experiments revealed tremendous differential growth/colonies of these organisms within standard incubation time 28. However, single method e.g. culturing or PCR to diagnose these organisms may not quite enough, therefore, both should be combined to allow confirmative, rapid and reliable diagnosis of *M. hominis* and *Ureaplasma urealyticum* infection.

Conclusion

Both, *Ureaplasma urearilyticum* and *Mycoplasma hominis* are frequently infect genitourinary tract of women particularly pregnant women. *U. urearilyticum* seams to be more frequent. PCR assay is not fully efficient in detection of genital mycoplasmas and/or ureaplasmas, consequently, rapid detection, confirmed identification and reliable diagnosis of these organisms require to combine culturing and PCR methods.

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