Multiplex PCR Assay: Comparison with Culture for Detection of Genital Mycoplasma

Mervat S. Mohamed, Somaia M. El Sheikh, Hosam El Dein El Sharkawy and Rehab El Tahlawy
Microbiology& Immunology Department, Faculty of Medicine, Zagazig University

ABSTRACT

Ureaplasma, spp, Mycoplasma genitalia and Mycoplasma hominis are associated with infection of genitourinary tract and reproductive failure. The aim of this work was to determine the sensitivity, specificity and predictive values of multiplex PCR versus culture for detection of genital Mycoplasmas in clinical samples. 95 patients (60 males and 35 females) seen at Gynecology and Urology clinics aged from 20 to 50 years. They were complaining from urinary, gynecological, sexual or infertility problems. The studied specimens were distributed as 40 urine samples, 36 urethral swabs and 19 endocervical swabs.. The specimens were cultured on PPLO media, the remaining material were frozen at -70 for multiplex PCR. The results of this study showed that: 45.3% and 51.6% of studied samples were positive for genital mycoplasmas by culture and PCR. respectively. Recovery of genital mycoplasmas by biochemical reactions (i.e., pH change in PPLO broth) was significantly higher than that by culture and staining. There was statistically significant agreement between culture and multiplex PCR in: Recovery of genital mycoplasmas from urine samples and urethral swabs. Recovery of Mycoplasma spp from urine samples and endocervical swabs. Recovery of Ureaplasma urealyticum from urine samples and urethral swabs. Recovery of Mycoplasma hominis from cases of cystitis and female infertility. Recovery of Ureaplasma urealyticum from cases of cystitis, vaginal discharge and male infertility. Multiplex PCR assay was found to have the following values regarding sensitivity, specificity, PPV and NPV of compared with culture: 81.4%, 73.1%, 71.4% and 82.6% in detection of genital mycoplasmas in urogenital specimens. 73.1%, 89.9%, 73.1% and 89.9% respectively in detection of M. hominis in urogenital specimens. 80.6%, 84.4%, 71.4% and 90.0% respectively in detection of U. urealyticum in urogenital specimens. In conclusion, Multiplex PCR assay is a rapid and accurate alternative of culture for detection of genital Mycoplasma species in clinical samples. We recommended that further studies should be done to identify M. genitalium, an extremely fastidious organism that could not be detected in the present study while it is reported to be implicated in several infections especially non-gonococcal urethritis and cervicitis.

INTRODUCTION

Mycoplasmas are the smallest and simplest self-replicating bacteria. They are prokaryotes but lack a cell wall; however, they are bound by a unique cell membrane that contains sterols, which are not present in either bacteria or viruses. Mycoplasmas were first described 100 years ago, and now over 190 species, widely distributed among humans, animals, insects and plants, are known. These microorganisms are included in a separate class named *Mollicutes*. Most human and animal mollicutes are *Mycoplasma* and *Ureaplasma* species of the family *Mycoplasmataceae* [(9)].

Genital Mycoplasmas include, *Mycoplasma hominis* (*M. hominis*), *Mycoplasma genitalium* (*M. genitalium*), *Ureaplasma urealyticum* (*U. urealyticum*) and *Ureaplasma parvum* (*U. parvum*). They have been associated with a wide array of infectious diseases in adults and infants. *M. fermentans* is an organism which may play a role in human disease in immunocompromised persons [(41)].

The lack of conclusive knowledge regarding the pathogenic potential of *Mycoplasma* and *Ureaplasma* spp. in many conditions is due to a general unfamiliarity of physicians and microbiology laboratories with their fastidious growth requirements which makes it difficult to detect them. The situation is now changing because of a greater appreciation of the genital mycoplasmas as perinatal pathogens and improvements in laboratory detection, particularly with regard to the development of powerful molecular nucleic acid amplification tests [(21)].

The main method of detecting mycoplasmas is by culture, but the organisms are difficult to isolate and require special culture

media. PCR is revolutionalizing the diagnosis of many infectious disease particularly those caused by organisms that are difficult to cultivate[6]. The aim of the present study was to determine sensitivity, specificity and predictive values of multiplex PCR assay versus culture for the detection of genital mycoplasmas.

SUBJECTS, MATERIALS & METHODS

This study was conducted during the period from February 2007 to May 2008 in Gynecology, Urology Clinics and Microbiology & Immunology Departments, Faculty of Medicine Zagazig University. 95 patients (60 males and 35 females) their ages ranged from 20 to 50 years, they were complaining of urinary, gynecological, sexual or infertility problems. Some patients were excluded from the study due to history of antibiotic treatment within the previous 2 weeks, history of urination within the previous one hour or past history of other sexually transmitted diseases (STDs)[28]. The selected cases were subjected to full history taking and complete physical examination.

Clinical specimens [12] :

- Urethral samples were collected (from cases of urethritis, epididymoorchitis and male infertility) with a Dacron swab placed into the urethra 2-3 cm and rotated to obtain as many cells as possible after cleaning the external meatus with 70% ethanol without using antibiotics (urethral discharge can be collected by the swab).
- Vaginal samples were taken (from cases of abnormal vaginal discharge and female infertility) from the endocervical region after exocervical mucus had been cleaned without using local antiseptic by the same manner as urethral swab.
- Urine samples: 20 ml of clean-catch urine were collected at different stages of urination in a sterile plastic container.

The swab taken from the patient was broken off into screw cap test tube containing 3 ml of tryptic soy broth (Oxoid).

Laboratory processing and culture:

- The transport medium containing the swab was vortexed for 30 seconds. 200 µl were inoculated in each of two tubes containing 1.8 ml of arginine broth and ureaplasma broth respectively (Difco USA).
- Urine sample was vortexed, divided into 3 tubes and concentrated by centrifugation for 30 minutes at 16000 Xg prior to testing. Each sediment of the 3 tubes was dissolved in 0.2 ml of the supernatant. One reconstituted sediment was added to 1.8 ml of arginine broth, while the second was added to 1.8 ml of ureaplasma broth and whereas the third was added to 2 ml of tryptic soy broth.
- Both arginine and ureaplasma broth tubes were incubated aerobically at 37°C overnight and subcultured the following morning by spreading 100 µl of the broth across the surface of PPLO agar and ureaplasma agar plates respectively.
- The incubated broths were observed daily for up to 5 days and subcultured again when any pH change was detected. The inoculated agar plates were incubated at 37°C at 5-10% CO2 in humidified candle jar for one week. The plates were examined every other day using the inverted microscope (32 X magnification).
- Agar plates were flooded with Dienes stain diluted 1 to 10 in distilled water. The plates were examined microscopically using a low power objective. Mycoplasmas stained an intense royal blue.
- Suspected colonies of U. urealyticum were confirmed by flooding the ureaplasma agar plates with manganese chloride-urea solution. Deposition of golden brown precipitate in ureaplasma colonies occurred within few minutes.
- The remaining materials (specimen or transport media) were frozen at -70°c for PCR testing.

Multiplex PCR

DNA extraction: DNA was extracted using phenol–chloroform method[35] and the concentration was determined [36].

DNA amplification: Taq PCR master mix (Qiagen, GmbH, Germany) it is ready to use premixed solution containing Taq DNA polymerase, PCR buffer and dNTps.

Primers[7,8]: Sets of primers specific for highly conserved region in the

- Urease gene of *U. urealyticum.*
  Forward 5' ACGAC GTCCA TAAGC AACT 3'
  Reverse 5' CAATC TGCTC GTGAA GTATT AC 3'
- 140 KDa adhesion protein gene of *M. genitalium*.
  Forward 5' AGGTT ATGGA AACCT TAACCC CTTGG3'
  Reverse 5' CCGTT GAGGG GTTTT CCATT TTTGC 3'

- 16S rRNA gene of *M. hominis.*
  Forward 5' CAATG GCTAA TGCCG GATAC GC 3'
  Reverse 5' GGTAC CGTCA GTCTG CAAT 3'

5 ul DNA extract in 50 ul reaction mixture containing 25 ul of the master mix in each PCR tube . Except the negative control tube where 5 ul of distilled water was added instead of the sample, 25 pmol of each primer were added. The amplification was performed in a Gene Amp PCR system 9600 (Perkin- Elmer Norwalk). 1 cycle of 10 min. at 95°C, followed by 35, 2-step cycles of 95°C for 15s and 60°C for 60s, followed by 5 min. at 72°C. Amplification products were detected by agarose gel electrophoresis in parallel with a molecular weight marker.

RESULTS

43 (45.3%) and 49 (51.6%) samples were positive for genital mycoplasmas by culture and multiplex PCR respectively. *M. hominis* (either alone or with *U. urealyticum*) was recovered from 26 (27.4%) samples by both culture and multiplex PCR while *U. urealyticum* (either alone or with *M. hominis*) was recovered from 31 (32.6%) samples by culture and from 35 (36.8%) samples by multiplex PCR. 14 (14.7%) and 12 (12.6%) samples yielded both organisms by culture and multiplex PCR respectively. *No specimen was shown to contain *M. genitalium* (Table 1)

Recovery of genital mycoplasmas by BR was significantly higher than culture and staining (P 0.0002, 0.002 for *M. hominis*. and *U. urealyticum* respectively). (Table 2)

There was excellent agreement between culture and multiplex PCR for urine specimens (P <0.001) and good agreement between them for urethral swabs (P = 0.04). (Table 3)

There was excellent agreement between the 2 methods in cases of cystitis (P <0.001) and good agreement between both in cases of female infertility (P = 0.01). (Table 4)

There was excellent agreement in cases of cystitis (P <0.001) and vaginal discharge (P = 0.008) and good agreement in cases of male infertility (P = 0.04). (Table 5)

Multiplex PCR had sensitivity 81.4%, specificity 73.1%, PPV 71.4% and NPV 82.6%. There was excellent agreement between culture and multiplex PCR in detection of genital mycoplasmas (P <0.001). (Table 6)

Multiplex PCR was found to have sensitivity 73.1%, specificity 89.9%, PPV 73.1 and NPV 89.9%. There was excellent agreement between culture and PCR (P <0.001) in detection of *M. hominis*. Sensitivity of multiplex PCR was 80.6%, specificity 84.4%, PPV 71.4% NPP 90.0% there was excellent agreement between culture and multiplex PCR in detection of *U. urealyticum* (P <0.001). (Table 7)

Table (1): Frequency of positive specimens for genital mycoplasmas by culture and/or multiplex PCR

Specimens (No = 95)	Culture No (%)	Multiplex PCR No (%)
+ ve for any one	43 (45.3)	49 (51.6)
+ ve for *M. hominis**	26 (27. 4)	26 (27.4)
+ ve for *U. urealyticum*	31 (32.6)	35 (36.8)
+ ve for both	14 (14.7)	12 (12.6)
- ve for both	52 (54.7)	46 (48.4)

Table (2): Recovery of genital mycoplasmas by BR, inverted microscope and Diene's stain

	M. hominis			*U. urealyticum*		
	No of +ve specimens	% + ve	P	No of +ve specimens	% + ve	P
BR	26	100	0.0002***	31	100	0.002***
Agar	15	57.7		23	74.1	
Diene's stain	20	77		27	87	
Total	26	100		31	100	

Test used is chi-square for trend

Table (3): Frequency of detection of genital mycoplasmas by culture and multiplex PCR in different clinical samples

Sample	Culture +ve N (%)	Multiplex PCR +ve N (%)	+ ve by both culture and PCR N (%)	P
Urine (40)	20 (50.0)	17 (42.5)	17 (42.5)	0.001
Urethral swab(36)	14 (38.9)	21 (58.3)	11 (30.5)	0.04
Endocervical swab (19)	9 (47.4)	11 (57.9)	7 (36.8)	0.09
Total (95)	43 (45.3)	49 (51.6)	35 (36.8)	

Test used is kappa agreement

Table (4): Comparison between culture and multiplex PCR in detection of *M. hominis* in different clinical conditions

M. hominis					
Clinical diagnosis	**Specimen**	**Culture + ve N (%)**	**Multiplex PCR + ve N (%)**	**+ve by both culture andPCR N (%)**	**P**
Cystitis (40)	Urine	13 (32.9)	10 (25.0)	10 (25.0)	0.001
Prostatitis (19)	Uretheral swab	0	3 (15.7)	0	–
Urethritis (10)		3 (30.0)	3 (30.0)	1 (10.0)	0.88
Epidedimoorchitis (3)		1 (33.3)	1 (33.3)	1 (33.3)	0.88
Male infertility (4)		0	0	0	-
Vaginal discharge (7)	Endocervical	3 (42.9)	5 (71.4)	3 (42.9)	0.14
Female infertility (12)	swab	6 (50.0)	4 (33.0)	4 (33.0)	0.01
Total (95)		26 (27.4)	26 (27.4)	19 (20.0)	

Test used is Kappa agreement

Table (5): comparison between culture and multiplex PCR in detection of *U. urealyticum* in different clinical conditions

U. urealyticum					
Clinical diagnosis	**Specimen**	**Culture +ve N (%)**	**Multiplex PCR + ve N (%)**	**+ve by both culture and PCR N (%)**	**P**
Cystitis (40)	Urine	16 (40.0)	12 (30.0)	12 (30.0)	0.001
Prostatitis (19)	Uretheral swab	5 (26.3)	9 (47.4)	4 (21.0)	0.88
Urethritis (10)		2 (20.0)	4 (40.0)	2 (20.0)	0.053
Epidedimoorchitis (3)		2 (66.7)	3 (100.0)	2 (66.7)	–
Male Infertility (4)		3 (75.0)	3 (75.0)	3 (75.0)	0.04
Vaginal discharge (7)	Endocervical	1 (14.3)	1 (14.3)	1 (14.3)	0.008
Female Infertility (12)	swab	2 (16.7)	3 (25.0)	1 (8.3)	0.37
Total (95)		31 (32.6)	35 (36.8)	25 (26.3)	

Test used is Kappa agreement

Table (6): Validity of multiplex PCR in detection of genital mycoplasmas in comparison with culture

	Culture		**Total**
	+ve	**– ve**	
Multiplex PCR			
+ ve	35	14	49
– ve	8	38	46
Total	43	52	95

Table (7): Validity of multiplex PCR in detection of *M. hominis and U. urealyticum* in comparison with culture

	Culture					
	M. hominis			***U. urealyticum***		
	+ ve	**- ve**	**Total**	**+ ve**	**- ve**	**Total**
Multiplex PCR						
+ ve	19	7	26	25	10	35
– ve	7	62	69	6	54	60
Total	26	69	95	31	64	95

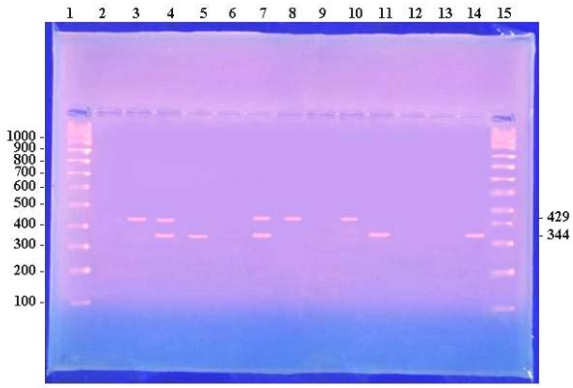

Figure (1) : Electrophoretic analysis of amplified products of Multiplex PCR performed for *M. hominis* and *U. urealyticum* in different urogenital samples
Lane 1,15: 100-bp DNA ladder.
Lanes 2: Negative control containing distilled water
Lanes 5,11,14: Samples positive for 344-bp 16S rRNAgene of *M. htmdtds*
Lanes 3, 8, 10: Samples positive for 429-bp urease gene of *U. urealyticum*
Lanes 4, 7: Samples positive for both *M. hominis* and *U. urealyticum*

DISCUSSION

The genital mycoplasmas represent a complex and unique group of microorganisms that have been associated with a wide variety of infectious diseases in adults and infants[(42)]. Urethral or vaginal discharges, burning or pain during urination and primary or secondary infertility were common complaints in patients. Comparison of the prevalence of genital mycoplasmas in asymptomatic individuals was not possible because urogenital samples were not collected from the cohort. Other studies revealed that the frequency of genital mycoplasmas was significantly higher in symptomatic than asymptomatic persons [(17)]

In the present study, 45.3% and 51.6% of tested specimens were +ve for genital mycoplasmas by culture and multiplex PCR respectively. On the other hand, in the study of **Stellrecht et al**,[(35)] 25% of specimens were culture +ve and 33% were multiplex PCR +ve. The rate may be higher in this study due to a number of factors including (i) the high risk population sampled (ii) the method of sample collection in that specimen was inoculation into tryptic soy broth for culture and PCR testing. The lower level of detection by culture compared to PCR may be attributed to difficulties of culturing and isolating genital mycoplasmas. Organisms which die before incubation can't be cultured whereas DNA of dead organisms can still be detected by PCR [(1)].

In the present study, *M. hominis* and *U. urealyticum* were isolated in culture from 27.4% and 32.6% of examined urogenital specimens respectively. This result was comparable to that of **Vaquez et al,**[(40)] but was different from that of **Karabay et al**,[(22)] who detected *M. hominis* and *U. urealyticum* in 4.4% and 48.4% of cultures respectively**.**

In the present work, multiplex PCR yeilded *M. hominis* and *U. urealyticum* in 27.4% and 36.8% of studied specimens respectively. This result was matched with that of **Serin et al,**[(34)]

who detected *M. hominis* by PCR in 33% of clinical samples and with that of **Stellrecht et al,**[35] who detected *U. urealyticum* in 29.4% of examined urogenital specimens.

In this work, 26 and 31 +ve specimens of *M. hominis* and *U. urealyticum* respectively detected by biochemical reactions in broth media represented all infections, then examination of PPLO agar plates by inverted microscope has recovered 57.7% and 74.1% of *M. hominis* and *U. urealyticum* respectively. Over growth of common urinary tract organisms was responsible for the majority of false -ve results on PPLO agar. In addition, the broth frequently demonstrated a color change after 24–48 hrs of incubation (form yellow to red), and these organisms are very sensitive to changes in pH of the broth media and exposure to an alkaline pH can be lethal to these organisms. So, initial inoculation of specimens into broth medium as well as onto a solid medium to increase the isolation rate[10]. Broth media provide a valuable diagnostic aid by indicating early growth of genital mycoplasmas and death of organisms must be avoided by rapid subculture [19].

As regards prevalence of genital mycoplasmas in different clinical samples, isolates of genital mycoplasmas were identified in 50% and 42.5% of urine samples, in 38.9% and 58.3% of urethral swabs and in 47.4% and 57.9% of endocervical swabs by both culture and multiplex PCR respectively. This order of prevalence was in accordance with **Stellrecht et al,** [35] who detected genital mycoplasmas in 33% and 50% of urine samples and in 23% and 50% of cervical swabs by culture and multiplex PCR, respectively.

In this work, *M. hominis* was isolated by culture in 32.9% and by multiplex PCR in 25% of urine samples. The most likely explanation for this finding is the presence of inhibitors often found in urine[20]**.** This rate of isolation was higher than that published by others[13,38,29] who detected DNA of *M. hominis* in 7%, 4% and 3% of urine samples, respectively.

In the present study, *U. urealyticum* was isolated by culture in 40% and by multiplex PCR in 30% of samples. This result was comparable with **Mobarak and Tharwat**[27] who reported that culture of midstream urine revealed *U. urealyticum* in 26.7% of samples collected from patients having urinary calculi. This result also agreed with that of **Afacan et al.**[3] who isolated *U. urealyticum* from 29.8% of cases with sterile pyuria.

In this work, patients with chronic prostatitis, *M. hominis* was not isolated by culture but detected in 15.7% of cases by multiplex PCR. *U. urealyticum* was recognized by both culture and PCR in 26.3% and 47.4% of cases respectively. This result was in accordance with **Peeters et al.** [32] who recovered *M. hominis* and *U. urealyticum* from 11.8% and 47.1% respectively of patients with chronic prostatitis. **Mandar et al.**[26] concluded that all genital mycoplasmas occur significantly more frequently in prostatitis patients than in controls.

In the current study, *M. hominis* was isolated by both culture and multiplex PCR in 30% of cases of urethritis. *U. urealyticum* was isolated in 20% and 40% of urethritis patients by culture and PCR, respectively. **Kilic et al.**[24] reported lower isolation rate (16%) for *M. hominis*, but similar rate (48%) for *U. urealyticum*. **Zdrodowska-Stefanow et al.**[44] added that mycoplasmal infections were the most frequently found in urethritis patients aged 30 to 39 years and diagnosed with epididymitis.

In this work, among 7 patients complaining of vaginal discharge, *M. hominis* was detected in 42.9% and 71.4% of cases by culture and multiplex PCR, respectively, while *U. urealyticum* was isolated from one patient (14.3%). These rates were relatively different from that of **Domingues et al.**[15] who isolated *M. hominis* and *U. urealyticum* from 31.5% and 27.8% of infected females respectively. In contrast, **Zdrodowska-Stefanow et al.** [45] and **Kechagia et al.** [23] reported that the incidence rate of genitourinary infection due to *U. urealyticum* was significantly high as compared to *M. hominis* infection (29.8% vs 3.7% and 18% vs 1% respectively) among examined women.

In the present study, of 4 male patients complaining of infertility, *U. urealyticum* was detected in 3 (75%). This frequency is much higher than that reported by **Andrade-Rocha et al.** [4] and **Rosemond et al.** [33] who isolated *U. urealyticum* from 28.4% and 3% of infertile males, respectively**.** This difference may be due to small number of patients included in the present study. On the other hand, *M. hominis* was not isolated from infertile males. This result was matched with that of **Rosemond et al.** [33]**,** but was not comparable to that reported by **Andrade-Rocha et al.** [4] and **Gdoura et al.** [16] who detected *M. hominis* in 13.3% and 10% of examined specimens from infertile males, respectively.

In the current study, *M. hominis* was detected by culture in 50% and by multiplex PCR in 33% of endocervical swabs collected from infertile females with good agreement between culture and PCR. This result was not matched with that of **Baczynska et al.**[5] who identified *M. hominis* in 2.4% of examined cervical swabs by both culture and PCR. This discrepancy may be explained by the study of **Hellberg et al.** [18] who found that *M. hominis* was isolated from 38.5% of cases with bacterial vaginosis compared to 8.3% with normal microbial flora. The association between high prevalence of genital mycoplasmas and presence of bacterial vaginosis was also demonstrated by **Domingues et al.**[15]. Bacterial vaginosis was not excluded in the current work.

U. urealyticum was found by culture in 16.7% and by multiplex PCR in 25% of endocervical swabs from infertile females with no significant agreement between culture and PCR. This result was consistent with that of **Teng et al.** [39].

In this work, no specimens were shown to contain *M. genitaluim.* Similar results have been demonstrated by others[2,25]. In contrast, **Yoshida et al.**[43] and **Cohen et al.**[11] reported higher frequency of identification of *M. genitaluim* DNA in clinical samples (11% and 7%, respectively). A quantitative real-time PCR was developed to determine *M. genitaluim* load and found to be sensitive, specific and rapid to minimize risk of contamination [37].

In this study, 8 patients had a positive culture but a negative PCR assay result. Similar observations have been described by other investigators[25,31], and have been attributed to degradation of bacterial DNA or inhibitors of the PCR reaction in clinical samples, e.g., blood contamination of endocervical swabs and acidity of urine samples. On the other hand, 14 of the patients had a positive PCR and a negative culture. This is a major concern when considering the results of any study in which PCR is compared to the inherently less sensitive culture technique is how to interpret findings in which the PCR is positive and the culture is negative to evaluate the specificity of the PCR assay and ensure the results were not due to a false-positive reaction due to contamination. Use of a second gene target and/or assessing repeat specimens may help to resolve such cases but this has not been done consistently [42].

In this study, *the* sensitivity, specificity, PPV, and NPV of PCR for genital mycoplasmas was 81.4%, 73.1%, 71.4% and 82.6% respectively with excellent agreement between culture and multiplex PCR for detection of genital mycoplasmas in clinical samples. Of the 95 patients' results, 35 specimens were + ve by both culture and PCR. This result was consistent with that of others[14,30].

On evaluating *the* validity of multiplex PCR versus culture in detection of *M. hominis*, the multiplex PCR was found to have sensitivity of 73.1%, specificity of 89.9%, PPV of 73.1% and NPV of 89.9%. This result was comparable with that of **Serin et al** [34] .

On evaluating the *validity* of multiplex PCR versus culture in detection of *U. urealyticum*, the multiplex PCR showed sensitivity of 80.6%, specificity of 84.4%, PPV of 71.4% and NPV of 90.0% This result was comparable with that of **Dhawan et al** [14].

In conclusion, members of genital mycoplasmas are prevalent in different urogenital specimens and different clinical conditions. Multiplex PCR assay is a rapid and accurate alternative of culture for detection of genital *Mycoplasma* species in clinical samples. We recommended that: further studies should be done to identify *M. genitaluim*, an extremely fastidious organism that could not be detected in the present study while it is reported to be implicated in several infections especially non gonococcal urethritis and cervicitis.

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تفاعل إنزيم البلمرة المتسلسل والمتعدد : مقارنة مع المزرعة فى الكشف عن الميكوبلازما التناسلية

ميرفت سليمان محمد ، سمية محمد الشيخ ، حسام الدين الشرقاوى ، رحاب الطحلاوى
قسم الميكروبيولوجيا والمناعة – كلية الطب – جامعة الزقازيق

تعتبر الميكوبلازما التناسلية من الميكروبات التى تتسبب في التهابات الجهاز البولى و الجهاز التناسلي مثل التهاب قناة مجرى البول، غدة البروستاتا و بطانة الرحم بالا ضافة الى العقم و المشاكل الصحية للاطفال حديثى الولادة. اما عن طرق التشخيص التقليدية و تشمل الزراعة على مستنبتات خاصة فتتطلب الكثير من الوقت و المال و الخبرة و لذلك يلجأ في التشخيص الي البحث عن الحامض النووي بواسطة تفاعل البلمرة المتسلسل لاكثر من جين

وتهدف هذه الدراسة إلى :

تعيين الحساسية والخصوصية والقيم التنبئوية السالبة و الموجبة لتفاعل البلمرة المتسلسل والمتعدد بالمقارنة بالمزرعة في الكشف عن الميكوبلازما التناسلية في العينات الاكلينيكية .

وقد تمت هذه الدراسة في معامل قسم الميكروبيولوجيا والمناعة بكلية الطب جامعة الزقازيق علي ٩٥ عينة تم جمعها من عيادات المسالك البولية والنساء والتوليد بمستشفيات جامعة الزقازيق العينات شملت ٦٠ رجل، ٣٥ امرأة تتراوح أعمارهم من ٢٠: ٥٠ سنة ويعانون من مشاكل بولية، تناسلية، جنسية أو عقم والعينات التى تمت عليها الدراسة انقسمت إلى ٤٠ عينة بول، ٣٦مسحة من مجرى البول و ١٩ مسحة من عنق الرحم.

تم نقل العينات إلى المعمل فى مزرعة"التربتيك صوى" السائلة وفى المعمل، نقلت العينات إلى مزرعة "البيلو" السائلة وعند ظهور أول علامة لنمو الميكروب بتغير لون المزرعة يتم نقلها بسرعة إلى أطباق مزرعة البيلو وتوضع الأطباق فى الحضانة عند درجة ٣٧° فى وجود ٥-١٠% ثانى أكسيد الكربون وتفحص الأطباق يوماً بعد يوم تحت الميكرسكوب المعكوس لرؤية مستعمرات الميكوبلازما المميزة التى تشبه البيض المقلى. تم استخدام صبغة "الديين" ومحلول" كلوريد المنجنيز" فى التأكد من وجود الميكروب

الجزء الباقى من العينات حفظ فى درجة -٧٠°م فى الفريزر لحين عمل تفاعل البلمرة المتسلسل والمتعدد و الذى تم إجراؤه على الجينات التالية:

- الجين الذى يكون أنزيم اليوريز فى اليوريابلازما.
- الجين الذى يكون بروتين الالتصاق فى الميكوبلازما جينيتاً ليام.
- الجين الذى يكون جزء خاص من الريبوسوم فى الميكوبلازما هو مينيز.

نواتج تفاعل البلمرة تم فصلها على جيل الأجاروز المصبوغ بالإيثيديام بروميد باستخدام التيار الكهربائى ثم تم فحصها وتحديدها على شاشة مضيئة بالأشعة فوق البنفسجية.

وقد أظهرت نتائج البحث أن :

- الميكوبلازما التناسلية تم فصلها من ٤٥,٣% من العينات بالمزرعة ومن ٥١,٦% من العينات بتفاعل البلمرة المتسلسل والمتعدد . كان معدل الكشف عن وجود الميكوبلازما التناسلية بتغير لون مزرعة البيلو السائلة نتيجة التفاعلات الكيميائية للميكروب أعلى منه بالبحث عن المستعمرات المميزة تحت الميكروسكوب بعد زراعتها على المستنبتات الصلبة. كان هناك توافق إحصائي معتبر بين المزرعة التقليدية وتفاعل البلمرة المتسلسل والمتعدد في الكشف عن الميكوبلازما التناسلية . بالنسبة لتفاعل البلمرة المتسلسل والمتعدد مقارنة بالمزرعة فى الكشف عن الميكوبلازما التناسلية فقد حصلنا على القيم الآتية :
- حساسية ٨١,٤%- خصوصية ٧٣,١%- القيمة التنبؤية الموجبة ٧١,٤% والقيمة التنبؤية السالبة % ٨٢,٦ في الكشف عن الميكوبلازما التناسلية في العينات الالكينيكية . حساسية ٧٣,١%- خصوصية ٨٩,٩%- القيمة التنبؤية الموجبة ٧٣,١% والقيمة التنبؤية السالبة ٨٩,٩% في الكشف عن ميكروب الميكوبلازما هومينيز في العينات الالكينيكية . حساسية ٨٠,٦%- خصوصية ٨٤,٤%- القيمة التنبؤية الموجبة ٧١,٤% والقيمة التنبؤية السالبة ٩٠% في الكشف عن ميكروب اليوريابلازما يورياليتيكام في العينات الالكينيكية .
- من هذه الدراسة نستنتج أن الميكوبلازما التناسلية منتشرة فى العينات الإكلينيكية المأخوذة من المسالك البولية والتناسلية فى الحالات المرضية المختلفة وأن محاولات عزل هذه الميكروبات بالمزرعة التقليدية صعبة وتحتاج إلى خبرة عالية ووقت طويل ولذلك يعد تفاعل البلمرة المتسلسل والمتعدد بديل سريع ودقيق للكشف عن الميكوبلازما التناسلية. ونوصى بمتابعة الدراسة على ميكروب الميكوبلازما جينيتاليام الذى لم نتمكن من فصله فى هذا البحث فى حين أظهرت أبحاث أخرى وجوده بنسب معتبرة فى أنواع مختلفة من عدوى الجهاز البولى والتناسلى .