· Advances in Medical Sciences · Vol. 53(1) · 2008 · pp 80-86 · DOI: 10.2478/v10039-008-0020-1

Detection of *Chlamydia trachomatis* and *Mycoplasma hominis*, *genitalium* and *Ureaplasma urealyticum* by Polymerase Chain Reaction in patients with sterile pyuria

Fadel A. Nassar[1], Farid H Abu-Elamreen[2] , Mohammad E. Shubair[1], Fadel A Sharif[1]

1 Department of Medical Technology Islamic University of Gaza, Palestine
2 Department of Medical Microbiology, Central Laboratory and Blood Bank, AlShifa Hospital, Palestinian Ministry of Health, Gaza, Palestine

* CORRESPONDING AUTHOR:
Medical Microbiology Department,
Central Laboratory and Blood Bank,
AlShifa Hospital, Ministry of Health,
Gaza, Palestine
telephone: 972-599808600; fax: 972-082834007
e-mail: Farid1212@yahoo.com (Farid H. Abu-Elamreen)

Received 26.09.2007
Accepted 07.05.2008
Advances in Medical Sciences
Vol. 53(1) · 2008 · pp 80-86
DOI: 10.2478/v10039-008-0020-1

ABSTRACT

Purpose: *Chlamydia trachomatis* and *Mycoplasma hominis*, *Mycoplasma genitalium*, and *Ureaplasma urealyticum* are associated with various diseases of the urogenital tract, but they are usually not detected by routine microbiological diagnosis. To determine the occurrence of *Chlamydia trachomatis*, *Mycoplasma hominis*, *Mycoplasma genitalium*, and *Ureaplasma urealyticum* in patients with sterile pyuria.

Material/Methods: Sterile pyuria urine samples collected during the period from February 2006 to April 2007 were tested by polymerase chain reaction (PCR) for the presence of *C. trachomatis*, *M. hominis*, *M. genitalium*, and *U. urealyticum* using specific primers for each species. A total of 200 sterile pyuria samples selected from about 2400 urine samples attending the genitourinary clinic at Al-Shifa hospital, Gaza, during the period February 2006 to April 2007 and were analyzed for routine urine examination and cultured on MacConkey agar, blood agar, and sabouraud agar to detect the presence of bacteria and *Candida*. The 200 samples (96 male, 104 female; aged ≥18 years) containing more than 10 leukocytes / HPF and negative for culture (showing no significant growth after 24 hr) were tested by PCR for *C. trachomatis* and *M. hominis*, *M. genitalium*, and *U. urealyticum*.

Results: *C. trachomatis* was detected in 20 samples (10%), *U. urealyticum* in 10 samples (5%), *M. hominis* in 6 samples (3%) and *M. genitalium* in 2 samples (1%). The difference in occurrence of *C. trachomatis* was statistically insignificant between males and females (P=0.509), but it was significant (P=0.008) for *U. urealyticum*. *M. hominis* was detected only in samples collected from female patients. On the other hand, *M. genitalium* was detected only in men.

Conclusion: PCR testing of sterile pyuria showed a significant number of *C. trachomatis*, *Mycoplasma*, and *Ureaplasma infections*. Consequently, PCR is recommended for the detection of those microorganisms in the urine samples of sterile pyuria patients.

Key words: Sterile pyuria, Polymerase Chain Reaction, *Chlamydia trachomatis, Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealyticum*

INTRODUCTION

Sterile pyuria is a condition in which white blood cells (WBCs) are present in the urine (≥10/HPF) without bacteria growing in standard cultures [1].

Sterile pyuria is associated with a number of infective agents including viruses, fungi and atypical or fastidious organisms such as *Chlamydia trachomatis*, Mycoplasmas, and Ureaplasmas [2]. Mycoplasmas, Ureaplasmas and *C. trachomatis* are associated with various diseases of the genitourinary tract, but they are usually not detected by routine microbiological diagnosis [3]. Molecular genetic techniques, such as polymerase chain reaction (PCR), are useful for the identification of microorganisms that are difficult to cultivate and for those that grow slowly [4].

Table 1. **Sequences of the primers used in PCR.**

Organism	Primer name	Sequence 5' to 3'	Reference
M. genitalium	MgPaW1*	5-AAG TGG AGC GAT CAT TAC TAA C-3	[39]
	MgPaR1*	5-CCG TTG TTA TCA TAC CTT CTG A-3	
M. hominis	MHF*	5-ATA CAT CGA TGT CGA GCG AG-3	[43]
	MHR*	5-CAT CTT TTA GTG GCG CCT TAC-3	
C. trachomatis	CTR	5-GCA AGA TAT CGA GTA TGC GTT GTT AGG-3	**
	CTF	5-TTC ATT GTA CTC ATT AAA CGA GGC GG-3	
U. urealyticum	U5*	5-CAA TCT GCT CGT GAA GTA TTA C-3	[22]
	U4*	5-ACG ACG TCC ATA AGC AAC T-3	

*(All primers were synthesized by operon Biotechnologies, Germany).
**(These primers were included in C. trachomatis cryptic plasmid, primer set kit) Maxim Biotech, Inc. San Francisco USA

C. trachomatis infection of the lower genital tract is one of the most prevalent sexually transmitted diseases (STDs) worldwide [5]. According to the World Health Organization (WHO), approximately 89 million people are newly infected with *C. trachomatis* infections annually worldwide [6]. *C. trachomatis* infection, if not treated in an early stage, can lead to severe sequelae, such as pelvic inflammatory disease (PID), ectopic pregnancy and tubal infertility [7].

However, 50-80% of infected men and women are asymptomatic [8]. This high number of unrecognized infected individuals provide the reservoir for spreading the infection to other men and women via sexual intercourse.

Several laboratory methods are used for the diagnosis of *C. trachomatis*, these include cytological tests for the detection of intracytoplasmic inclusion bodies, cell culture, Enzyme-linked immunosorbent assay (ELISA), Direct immuno- fluorescence (DIF) and DNA amplification via PCR [9].

PCR is more sensitive than cell culture, it has a high sensitivity and specificity when compared to other tests used for *C. trachomatis* diagnosis, such as direct IF and ELISA which may give false positive results [6].

PCR procedure to detect *C. trachomatis* targets a cryptic chlamydial plasmid (pCT) or the gene of the major outer membrane (MOMP) as templates for PCR amplification [10]. Mycoplasmas and ureaplasmas, members of the family *Mycoplasmataceae* of the class Mollicutes, are widely distributed in humans, mammals, birds, reptiles, fish and other vertebrates as well as plants [11].

Up to now,13 species of *Mycoplasma* and 2 species of *Ureaplasma* have been isolated in humans [12,13]. Three of these species *Mycoplasma genitalium*, *Ureaplasma parvum* and *Ureaplasma urealyticum*, are thought to be associated with genitourinary infections [12,14].

M. genitalium was first isolated from men with urethritis, but studies that attempted to assess its association with disease were inhibited by the difficulty of growing the organism in culture. Currently the microorganism is detected mainly by PCR [15,16].

M. hominis has been associated with bacterial vaginosis, PID, post-partum fever and post-abortal fever, as well as a number of gynecological infections [12,17,18].

Detection of Mycoplasmas employs the 16s rRNA gene for PCR amplification [19].

There is an increasing evidence that *U. urealyticum* is associated with a range of human disorders including non-gonococcal urethritis (NGU) [20]. Previously *U. urealyticum* has been differentiated into biovars 1 and 2. In 1998 *U. urealyticum* biovars 1 and 2 were classified into *U. urealyticum* and *U. parvum,* respectively [21].

At present, the main method of detecting Ureaplasmas is by culture, but the organism is difficult to isolate and requires special culture media [21].

Detection of ureaplasmas by PCR employs urease gene as template for amplification [22].

The goal of the present study was to detect *C. trachomatis*, *M. genitalium*, *M. hominis* and *U. urealyticum* in urine specimens of 200 patients with sterile pyuria by PCR. It is worth mentioning that no previous studies have been conducted on this topic in Gaza strip. This study is important in identifying the etiological agents for many of sterile pyuria cases and would be helpful in choosing the correct treatment.

MATERIALS AND METHODS

Patient selection: Between February 2006 to April 2007, all mid-stream specimens of urine received by the microbiology laboratory of the Al-Shifa hospital, Gaza. Most patients had symptoms and signs compatible with urethral discharge and urinary tract infection including dysuria, pollakiuria, and nocturia. All samples were tested for routine urinalysis and culture were considered for inclusion in the study. Urine samples from which bacteria were isolated were excluded. All other sterile pyuria samples from men and women patients aged between 16 and 65 years were included. All urine samples were analyzed for routine urine examination, any sample containing more than 10 leukocytes / HPF was cultured on MacConkey agar, blood agar, and sabouraud agar to detect the presence of bacteria and *Candida*. A total of 200 sterile pyuria samples were negative for culture (showing no significant growth after 24 hr) were included in the study.

Figure 1. **Aphotograph of ethidium bromide stained 2% agarose gel.**

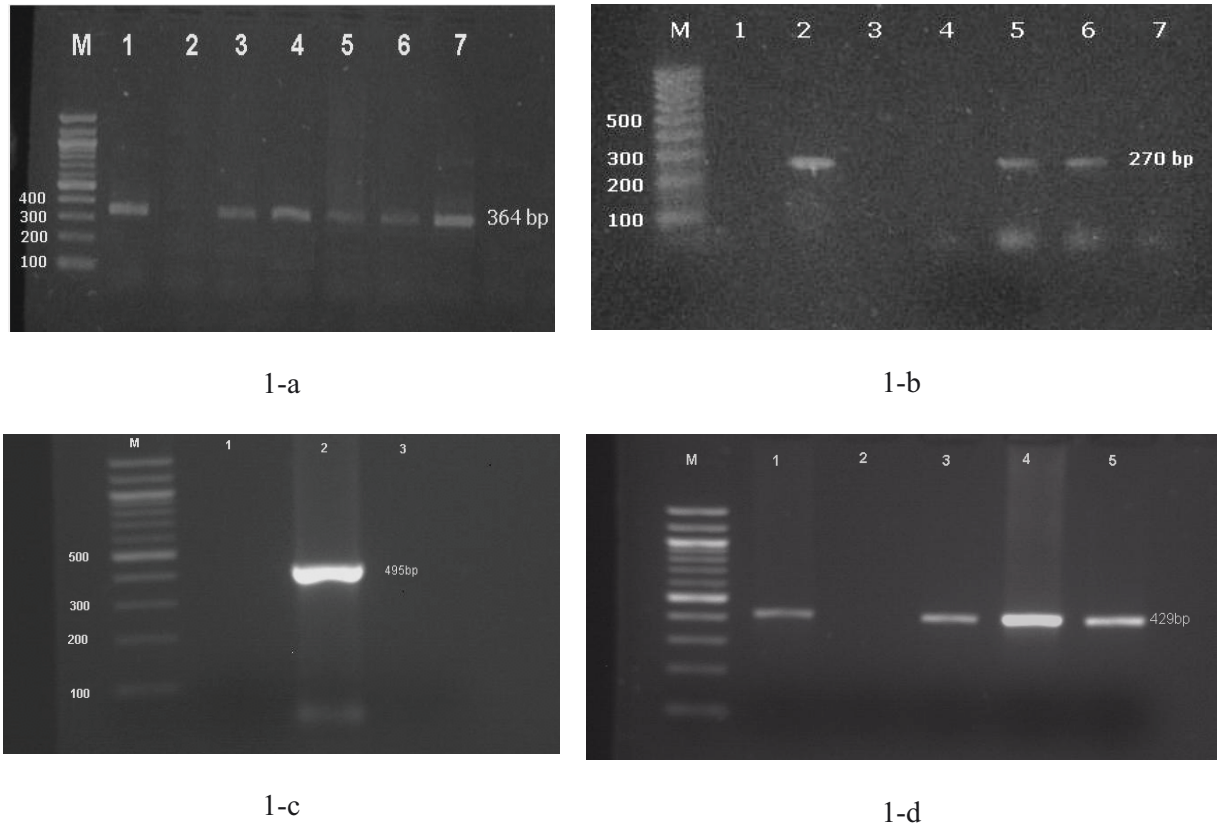

Figure 1-a. A PCR result for identification of *C. trachomatis* targeting cryptic plasmid. M:100bp DNA marker, lane1: positive control, lane2: negative control, lane3 to lane7: positive samples with 364bp product. Figure 1.b. A PCR result for identification of *M. hominis* targeting 16s rRNA gene. M: 100bp DNA marker, lane1: negative control, Lanes 3, 4, 7: negative samples, lanes2, 5, 6: positive samples with 270bp product . Figure 1.c. A PCR result for identification of *M. genitalium*. M : 100bp DNA marker, lane1: negative control, lane2 : positive sample with 495bp product, lane3 : negative sample Figure 1.d. A PCR result for identification of *U. urealyticum* targeting the urease gene. M : 100 bp DNA marker, lan2: negative control, lanes1, 3, 4, 5 : positive samples with 429bp product

Collection of samples: Subjects were asked not to void urine for 1 hour before urine collection and then to collect 20-50 ml of the first portion of voided urine (FVU) in a sterile plastic, screw-capped cups. The urine specimens were stored frozen at (-70^{0}C) for 4 to 6 days until processing.

DNA extraction: The urine specimens were thawed at room temperature. The urine specimens were briefly vortexed before being processed into the respective assay reagents. For DNA isolation, 1 ml of urine was transferred to a 1.5-ml microcentrifuge tube and centrifuged at 13000 rpm for 30 min at room temperature. After removal of the supernatant, the pellet was resuspended in 1 ml of urine resuspension buffer provided with the *MasterPure DNA Purification Kit* (*EPICENTRE* Technologies, USA) and the specimens were processed following the manufacturer's instructions.

PCR primers: Oligonucleotide primers for the PCR were adapted from the published nucleotide sequences, and are shown in *Tab. 1*.

Detection of *Chlamydia trachomatis* by PCR: The DNA specimens were tested by the *Chlamydia trachomatis* cryptic plasmid, using primer set kit *(Tab. 1)*, (10.0 µl) of the prepared DNA template or control DNA were added to 40.0 µl of Master Mixture in 0.2 ml thin walled microfuge tube. DNA amplification was carried out in a Eppendorf Mastercycler using an initial denaturation step of 96°C for 1 min, followed by 35 cycles of denaturation at 94°C for 60 s, annealing at 58°C for 60 s, and extension at 72°C for 60 s, ending with a final extension at 72°C for 10 min. Ten microliter of the amplified samples were subjected to electrophoresis in 1.5% agarose gel containing 0.5 µg/ml ethidium bromide. A band of 364 base pairs in size was considered a positive result.

Detection of of *M. genitalium* by PCR: Five microliters of DNA was added to 25µl PCR master mix, 4µl Primer1 MgpaW1 and 4µl Primer2 MgpaR. DNA amplification was carried out using an initial denaturation at 95° C for 10 minutes, 35 cycles of: denaturation at 94° C for 40 seconds, annealing at 56° C for 40 seconds, elongation at 72° C for 40 seconds and final extension at 72° C for 15 minutes. Upon completion of PCR, 20µl of the PCR products were analyzed by ethidium bromide stained 2% agarose gel electrophoresis. The expected size of DNA fragment amplified using the specific primers in this kit is 495 bp.

Detection of *M. hominis* by PCR: Reaction mixture was prepared in a PCR tube by combining the reagents as described

Table 2. **Microorganisms identified in the 200 studied specimens.**

Microorganisms	male		female		total		P value
	n	(%)	n	(%)	n	(%)	
C. trachomatis	11	(5.5)	9	(4.5)	20	(10.0)	0.509
U. urealyticum	1	(0.5)	9	(4.5)	10	(5.0)	0.008*
M. hominis	0	(0.0)	6	(3.0)	6	(3.0)	0.005*
M. genitalium	2	(1.0)	0	(0.0)	2	(1.0)	0.085

for *M. genitalium* except that primers MHR and MHF were used instead. PCR tubes were then placed in a thermal cycler and PCR amplification was done as described for *M. genitalium* except that the annealing temperature was 58° C. A positive PCR test should yield a 270 bp DNA fragment.

Detection of *U. urealyticum* by PCR: Reaction mixture was prepared in a PCR tube by combining the reagents as described for *M. genitalium* but using primers u4 and u5. DNA amplification was carried out using an initial denaturation at 94° C for 2 minutes, 50 cycles of denaturation at 94° C for 30 seconds, annealing at 56° C for 1 minute, elongation at 72° C for 45 seconds and final extension at 72° C for 5 minutes A positive PCR test should yield a 429 bp DNA fragment.

Data analysis: The data were analyzed by a personal computer using SPSS 8.0. Differences in proportions were assessed by Chi–square test and p values < 0.05 were considered statistically significant.

RESULTS

In the present study 200 urine samples from patients with sterile pyuria (96 male , 104 female) aged > 18 years in Gaza, Palestine were subjected to PCR for detection of *C. trachomatis, U. urealyticum*, *M. hominis* and *M. genitalium*. The results can be summarized as follows: Occurrence of *C. trachomatis, U. urealyticum, M. hominis and M. genitalium C. trachomatis* was detected in 20 samples (10%) *(Fig. 1-a)*, *U. urealyticum* in 10 samples (5%) *(Fig. 1-b)*, *M. hominis* in 6 samples (3%) *(Fig. 1-c)* and *M. genitalium* in 2 samples (1%) *(Fig. 1-d)*, *(Tab. 2)*.

Ninety six samples (48%) were from male patients and 104 (52%) from female patients. The occurrence of the investigated microorganisms with regard to gender of patients is presented in *Tab. 2.* The difference in occurrence of *C. trachomatis* was statistically insignificant between males and females but, that of *U. urealyticum* was significant. *M. hominis* was detected only in samples collected from female patients. On the contrary, *M. genitalium* was detected only in men.

DISCUSSION

Sterile pyuria in patients with clinical symptoms consistent with UTI can be a diagnostic challenge and warrants further investigation for detection of fastidious and atypical microorganisms such as *C. trachomatis*, *M. hominis*, *M. genitalium*, and *U. urealyticum*. These microorganisms are associated with various diseases of the genitourinary tract, but they are usually not detected by routine microbiological diagnosis.

C. trachomatis, *M. hominis*, *M. genitalium*, and *U. urealyticum* organisms are not screened by routine examination of urine samples in health laboratories in Gaza strip, and this is the first study that employed PCR assay for detection of these organisms in sterile pyuria specimens in Gaza strip.

Findings of this study showed that PCR testing of sterile pyuria could identify a significant number of causative microorganisms and should demonstrate to the clinicians the advantage of detection of the fastidious microorganisms in urine from the patients with UTI symptoms, when standard cultures fail to detect microbial infection. Since the detection of those microorganisms should constitute an essential part of the diagnosis and management of patients. The association of sterile pyuria with these organisms infections, however, ought to be discussed.

C. trachomatis infections are the most prevalent sexually transmitted bacterial infections among women and men worldwide [23]. Screening for these infections is important not only to identify infected symptomatic individuals for the diagnosis and management of their infections but also to identify asymptomatic individuals who serve as reservoirs for *C. trachomatis* infections [23].

Traditionally, the gold standard for the identification of *C. trachomatis* is culture, however, culture is time-consuming and labor-intensive. It takes 3 to 6 days to complete, and it requires access to specialized facilities and trained personnel [24].

ELISAs have also been evaluated as screening tests for the rapid identification of infected individuals by using first-catch urine [25]. ELISAs are relatively fast and easy to complete, but sensitivities of the tests for urine specimens remain relatively low [26].

In the current study, the occurrence of chlamydial infection was 10% in the 200 urine specimens collected

from symptomatic patients with sterile pyuria. In samples from women (104 samples) *C. trachomatis* was detected in 9 samples (8.7%) and in 11 of the 96 male specimens (11.5%). The difference in occurrence was statistically insignificant (P=0.509) between males and females.

Our results revealed that the occurrence of *C. trachomatis* was higher than the occurrence of the other microorganisms detected in this study. These results suggest that *C. trachomatis* infection should be strongly considered in urine samples that show sterile pyuria.

This is congruent with the conclusion of Basarab *et al.* (2002) who showed that a significant number of *C. trachomatis* could be detected in urine specimens from sterile pyuria [27]. When compared to other studies from other countries, our results are still lower than that reported in Egypt (31%), Iran (22%), India (30.8%) and the UK (21%). However, our results is higher than that reported in Jordan (4.6%), and Ghana (3.0%) [28-32].

Variations of *Chlamydia* prevalence between countries and studies could be due to several factors such as, study population (selection of high risk group, symptoms, education level, sample size, etc.), rate of infection in the study area, hygiene level and socioeconomic status of the study area, culture of the society whether it is open or conservative, and the technique and the DNA target of PCR used.

Mycoplasma hominis is a heterogeneous genital mycoplasma [33] found in at least two-thirds of women with bacterial vaginosis (BV), compared to 10% of healthy women [34]. However, its role as a primary pathogen is doubtful since it co-exists with many other bacteria in BV [35].

Culture is the most commonly employed method for detection of genital *Mycoplasma,* but it requires special handling, complex media, and cultivation. Furthermore, positive samples need further testing to determine the species [36].

Comparison between culture method and PCR has been performed and showed that a PCR assay was as sensitive as culture for detection of *M. hominis* from clinical samples. In addition it was very specific [37].

The results showed that *M. hominis* was present in 6 samples (3%). The occurrence of *M. hominis* among women was 5.8% and it was not detected in men. This finding deserves further investigation to determine whether *M. hominis* has a role in disease or just in colonization and to improve the association of sterile pyuria with urogenital *M. hominis* infection or colonization.

M. genitalium was first isolated in urethral cultures from two men with NGU in 1980 [13]. Although *M. genitalium* has been proposed as a cause of human NGU, the precise role of this mycoplasma in the etiology of NGU has not been established because of the immense difficulty of isolating it from clinical samples.

The results showed that the occurrence of *M. genitalium* was 1% and it was detected in men only. The detection of *M. genitalium* in urine samples of 2 of 96 men (2.1%) with UTI symptoms and sterile pyuria by PCR in this study and previous studies that used these assays should be considered an evidence for the association between urethritis in men and *M. genitalium* infection.

When compared to the results of other studies, our findings are similar to those reported by Takahashi *et al.* (2006) in Japan, where they recorded a 1% prevalence of *M. genitalium* among men [38].

Our results, however, are considerably lower than reported by Leandro *et al.* (2002) in the USA, where they examined the prevalence of *M. genitalium* in men with urethritis at (STDs) clinic in New Orleans (97 men with urethritis and 184 asymptomatic men) by PCR, the results of their study showed that *M. genitalium* infection rates in symptomatic and asymptomatic men were 25% and 7%, respectively. The authors concluded that *M. genitalium* is associated with (NGU) in that population [39].

In Japan, Maedo *et al.* (2004) reported that the prevalence of *M. genitalium* was 17.0% in first-voided urine specimens from153 male patients with NGU [40].

The difference between our results and those reported by previous studies may be due to the difference in the populations studied and the prevalence of the microorganism in the study area.

Another issue that needs further investigation is the role of *M. genitalium* in genital tract inflammatory disease in women.

U. urealyticum has been shown to be associated with NGU [20]. At present, the main method of detecting *U. urealyticum* is by culture, but the organism is difficult to isolate and requires special culture media. *U. urealyticum* can also be detected rapidly and accurately by PCR such that treatment may be established in the early stages of infection, also this method avoids the problems associated with culturing.

In the present study *U. urealyticum* was detected in 9 (8.7%) of female samples and in only one (1%) of the male sample. The difference in occurrence was statistically significant (P=0.008) between males and females.

Detection of the microorganism in urine specimens leaves the clinical significance of *U. urealyticum* open to question. It should be noted that 60% of healthy women carry *U. urealyticum* in their urogenital tract. Unlikely, in male urogenital tract this microorganism should not appear. If present in male urethra, it should always be treated as a pathogen [41].

It is known that other organisms can cause the clinical disorders observed and also that not all serotypes of *U. urealyticum* are pathogenic.

When we compared our results to other studies, our results are still lower than those reported in Japan (16.3%) , China (24%) and Austria (20%) [4,40,42].

These variations could be explained by differences in the study population and rate of infection in the study area. Previous studies have included populations that have much higher incidences and prevalences of STD than does the population of our study.

Finally, Mycoplasmas, Ureaplasmas and *C. trachomatis* are associated with sterile pyuria in Gaza Strip , PCR assay provide a rapid and effective measure to detect fastidious and atypical microorganisms in sterile pyuria cases which is useful for identification of etiological agents and the consequent management and treatment of patients.

Further investigations should be done including asymptomatic cases and symptomatic without sterile pyuria to compare the prevalence of these microorganisms among these groups. STDs clinics are not available, it recommended to establish such clinics to minimize the danger of such diseases. We also recommend the establishment of a database for STDs in Gaza strip.

ACKNOWLEDGMENT

This research was funded by the Deanery of Scientific Research at the Islamic University of Gaza.

REFERENCES

1. Dieter RS.Sterile pyuria: a differential diagnosis.Compr Ther. 2000 Fall;26(3):150-2.

2. Tayal SC, Pattman RS.Sterile pyuria: consider chlamydial infection. Br J Clin Pract. 1996 Apr-May;50(3):166-7.

3. Piémont Y, Jaulhac B. [Value of molecular biology methods for diagnosis in bacteriology] Ann Dermatol Venereol. 1995;122(4):206-12.

4. Daxboeck F, Zitta S, Stadler M, Iro E, Krause R. Mycoplasma hominis and Ureaplasma urealyticum in patients with sterile pyuria. J Infect. 2005 Jul;51(1):54-8.

5. Gerbase AC, Rowley JT, Heymann DH, Berkley SF, Piot P. Global prevalence and incidence estimates of selected curable STDs. Sex Transm Infect. 1998 Jun;74 Suppl 1:S12-6.

6. Rowe PJ. Reproductive tract infections. Annual Technical Report. Human Reproduction Program, WHO; 1998. 186-190.

7. Marrazzo JM, Stamm WE. New approaches to the diagnosis, treatment, and prevention of chlamydial infection. Curr Clin Top Infect Dis. 1998;18:37-59.

8. Stamm WE. Chlamydia trachomatis Infections of the Adult. In Sexually Transmitted Diseases. 3rd ed. Holmes KK, Sparling PF, Mardh PA, Lemon SM, Stamm WE, Piot P, Wasserheit JN. New York: McGraw-Hill; 1999. 407–22. Stamm WE. Chlamydia trachomatis Infections of the Adult. In Sexually Transmitted Diseases. 3rd ed. Holmes KK, Sparling PF, Mardh PA, Lemon SM, Stamm WE, Piot P, Wasserheit JN. New York: McGraw-Hill; 1999. 407–22.

9. Santos C, Teixeira F, Vicente A, Astolfi-Filho S. Detection of Chlamydia trachomatis in endocervical smears of sexually active women in Manaus-AM, Brazil, by PCR. Braz J Infect Dis. 2003 Apr;7(2):91-5.

10. Mahony JB, Luinstra KE, Sellors JW, Chernesky MA. Comparison of plasmid- and chromosome-based polymerase chain reaction assays for detecting Chlamydia trachomatis nucleic acids. J Clin Microbiol. 1993 Jul;31(7):1753-8.

11. Razin S, Yogev D, Naot Y. Molecular biology and pathogenicity of mycoplasmas. Microbiol Mol Biol Rev. 1998 Dec;62(4):1094-156.

12. Taylor-Robinson D, Furr PM. Genital mycoplasma infections. Wien Klin Wochenschr. 1997 Aug 8;109(14-15):578-83.

13. Tully JG, Taylor-Robinson D, Cole RM, Rose DL. A newly discovered mycoplasma in the human urogenital tract. Lancet. 1981 Jun 13;1(8233):1288-91.

14. Schwartz MA, Hooton TM. Etiology of nongonococcal nonchlamydial urethritis. Dermatol Clin. 1998 Oct;16(4):727-33.

15. Palmer HM, Gilroy CB, Furr PM, Taylor-Robinson D. Development and evaluation of the polymerase chain reaction to detect Mycoplasma genitalium. FEMS Microbiol Lett. 1991 Jan 15;61(2-3):199-203.

16. Jensen JS, Uldum SA, Søndergård-Andersen J, Vuust J, Lind K. Polymerase chain reaction for detection of Mycoplasma genitalium in clinical samples.J Clin Microbiol. 1991 Jan;29(1):46-50.

17. Hillier SL, Nugent RP, Eschenbach DA, Krohn MA, Gibbs RS, Martin DH, Cotch MF, Edelman R, Pastorek JG 2nd, Rao AV, McNellis D, Regan JA, Carey JC, Klebanoff MA. Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. The Vaginal Infections and Prematurity Study Group. N Engl J Med. 1995 Dec 28;333(26):1737-42.

18. Shimada M, Kotani T, Ohtaki S, Sameshima H, Ikenoue T, Sasaki T, Kenri T. [Clinicobacteriological studies on the nine cases with upper genital tract Mycoplasma hominis infection] Kansenshogaku Zasshi. 1999 Jul;73(7):646-51.

19. Yoshida T, Maeda S, Deguchi T, Ishiko H. Phylogeny-based rapid identification of mycoplasmas and ureaplasmas from urethritis patients. J Clin Microbiol. 2002 Jan;40(1):105-10.

20. Taylor-Robinson D, McCormack WM. The genital mycoplasmas (first of two parts). N Engl J Med. 1980 May 1;302(18):1003-10.

21. Kong F, James G, Ma Z, Gordon S, Bin W, Gilbert GL. Phylogenetic analysis of Ureaplasma urealyticum--support for the establishment of a new species, Ureaplasma parvum. Int J Syst Bacteriol. 1999 Oct;49 Pt 4:1879-89.

22. Povlsen K, Jensen JS, Lind I. Detection of Ureaplasma urealyticum by PCR and biovar determination by liquid hybridization. J Clin Microbiol. 1998 Nov;36(11):3211-6.

23. White R, Perry K. MHRA 04122. Chlamydia trachomatis. Nucleic acid amplification testes (NAATs): Review and evaluation of literature. [Internet] London: MHRA; 2004.

24. Jaschek G, Gaydos CA, Welsh LE, Quinn TC. Direct detection of Chlamydia trachomatis in urine specimens from symptomatic and asymptomatic men by using a rapid

polymerase chain reaction assay. J Clin Microbiol. 1993 May;31(5):1209-12.

25. Chernesky M, Castriciano S, Sellors J, Stewart I, Cunningham I, Landis S, Seidelman W, Grant L, Devlin C, Mahony J. Detection of Chlamydia trachomatis antigens in urine as an alternative to swabs and cultures. J Infect Dis. 1990 Jan;161(1):124-6.

26. Jensen IP. A comparison of urine sample to urethral swab for detection of Chlamydia trachomatis in asymptomatic young men using two enzyme immunoassays. Sex Transm Dis. 1992 May-Jun;19(3):165-9.

27. Basarab A, Browning D, Lanham S, O'Connell S. Pilot study to assess the presence of Chlamydia trachomatis in urine from 18-30-year-old males using EIA/IF and PCR. J Fam Plann Reprod Health Care. 2002 Jan;28(1):36-7.

28. Mohamed N, Sharaf T. Evaluation of different techniques in diagnosing chlamydial endocervical infection among Egyptian females. The Egyptian J. Hosp. Med. 2001; 2:138-47.

29. Badami N. Isolation and serotyping of Chlamydia trachomatis from patients with urethritis and cervicitis in Tehran and Bandar abbas (PhD Thesis). School of Public Health, Tehran University of Medical Sciences, Iran 1991.

30. Singh V, Salhan S, Das BC, Mittal A. Predominance of Chlamydia trachomatis serovars associated with urogenital infections in females in New Delhi, India. J Clin Microbiol. 2003 Jun;41(6):2700-2.

31. Awwad ZM, Al-Amarat AA, Shehabi AA. Prevalence of genital chlamydial infection in symptomatic and asymptomatic Jordanian patients. Int J Infect Dis. 2003 Sep;7(3):206-9.

32. Apea-Kubi KA, Yamaguchi S, Sakyi B, Kishimoto T, Ofori-Adjei D, Hagiwara T. Neisseria gonorrhoea, Chlamydia trachomatis, and Treponema pallidum infection in antenatal and gynecological patients at Korle-Bu Teaching Hospital, Ghana. Jpn J Infect Dis. 2004 Dec;57(6):253-6.

33. Christiansen G, Jensen LT, Boesen T, Emmersen J, Ladefoged SA, Schiøtz LK, Birkelund S. Molecular biology of Mycoplasma. Wien Klin Wochenschr. 1997 Aug 8;109(14-15):557-61.

34. Taylor-Robinson D, Furr PM. Update on sexually transmitted mycoplasmas. Lancet. 1998;351 Suppl 3:12-5.

35. Mårdh PA, Weström L. Tubal and cervical cultures in acute salpingitis with special reference to Mycoplasma hominis and T-strain mycoplasmas. Br J Vener Dis. 1970 Jun;46(3):179-86.

36. Cassell GH, Waites KB. Mycoplasma infections. Infectious diseases of the fetus and newborn infant. 4 th ed. Remington J, Klein J. Philadelphia: Saunders WB; 1995. 619–655p.

37. Luki N, Lebel P, Boucher M, Doray B, Turgeon J, Brousseau R. Comparison of polymerase chain reaction assay with culture for detection of genital mycoplasmas in perinatal infections. Eur J Clin Microbiol Infect Dis. 1998 Apr;17(4):255-63.

38. Takahashi S, Takeyama K, Miyamoto S, Ichihara K, Maeda T, Kunishima Y, Matsukawa M, Tsukamoto T. Detection of Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma urealyticum, and Ureaplasma parvum DNAs in urine from asymptomatic healthy young Japanese men. J Infect Chemother. 2006 Oct;12(5):269-71.

39. Mena L, Wang X, Mroczkowski TF, Martin DH.Mycoplasma genitalium infections in asymptomatic men and men with urethritis attending a sexually transmitted diseases clinic in New Orleans.Clin Infect Dis. 2002 Nov 15;35(10):1167-73.

40. Maeda S, Deguchi T, Ishiko H, Matsumoto T, Naito S, Kumon H, Tsukamoto T, Onodera S, Kamidono S. Detection of Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma parvum (biovar 1) and Ureaplasma urealyticum (biovar 2) in patients with non-gonococcal urethritis using polymerase chain reaction-microtiter plate hybridization. Int J Urol. 2004 Sep;11(9):750-4.

41. Voiko R, Remmet J. Detection by PCR method of fastidious microorganisms in urine from patients with UTI symptoms. European Society of Clinical Microbiology and Infectious Diseases. 16th European Congress of Clinical Microbiology and Infectious Diseases. 2006. April 1-4. Nice, France.

42. Teng K, Li M, Yu W, Li H, Shen D, Liu D. Comparison of PCR with culture for detection of Ureaplasma urealyticum in clinical samples from patients with urogenital infections. J Clin Microbiol. 1994 Sep;32(9):2232-4.

43. Mygind T, Birkelund S, Christiansen G. DNA sequencing reveals limited heterogeneity in the 16S rRNA gene from the rrnB operon among five Mycoplasma hominis isolates. Int J Syst Bacteriol. 1998 Jul;48 Pt 3:1067-71.