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

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**ABSTRACT**

**Purpose:** *Chlamydia trachomatis* and *Mycoplasma hominis*, *Mycoplasma genitalium*, and *Ureaplasma urealyticum* are associated with various diseases of the genitourinary 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].
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 immunofluorescence (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].

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

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

*(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*
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°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 40 seconds, annealing at 58°C for 40 seconds, and extension at 72°C for 40 seconds. Upon completion of PCR, 20µl of the PCR products were analyzed by ethidium bromide stained 1.5% agarose gel containing 0.5 µg/ml ethidium bromide. A band of 364 base pairs in size was observed.

**Detection 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, and extension at 72°C for 40 seconds. 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...
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 429 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 270 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* was detected in 20 samples (10%) (Tab. 2).

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

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>male</th>
<th>female</th>
<th>total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>(%)</td>
<td>n</td>
<td>(%)</td>
</tr>
<tr>
<td><em>C. trachomatis</em></td>
<td>11 (5.5)</td>
<td>9 (4.5)</td>
<td>20 (10.0)</td>
<td>0.509</td>
</tr>
<tr>
<td><em>U. urealyticum</em></td>
<td>1 (0.5)</td>
<td>9 (4.5)</td>
<td>10 (5.0)</td>
<td>0.008*</td>
</tr>
<tr>
<td><em>M. hominis</em></td>
<td>0 (0.0)</td>
<td>6 (3.0)</td>
<td>6 (3.0)</td>
<td>0.005*</td>
</tr>
<tr>
<td><em>M. genitalium</em></td>
<td>2 (1.0)</td>
<td>0 (0.0)</td>
<td>2 (1.0)</td>
<td>0.085</td>
</tr>
</tbody>
</table>

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 of 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.

**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 are 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 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 asymptomatic 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**

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