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Detection of urogenital pathogens in sterile pyuria samples by polymerase chain reaction

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Abstract

Background: Patients with sterile pyuria may be infected with sexually transmitted diseases or have renal tuberculosis. This study investigated the possibility of detecting sexually transmitted diseases and *Mycobacterial tuberculosis* in sterile pyuria samples with polymerase chain reaction.

Methods: Forty-four day-3 negative urine culture samples were investigated for the presence of sexually transmitted diseases and *Mycobacterial tuberculosis*.

Results: Among the 44 samples, 61.4% were positive by polymerase chain reaction (PCR) for bacterial DNA (either sexually transmitted diseases or Mycobacteria). Among the 27 positive samples, 37% were positive for *Ureaplasma urealyticum*, 26% were positive for *Chlamydia trachomatis*, 14.8% were positive for *Neisseria gonorrhoeae*, 11.1% were positive for *Mycoplasma genitalium*, 7.4% were positive for *Mycoplasma hominis*, and only one sample (3.7%) was positive for *Mycobacterial tuberculosis*. No significant associations were found between PCR-positive urine samples and patient characteristics.

Conclusions: It was concluded that *Ureaplasma urealyticum* was predominant in sterile pyuria followed by *Chlamydia trachomatis*. There were no significant associations between PCR-positive samples and sex, symptomatic patients, or antibiotic use. PCR is an instant diagnostic tool for sexually transmitted diseases in sterile pyuria; hence, it is advised to be performed on negative culture samples as a routine laboratory screening test whenever possible.

Keywords: Sterile pyuria, PCR, Urogenital, Pathogens, Sexually transmitted diseases

1 Background

Pyuria is the presence of three or more pus cells per high-power field of centrifuged urine, 10 or more pus cells per cubic millimetre in a urine specimen, or a positive leukocyte esterase urine dipstick test [1]. The presence of more than 5–8 leucocytes per high-power field on microscopy is classified as sterile pyuria (SP) in the absence of positive urine cultures. The diagnosis of SP can be complicated by the presence of casts, proteinuria, and haematuria. It can generally be categorized as either infectious or noninfectious. Noninfectious SP could be caused by radiotherapy, pelvic inflammation, appendicitis, urinary stones, tumours, or physiological changes (pregnancy) [2]. Infectious SP is identified by aerobic laboratory procedures (on a 5% sheep-blood agar plate and MacConkey agar plate) as the prolonged presence of white cells in the urine in the absence of bacteria. Population-based studies suggest that 13.9% of women and 2.6% of men have sterile pyuria, a very common disorder [3]. Historically, colony counts larger than 100,000 colony-forming units (CFU) per millilitre of void urine have been used to distinguish between colonization and bacterial urinary tract infection [3].

Sexually transmitted diseases (STDs) are one of the main causes of SP, and 500 million people worldwide have been reported to be infected with STDs such as gonorrhoea, chlamydia, syphilis, mycoplasma, trichomoniasis, or sexually transmitted viruses [4]. Additionally,

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SP has been linked to widespread viruses such as adenoviruses and parasitic illnesses such as schistosomiasis [5]. The majority of STDs in men result in symptomatic urethritis, with epididymitis occurring less frequently. Many women may initially experience no symptoms, and pelvic inflammatory disease can progress symptomlessly.

Leukocyte esterase-detecting urine tests in asymptomatic men have a sensitivity of 66.7% for gonorrhoea and 60.0% for chlamydia. *Chlamydia trachomatis* is the most typical bacterium found in cultures from sexually active populations presenting with sterile pyuria [6]. *C. trachomatis* and *Neisseria gonorrhoeae* can be quickly identified using commercially available nucleic acid techniques [7]. *Mycoplasma hominis* and *Ureaplasma urealyticum* are among the diseases associated with SP [8].

Atypical infections, in particular renal tuberculosis, should also be taken into consideration in individuals with chronic SP [9]. Patients with chronic sterile pyuria may develop the condition from an unusual infection, such as kidney tuberculosis, that causes the disorder. After lymphadenopathy, genitourinary tuberculosis is the most prevalent type of nonpulmonary tuberculosis, accounting for 27% of cases.

When SP is found, doctors may take a medical history and request laboratory tests, including urine tests for microscopy (fungi, Schistosoma ova), STDs, and *Mycobacterial tuberculosis* infection [10]. To avoid using unnecessary antibiotics and to encourage antibiotic stewardship, early identification of the cause of SP is crucial; hence, a quick and affordable method for identifying pathogens is necessary, such as polymerase chain reaction (PCR) [11]. According to numerous studies, multiplex PCR compares highly with conventional urine culture in terms of accuracy. Significant time savings are also achieved with multiplex PCR relative to traditional urine culture. This study investigated the presence of STDs and *M. tuberculosis* in sterile pyuria samples with PCR.

2 Methods

2.1 Study setting

A descriptive cross-sectional study was conducted at a tertiary hospital (in Makkah) in 2022.

2.2 Study design

This is a laboratory-based study.

2.3 Inclusion criteria

Urine samples showing pus cells (⩾10 in the mid-stream urine per cubic millimetre in a urine specimen, or 3 or more white cells per high-power field of unspun urine) and no bacterial growth after 3 days of incubation.

2.4 Exclusion criteria

Urine samples showing bacterial growth within 3 days of incubation.

2.5 Sample size and sampling type

Only urine specimens were used in this study. The Yamane formula used for determining the necessary sample size is as follows: $n = N(1 + Ne^2)$, where n = sample size (= 44), N = population size (50 based on the total visits of the patients), and e (significance) = 0.05.

2.6 Patients and clinical samples

In this study, 44 clinical urine samples obtained in 2022 from 44 patients in a tertiary hospital (in Makkah) were analysed. The samples were selected from among those with culture negativity according to standard microbiological methods used in the microbiology laboratory. Only those samples showing no bacterial growth after 3 days of incubation were studied for STDs and *M. tuberculosis* with PCR. Ethical approval (No. HAPO-02-K-012-2022-09-1171) was obtained from the biomedical research ethics committee at the Faculty of Medicine-Umm Al-Qura University.

2.7 DNA extraction and PCR

Ten millilitres of urine was centrifuged at 10,000 × g for 5 min. Total DNA was then extracted from the pellet using a conventional boiling method. In brief, after being resuspended in 100 µl of water suitable for molecular biology, the samples were centrifuged at 15,000 × g for 10 min. The pellets were reconstituted in 100 µl of water, boiled in a water bath for 15 min at 95 °C, cooled on ice, centrifuged for 2 min at 15,000 g, and then stored at − 20 °C until analysis [12].

PCR was performed using primers (Table 1) selected to detect *U. urealyticum*, *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium*, *M. hominis*, and *M. tuberculosis,* as shown in Table 1. The reaction mixture used for each standard PCR amplification contained deoxynucleoside triphosphates (dNTPs), internal control, primer, DNA polymerase, and a contaminant-prevention solution (Qiagen, USA). Amplification was performed using an Eppendorf Mastercycler PCR machine (Eppendorf, Germany). The cycling conditions included a 5-min initial denaturation period at 95 °C, 30 cycles of 30-s denaturation at 95 °C, 30-s annealing at the proper annealing temperature (Table 1), and a 45-s extension period at 72 °C followed by a final 10-min extension period. After electrophoresis on a 1.5% agarose gel and ethidium bromide staining, the

Table 1 Primers used in this study

Pathogen	Gene	Sequence	Size	References
C. trachomatis	KL1	TCCGGAGCGAGTACGAAGA	241	[13]
	KL2	AATCAATGCCCGGGATTGGT		
N. gonorrhoeae	HO1	GCTACGCATACCCGCGTTGC3	390	[14]
	HO2	CGAAGACCTTCGAGCAGAC3		
U. urealyticum	U4	ACGACGTCCATAAGCAACT3	429	[15]
	U5	CAATCTGCTCGTGAAGTATTAC3		
M. genitalium	MgPa1	AGTTGTGAAACCTTAACCCCTTGG3	281	[16]
	MgPa-3	CCGTTGAGGGGTTTTCCATT TTTTGC3		
M. hominis	RNAH1	CAA TGG CTA ATG CCG GAT ACG C	*344*	[17]
	RNAH2	GGT ACC GTC AGT CTG CAA T		
M. tuberculosis	*MTB1*	CCT GCG AGC GTA GGC GTC GG	*123*	[18]
	MTB2	CTC GTC CAG CGC CGC TTC GG		

Table 2 Demographic features of the patients

Demographic features	*N* (%)
Male	40 (90.9)
Female	4 (9.1)
< 20	1 (2.3)
20–40	22 (50)
41–60	12 (27.3)
> 61	9 (20.4)
Symptomatic	37 (84.1)
Asymptomatic	7 (15.9)
Antibiotics use	11 (25)
No antibiotics use	33 (75)
Total	44 (100)

amplification products were observed and photographed using a UVP BioDoc It Imaging System (Cambridge, UK).

2.8 Statistical analysis

The statistical analysis was performed using SPSS 25 for Windows. The Chi-square (χ2) test was used to find an association between the presence of organisms and patient characteristics (p value < 0.05).

3 Results

Forty-four day-3 negative urine culture samples were studied. Table 2 shows descriptive statistics of the demographic features of the patients. Thus, 40 (91.0%) of the patients were men, while 4 (9%) were women; 22 (50%) were aged 20–40 and 12 (27.3%) were aged 41–60; 7 (15.9%) were asymptomatic and 37 (84.1%) were symptomatic; 11 (25%) were using antibiotics during the study period, while 33 (75%) were not.

Table 3 Detection of urogenital pathogens from sterile pyuria by PCR

Pathogens	*N* (%)
U. urealyticum	10 (37)
C. trachomatis	7 (26)
N. gonorrhoeae	4 (14.8)
M. genitalium	3 (11.1)
M. hominis	2 (7.4)
M. tuberculosis	1 (3.7)
Total	27 (100)

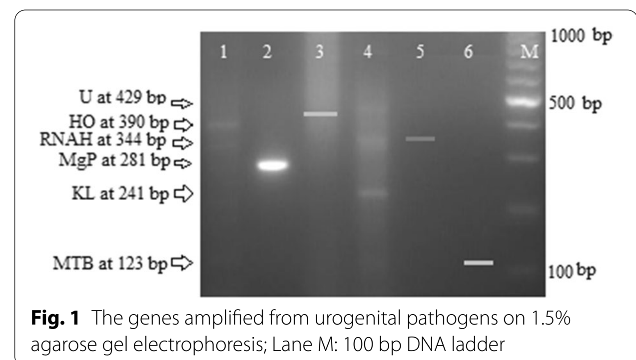

Fig. 1 The genes amplified from urogenital pathogens on 1.5% agarose gel electrophoresis; Lane M: 100 bp DNA ladder

The results showed that 27 (61.4%) urine culture samples were positive by PCR for bacterial DNA. Table 3 shows the distribution of the urogenital pathogens in the samples positive for bacterial DNA. Of the 27 positive samples, 10 (37%) were positive for *U. urealyticum*, 7 (26%) were positive for *C. trachomatis*, 4 (14.8%) were positive for *N. gonorrhoeae*, 3 (11.1%) were positive for *M. genitalium*, 2 (7.4%) were positive for *M. hominis*, and

1 (3.7%) was positive for *M. tuberculosis* (Fig. 1). No significant associations were found between PCR-positive urine culture and sex ($P = 0.557$), the presence of symptomatic patients ($P = 0.551$), or antibiotic use ($P = 0.371$).

4 Discussion

The traditional diagnostic tools for detecting urine pathogens are culture, antigen detection, and serology; however, the cultivation of organisms such as *U. urealyticum, C. trachomatis, N. gonorrhoeae, M. genitalium, M. hominis, and M. tuberculosis* is laborious, time-consuming, and requires specific expertise [19, 20]. Such organisms could be diagnosed by molecular testing, which is more sensitive, specific, rapid, and valuable in diagnosing STDs [21, 22]. The high sensitivity of PCR allows the detection of bacterial DNA even when it is present at extremely low concentrations.

The aim of this study was to detect organisms responsible for STDs and *M. tuberculosis* in sterile urine samples. In this study, 44 day 3 negative urine culture samples were studied. The results showed that 27 (61.4%) urine samples were positive for STDs by PCR. Of the 27 positive samples, 10 (37%) were positive for *U. urealyticum*, 7 (26%) were positive for *C. trachomatis*, 4 (14.8%) were positive for *N. gonorrhoeae*, 3 (11.1%) were positive for *M. genitalium*, 2 (7.4%) were positive for *M. hominis*, and 1 (3.7%) was positive for *M. tuberculosis*. Statistically, there were no significant associations found ($P > 0.05$) between PCR-positive urine culture and sex, symptomatic patients, or antibiotic use.

Asymptomatic pyuria is the presence of pus cells (greater than 5–9 white blood cells/high-power field) in a properly collected urine sample of a patient who has no signs or symptoms of a urinary tract infection. It was previously found that asymptomatic pyuria may increase the risk of developing overt UTI [23]. In a study of sterile pyuria among admitted patients, only 18.8% of patients with pyuria had a positive culture [24]. In one study, 46.6% of urogenital samples were found to be positive for STDs using PCR, in which *U. urealyticum* represented 60.7% [25]. Similar studies have reported a higher rate (more than 40%) for *U. urealyticum* than for *M. hominis* (more than 20%) [26, 27]. To our knowledge, this is the first study in our area using PCR as a diagnostic tool for STDs. In contrast, other studies reported *C. trachomatis* as the most prevalent bacterial STD [21, 28]. Such genital pathogens (mycoplasmas) may be related to a low socioeconomic background, such as poverty, and the use of contraceptive drugs [29]. Gonorrhoea has been reported as a cause of sterile pyuria, according to past and present investigations [3, 30]. In one study, more than 1200 symptomatic men with pyuria were found to be infected with *C. trachomatis* (31%) and *M. genitalium* (10%) [31]. Another study showed that *U. urealyticum* was associated with the presence of symptoms and higher rates of pyuria. It was also reported that 37% of patients had mycobacteria isolated from urine with acid-fast bacilli staining, 46% had positive histopathology (bladder biopsies), and 93% had urinary PCR results for *M. tuberculosis* [32]. The diagnosis of urinary tuberculosis is usually made very late, since it manifests asymptotically with nonspecific signs; hence, it is very important to use a more rapid and sensitive method, such as PCR, for diagnosis [33]. Gonorrhoea has been reported as a common sexual disease in the USA [34]. Additionally, in the WHO statistics for the European Region, in 2008, there were 3.4 million new cases of *N. gonorrhoeae* [35], placing gonorrhoea in third place after chlamydiosis and syphilis.

Sterile pyuria may be caused by STDs; hence, young, sexually active patients should undergo culture for organisms such as *C. trachomatis,* which can cause 10% of cases of sterile pyurias [36]. In addition, the fact that bacterial DNA is found in a clinical sample does not necessarily mean that there is an infection. Bacteria may be detected at various sites as a result of colonization or translocation, especially if sensitive methodologies are used. For the detection and identification of live or dead bacteria in situations of suspected infection in patients receiving antibiotic therapy and in symptomatic patients with a culture-negative microbiological report, PCR has proven beneficial.

5 Conclusions

The present study identified 61.4% of negative urine culture samples as positive for STDs with PCR. *U. urealyticum* was the most frequent (37%), followed by *C. trachomatis* (25.9%), *N. gonorrhoeae* (14.8%), *M. genitalium* (11.1%), *M. hominis* (7.4%), and *M. tuberculosis* (3.7%). There were no significant associations found between PCR-positive urine culture and sex, symptomatic patients, or antibiotic use. PCR is an instant diagnostic tool for STDs; hence, it is advised to be performed on negative culture samples as a routine laboratory screening test whenever possible.

Abbreviations

C. trachomatis: *Chlamydia trachomatis*; CFU: Colony-forming units; dNTP: Deoxynucleoside triphosphate; *M. tuberculosis*: *Mycobacterial tuberculosis*; *M. hominis*: *Mycoplasma hominis*; *N. gonorrhoeae*: *Neisseria gonorrhoeae*; PCR: Polymerase chain reaction; STDs: Sexually transmitted diseases; SP: Sterile pyuria; *U. urealyticum*: *Ureaplasma urealyticum*.

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Author contributions
OA helped in formulation of hypothesis, conceptualization, methodology, data collection, writing—original draft and supervision. FB contributed to data collection, writing—review, editing and resources, manuscript revision. Both authors have read and approved the manuscript.

Availability of data and materials
The datasets generated in the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and Consent to participate
The study was approved by the biomedical research ethics committee at Faculty of Medicine-Umm Al-Qura University (Approval No. (HAPO-02-K-012–2022-09–1171). An informed written consent to participate in the study was provided by all participants.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests

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