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# Molecular Detection of *Mycoplasma genitalium* and *Chlamydia trachomatis* infections among Pyuria Patients attending Urology Clinics in Khartoum State

Eshraga S. Alhaj[1], Al Fadhil A. Omer[1], Wafa I. Alhaj[1], Nawal Taj Alsir[2]
[1]*Al Neelain University, Khartoum- Sudan.*
[2]*National Health Laboratory, Khartoum, Sudan*

## Abstract

**Background:** Sterile pyuria is a condition in which leucocytes (pus cells) are present in urine (≥ 10/HPF) without bacterial growth in a standard culture. Sterile pyuria is associated with a number of infective agents including viruses, fungi and a typical or fastidious organisms such as *Mycoplasma* (*M. genitalium*), *Ureaplasma* and *Chlamydia trachomatis* (*C. trachomatis*).

**Objective**: To perform molecular detection of *Mycoplasma genitalium* and *Chlamydia trachomatis* infections among pyuria patients attending urology clinics in Khartoum State.

**Materials and methods**: Urine specimens were collected from 300 patients (150 males and 150 females), aged ≥ 20 years, and attending urology clinics in Khartoum State. Real time PCR technique was used to amplify DNA extracted from all 300 urine specimens collected from urology patients with pyuria and non-pyuria .

**Results:** The frequency rate of *C. trachomatis* in patients with sterile pyuria was 5% out of the 300 patients investigated. *M. genitalium* was not detected in all specimens collected. The highest incidence rate of infected *C. trachomatis* was among patients aged 31-40 years. Also, the frequency rate of positive *C. trachomatis* patients was (12/8%) in females and (3/2%) in males.

**Conclusion:** Real time PCR assay is a rapid, specific technique for detection of *C. trachomatis* associated with urogenital tract infections.

**Keywords:** Real time PCR, *Mycoplasma genitalium, Chlamydia trachomatis* Pyuria patients.

## Introduction

Sterile pyuria is a condition in which WBC are present in urine (≥ 10/HPF) without bacterial growth in a standard culture. Sterile pyuria is associated with a number of infective agents including viruses, fungi and typical or fastidious organisms such as *Mycoplasmas, Ureaplasma* and *Chlamydia* infections. *C. trachomatis* is a non-infectious condition leading to deposit renal calculi, anatomic nephro-calcinosis and polycystic kidney disease. *Mycoplasma* and *Chlamydia trachomatis* are associated with viral diseases and genitourinary tract infection. In males and

females, *C. trachomatis* causes urinary tract infections, and males it causes chronic prostatitis, prostate cancer, and non-gonococcal urethritis, and male infertility[1].
In females, *C. trachomatis* causes pelvic inflammatory disease (PID) and tubal factor infertility. However, they are usually not detected by routine microbiological diagnosis methods. Molecular techniques such as real time polymerase chain reaction (RT-PCR) are useful for the identification of microorganisms that are difficult to cultivate and for slow-growing organisms. In this study real time PCR is used for direct detection of *Mycoplasma genitalium* and *Chlamydia trachomatis*, which are commonly authenticated as urethral pathogens and independently associated with non-gonococcal urethritis (NGU). These bacteria are also sexually transmitted. These infections affect both men (50%) and women (80%). Nearly all men may be asymptomatic. The prevalence rate of *C. trachomatis* and *M. genitalium* infections among Sudanese pyuria patient is not fully documented[2].

## Materials and methods

This was a cross-sectional, case control study conducted during the period from October 2015 to October 2016. Patients investigated were suffering from genital discharge, dysuria, pain. They were attending urology clinics in Khartoum State. The study was approved by the Scientific Research Committee of Al Neelain University. Permission to collect the specimens was granted by authorities of the urology clinics where the study was conducted. All study participants were informed with the aims of the study and its importance, and a verbal consent was obtained from each participant. Data was analyzed using the SPSS program, version 14. The results were expressed as percentages, mean, and standard deviation (SD). Independent T-test was performed to compare the study parameters in cases versus control groups. Correlation was done to study the relationship between study parameters and study variables. A p-value less than 0.05 was considered as significant. A well-structured questionnaire was used to collect clinical and demographical data of all participants.
A total of 300 sterile pyuria specimens (150 males + 150 females) were collected from patients aged more than 20 years. 15- 20 ml of first voided urine (FVU) were collected in sterile plastic, screw-capped containers. All specimens were subjected to 24 hrs culture using the conventional, standard, bacteriological methods. Positive specimens (with more than 10 leukocytes / HPF) were enrolled in the study and molecular detection was performed.
Molecular procedure: DNA was extracted from each specimen according to manufacturer's instructions (CheLex Biotechnologies, China). Urine sediments were thawed at room temperature then 500µl of sample were transferred to 1.5 ml tubes, 900µl 1x PBS were added for washing. After that 200µl instigate matrix (chelex6%) were added, vortexed at high speed for 10 sec and incubated at 100° C for 15min. Vortexing was performed during the incubation and after incubation. Then specimens were centrifuged at 12,000 rpm for 2 min. The supernatants were transferred to sterile clean 1.5 ml tubes, and the DNA extracted was labeled.
Detection of *Chlamydia trachomatis* and *M. genitalium* genomes was performed using kits of Sacace Biotechnology Company (Italy); where the pathogen genome was amplified by specific region primers.
Detection of *C. trachomatis*: The PCR-mix-2-FRT tube was thawed, all reagents tubes (including

2 controls) were vortexed, centrifuged briefly, labeled, and 10 µl of PCR-mix-1-FL of *Chlamydia trachomatis* was transferred to sterile Eppendorff tube, then 5.0 µl of PCR-mix-2-FRT and 0.5 µl of polymerase (TaqF) were added. After that all tubes were vortexed for 2-3 sec, 15 µl of the prepared mixture were transferred to each tube by using tips with aerosol barrier. Then 10 µl of *Chlamydia trachomatis* DNA samples and controls were add for amplification reactions. This was done by adding 10 µl of DNA-buffer to the tube labeled NCA (negative control of amplification) and to the tube labeled PCA (positive control of amplification). All tubes were inserted in the Rotor gene 6000 Real time PCR thermo cycler that was equipped with 36 well-blocked tubes, and 36 well-MicroAmp optical reaction plates. Plates were covered with optical adhesive covers and entered the thermal cycler. Denaturation, annealing, and extension were performed by the following steps:

* Step (1): was at 95°C for15 min in one cycle.
* Step (2): was at 95°C for 5 sec.
* Step (3): Five cycles of annealing at 60°C for 20 sec, and at 72°C for 15 sec, and extension at 95°C for 5 sec for 40 cycles at 60°C for 20 sec and at 60°C for 30 sec.

Fluorescence of *Chlamydia trachomatis* genome was detected by appearance of the FAM (Green) channel (*IC DNA* on the JOE (Yellow)/HEX/Cy3 channel).

Detection of *M.. genitalium:* New sterile Eppendorff tubes were prepared for each sample and controls. 10 µl of PCR-mix-1-FRT,were transferred to Eppendorff tubes and 5 µl of PCR-mix-2 and 0.5 TaqF polymerase were added. All tubes were vortexed, centrifuged for 2-3 sec, and 15 µl of Reaction Mixed and 10 µl of the extracted *M.. genitalium* DNA were pipetted to each tube. For each run 2 controls were prepared by adding 10 µl of DNA-buffer to the NCA and the PCA tubes, then 10 µl negative and positive controls were added respectively. All tubes were inserted in the Rotor gene 6000 Real time PCR thermo cycler that was equipped with 36 well-blocked tubes, and 36 well-MicroAmp optical reaction plates. Plates were covered with optical adhesive covers and entered the thermal cycler. Denaturation, annealing, and extension were performed by the following steps:

* Step (1): was at 95°C for15 min in one cycle.
* Step (2): was at 95°C for 5 sec.
* Step (3): Five cycles of annealing at 60°C for 20 sec, and at 72°C for 15 sec, and extension at 95°C for 5 sec for 40 cycles at 60°C for 20 sec and at 60°C for 30 sec; ending with, a final extension at 72°C for 15 sec.

Fluorescence was detected by the appearance of the FAM (Green) channel (*IC DNA* on the JOE (Yellow)/HEX/Cy3 channel). Fig. (1).

## Results

In the present study 300 urine samples from patients with sterile pyuria (150 males and 150 females) aged more than 20 years attending urology clinics in Khartoum State. The specimens were subjected to real time PCR technique for detection of *C. trachomatis* and *M. genitalium* genomes. *M. genitalium* genome was not detected in all specimens investigated. From the 300 specimens investigated, *C. trachomatis* genome was detected in 15 cases (5%) and was negative in 285 cases (95%). Among the male patients investigated (150 males), *C. trachomatis* was detected in 3 males (2%) and negative in 147 males (98%). Also, among the female patients

investigated (150 females), *C. trachomatis* was detected in 12 females (8%) and negative in 138 females (92%). This finding showed a higher frequency rate in females than males.

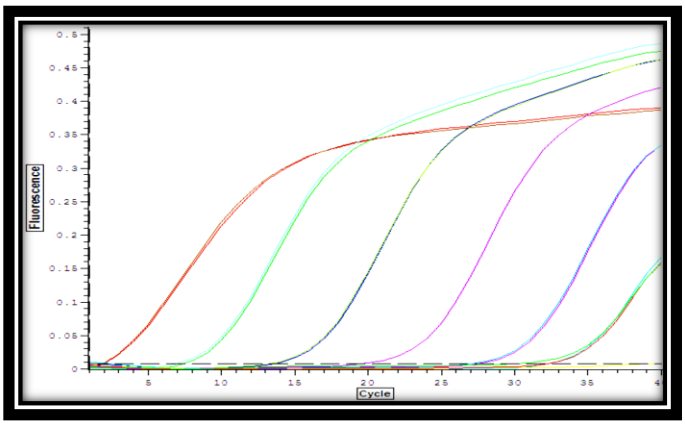


Fig. (1): Positive fluorescence of the FAM (Green) channel

According to the age incidence, the frequency rate of *C. trachomatis* was 5 cases (1.6%) among patients aged 20-30 years, 8 cases (2.6%) among patients aged 31-40 years, and 2 cases (0.6%). As seen in Table (1), leucocytes count in 8 positive *C. trachomatis* cases (2.6%) was 21-30/HPF, leucocytes count in 6 positive *C. trachomatis* cases (2 %) was > 31/HPF, and leucocytes count in one positive *C. trachomatis* case (0.3 %) was 10-20/HPF. The association of leucocytes count in positive *C. trachomatis* urine specimens was found statistically significant ($p < 0.05$).

Table (1): Association of leucocytes count in positive *C. trachomatis* urine specimens

| Leucocytes count | Positive urine specimens | Total |
|---|---|---|
| 10-20/HPF | 1 (0.3%) | 9 (31%) |
| 21-30/HPF | 8 (2.6%) | 120 (40%) |
| > 31/HPF | 6 (2%) | 87 (29%) |
| Total | 15 (2.9%) | 300 (100%) |

The total number of patients investigated with genital discharge was 182 (61%) patients. Those without genital discharge were 118 (39%) cases. *C. trachomatis* genome was detected in 12 (7%) urine specimens out of 182 patients with genital discharge, and in 3 (3%) urine specimens out of 118 patients without genital discharge. Also, *C. trachomatis* genome was not detected in170 (93%) genital discharge cases and also not detected in115 (97%) cases without genital discharge. In this study, 160 (53%) patients out of the 300 cases investigated were found complaining of dysuria; and140 (47%) patients were not complaining of dysuria. *C. trachomatis* was detected in 10 (6%) patients with dysuria; and in 5 (4%) patients without dysuria. It was not detected in 150 (94%) patients with dysuria and in135 (96%) patients without dysuria.

## 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* and *M. genitalium*. These microorganisms are associated with various diseases of the genitourinary tract, but they are usually not detected by routine microbiological techniques. *C. trachomatis* and *M. genitalium* are not screened thoroughly by routine examination of urine samples in medical laboratories in Sudan. To our knowledge, this study was the first work employing RT-PCR for detection of these organisms in sterile pyuria. This context had revealed a higher frequency rate of *C. trachomatis* than that of *M. genitalium* which was not detected in this study. These findings suggest that *C. trachomatis* infection should be strongly considered in urine specimens that show sterile pyuria.

The findings of this study showed that real time PCR testing of sterile pyuria could identify a significant number of causative microorganisms and clinicians should be informed about the importance of detection of these fastidious microorganisms in urine specimens collected from patients with urinary tract infection; since standard cultures fail to detect microbial infection. Wu and his colleagues (1992)[3] found that the sensitivities of both PCR and ELISA were 90.9% for male urine compared with urethral culture.

Overall, for both males and females, the PCR was more sensitive (95.6%) than the ELISA (87.0%). PCR specificities were 97.7% for men and 95.7% for women. ELISA specificities were 99.4% for men and 96.5% for women[3] .

Basarab and his colleagues (2002)[4] reported that a significant number of *C. trachomatis* could be detected in urine specimens from sterile pyuria. Singh and his co-workers (2003)[5] from India investigated symptomatic men and women, and reported a 30.8% frequency rate which was higher than the frequency rate of this study.

In Jordan, Awaad and his co-authors (2003) reported a prevalence rate of 4.6% in symptomatic and asymptomatic Jordanian patients with genital chlamydial infection[6]. This finding was lower than the finding reported in the present study.

Another study conducted by Nassar and his colleagues (2007)[7] in Gaza (Palestine) who investigated *Chlamydia trachomatis*, *Mycoplasma hominis*, *Mycoplasma genitalium*, and *Ureaplasma urealyticum* by PCR technique in patients with sterile pyuria. Their findings showed detection of *C. trachomatis* in 20 specimens (10%), *U. urealyticum* in 10 specimens (5%), *M. hominis* in 6 specimens (3%), and *M. genitalium* in 2 specimens (1%). These findings were higher than the findings of our study.

Furthermore, Mohammed and his co-authors (2012)[8] in Khartoum investigated 200 endo cervical smears *to detect Chlamydia trachomatis* among gynecological patients. They reported 43 (51.2%) positive patients by PCR technique. In the present context, our finding among females was lower.

On the other hand, our findings in female patients was higher than the finding of the study performed by Ortashi and his co-workers (2004)[9] who reported a prevalence rate of 7.3% *Chlamydia* infection among Sudanese women attending an obstetric and gynecology clinic in Khartoum. Few studies were conducted in Sudan to detect the prevalence rate among male patients.

Kese and his colleagues (2005)[10] reported 19.5% and 10.7% prevalence rates of *C. trachomatis* in male and female patients respectively. The prevalence rate of the present study was lower in male patients (3%); however, it was higher in female patients (12%).
In comparison with reported data[11], an unusually low prevalence of *M. genitalium* was found in infected urine specimens. The reasons for this unexpected result are not known; possibly, local demographic and social characteristics of the population influenced the result. Further studies to investigate *M. genitalium* in sterile pyuria and other urinary tract infections are needed.
**Recommendations:** It may be recommended that the association of *Chlamydia trachomatis* and *M. genitalium* with urogenital infections should be considered and proper identification of these microorganisms should not be neglected to provide effective treatment and control strategies. Clinicians need to be aware of *Chlamydia trachomatis* and *M. genitalium* on managing sterile pyuria patients.
**Conclusion:** Real time PCR assay is a rapid, specific technique for detection of *C. trachomatis* associated with urogenital tract infections.

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