

# Detection of Ureaplasma Species in Urogenital Samples by Real-Time PCR in Suspected Sexually Transmitted Infection Patients Attended in Evercare Hospital Dhaka in 2022

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## ABSTRACT

### Background

Sexually Transmitted Infections (STIs) are a continual and under-diagnosed disease. *Mycoplasma hominis* (*M. hominis*), *Ureaplasma urealyticum* (*U. urealyticum*), *Ureaplasma parvum* (*U. parvum*) are potential pathogens for urogenital infection of males and females. However, the association of these pathogens with STIs in our country is not yet reported. To determine the existence of *M. hominis*, *U. urealyticum*, *U. parvum* urogenital samples from suspected STIs patients who attended Evercare Hospital Dhaka were investigated.

### Materials and methods

Extracted DNA from urine, prostatic secretion, urethral swab, and high vaginal swab as per clinician's recommendation from 50 cases already tested negative for *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Trichomonas vaginalis* were used in this study from February 2022 to August 2022. Extracted and stored DNA were screened by multiplex real-time PCR for *M. hominis*, *U. urealyticum*, *U. parvum*.

### Result

Ureaplasma was detected in 12 (24%) samples, *U. parvum* in 9 (75%), and *U. urealyticum* in 3 (25%) samples. No pathogens were isolated by routine culture from any of these samples. The positivity rate was more frequent in males (66.6%) than females (33.4%) and in the age group between 19-40 years old.

### Conclusion

Our data show a significant association of Ureaplasma in STIs patients who attended this hospital. Large scale study is recommended for exploring the prevalence in the community and the role of this pathogen in STIs.

**Keywords:** STIs, *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Ureaplasma parvum*, multiplex PCR.

## INTRODUCTION

Sexually Transmitted Infections (STIs) are generally acquired by sexual contact. The bacteria, viruses, fungus, and parasites cause sexually transmitted diseases and may spread from person to person through semen, or vaginal and other body fluids<sup>1</sup>. Most sexually active individuals affected by STIs for at least once in their lives, being involved in the epidemiological chain of infection<sup>2</sup>. It is mentionable that, the role of genital mycoplasmas and ureaplasmas has been a contentious research topic over the past two decades<sup>3</sup>. Genital mycoplasma and ureaplasma (*Mycoplasma hominis*, *Ureaplasma urealyticum* and *Ureaplasma parvum*) belong to the class Mollicutes (soft skin) and Mycoplasmataceae family of bacteria, which are the smallest known free-living microorganisms<sup>4</sup>. *M. hominis*, *U. urealyticum*, and *U. parvum* have been associated with

some symptomatic and asymptomatic genital tract conditions in women and men including painful or burning urination, pain during sex, unusual discharge from urethra or vagina, lower abdominal pain etc<sup>5</sup>. A study in Australia showed that the prevalence of genital *U. parvum* infection among patients attending sexual health clinic was 72%<sup>6</sup>, and in a study in Bangladesh, it was 45.3% among young female sex workers<sup>7</sup>. However, there is neither any report yet about the prevalence of mycoplasma and ureaplasma infection in Bangladesh nor any incidence of these pathogens among patients who attended dermatology and venerology clinics or gynecology clinics probably due to a lack of any sensitive detection system.

The introduction of Polymerase Chain Reaction (PCR), as well as multiplex PCR, has improved the

knowledge regarding the detection of these microorganisms and allowed to differentiate their types<sup>3,8</sup>. As a result genital mycoplasma and ureaplasma (*M. hominis*, *U. urealyticum* and *U. parvum*) can be detected from the genitourinary tract of both males and females by these diagnostic tools<sup>9</sup>. The aim of this study was to determine the infection rate of *M. hominis*, *U. urealyticum*, and *U. parvum* among the suspected STIs patient attended at Evercare Hospital Dhaka between February 2022 and August 2022.

## MATERIAL AND METHODS

### Method of data collection:

The data of the patients were accumulated from the Hospital Information System (HIS) of Evercare Hospital Dhaka, Bangladesh. This study was carried out between February 2022 and August 2022. Age, sex, signs and symptoms, and types of specimens, were used for data analysis. To protect the patient's private information all samples were de-identified except for the age and sex, and patient consent was not needed as the stored left-over DNA after the routine test was used for PCR in this study.

### Clinical Samples:

A total of 50 samples were tested which identified negative for *N. gonorrhoeae*, *C. trachomatis*, *M. genitalium*, *T. vaginalis*. Urine, prostatic secretion, and urethral swabs were collected from male patients, and urine, high vaginal swabs were collected from female patients according to the clinician's advice. Commonly presented symptoms among these patients were painful or burning urination, pain during sex, discharge from the penis, vaginal discharge, swelling or ulceration, and itching in the genital area etc. All swabs were collected by trained medical personnel in sterile swab sticks and sent to the laboratory. Urine and semen samples have been collected in a 50 ml sterile plastic container. In the case of swab samples, we added 2-3 ml of sterile Phosphate Buffer Saline (PBS) to the swab stick tube and mixed it with the vortex mixture. All samples were stored at 2-8°C for no longer than 24 hours until DNA extraction and then extracted DNA was stored at -80°C. All the laboratory works have been performed in the molecular laboratory of Evercare Hospitals Dhaka.

### DNA extraction & Multiplex Real-Time PCR:

The DNA was isolated by using QIAamp DNA Mini (Qiagen, Germany) spin column-based extraction kit

according to the manufacturer's instructions. 200 µl of sample was used for DNA extraction. We added PCR kit recommended 5 µl of internal control during the DNA isolation into the lysis mixture. The elution volume was 50 µl.

We used CE-IVD approved Genproof MH /UU /UP commercial multiplex real-time PCR kit from Czech Republic for the detection of three bacterial pathogen-*M. hominis*, *U. urealyticum*, *U. parvum*. The total PCR volume was 20 µl where 15 µl master mix was dispensed for each sample, Negative control (NC) and Positive control (PC), and then 5 µl of extracted DNA, negative control and positive control were added respectively in 0.2 ml PCR strip tube. PCR amplification was done by Quantstudio-5Dx (Applied Biosystems) thermocycler according to the kit manufacturer's instruction which was programmed as follows: Hold 1 cycle of 37°C for 2 minutes, 1 cycle of 95°C for 10 minutes, then 45 cycles of 95°C for 5 seconds, 60°C for 40 seconds and 72°C for 20 seconds. The signal was acquired at 60°C, and analysis was performed on the linear scale. Thresholds were set manually in each run. The fluorescence was detected in FAM channel for *M. Hominis*, ROX channel for *U. urealyticum*, CY5 channel for *U. parvum*, and HEX/JOE/VIC channel for amplification of internal control. The recommendations of the manufacturer were strictly followed for DNA extraction and real-time PCR.

## RESULTS

This is a retrospective study. A total of 50 cases were selected based on routine PCR negative for *N. gonorrhoeae*, *C. trachomatis*, *M. genitalium*, *T. vaginalis*. Out of 50 cases, 41 (82%) were male and 9 (18%) were female patients. The age range of the patients was 13-68 years. The majority (58.4%, 7/12) belonged to the age group 19-40 years. Clinical history and other lab data were taken from the hospital information system. Burning sensation during urination or sexual intercourse was the most common symptom in male patients followed by urethral discharge (Table-1). The number of female patients in this study was few and vaginal discharge and burning sensation during urination or sexual intercourse were the common symptoms.

**Table 1:** Clinical presentation of STIs patients

Symptoms	Total =50 (M-41, F-9)	
	Male (%)	Female (%)
Burning sensation during urination/intercourse	23(56.1)	4(44.4)
Urethral discharge	7(17)	5(55.5)
Ulceration/Wart in genital area	4(9.7)	1(11.1)
Swelling/Pain in genital area	2(4.8)	0
Itching on genital area	3(7.3)	2(22.2)
Abdominal pain	0	0

Multiplex PCR detected 12 ureaplasma positive samples out of 50 and out of these 12 positive samples 9 (75%) were *U. parvum* and 3 (25%) were *U. urealyticum*. There were no *M. hominis* infections found in this study (Table:2). The overall PCR positivity rate for ureaplasma was 24% (12/50) among all age groups. The age specific positive rate was higher (58.4%, 7/12) in the 19-40 years group. Ureaplasma were more common in male with 66.6% (8/12) in comparison with female 33.4% (4/12).

**Table 2:** Distribution of STIs pathogens among different age groups & sex

Age (Years)	Total Case (%)	Positive (%)	MH (%)	UU (%)	UP (%)
	M- 41(82), F-9(18)	M-8(66.6), F-4(33.4)			
13-18	1(2)	0	0	0	0
19-40	30(60)	07(58.4)	0	1(8.4)	6(50)
41-60	18(36)	04(33.4)	0	2(16.7)	2(16.7)
>60	1(2)	01(8.4)	0	0	01(8.4)
Total (%)	50(100)	12(24)	0	3(25)	9(75)

**Abbreviations:** MH- Mycoplasma Hominis, UU- Ureaplasma Urealyticum, UP- Ureaplasma Parvum.

## DISCUSSION

There are limited studies on the prevalence of genital mycoplasmas and ureaplasmas. Most probably, it is due to the controversy of infection or colonization by these microorganisms<sup>10</sup>. In our study the overall ureaplasma positivity rate was 24% (12/50) of which 75 % are *U. parvum* and 25% *U. urealyticum*. Ureaplasma detection rate was found more in male than female. Ureaplasma was also detected at a higher rate than mycoplasma in both male and female urethritis patients in many other countries including India<sup>11-14</sup>. This data is similar to an Australian study in non-pregnant women where patients attended in sexual health clinic<sup>6</sup>. Another study in Australia also showed that *U. parvum*

was the most detected pathogen<sup>15</sup>.

Our data is also partially similar to another study in Bangladesh where *U. parvum* was the second commonest pathogen in young female sex workers while *M. hominis* was the commonest<sup>7</sup>. We did not find *M. hominis* in this study probably due to the different patient group and a small number of samples.

Multiplex real-time PCR method in our study detected 12(24%) positive cases of ureaplasma while none were detected by culture. The reason behind the no growth in culture is probably not using the ideal media/system for these slow-growing bacteria. It is also reported already that the sensitivity of PCR is higher than culture for the detection of ureaplasma and mycoplasma<sup>8,16</sup>. In addition, it is a rapid method and multiplex PCR can differentiate many STIs pathogens in a single assay. Therefore, PCR is the better laboratory method to detect STIs pathogen and can be used for routine laboratory though it is a little bit more expensive than routine culture.

The role of Ureaplasma as a STIs pathogen is still uncertain and needs clinical evaluation and research since these organisms are also isolated from non-symptomatic healthy women and men.<sup>17-18</sup>. The prevalence of *U. urealyticum* and *M. hominis* infections in pregnant women is reported high in some countries, and the resistance rate of antimicrobial agents tends to increase<sup>19</sup>.

## CONCLUSION

Our data show a significant association of Ureaplasma in STIs patients who attended this hospital. Large scale study is recommended for exploring the prevalence in the community and the role of this pathogen in STIs.

## LIMITATION

A major limitation of the study is the small number of samples (n=50) and the short duration (7 months) of the study period. A large number of symptomatic as well as asymptomatic patients may be included in future studies for exploring the prevalence of this pathogen and to understand the pathogen burden in the community.

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