

# Application Realtime-PCR for the Detection of Urogenital Infections in Vietnamese Men



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## Abstract

Sexually transmitted diseases (STDs) are now the commonest group of infectious diseases in most countries, particularly in the 15-50 age group. The worldwide incidence of major bacterial and viral STDs is estimated at over 125 million cases each year. STDs not only have a negative impact on health, especially reproductive health but also seriously affect socio-economic. Nowadays, exponential development of science and technology generates many modern methods for quick and accurate diagnosis STDs. This study was conducted by a cross-sectional descriptive method, on 300 men who were suspected of having urogenital tract infections. The samples were tested by realtime PCR for identifying 11 microorganisms. The result was that the rate of patients diagnosed with microorganisms was 72.9%. Gardnerella vaginalis was the most common pathogenic bacteria (45.7%). The rates of mono-infection and multi-infection were similar, at 49% and 51%, respectively. G. vaginalis was the most common cause of mono-infection and multi-infection. Patients in sexually active age (from 20 to 29 years old) accounted for the largest percentage of men suspected of having STDs (42.7%) as well as men who had urogenital tract infections (43.9%).

**Keywords:** Candida; Infections; Realtime PCR; STD; Urogenital

## Introduction

Sexually transmitted diseases (STDs) are a group of infectious diseases that have a negative influence on human health, especially reproductive health [1]. Despite the crucial progress in terms of diagnosis, treatment and prevention (e.g. vaccination), they remain the most widespread and hazardous infectious diseases [2]. According to the World Health Organization (WHO), about one million STDs are acquired every day worldwide. In developing countries in Africa and Asia, STDs are one of the five most common diseases [3]. Urogenital infections are also a worldwide shared problem that represent the most common reason for a woman to decide to visit to gynaecologist or urologist [4]. The term sexually transmitted diseases (STDs) refers to a variety of clinical syndromes and infections caused by pathogens transmitted from an infected person to an uninfected person through vaginal, anal, or oral sexual contact [5].

This group of diseases has a wide variety of clinical symptoms and treatments. However, up to 90% of sexually transmitted infections are asymptomatic that result in delayed diagnosis and treatment [6]. If not treated promptly and thoroughly, they may cause life-threatening complications including cancers, infertility, ectopic pregnancy, spontaneous abortions, stillbirth, low birth weight, neurologic damage, and even death [7]. Beyond the individual realm, STDs are also a problem that impacts society, representing a huge economic burden for healthcare systems [8]. For

example, in the US, the Centers for Disease Control and Prevention (CDC) estimate the costs for the US healthcare systems to treat the eight most common STDs amount to a total of approximately \$16 billion US dollars each year [9]. In recent years, the rapid growth of molecular biology allows people to detect many modern methods for quick and accurate diagnosis STDs and of these, real-time PCR is a common technique. This is a modern molecular biology method that allows accurate and simultaneous identification of many agents in the specimen.

In 2005, Caliendo AM and partners (Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA 30322, USA) conducted the study called "Realtime PCR improves detection of Trichomonas vaginalis infection compared with culture using self-collected vaginal swabs". 524 specimens that were obtained from adolescent and young adult African-American women participating in HIV-1 prevention programs, were tested by both culture and real-time PCR to detect Trichomonas vaginalis. The result was 36 were culture positive and 54 were positive in the real-time PCR assay; 16 of the 18 discrepant specimens were also positive in the confirmatory PCR assay [10]. In 2013, Zhang MJ and partners (State Key Laboratory for Infectious Disease Prevention and Control, and National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 102206, China) conducted the study namely

“Development and application of a realtime polymerase chain reaction method for *Campylobacter jejuni* detection”. 242 stool specimens were analyzed for the presence of *C. jejuni* by direct bacterial culture and real-time PCR.

The result was 20/242 (8.3%) *C. jejuni* strains were isolated from bacterial culture, while 41/242 (16.9%) samples were found to be positive by realtime PCR. In conclusion, it is clear that the sensitivity of detection of *C. jejuni* from stool specimens was much higher using this PCR assay than using the direct culture method [11].

So far, pathogen transmission via sexual contact is known for more than 30 different bacteria, viruses and parasites [3]. Thereof, three bacterial (*Gardnerella vaginalis*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*), a protozoan (*Trichomonas vaginalis*) and four viral (Human papilloma virus (HPV), Herpes simplex virus (HSV), Hepatitis B virus (HBV), and HIV) STIs account for the majority of infection cases [3,12]. Based on that, we selected 11 most common causative agents for identifying with realtime PCR. With the purpose of assessing the incidence of sexually transmitted infections in order to contribute to screening, early diagnosis and treatment STDs, we first applied realtime PCR to our research with the goal: determining the rate of infection of 11 bacteria causing urogenital tract infections in male patients at the Genetic Counseling Center - Hanoi Medical University Hospital (Table 1).

**Table 1:** 11 microorganisms were identified by realtime PCR.

| 11 Microorganisms Were Identified by Realtime PCR |                               |
|---|-------------------------------|
| Bacteria  | <i>Chlamydia trachomatis</i>  |
|   | <i>Neisseria gonorrhoeae</i>  |
|   | <i>Gardnerella vaginalis</i>  |
|   | <i>Mycoplasma hominis</i>     |
|   | <i>Mycoplasma genitalium</i>  |
|   | <i>Ureaplasma urealyticum</i> |
|   | <i>Ureaplasma pавum</i>       |
|   | <i>Ureaplasma spp</i>         |
| Parasite  | <i>Toxoplasma gondii</i>      |
|   | <i>Trichomonas vaginalis</i>  |
| Fungus  | <i>Candida albicans</i>       |

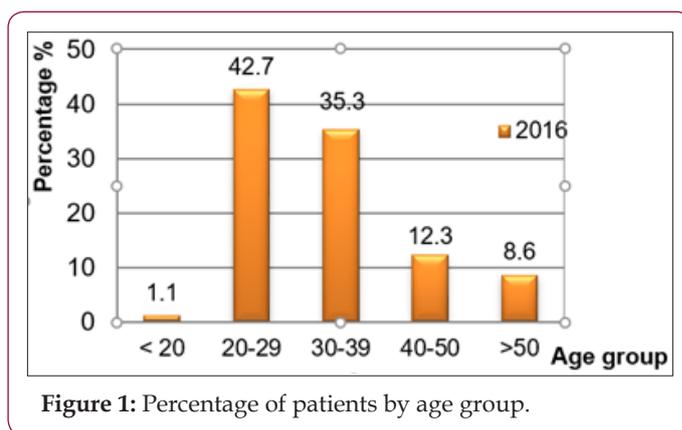
**Materials and methods**

We conducted a cross-sectional descriptive study over 300 male patients suspected of having urogenital tract infections came to the Genetic Counseling Center - Hanoi Medical University Hospital in 2016. We calculated the incidence of STDs based on the positive PCR test results for any of the 11 microorganisms.

**Results and Discussion**

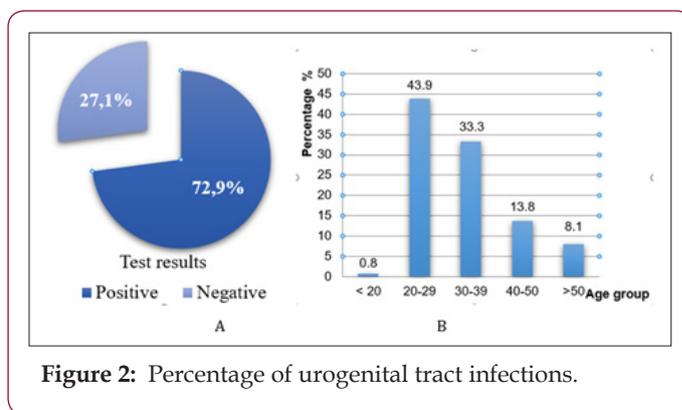
**Characteristics of Study Population**

Figure 1 The average age of the patients was 33,0 ± 7,26 years. The youngest patient was 19 years old, the oldest was 66 years old. The percentage of patients peaked at 42.7% in the 20-29 age group, which was the most sexually active group. This percentage underwent significant decreases in age groups after 30.



**Results of the identification of Urogenital Tract Infections by Realtime PCR**

Figure 2 The positive rate in the realtime PCR assay accounted for 72.9%, which was much higher than the rate of urogenital tract infections announced by the WHO in the Western Pacific Region (13%) [13]. This different was because our 300 subjects were male patients with symptoms of suspected urogenital tract infections and were assigned to test for pathogenesis. So, the prevalence of the disease in our study was higher than one in the general population and in the high-risk populations in particular. Another reason was due to the difference in test method. While WHO (2008) used PCR, we applied realtime PCR to our research. In the group of patients with positive results, it was noticeable that patients in sexually active age (from 20 to 29 years old) took up the largest percentage of men suspected of having STDs (42.7%) as well as men who had urogenital tract infections (43.9%).



Compared to N Fournet’s research (2006 - 2012) that indicated that the popular age group among STD patients was between 15 and 24 years (accounting for 44%) [14], the popular age group in our study was a little higher. The reason for that was due to differences in sexual habits between Vietnam and other countries. The study by Gutierrez JP et al. (2006) found that 23% of young people aged 15-18 years had had sexual intercourse, of which about 4% had the sex purchase act and only 14-36% of them used condoms during sex [15].

**Percentage of Eleven STIs Microorganism**

Figure 3 Overall, of all the causes of genital urinary tract infections, *Gardnerella vaginalis* was the most common cause

(45.7%). This result was different from the one reported by N Fourmet et al., which suggested that Chlamydia trachomatis and Neisseria gonorrhoeae were the most common [14]. This different could be explained by changes in study population characteristics over time and space. Another reason was the difference in test method, similarly, while N Fourmet et al. (2006 - 2012) used PCR, we applied realtime PCR to our study.

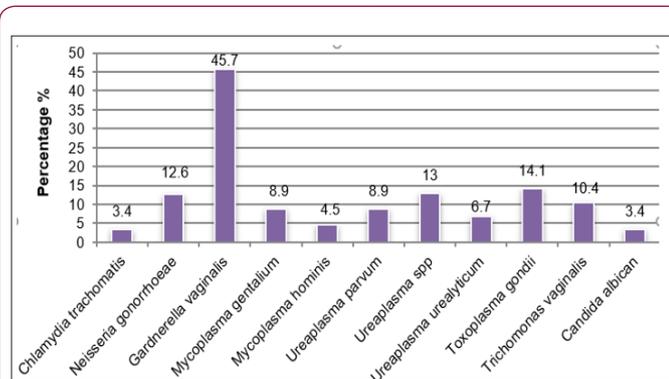


Figure 3: Percentage of microorganisms in STD patients.

Percentage of Mono-Infection and Multi-Infection

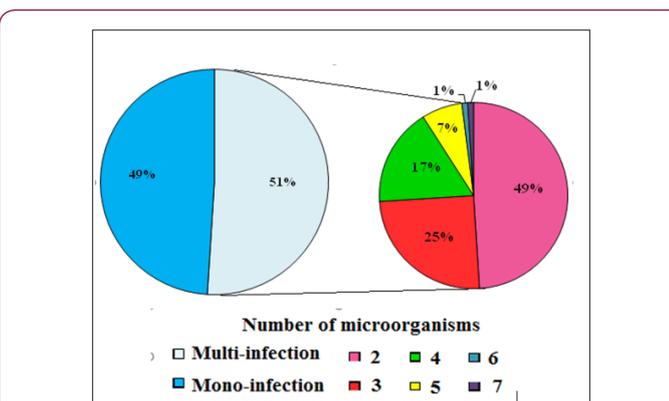


Figure 4: Percentage of mono-infection and multi-infection.

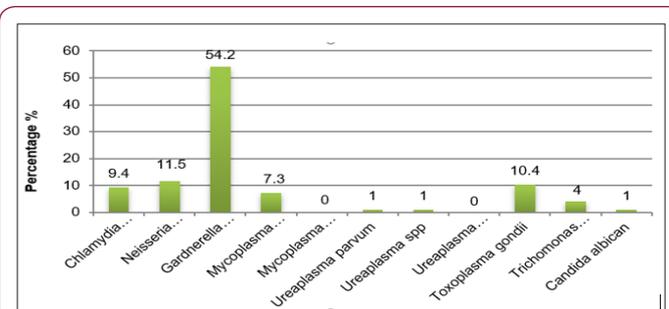


Figure 5: Percentage of microorganisms in mono-infected group.

Figure 4 It is clear that the percentages of mono-infection and multi-infection were similar, at 49% and 51%, respectively. In the multi-infected group, the number of co-infected patients was the highest. In addition, the higher the number of bacterial infections, the lower the percentage. In particular, 1% of patients was infected

with 7 microorganisms (Figure 5). In the mono-infected group, G. vaginalis still occupied the highest incidence (54.7%). The percentage of the remaining causes was not significantly different (Table 2). As indicated on the table above, Mycoplasmas did not often appear alone but in combination with other microorganisms, most commonly associated with G. vaginalis (36%). C. trachomatis and G. vaginalis were also one of the most common co-infections (21%).

Table 2: Popular group of microorganisms.

| Multi-infection            | Percentage |
|----------------------------|------------|
| G.vaginalis-Mycoplasma     | 36%        |
| G.vaginalis-C.Tracjomatics | 21%        |
| G.vaginalis-N.Gonorrhoeae  | 17%        |
| G.vaginalis-T.Gondii       | 17%        |
| T.Gondii-Mycoplasma        | 14%        |

Conclusion

From application of realtime PCR for detection of urogenital infections on 300 male patients, we came to the conclusion: The rate of patients diagnosed with microorganisms was 72.9%. Gardnerella vaginalis was the most common pathogenic bacteria (45.7%). The rates of mono-infection and multi-infection were similar, at 49% and 51%, respectively. G. vaginalis was the most common cause of mono-infection and multi-infection. Patients in sexually active age (from 20 to 29 years old) accounted for the largest percentage of men suspected of having STDs (42.7%) as well as men who had urogenital tract infections (43.9%).

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