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Original Article

Equivalency of First-Void Urine Culture with Prostatic Secretion Fluids

Kiumars Ghazisaidi[1], Yasaman Azimi[2], Ali Samadi[3], Seyed Mohammad Kazem Aghamir[4], Maryam Aghaii[4], Gholamreza Pourmand[4*]

[1]*Infectious Disease Research Center, Golestan University of Medical Sciences, Gorgan, Iran*

[2]*Department of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

[3]*Department of Genetics and Molecular Medicine, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran*

[4]*Urology Research Center, Tehran University of Medical Sciences, Tehran, Iran*

HIGHLIGHTS

- Urinary Tract Infections can cause kidney failure and prostatitis.
- First-void urine (VB1) culture can take the place of prostatic secretion (EPS) fluids.
- The bacterial contamination usually is done by prostatic secretion (EPS) fluids.

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ABSTRACT

Introduction
The purpose of the current study was to investigate the separation of the bacterial factors of men's urinary tract infections using the prostate massage technique and comparing them with the initial-stream urine, midstream urine, and end-stream urine in Tehran's Health Centers.

Methods
In this study, samples were collected from 50 men with genital-urinary tract defects who were referred to medical centers. During sampling, demographic information, medical history, and drugs are taken were asked from patients. Prostate secretions and first-void urine (VB1) were collected from the first 25 patients, while prostate secretions and VB1, midstream urine (VB2), and end-stream urine (VB3) were collected from the second 25 patients.

Results
Using prostate secretions culture, out of the first 25 samples, 7 samples (28%) were infected by *Mycoplasma hominis* and 9 samples (36%) were infected by *Ureaplasma urealyticum*. using VB1 culture it was analyzed that 6 samples (24%) were infected by *Mycoplasma hominis* and 8 samples (32%) were infected by *Ureaplasma urealyticum*. In the second 25 samples, Bacterial contamination was seen in 23 samples (92%) by prostate secretions culture. VB1 culture showed bacterial contamination in 14 samples (56%). VB2 and VB3 cultures showed bacterial contamination in 3 (12%) and only 2 samples (8%), respectively.

Conclusions
In conclusion, the results obtained from the prostate secretions culture were more accurate and precise than the VB1 culture, but they were close to each other. Therefore, instead of using prostate massage which can be irritating for the patients or if the patient does not have prostate secretions, VB1 culture can be used.

Keywords: Prostate Massage; Urinary Tract Infection; Bacterial Factors; Bacterial Prostatitis

***Corresponding Author**: Gholamreza Pourmand
Email: gh_pourmand@yahoo.com
Address: Urology Research Center (URC), Sina Hospital, Hassan Abad Sq., Tehran, Iran

Introduction

Urinary Tract Infections (UTIs) are severe health problems and are considered a very costly and serious health problem for society (1). UTIs are caused by a variety of pathogens, but most commonly by *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, and *Staphylococcus saprophyticus* (2). Anatomically, urinary infections can be divided into 2 groups; the first group was infections of the lower urinary tract (urethritis, cystitis, and prostatitis); and the second group was infections of the upper urinary tract (pyelonephritis) (3). The favorable factors of urinary tract infections consist of age, gender, inserting the device, surgery, pregnancy, neurogenic origin, diabetes mellitus, consumption of immunosuppressive drugs like steroids and cytotoxic drugs, genetic factors, and fatigue (4, 5). Prostatitis indicates inflammation of the prostate gland and usually, it occurs by the direct invasion of the pathogen through the urethra (5). The development of prostate cancer mostly is due to the inflammation caused by infection (6). Acute bacterial prostatitis is a common and clinically important genitourinary disorder. Acute bacterial prostatitis is commonly caused by an ascending urinary tract infection, and patients can present with a range of symptoms from local to systemic (7). Generally, prostatitis syndrome is divided into four categories: acute bacterial prostatitis, chronic bacterial prostatitis, prostatodynia, and chronic nonbacterial prostatitis (or idiopathic). Prostatitis may also have no symptoms (8). *Ureaplasma urealyticum* is one of the etiological factors in idiopathic prostatitis which the inflammation in urine voided immediately after prostatic massage (VB3) is visible; however, the infection cannot be detected (9). The role of *Ureaplasma urealyticum* in bacterial prostatitis has not been completely clarified. However, *Ureaplasma urealyticum* and other unusual pathogens found in expressed prostatic secretion (EPS) of some patients cannot be ignored (10). These patients suffer from dysuria, testicular pain, groin pain, frequent urination, nocturia, and sometimes hematospermia (11). Most of these complications with more than 10 bacterial organisms in the prostatic fluid have responded to the tetracycline treatment (12). Weidner and colleagues concluded that *Ureaplasma urealyticum* is the etiological factor in 9% of nonbacterial prostatitis (13). Among genital *Mycoplasmas*, *Ureaplasma urealyticum*, and *Mycoplasma hominis* are more abundant than others (14). These two organisms usually have commensal behavior, but in fact, they are opportunistic pathogens (15). *Ureaplasma* has an important role in the development of genital and urinary tract defects such as nongonococcal urethritis and chronic prostatitis, abortion, dead infants or infants with low birth rate, and pelvic inflammatory disorder (16). Local aggregation of some of the *Mycoplasma's* metabolites such as hydrogen peroxide enzymes, superoxide radicals, and ammonia may cause damage to tissues (17). Some studies have shown that patients with *Mycoplasma genitalium* have some symptoms of prostatitis (14, 18). It appears that *Mycoplasma hominis* does not affect men's urethritis. However, *Ureaplasma Urealyticum* is associated with chronic prostatitis, Non-chlamydial non-gonococcal urethritis, and sterility. *Ureaplasma* also has been removed from 50% to 80% of patients with nongonococcal urethritis that underlines the etiology and role of this microorganism (19). Therefore, analysis and diagnosis of these microorganisms in the male genital disease could be effective and beneficial.

Usually, the growth of *Mycoplasma* in a liquid environment is without turbidity, except for *Mycoplasma pneumonia* which causes slight turbidity in a liquid phase (20). It is worth mentioning that the replication speed for this bacteria is slower than others (21). The growth of *Mycoplasma* in the liquid environment happens with alteration of the color which leads to the initial identification of the organism (22, 23). This color change is due to the acidification and alkalinity of the environment which is specified by the indicator present in the environment. By centrifuging the liquid phase at high speed and applying the precipitate to the Giemsa color, the pleomorphic shapes are visible (24).

Mycoplasma hominis and *Ureaplasma urealyticum* are associated with many genital and urinary tract defects such as nongonococcal urethritis, prostatitis, and epididymitis (25). Urethritis is categorized into gonococcal urethritis and nongonococcal urethritis (NGU). The maximum outbreak of gonococcal urethritis was in 2011 and currently, the outbreak of NGU in the United States and developed countries is lower than the outbreak of gonococcal urethritis and NGU is probably the most common urinary tract infection in men of reproductive age (26).

If the current study shows that urine tests are as valuable as prostate massage, then the procedure suggested by this study will be more comfortable and significant for the patients, laboratory, and physician.

The purpose of the current study was to investigate the separation of the bacterial factors of men's urinary tract infections using the prostate massage technique and comparing them with the initial-stream urine, midstream urine, and end-stream urine in Tehran's Health Centers. If it could be proven by this study that the urine test can be used for diagnosis of bacterial prostatitis instead of using prostate massage which is irritating and difficult, then very valuable and influential work has been done.

Methods

In this study, samples were collected from 50 men with genital-urinary tract defects who were referred to medical centers. During sampling, demographic information, medical history, and drugs are taken were asked from patients. Prostate secretions and first-void

urine (VB1) were collected from the first 25 patients; prostate secretions and VB1, midstream urine (VB2), and end-stream urine (VB3) were collected from the second 25 patients. The study was under the Tehran University of Medical Sciences Ethical Committee (IR.TUMS.SINAHOSPITAL.1399.031), and all patients enter the study after signing the informed consent.

First, 10-15 cm^3 of the first droplets of urine were collected (VB1). Then 100-500 cm^3 urine was discharged and 10 cm^3 of this portion of the urine was gathered (VB2). Prostate secretions were collected after the prostate massage. Then again, 10 cm^3 urine was collected (VB3). The samples consisted of VB1, VB2, VB3, and prostate secretions. If VB1 was only one or two droplets, it was entered into the transport environment directly and if it exceeded this amount, first it was centrifuged and then entered into the transport environment. Due to the small amount of prostate secretions, they were directly entered into the transport environment. Also, VB2 and VB3 entered in blood agar and chocolate agar environment without being centrifuged. Only for direct detection VB2 and VB3 were centrifuged and their sediment was used. After the prostate massage, a couple of drops of prostate secretions are transmitted into a sterile container and entered into *Mycoplasma* transport environment. This environment was kept in laboratory conditions for 20-30 minutes. Afterward, by using a sterile filter 0.45 or 0.22 micron the only couple of drops were added into the urea environment to diagnose *Ureaplasma*, and a couple of drops were added into the arginine environment to separate *Mycoplasma hominis*. Next, samples were incubated at 20^{oc} in CO2 for a week. If *Ureaplasma urealyticum* exists in the samples, the environment would be alkalinized by breaking down the urea and producing ammonia and this would lead to a change in color from yellow to pink or purple. If *Mycoplasma hominis* exists in the sample, the environment will be alkalinized by breaking down the arginine and producing citrulline and eventually ammonia. This will result in a color change from yellow to pink or purple. To separate other bacteria from VB2 and VB3 urine culture was used. To examine the urine sample directly, 3-5 cm^3 urine was centrifuged and a slide was collected from its sediment and checked with a 40 magnifying glass lens. Also, a lope from prostate secretion and a lope for separating bacteria from VB1 in a blood agar environment were cultured, and to observe directly gram stain was used.

Variables were reported by frequency and percentage for categorical data. Data were analyzed by SPSS version 12, and P-value less than 0.05 indicated a statistically significant difference.

Results

50 samples were collected from men who were referred to the laboratory. The first 25 samples were analyzed by first-catch urine culture (VB1) and prostate secretions culture. The second 25 samples were analyzed by first-catch urine culture (VB1), prostate secretions culture, midstream urine culture (VB2), and end-stream urine culture (VB3). Using prostate secretions culture, out of the first 25 samples, 7 samples (28%) were infected by *Mycoplasma hominis* and 9 samples (36%) were infected by *Ureaplasma urealyticum*. Using VB1 culture it was analyzed that 6 samples (24%) were infected by *Mycoplasma hominis* and 8 samples (32%) were infected by *Ureaplasma urealyticum*.

In the second 25 samples, Bacterial contamination was seen in 23 samples (92%) by prostate secretions culture. VB1 culture showed bacterial contamination in 14 samples (56%). VB2 and VB3 cultures showed bacterial contamination in 3 (12%) and only 2 samples (8%), respectively. Furthermore, in the second 25 samples, prostate secretions culture showed that 19 samples (76%) were contaminated by *Staphylococcus saprophyticus*; 6 samples (24%) were contaminated by *Staphylococcus epidermidis*; 7 samples (28%) were contaminated by viridans streptococci; 5 samples (20%) were contaminated by diphtheria, and 3 samples (12%) were contaminated by *E.coli*. VB1 culture showed that 8 samples (32%) were contaminated by *Staphylococcus saprophyticus*, 2 samples (8%) were contaminated by viridans *streptococci*, and 2 samples (8%) were contaminated by *E.coli*. VB2 culture only showed 1 sample (4%) contaminated by *Staphylococcus saprophyticus* and 1 sample (4%) contaminated by *E.coli*. VB3 culture showed 1 sample (4%) contaminated by *Staphylococcus saprophyticus* and 1 sample (4%) contaminated by *E.coli* (Table 1-3).

Discussion

Based on this study, the results obtained from the prostate secretions culture and VB1 culture are close to each other. Therefore, instead of using prostate massage which can be irritating for the patients or if the patient does not have prostate secretions, VB1 culture can be used. Of course, it should be noted that the results obtained from the prostate secretions culture are more accurate and precise.

However, due to the slight difference between these two methods, to identify *Mycoplasma hominis* and *Ureaplasma urealyticum* VB1 culture can be used instead of the prostate secretions culture. Nickel JC et al., suggested a new and fast test called the Pre and Post massage test (PPMT). PPMT is a simple and cost-effective test that is used for clinical purposes when patients refer with chronic prostatitis diagnosis with no signs and symptoms of urinary tract infection (no tract secretion, no painful urination, no urinary tract inflammation alone or with painful urination) (27). In Pre-massage, the patient cleans the outer part of the urinary tract precisely and provides

Table 1. Absolute frequency and relative frequency of *Mycoplasma hominis* and *Ureaplasma urealyticum*

	Ureaplasma urealyticum				Mycoplasma Hominis				Total Sum
	Positive		Negative		Positive		Negative		
	Quantity	Percentage	Quantity	Percentage	Quantity	Percentage	Quantity	Percentage	
VB1	6	24%	19	76%	8	32%	17	68%	25
Prostate	7	28%	18	72%	9	36%	16	64%	25

VB1: First-void urine

Table 2. Absolute frequency and relative frequency of microbial contamination

	Microbial contamination				Total Sum
	Positive		Negative		
	Quantity	Percentage	Quantity	Percentage	
VB1	14	56%	11	44%	25
Prostate	23	92%	2	8%	25
VB2	3	12%	22	8%	25
VB3	2	8%	23	92%	25

VB1: First-void urine; VB2: Midstream urine; VB3: End-stream urine

Table 3. Absolute frequency and relative frequency of different bacterial urinary tract infections

	Staphylococcus saprophyticus		Staphylococcus epidermidis		viridans streptococci		Corynebacterium diphtheriae		Escherichia coli		Total Sum
	Quantity	Percentage	Quantity	Percentage	Quantity	Percentage	Quantity	Percentage	Quantity	Percentage	
Prostate culture	19	76%	6	24%	7	28%	5	20%	3	12%	25
VB1	8	32%	-	-	2	8%	-	-	2	8%	25
VB2	1	4%	-	-	-	-	-	-	1	4%	25
VB3	1	4%	-	-	-	-	-	-	1	4%	25

VB1: First-void urine; VB2: Midstream urine; VB3: End-stream urine

a urine sample (~10 ml). Afterward, the prostate will be massaged (from outer parts towards the middle) and again the patient is asked to give another urine sample (~ 10 ml) which is composed of the secretions from the prostate gland. This is considered as post-massage. These two samples will be centrifuged and the sediment will be used for quantitative culture and microscopic analysis. If the amount of leucocyte in the post-massage is more than 10 in high power field, with an increase of 1 log (11 times) in contrast with the pre-massage sample, then prostatitis is diagnosed. Detecting bacteria in the post-massage prostate sample (>10) or counting more than 1 log colony in each milliliter in comparison with the pre-massage sample suggests chronic bacterial prostatitis. Diagnosing bacteria in urine in both samples indicates prostate inflammation. However, this can be related to bacterial bladder infection or upper urinary tract inflammation (27).

The accuracy and sensitivity of the calculated PPMT in the chosen population is 41% and using this test for patients suggested to urologists on the first patient's visit. For the second 25 samples, the amount of bacterial contamination seen by first-catch urine culture VB1, prostate secretions culture, VB2, and VB3 were analyzed. The bacterial contamination rate using prostate culture was 23 (92%); using first-catch urine culture was 14 (56%); using VB2 culture was 3 (12%) and using the end section urine culture was 2 (8%). Furthermore, analyzing the other bacterial agents using prostate culture in the second 25

sample showed that the results obtained from VB2 and VB3 culture are not accurate and sensitive enough and they cannot be used for *Mycoplasma*, *Ureaplasma*, and other bacterial agents' diagnosis in diagnostic tests.

Conclusions

In conclusion, the results obtained from the prostate secretions culture were more accurate and precise than the VB1 culture, but they were close to each other. Therefore, instead of using prostate massage which can be irritating for the patients or if the patient does not have prostate secretions, VB1 culture can be used.

Authors' contributions

SMKA was responsible for study conception and design. KGH provided data, and MA was responsible for statistical analysis. AS wrote the manuscript, and GHP was supervisor of the study.

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Conflict of interest

All authors declare that there is not any kind of conflict of interest.

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Ethical statement

The study was under the Tehran University of Medical Sciences Ethical Committee (IR.TUMS.SINAHOSPITAL.1399.031).

Data availability

Data will be provided by the corresponding author on request.

Abbreviation

EPS	Expressed prostatic secretion
NGU	Nongonococcal urethritis
PPMT	Pre and post massage test
UTIs	Urinary tract infections

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Author (s) biosketches
Ghazisaidi K, Assistant Professor, Infectious Disease Research Center, Golestan University of Medical Sciences, Gorgan, Iran. Email: kiumarsghazisaidi@yahoo.com
Azimi Y, MD, Department of Medicine, Tehran University of Medical Sciences, Tehran, Iran. Email: azimiyasaman4@gmail.com
Samadi A, MSc, Department of Genetics and Molecular Medicine, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran. Email: a.samadi99@yahoo.com
Aghamir SMK, Associate Professor, Urology Research Center, Tehran University of Medical Sciences, Tehran, Iran. Email: mkaghamir@tums.ac.ir
Aghaii M, PhD, Urology Research Center, Tehran University of Medical Sciences, Tehran, Iran. Email: ma.aghaii@yahoo.com
Pourmand G, Professor, Urology Research Center, Tehran University of Medical Sciences, Tehran, Iran. Email: gh_pourmand@yahoo.com

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