

Detection of bacterial agents causing prostate infection by culture and molecular methods from biopsy specimens

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ABSTRACT

Background and Objectives: Prostatitis affects about 16% of men in their lifetime and sometimes leading to prostate cancer. Bacterial infections are the most common causes of prostatitis. Diagnosis of the causative agents of bacterial prostate infections plays an essential role in timely treating and preventing secondary complications. This study isolated bacterial infectious agents in patients' surgical prostate and evaluated them by routine and molecular microbiological methods.

Materials and Methods: In this cross-sectional study, 72 prostate biopsy specimens were collected from the Urology Department of hospitals of Qazvin University of Medical Sciences. All samples were cultured in aerobic and anaerobic conditions. Antibiotic susceptibility test by Kirby-Bauer standard method was performed for all isolated bacteria. In addition, all isolated bacteria were identified using 16S rDNA PCR and sanger sequencing methods. Also, TaqMan real-time PCR was applied to detect *Ureaplasma aerealyticum*, *Mycoplasma hominis*, and *Mycoplasma genitalium*.

Results: In conventional culture method, out of 18 positive samples, 15 samples (83.3%) were Gram-negative bacteria and 3 samples (16.6%) were Gram-positive bacteria, containing *Escherichia coli* (55.5%), *Klebsiella pneumoniae* (11.1%), *Enterobacter cloacae* (5.5%), *Pseudomonas aeruginosa* (11.1%), *Staphylococcus aureus* (11.1%), and *Enterococcus faecalis* (5.5%). The results of molecular identification methods were the same as conventional culture results. Also, four patients were *Ureaplasma aerealyticum*, and three patients were positive for *Mycoplasma hominis*.

Conclusion: Most bacteria isolated from prostate specimens belonged to the *Enterobacteriaceae* family, especially *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*. *Staphylococcus aureus* and *Enterococcus faecalis* were cocci isolated in the specimens too. Also, *Ureaplasma aerealyticum*, and *Mycoplasma hominis* were identified in prostatitis.

Keywords: Prostatitis; Pathogens; Enterobacteriaceae; 16s rDNA; Real-time polymerase chain reaction

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INTRODUCTION

Prostate is part of the male reproductive system that plays a role in semen production (1). The natural volume of this gland is 30 ml to 40 ml. One-third of men over the age of 50 years old affect the swelling and magnitude of the prostate. As an inflammatory condition in the prostate gland, prostatitis affects about 16% of men in their lifetime (2). Prostatitis varies from a specific clinical state to a complex and debilitating condition, including bacteremia, prostate abscess, semen disorder, infertility, and elevated prostate-specific antigen. Also, prostatitis is a vital risk factor for prostate cancer (3). Prostatitis is categorized in four major groups, including acute bacterial prostatitis (ABP), chronic bacterial prostatitis (CBP), chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS), and asymptomatic inflammatory prostatitis (AIP) (4). Aerobic and anaerobic bacterial infections are the most common causes of prostatitis. In addition, viral, fungal, and parasitic agents are involved in prostatitis. The entry of bacteria occurs through ascending transmission through the urethra, rejection of the infectious urine into the prostate, and direct spread from the rectum through the lymphatic blood system. Other side-effects include bacteremia, prostate abscess, semen disorder, infertility, elevated prostate-specific antigen. Prostatitis is one of the risk factors for prostate cancer (5, 6).

Most ABP patients have a bladder infection at the time of referral, and their urine culture shows pathogenic bacteria. CBP is the most common urological problem in men under 50 years old, associated with various symptoms in the pelvis, perineum, scrotum, rectum, testicles, and penis. The prevalence of CBP in the male population is estimated at up to about 50%. Other manifestations of CBP are asymptomatic bacteriuria, painful ejaculation, and blood in the semen (4, 7).

Numerous pathological organisms lead to prostatitis and induction of inflammatory responses. The essential prostatitis pathogens are Gram-negative organisms of the *Enterobacteriaceae* family, such as *Escherichia coli*, *Klebsiella* spp. *Pseudomonas* spp. and *Enterococcus faecalis*. Due to the insufficient penetration of the drug into the prostate tissue, it is complicated to treat infections of this gland. Prostate infections are more limited to the peripheral parts of the prostate because the ducts that drain the peripheral portions of the prostate are more prone to reflux (6).

The use of antibiotics has a wide range of side effects and cellular toxicity. Also, misdiagnosis of pathogenic bacteria and incorrect administration of antibiotics cause prostatitis to enter the chronic phase and create secondary therapeutic challenges (8). Detection of the dominant pathogen in the population with prostatitis is critical to design a specific therapeutic protocol in the communities. This study evaluated bacterial pathogen in prostate biopsy via conventional culture and molecular methods in surgery patients suspected of prostate diseases.

MATERIALS AND METHODS

Sampling. In this cross-sectional study, 72 prostate biopsy specimens were collected from the Urology Departments of Qazvin University of Medical Sciences Hospital, Iran. Patients with a history of urinary catheterization and a history of antibiotic use within the past month were excluded from the study. The ethical status was approved by the ethics committee of Qazvin University of medical sciences [IR.QUMS.REC.1397.232].

The biopsy tissues were transferred into the thioglycolate medium immediately after extraction from the patient's body. Thioglycolate medium were rapidly transferred to a microbiology laboratory. Each biopsy sample was removed from the thioglycolate medium and divided into three sections with a sterile scalpel. A section was sent to the Routine Microbiology Laboratory for the culture of aerobic bacteria. Another part was sent for culturing anaerobic bacteria, and the third part was sent for molecular identification and detection of bacteria. All three laboratories worked independently to isolate and identify possible bacterial agents causing prostate infection in patients.

Aerobic culture identification method. The first part of the biopsy was quickly prepared as a homogeneous suspension under aseptic conditions. Identification of aerobic isolates was performed based on standard microbiological methods with API tests. The antibiogram test was performed on all isolated bacteria by the Kirby-Bauer standard method according to the Clinical Laboratory Standards Institute (CLSI). One colony of a pure culture of each sample was glycerine stocked in -70°C for further steps.

Anaerobic culture identification method. The second part of the biopsy was quickly prepared as a homogeneous suspension under aseptic conditions. The suspensions were inoculated into a suitable culture medium for anaerobic bacteria. The plates were transferred to anaerobic jars, and anaerobic conditions were created using an automatic anoxomat system. After one week of incubation, the culture results of the samples were evaluated.

Molecular identification method. The third part of the prostate biopsies was entered into the bacterial molecular detection reaction. The 16S rDNA Polymerase Chain Reaction (PCR) method was used for the molecular identification of isolates.

16S rDNA PCR. The biopsies of related tissue were used to extract DNA by High Pure PCR Template Preparation Kit (Roche, Germany). To amplify 16S rDNA, 5'-TGTCCTGGCTCAGATTG-3' oligonucleotide was used as the forward 5'-GTTACCTGT-TACGACTTCAC-3' oligonucleotide was used as the reverse primer.

***Ureaplasma* and *Mycoplasma* identification.** Due to the importance of pathogenicity of *Ureaplasma urealyticum*, *Mycoplasma hominis* and *Mycoplasma genitalium* prostatitis, and inability to grow via conventional culture methods, the TaqMan real-time PCR was performed for detection and identification. TaqMan probes and primers were as mentioned in Table 1. To controlling the performance of the probes and primers, the standard strains of *Mycoplasma hominis*, *Ureaplasma urealyticum* and *Mycoplasma genitalium* (Pasteur Institute of Iran) were used as a positive control.

DNA sequencing. All PCR-positive amplicons were sequenced (Macrogen, South Korea). The results were checked with sequence analysis software. Then, the sequences were blasted and aligned with the genes of standard strains registered in the National Center for Biotechnology Information (NCBI).

Statistical analysis. Data analysis was performed by SPSS software ver.25 and Crosstabs and Chi-square statistical tests to evaluate the qualitative variables and compare the percentage of variables. The significance level was considered 95%.

RESULTS

Demographic results. In this cross-sectional study on 72 prostate disease patients aged 70.81 ± 9.12 -year-old (from 52 to 88), hematuria (in 33.3%), pelvic pain (in 22.2%), urinary tract secretion (in 18.1%), frequent urination (87.5%), urinary retention (94.4%), dysuria (in 37.5%), and urinary incontinence (in 87.5%) were observed in patients.

Conventional culture results. In aerobic culture conditions, 18 samples (25.00%) were grown, and 52 samples (75.00%) were negative. Out of 18 positive samples, 15 samples (83.3%) were Gram-negative bacteria, and 3 (16.6%) were Gram-positive bacteria. The frequency of isolates and their antibiotics resistance pattern are summarized in Table 2. Gram-negative bacteria species were tested for extended-spectrum beta-lactamases (ESBL) and only one isolate was ESBL positive.

In anaerobic culture conditions, all samples did not show growth on specific anaerobic media.

Table 1. Specific primer and probs for genome of *Ureaplasma urealyticum*, *Mycoplasma hominis* and *Mycoplasma genitalium*

| Microorganism | Oligonucleotide | Sequence |
|-------------------------------|-----------------|--|
| <i>Ureaplasma urealyticum</i> | Forward primer | 5'-CTAGATGCTTAACGTCTAGCTGTATCAA-3' |
| | Reverse primer | 5'-GCCGACATTTAATGATGATCGT-3' |
| | TaqMan probe | 5'-(FAM)-AAGGCGCCAACCTGGACTATCACTGAC-(TAMRA)3' |
| <i>Mycoplasma hominis</i> | Forward primer | 5'-TTTGGTCAAGTCCTGCAACGA-3' |
| | Reverse primer | 5'-CCCCACCTTCCTCCCAGTTA-3' |
| | TaqMan probe | 5'-(FAM)-TACTAACATTAAGTTGAGGACTCTA-(TAMRA)-3' |
| <i>Mycoplasma genitalium</i> | Forward primer | 5'-CCTAGTCGGGTAAATTCC-3' |
| | Reverse primer | 5'-CATGGTGGTGTGTTTGTATC-3' |
| | TaqMan probe | 5'-(FAM)-CCATCTCTGACTGTCTCGGCT-(TAMRA)-3' |

Table 2. Conventional culture-achieved frequency of isolated species and their antibiogram pattern.

| Isolated Bacteria | Frequency | Resistance status | Antibiotics | | | | | | | | | | | | | | | | | | | |
|-------------------------------|-----------|-------------------|-------------|-------------|-------------|-----------|------------|-----------|-------------|----------|------------|----------|---------------|---------------|----------------|-----------------|------------|-----------|------------|--------------|------|------|
| | | | Ampicillin | Amoxicillin | Cephalothin | Cefazolin | Cefotaxime | Cefoxitin | Ceftazidime | Imipenem | Gentamicin | Amikacin | Ciprofloxacin | Cotrimoxazole | Nitrofurantoin | Clavulanic acid | Penicillin | Oxacillin | Vancomycin | Tetracycline | | |
| <i>Escherichia coli</i> | 10 | Sensitive | 0% | 0% | 0% | 10% | 10% | 20% | NA | NA | 0% | 30% | 100% | 20% | 20% | 90% | 10% | NA | NA | NA | NA | |
| | 55.5% | Moderate | 0% | 0% | 0% | 0% | 0% | 20% | NA | 10% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | NA | NA | NA | NA |
| <i>Klebsiella pneumoniae</i> | 2 | Resistant | 100% | 100% | 100% | 90% | 90% | 60% | NA | 90% | 70% | 0% | 80% | 80% | 10% | 90% | NA | NA | NA | NA | NA | |
| | 11.1% | Sensitive | 0% | 0% | 0% | 0% | 0% | 0% | NA | 0% | 100% | 100% | 0% | 0% | 0% | 100% | NA | NA | NA | NA | NA | NA |
| <i>Enterobacter cloacae</i> | 1 | Resistant | 100% | 100% | 100% | 100% | 100% | 0% | NA | 100% | 0% | 0% | 100% | 100% | 0% | NA | NA | NA | NA | NA | NA | NA |
| | 5.5% | Sensitive | 0% | 0% | 50% | 0% | 100% | 50% | NA | 50% | 0% | 100% | 50% | 50% | 100% | NA | NA | NA | NA | NA | NA | NA |
| <i>Pseudomonas aeruginosa</i> | 2 | Moderate | 0% | 0% | 0% | 0% | 0% | 0% | NA | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| | 11.1% | Resistant | 100% | 100% | 50% | 100% | 50% | NA | 50% | NA | 100% | 0% | 50% | 50% | 0% | NA | NA | NA | NA | NA | NA | NA |
| <i>Staphylococcus aureus</i> | 2 | Sensitive | NA | NA | NA | NA | NA | 0% | 50% | NA | 0% | 0% | 0% | 0% | 0% | NA | NA | NA | NA | NA | NA | NA |
| | 11.1% | Moderate | NA | NA | NA | NA | NA | NA | NA | NA | 50% | 0% | 50% | 100% | 0% | NA | NA | 0% | 0% | 0% | 0% | 0% |
| <i>Enterococcus faecalis</i> | 1 | Resistant | NA | NA | NA | NA | NA | NA | NA | NA | 50% | 0% | 100% | 50% | 0% | NA | NA | 100% | 50% | 100% | 100% | 100% |
| | 5.5% | Sensitive | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Total | 18 | Resistant | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| | 100% | | | | | | | | | | | | | | | | | | | | | |

Molecular identification results. Out of 72 prostate biopsy samples, 18 samples (15.28%) were positive in the 16S rDNA PCR assay. Sequences of PCR-positive amplicons were analyzed at the NCBI site, and it was determined that all positive cases were infected with aerobic bacteria, including 10 samples of *Escherichia coli*, two samples of *Klebsiella pneumoniae*, one sample of *Enterobacter cloacae*, two samples of *Pseudomonas aeruginosa*, two samples of *Staphylococcus aureus*, and one sample of *Enterococcus faecalis*. Also, the results showed that four patients (5.6%) were *Ureaplasma urealyticum*-positive and three (4.2%) were positive for *Mycoplasma hominis*. None of samples was positive for *Mycoplasma genitalium*.

DISCUSSION

It has previously been reported that prostatitis and the symptoms of inflammatory prostate are associated with an increased risk of prostate cancer (1, 2). Bacterial infections may cause chronic inflammation in the prostate, leading to increased production of inflammatory cytokines. Both neutrophils and macrophages may help prevent inflammation. Molecules such as nitric oxide that tend to cause genetic damage can pave the way for cell proliferation and cancer (3). It has been reported that many pathogenic microorganisms can induce symptomatic and asymptomatic inflammatory reactions in the prostate, i.e., *Enterobacteriaceae*, especially *Escherichia coli*, *Klebsiella* spp., *Proteus mirabilis*, *Serratia* spp., *Enterobacter* spp. and Gram-positive organisms such as *Staphylococcus aureus* and *Enterococcus* spp. Microbial entry occurs through ascending transmission from the urethra, rejection of infected urine into the prostate, and direct diffusion from the rectum through the blood-lymph (6). Therefore, the diagnosis of the causative agents of bacterial prostatitis and treatment of infection in early phases plays an essential role in the prevention of prostate cancer.

In the present study, for molecular and conventional identification of aerobic and anaerobic bacteria isolated from surgical prostate tissues, 72 prostate tissue samples were collected from QUMS hospitals for 13 months. After culturing the samples, 27.8% of the samples were positive. All isolates were aerobic bacteria. All the necessary points were performed for creating anaerobic conditions and the culture medium, and the necessary supplements were provided

for the growth of anaerobic bacteria, but no anaerobic bacteria grew. For quality control, anaerobic bacteria were well grown under anaerobic conditions on a culture medium with supplements.

A 2017 study by Benelli et al. showed that the most common pathogens in bacterial prostatitis are *Escherichia coli*, *Klebsiella* spp., *Proteus mirabilis*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Mycoplasma hominis*, *Mycobacterium tuberculosis*, and *Candida* spp. (9). In Busetto et al. study, it found that the common pathogens involved in prostate inflammation and CBP, who receive fluoroquinolones, are Gram-negative bacteria (53.9%, i.e., *Escherichia coli* (50.7%), *Enterobacter* spp. (30.7%), *Klebsiella* spp. (10.7%), *Proteus mirabilis* (4.6%), and *Serratia* spp. (3%) and Gram-positive bacteria (46.1%, i.e., *Enterococcus* spp. (56%), *Staphylococcus saprophyticus* (22.7%), *Staphylococcus epidermidis* (4.5%), *Staphylococcus aureus* (2.3%), and *Streptococcus* B group (13.6%) (10). In a study by Stamatiou et al. the common isolates from CBP (249 samples) were *Escherichia coli* (90 isolates, 36%), coagulase-negative *Staphylococcus* spp. (71 isolates, 28.5%), *Enterococcus faecalis* (70 isolates, 28.1%), *Streptococcus* spp. (21 isolates, 8.4%), *Proteus mirabilis* (20 isolates, 8%), *Staphylococcus aureus* (17 isolates, 6.8%), and *Klebsiella pneumoniae* (4 isolates, 1.6%). The pathogens isolated from ABP were *Escherichia coli* (52 isolates, 41.2%), coagulase-negative *Staphylococcus* spp. (38 isolates, 15.2%), *Enterococcus faecalis* (31 isolates, 24.6%), *Streptococcus* spp. (10 isolates, 7.9%), *Proteus mirabilis* (8 isolates, 6.3%), *Staphylococcus aureus* (5 isolates, 3.9%), and *Klebsiella pneumoniae* (5 isolates, 3.9%) (11). In a study on 332 patients with CBP by Heras-Cañaset et al. *Enterococcus faecalis* was the most common species with a prevalence of 37.7%, followed by *Escherichia coli* with a prevalence of 22.2% (12). A 2017 study by Delcaru et al. aimed at antibiotic resistance and pathogenic biotypes of bacterial species isolated from UTI in elderly patients with the prostate disease (13). In this study, 85 patients with benign prostatic hyperplasia participated, and 70% of them were positive for urine culture. The isolated microorganisms include *Escherichia coli* (60%), *Klebsiella* spp. (8.2%), *Proteus* spp. (7%), *Enterobacter* spp. (5%), *Serratia* spp. (1.1%), *Morganella morganii* (1.1%), *Enterococcus* spp. (15%) and *Streptococcus agalactiae* (2.3%).

Out of 72 prostate biopsy samples, 18 samples

(15.28%) were positive in the 16S rDNA PCR assay. All PCR-positive isolates were sequenced for molecular identification. The results of molecular identification showed that all isolates are aerobic species. Our findings showed that conventional culture and molecular identification methods are similar in identifying prostate infection pathogens. In a study conducted in Sweden, 402 prostate specimens were collected to detect the bacteria via 16S rDNA PCR. This study showed that in 96 out of 325 positive samples. After sequencing of *Propionibacterium acnes*, it was introduced as the significant known microorganism involved in prostatitis. Also, *Escherichia coli* was found in 12 samples (14). In 2006, a molecular study was performed on 352 prostate specimens of patients with benign prostate hyperplasia. According to this study, the most common isolated strain is *Propionibacterium acnes*, followed by *Escherichia coli* (23% and 12% of 96 positive samples, respectively). The diagnosis of *Propionibacterium acnes* in tissue was associated with cancer. Also, strains such as *Pseudomonas* spp. (3 patients), *Actinomyces* spp. (2 patients), *Streptococcus mutans* (1 patient), *Corynebacterium* spp. (2 patients), *Nocardioideis* spp. (1 patient), *Rhodococcus* spp. (1 patient) were found (15). Eslami et al. began a genotyping study to determine the role of *Ureaplasma urealyticum* and *Mycoplasma genitalium* in 62 samples of prostate cancer and 62 samples of benign prostate hyperplasia. In this study, *Ureaplasma urealyticum* was found in 1.6% of 62 prostate cancer specimens. Also, *Mycoplasma genitalium* was not found in any of the 124 specimens (16).

CONCLUSION

The diagnosis and on-time treatment of microbial pathogens of prostatitis are critical in the prevention of prostate cancer. Our results showed that the conventional method is as good as molecular detection of specimens in prostatitis, but the molecular method can help to diagnose the patient earlier. Also, we found that *Escherichia coli* is the most common pathogen in prostatitis. Also, anaerobic bacteria are not isolated from prostate tissue in microbial-infected prostatitis. For further study, we suggest investigating a non-invasive method to identifying prostate specimens with further samples.

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