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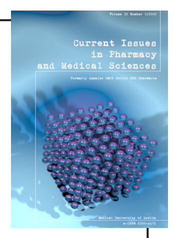

Profile of antibiotic sensitivity pattern and detection some virulence gene of bacteria isolated from patients with chronic bacterial prostatitis in Hilla city and its suburbs

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ABSTRACT

This study aimed to evaluate the spectrum of bacterial pathogens responsible for chronic bacterial prostatitis among patients from Babylon Province and Al-Mahawel District, Iraq, and to assess their antimicrobial resistance patterns as well as the prevalence of selected virulence genes. A total of 150 bacterial isolates were recovered from urine cultures, expressed prostatic secretions, and post-massage urine samples. Patients were categorized into three age groups, with individuals aged 40–60 years representing the largest group (83 patients). The results showed that *Staphylococcus aureus* was the predominant Gram-positive pathogen, accounting for 73 of 150 isolates (48.66%), followed by *Streptococcus pyogenes* with 5 isolates (3.33%). Among Gram-negative bacteria, *Escherichia coli* was the most frequently isolated species, accounting for 48 isolates (32%), followed by *Pseudomonas aeruginosa* (9 isolates; 6%), *Klebsiella pneumoniae* (5 isolates; 3.33%), *Proteus mirabilis* (4 isolates; 2.66%), and *Enterobacter aerogenes* (2 isolates; 2%). Antimicrobial susceptibility testing revealed that *S. aureus* exhibited the highest resistance to cefotaxime (97.27%), followed by ceftriaxone (95.9%) and penicillin (93.16%). In contrast, gentamicin showed the greatest antibacterial activity, with a susceptibility rate of 94.52%, followed by imipenem (93.15%) and vancomycin (91.78%). Molecular analysis of biofilm-associated genes demonstrated that 76% of *S. aureus* isolates carried the *icaA* gene, whereas the *icaD* gene was detected in 66% of the isolates. These findings highlight the high prevalence of multidrug-resistant pathogens in chronic bacterial prostatitis and emphasize the importance of molecular characterization in guiding effective therapeutic strategies.

INTRODUCTION

Etiological agents and pathogenicity of prostate gland infections

Prostatitis is an inflammatory condition of the prostate gland that may result from either bacterial infection or non-bacterial inflammatory processes. The condition can be extremely painful and significantly impair patients' quality of life. It is estimated that one in six men will experience prostatitis at some point during their lifetime, and the disease affects men of all ages [1].

In the history of clinical microbiology, *Staphylococcus aureus* was one of the first microorganisms recognized to form biofilms. In 1982, a 56-year-old man with a history of chronic bacteremia who had undergone multiple courses of antibiotic therapy was investigated. Electron microscopy revealed that the persistent infection was associated with the formation of an *S. aureus* biofilm on the endocardial surface surrounding the patient's pacemaker. Since then, *S. aureus* biofilms have been recognized as a major cause of persistent infections associated with implanted medical devices, including arteriovenous shunts, prosthetic heart valves, and orthopedic implants [2].

Staphylococcus aureus possesses numerous virulence factors that contribute to the pathogenesis of associated diseases. The most important include capsular polysaccharides, peptidoglycan, catalase, teichoic acids, clumping factor, fibrinolysins, coagulase, hyaluronidase, lipases, phosphatidylinositol-specific phospholipase C, nucleases,

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phosphodiesterases, and β-lactamase enzymes. Some of these virulence determinants also act as superantigens, activating host immune cells and stimulating cytokine release, which may result in systemic effects [3].

Biofilm formation is one of the most important pathogenic mechanisms of *S. aureus*, as it markedly enhances bacterial resistance to antimicrobial agents and provides protection against host immune defenses [4]. Therefore, the identification of virulent strains requires assessment of their biofilm-forming ability. Almost all *S. aureus* strains produce several extracellular enzymes that contribute to tissue destruction and the inactivation of host antimicrobial defense mechanisms [5].

Staphylococcus aureus is one of the most common bacterial species associated with biofilm-related infections. Biofilm formation enables bacteria to withstand environmental stresses, evade host immune responses, and survive antimicrobial therapy, thereby posing a major challenge to clinical management [6]. It is now widely accepted that bacterial biofilms are involved in more than 80% of chronic infections [7].

In healthcare settings, *S. aureus* readily adheres to and persists on host tissues and implanted medical devices, causing a wide spectrum of infections, including osteomyelitis, endocarditis, pneumonia, bacteremia, and skin and soft tissue infections [8,9].

Therefore, the primary aim of the present study was to investigate the relationship between biofilm-forming capacity and antibiotic resistance in *S. aureus* using both phenotypic and genotypic approaches under in vitro conditions.

METHODS

Collection of urine samples

Clean-catch post-prostatic massage urine (VB3) samples were collected in sterile wide-mouth containers.

Isolation and identification of bacterial isolates

The present study included 150 clinical specimens obtained from patients and 50 specimens collected from healthy individuals, which served as controls. The study samples consisted of urine specimens collected from Al-Hilla Teaching Hospital, Al-Imam Al-Sadek Hospital in Hilla City, Al-Mahawel Hospital, and several private clinics between January 2021 and September 2022.

All specimens were subjected to morphological, microscopic, and biochemical analyses, and bacterial identification was confirmed using the VITEK® 2 system. Molecular analysis was also performed for isolates obtained from both patients and healthy individuals. In addition, antimicrobial susceptibility testing was carried out for bacteria isolated from patients with prostatic infections.

The specimens were inoculated onto various culture media, including blood agar, Mannitol Salt Agar, MacConkey agar, and eosin methylene blue (EMB) agar, using sterile inoculating loops. The inoculated plates were incubated at 37°C for 24 h and subsequently examined for bacterial growth. Single, well-isolated colonies were subcultured onto Brain Heart Infusion (BHI) medium for preservation and further characterization. Morphological evaluation was performed by Gram staining, and additional biochemical tests were used to confirm the identity of the isolates [10].

Antibiotic susceptibility profile of *Staphylococcus aureus* isolates

Antimicrobial susceptibility testing was performed using the disc diffusion method according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI). Commercial antibiotic discs supplied by Bioanalyse Company (Turkey) were used.

Overnight cultures of each isolate were prepared in Brain Heart Infusion (BHI) broth. The turbidity of the bacterial suspension was adjusted to the 0.5 McFarland standard. Each suspension was inoculated onto Mueller-Hinton (MH) agar using sterile cotton swabs. After 15 min, antibiotic discs were placed on the agar surface, maintaining a minimum distance of 15 mm between discs. The plates were incubated at 37°C for 18–20 h. The diameters of the inhibition zones were measured and interpreted according to CLSI guidelines.

DNA extraction

Genomic DNA was extracted from bacterial isolates using the boiling method. DNA concentration and purity were determined using a BioDrop RNA/DNA spectrophotometer. The purity of the extracted DNA ranged from 1.8 to 2.0. Agarose gel electrophoresis was performed to confirm the quality and integrity of the extracted DNA.

Detection of biofilm formation

Biofilm formation was assessed semi-quantitatively using tissue culture-treated 96-well polystyrene microtiter plates (MTPs) according to the method described by Lizcano *et al*. [11].

PCR amplification of *icaA* and *icaD* genes

The primer sequences used for amplification of the *icaA* gene were as follows: forward primer 5′-TCTCTTGCAGGAGCAATCAA-3′ and reverse primer 5′-TCAGGCACTAACATCCAGCA-3′, yielding an amplicon of 188 bp. For the *icaD* gene, the forward primer sequence was 5′-ATGGTCAAGCCCAGACAGAG-3′ and the reverse primer sequence was 5′-CGTGTTTTCAACATTTAATGCAA-3′, yielding an amplicon of 198 bp [12].

PCR amplification was performed under the following conditions: initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 60.3°C for 30 s, and extension at 72°C for 30 s. A final extension step was carried out at 72°C for 7 min, after which the reaction mixtures were maintained at 4°C until further analysis.

Amplification reactions were performed using a Veriti™ Thermal Cycler. The PCR products were analyzed by electrophoresis on 1% agarose gels stained with ethidium bromide and visualized using a gel documentation system.

RESULTS AND DISCUSSION

A total of 150 male patients were categorized into three age groups: 20–40 years (3 patients), 41–60 years (83

patients), and 61–80 years (64 patients), as shown in Table 1. The highest proportion of prostatitis cases was observed among patients aged 41–60 years, accounting for 55% of all cases, followed by the 61–80-year age group (43%). In contrast, the lowest proportion of cases (2%) was recorded among individuals aged 20–40 years.

Table 1. Distribution of patients with prostatitis according to age

Age/Years	Number of patients	Percentage (%)
20-40	3	2
41-60	83	55
61-80	64	43
Total	150	100

A total of 150 clinical specimens were collected during the study. The specimens were cultured on Mannitol Salt Agar and blood agar media. Mannitol Salt Agar is widely used as a selective and differential medium for the isolation of *Staphylococcus* spp. because of its high sodium chloride concentration (7–10%) and the presence of mannitol [13].

Among the 150 isolates recovered, 78 (51.99%) were Gram-positive bacteria, including 73 isolates of *Staphylococcus aureus* (48.66%) and five isolates of *Streptococcus pyogenes* (3.33%).

Non-mannitol-fermenting isolates produced small white colonies without causing any color change in the medium. In contrast, mannitol-fermenting isolates formed large golden-yellow colonies surrounded by yellow zones, resulting in a color change of the medium from pink to yellow [14].

Overall, 73 isolates of *S. aureus* (48.67%) were recovered from patients with prostatitis, making this species the predominant pathogen associated with prostate gland infections. As shown in Table 2, the distribution of Gram-positive bacteria included *S. aureus* (73/150; 48.67%) and *S. pyogenes* (5/150; 3.33%).

Table 2. Number and percentage of Gram-positive and Gram-negative bacteria isolated from patients with prostatitis

Bacterial species	Number of isolates	Percentage (%)	Chi square (p-value)
Gram-positive bacteria			p = 0,220
Staphylococcus aureus	73	48.67	
Streptococcus pyogenes	5	3.33	
Total	78	52.00	
Gram-negative bacteria			
Escherichia coli	48	32.00	
Pseudomonas aerogenosa	9	6.00	
Enterobacter aerogenes	6	4.00	
Kliebsiella pnemoniae	5	3.33	
Proteus mirabilis	4	2.67	
Total	72	48.00	

Gram-negative bacteria were isolated less frequently. *Escherichia coli* was the most prevalent Gram-negative species, accounting for 48 isolates (32%), followed by *Pseudomonas aeruginosa* (9 isolates; 6%), *Enterobacter aerogenes* (6 isolates; 4%), *Klebsiella pneumoniae* (5 isolates; 3.33%), and *Proteus mirabilis* (4 isolates; 2.67%).

These findings are consistent with those reported by Ibrahim et al. [15], who documented comparable isolation rates for *Escherichia coli* (13.7%), *Proteus mirabilis* (1.0%), and *Klebsiella pneumoniae* (2.0%). Similar observations were reported by Issa *et al.* [1], who isolated 10 strains of *E. coli* and one strain of *Enterobacter aerogenes* from patients with prostatitis.

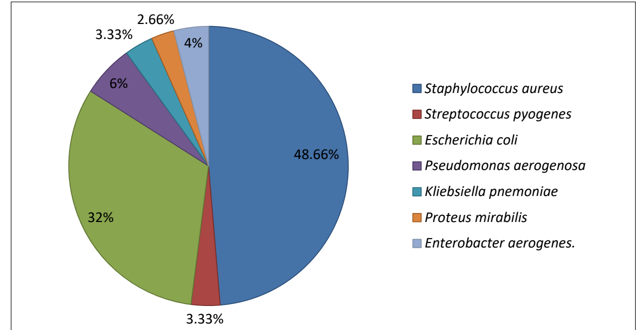


Figure 1. Distribution of bacterial species isolated from patients with prostate gland infections.

Bacteriological study

Identification of *Staphylococcus aureus*

Men with high leukocyte counts and bacterial growth in expressed prostatic secretion (EPS) and post-prostatic massage urine (VB3) samples, but with negative first-voided urine (VB1) and midstream urine (VB2) samples, were included in the study. Diagnosis was based on standard primary diagnostic procedures. Each specimen was cultured on primary and selective media and subjected to colony morphology assessment, microscopic examination, and biochemical testing, including oxidase, catalase, and coagulase assays.

The morphology of *S. aureus* colonies on blood agar was characterized by opaque white to cream-colored colonies measuring 1–2 mm in diameter. Hemolysin production was detected by culturing the isolates on blood agar. All *S. aureus* isolates showed complete lysis of red blood cells due to β-toxin production, resulting in a clear zone surrounding the colonies [16].

Seventy-three isolates tested positive for coagulase, confirming their identification as *S. aureus*. This enzyme is used to distinguish *S. aureus* from coagulase-negative staphylococci (CoNS). Coagulase is produced by *S. aureus* and converts soluble fibrinogen in plasma into insoluble fibrin [17].

In the current study, *S. aureus* isolates exhibited the following characteristics: Gram-positive cocci, typically arranged in grape-like clusters; catalase-positive; non-motile; and forming small, shiny colonies on blood agar. All *S. aureus* isolates showed β-hemolysis after 24 h of

Table 3. Biochemical characteristics of *Staphylococcus aureus* isolates

Bacterium	Biochemical test						
	Mannitol fermentation	Pigment production	Hemolysis	Catalase	Coagulase	Bacitracin sensitivity test	Novobiocin sensitivity test
Staphylococcus aureus	+	Yellow	β-hemolysis	+	+	R	S

R - Resistant; S - Susceptible

incubation at 37°C, were able to grow on Mannitol Salt Agar, were resistant to bacitracin, and were susceptible to novobiocin.

Antibiotic susceptibility and resistance patterns of bacterial isolates

A range of antibiotics was used to evaluate the susceptibility of *S. aureus*, the predominant bacterium isolated from patients with prostatitis. The disc diffusion method was used to assess bacterial susceptibility to antimicrobial agents.

Seventeen antibacterial agents were selected to determine the susceptibility profile of the isolates. All antibiotics were used and interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2020) guidelines for *S. aureus*. The tested antimicrobial agents included imipenem (IPM 10), trimethoprim (TMP 10), chloramphenicol (C 10), tetracycline (TE 10), nalidixic acid (NA 30), nitrofurantoin (F 300), vancomycin (VA 30), erythromycin (E 15), amoxicillin (AX 30), azithromycin (AZM 30), bacitracin (B 10), azithromycin (AZ 15), penicillin (P 10), ciprofloxacin (CIP), ceftriaxone (CRO), cefotaxime (CFM 5), and gentamicin (CN).

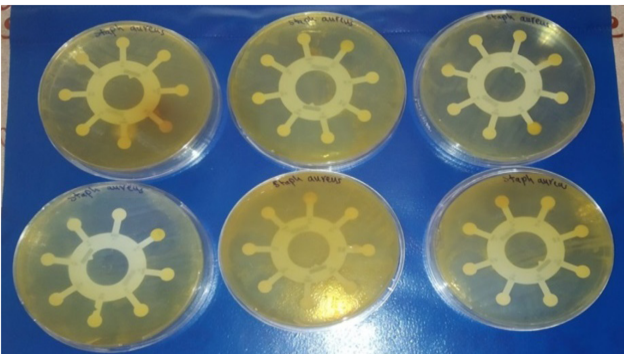


Figure 2. Antibiotic susceptibility testing using the multi-disc diffusion method.

Table 4. Antibiotic susceptibility patterns of *Staphylococcus aureus* isolates recovered from patients with prostate gland infections

Antibiotic class	Antibiotic	Susceptible, n (%)	Resistant, n (%)
Penicillins	Penicillin (P 10)	5 (6.84%)	68 (93.16%)
β-Lactam/β-lactamase inhibitor	Amoxicillin (AX 30)	39 (53.42%)	34 (45.58%)
Aminoglycosides	Gentamicin (CN 10)	69 (94.52%)	4 (5.84%)
Macrolides	Azithromycin (AZM 30)	40 (54.79%)	33 (45.21%)
Macrolides	Azithromycin (AZ 15)	30 (41.09%)	44 (58.91%)
Macrolides	Erythromycin (E 15)	25 (36.98%)	48 (63.02%)
Tetracyclines	Tetracycline (TE 10)	36 (49.31%)	37 (50.69%)
Fluoroquinolones	Ciprofloxacin (CIP 10)	53 (72.60%)	20 (27.40%)
Cephalosporins	Ceftriaxone (CRO 30)	3 (4.10%)	70 (95.90%)
Quinolones	Nalidixic acid (NA 30)	16 (21.91%)	53 (78.09%)
Phenicols	Chloramphenicol (C 10)	56 (76.71%)	17 (32.29%)
Nitrofurans	Nitrofurantoin (F 100/F 300)	64 (87.67%)	9 (12.33%)
Cephalosporins	Cefotaxime (CFM 5)	2 (2.73%)	71 (97.27%)
Diaminopyrimidines	Trimethoprim (TMP 10)	54 (73.97%)	19 (26.03%)
Polypeptides	Bacitracin (B 10)	24 (32.87%)	49 (67.13%)
Carbapenems	Imipenem (IPM 10)	68 (93.15%)	5 (6.85%)
Glycopeptides	Vancomycin (VA 30)	67 (91.78%)	6 (8.22%)

The table presents the susceptibility and resistance patterns of *S. aureus* isolates associated with prostatitis against a panel of antibiotics. All isolates were tested using the disc diffusion method. *Staphylococcus aureus* exhibited high resistance to several antibiotics, including penicillin (68 isolates; 93.16%), azithromycin AZ 15 (44 isolates; 58.91%), erythromycin (48 isolates; 63.02%), tetracycline (37 isolates; 50.69%), ceftriaxone (70 isolates; 95.90%), nalidixic acid (53 isolates; 78.09%), cefotaxime (71 isolates; 97.27%), and bacitracin (49 isolates; 67.13%).

These findings are partially consistent with those reported by [18], who observed resistance of *S. aureus* to gentamicin (7 isolates; 25.93%), cephalothin (9 isolates; 15.52%), tetracycline (8 isolates; 17.39%), rifampin (6 isolates; 12.24%), trimethoprim (6 isolates; 13.04%), cefotaxime (10 isolates; 18.87%), penicillin G (6 isolates; 13.64%), and clindamycin (6 isolates; 13.04%).

Conversely, *S. aureus* isolates showed susceptibility to several antimicrobial agents, including amoxicillin (39 isolates; 53.42%), gentamicin (69 isolates; 94.52%), azithromycin AZM 30 (40 isolates; 54.79%), ciprofloxacin (53 isolates; 72.60%), chloramphenicol (56 isolates; 76.71%), nitrofurantoin (64 isolates; 87.67%), trimethoprim (54 isolates; 73.97%), imipenem (68 isolates; 93.15%), and vancomycin (67 isolates; 91.78%).

These results are in agreement with those of [19], who reported that *S. aureus* isolates showed 93% susceptibility to gentamicin, 78% susceptibility to nitrofurantoin, and 71% susceptibility to chloramphenicol. Most Gram-positive isolates, including *S. aureus*, showed high resistance to azithromycin.

Vancomycin, an older antibiotic that remains in clinical use, is still particularly effective against a wide range of Gram-positive bacteria. It is commonly used to treat infections caused by methicillin-resistant *S. aureus* (MRSA) and in patients with allergies to cephalosporins or penicillins [20]. Imipenem was also highly effective in the present study, with 68 isolates (93.15%) classified as susceptible and 5 isolates (6.85%) classified as resistant. These findings differ from those reported in [21,22].

Multidrug-resistant (MDR) strains are commonly defined as bacteria resistant to at least one agent in three or more antimicrobial categories. In this study, MDR *S. aureus* accounted for 17 isolates (23.3%). The reported resistance pattern included erythromycin (69.5%), clindamycin (57.8%), trimethoprim-sulfamethoxazole (SXT; 47.38%), vancomycin (2.74%), ciprofloxacin (71.6%), gentamicin (42.5%), and minocycline (38.5%). All isolates were susceptible to linezolid [23].

The susceptibility of *S. aureus* to azithromycin varied depending on the disc concentration used: AZM 30 µg showed susceptibility in 40 isolates (54.79%), AZ 15 µg in 30 isolates (41.09%), and erythromycin 15 µg in 25 isolates (36.98%). Microorganisms have developed various mechanisms of resistance to antibiotics, particularly macrolides [24,25]. Previous studies have shown that macrolides may be ineffective against some *S. aureus* isolates, even at MIC values of 256 µg/mL for the tested macrolides, emphasizing the need for strategies to prevent the spread of macrolide resistance [26].

Tetracycline has been widely used for the treatment of bacterial infections [27]. In this study, 37 *S. aureus* isolates (50.69%) were resistant to tetracycline, which is consistent with [15], who reported 36 tetracycline-resistant isolates (43.4%). Susceptibility to nitrofurantoin was observed in 64 isolates (87.67%), which is consistent with findings reported in [19,22] but differs from those in [18,28].

Fluoroquinolones are highly effective against a broad spectrum of bacteria and are widely used worldwide in the treatment of prostatitis [29,30]. In the present study, resistance was observed to nalidixic acid (53 isolates; 78.09%), whereas ciprofloxacin remained effective against 53 isolates (72.60%). Similarly, [31] reported an 80.4% resistance rate to ciprofloxacin among MRSA isolates.

Biofilm test

Biofilm formation was assessed using microtiter plates (MTPs) and Brain Heart Infusion (BHI) broth. A total of 73 *S. aureus* isolates were tested for their biofilm-forming ability. Biofilm production was quantified by measuring the absorbance of stained biofilms at 630 nm using a plate reader. The results showed that 53 isolates (73%) were strong biofilm producers, 7 isolates (9%) exhibited moderate biofilm formation, and 13 isolates (18%) showed weak biofilm formation (Table 5). These results are consistent with those reported by Abbas and Motaweq [32].

Table 5. Distribution of *Staphylococcus aureus* isolates according to biofilm-forming ability

Bacterium	Weak n (%)	Moderate n (%)	Strong n (%)
Staphylococcus aureus	13 (18 %)	7 (9%)	53 (73 %)

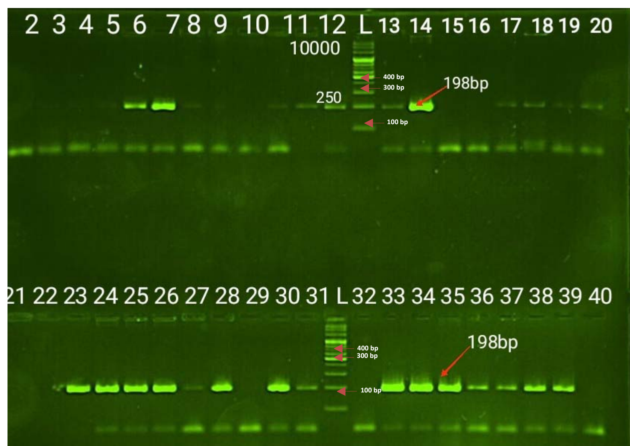
Molecular analysis of *Staphylococcus aureus*

Detection of slime-layer-associated genes (*icaA* and *icaD*)

PCR amplification of the *icaA* and *icaD* genes revealed that 76% of *S. aureus* isolates were positive for *icaA*, producing an amplicon of 188 bp (Figure 3). In addition, 66% of *S. aureus* isolates were positive for the *icaD* gene, with an expected amplicon size of 198 bp (Figure 4).

PCR products were separated by electrophoresis on a 2.3% agarose gel at 85 V for 90 min, with 5 μL of PCR product loaded per well. Lane M: DNA ladder (10,000 bp). Lanes 1–73: PCR products of S. aureus isolates showing a positive band at 188 bp

Figure 3. PCR amplification of the *icaA* gene



PCR products were separated by electrophoresis on a 2% agarose gel at 85 V for 90 min, with 5 μL of PCR product loaded per well. Lane M: DNA ladder (10,000 bp). Lanes 1–73: PCR products of S. aureus isolates showing a positive band at 198 bp

Figure 4. PCR amplification of the *icaD* gene

Slime-layer formation, an important component of biofilm production, plays a key role in bacterial colonization and persistence on biotic and abiotic surfaces [33]. Several methods have been used to evaluate slime-layer production, including the Congo Red Agar (CRA) method, the standard tube method, and Christensen's tube method [34,35].

CONCLUSIONS

Antibiotic resistance represents a significant public health challenge that requires coordinated action by local health authorities. Understanding the spectrum of pathogens and their resistance profiles is essential for developing and updating local treatment guidelines for prostatitis.

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