Indian Journal of Modern Research and Reviews

This Journal is a member of the *'Committee on Publication Ethics'*

Online ISSN:2584-184X

Research Paper

Prevalent Bacterial Causes and The Development of Antibiotic Resistance in Individuals with Chronic Prostate Disease In AL-Najaf Province

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DOI: https://doi.org/10.5281/zenodo.17609686

ABSTRACT

Chronic prostatitis (CP) or Persistent prostatitis affecting the male urinary system is the hallmark of Chronic Pelvic Pain Syndrome (CPPS), an illness that is sometimes challenging to diagnose and cure. Symptoms of CPPS include discomfort and lower urinary tract abnormalities. To determine the frequency of bacterial isolates causing prostatitis and their antimicrobial resistance profile, examine the spectrum and rates of resistance to antibacterial agents in the pathogens of bacterial prostatitis in patients, assess the occurrence of multidrug-resistant (MDR) cases, and identify different risk factors (age group) associated with prostatitis infection, the current study was designed. After clinical samples were cultivated, standard microbiological techniques were used to identify the bacterial isolates, which VITEK subsequently validated. 131 isolates from cultures of urine and seminal fluid from patients aged 25-85 years. The age range of 46–55 years old had the greatest number of positive cases. There were 26 (19.84%) Gram-positive and 105 (80.15%) Gram-negative pathogenic bacteria. While Staphylococcus aureus (73.07%) was the most common Gram-positive strain, Escherichia coli (E. coli) was the most isolated Gram-negative strain (41.9%). Gram-negative bacteria were highly resistant 100% to Cefuroxime, Amoxicillin+clavulanic acid and Amoxicillin due to their increased resistance to widely used antibiotics, these pathogens reduced the number of available treatments and increased the risk of recurring or chronic infections. This result emphasises the critical need for enhanced infection control methods, sensitivity-test-based customised antibiotic therapy, and ongoing antimicrobial surveillance. However, co-trimoxazole was not effective against Gram-positive bacteria. Imipenem, Erythromycin, Pristinamycin, Chloramphenicol, Rifampicin and Ciprofloxacine increase the risk of persistent or recurrent infections and emergence of multidrug-resistant *Staphylococcus aureus* and *Enterococcus spp*. Targeted interventions are critical to manage resistance and improve patient outcomes in chronic prostatic infections. In order to control resistance and enhance patient outcomes in cases of chronic prostatic infections, targeted therapies are essential.

Manuscript Info.

- ✓ **ISSN No:** 2584- 184X
- ✓ **Received:** 12-10-2025
- ✓ **Accepted:** 02-11-2025
- ✓ **Published:** 14-11-2025
- ✓ **MRR:3(11):**2025;30-36

- ✓ **Peer Review Process:** Yes
- ✓ **Plagiarism Checked:** Yes

How To Cite this Article

Hashim HS. Prevalent Bacterial Causes and The Development of Antibiotic Resistance in Individuals with Chronic Prostate Disease In AL-Najaf Province. Indian J Mod Res Rev. 2025;3(11):30-36.

KEYWORDS: Chronic prostatitis (CP), pathogenic bacteria, multidrug-resistant (MDR)

1. INTRODUCTION

In Western countries, prostate disorders are a prevalent health problem that significantly affects older men's quality of life and increases male morbidity and death [1]. Prostate cancer, benign prostatic hyperplasia, and chronic prostatitis are among the illnesses that fall under the category of chronic prostate diseases. Prostate cancer is largely influenced by persistent inflammation. [2]. Both chronic and acute inflammatory infiltrates can occur in the prostate. These infiltrates might be "silent," meaning they don't cause any symptoms, or they can be accompanied by noticeable symptoms including lower pelvic pain or discomfort, trouble urinating, and frequent urination, and it can cause infertility through several methods, including preventing sperm transit, inhibiting sperm production, and decreasing accessory gland activity [3]. These mechanisms could lead to lower-quality semen during the inflammatory period, which could diminish fertility [4]. These processes have the potential to impair fertility by causing lower-quality semen during the inflammatory phase. [5] Younger and older men are both susceptible to bacterial prostatitis (BP), a bacterial infection that affects the prostate gland. It is a prevalent ailment that impacts 10% of men globally [6]. If left untreated, bacterial prostatitis can cause serious consequences and manifest as either acute (ABP) or chronic (CBP) [7]. The role of the urinary microbiota in influencing prostate cancer development and Alterations in microbiota composition may contribute to prostate cancer risk and progression [8]. The most common bacteria that cause prostate disease include *Escherichia coli* is the most frequently isolated gram-negative bacterium in prostate infections. *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter* and *Proteus mirabilis are* other notable Gram-negative bacteria found in prostate specimens. Identified in prostatitis cases, indicating a broader spectrum of bacterial involvement [9]. *Staphylococcus aureus* is the most common bacterial isolate of Gram-positive bacteria [10].

Persistent and severe resistance to antibiotics has an impact on how infections are diagnosed and treated in hospitals and the community [11]. Antibacterial resistance in bacteria causing prostate disease, particularly chronic bacterial prostatitis, is a significant concern [12]. Various studies highlight the prevalence of resistant strains, primarily focusing on Gram-positive and Gram-negative bacteria [13]. Therefore, this study was carried out to determine the frequency of bacterial isolates associated with Chronic prostatitis and to investigate their antimicrobial resistance profile. Thus, the purpose of this investigation was to ascertain the prevalence of bacterial isolates linked to chronic prostatitis and to look into the profile of antibiotic resistance in these isolates.

2. MATERIALS AND METHODS

2.1. Study design

The study was conducted for six months, from March to August 2024, in AL-Saddar Medical City in the province of Al-Najaf. Using purposive standard sampling technique included a total of 131 male patient who attended the infertility departments at different ages; their ages ranged between 25-85 years old.

2.2. Sample collection

To identify bacterial infection, two types of samples were collected for each patient: a urine sample and a semen sample. After being collected in sterile containers, the urine samples were processed in less than an hour and delivered to the analysis location. As soon as the samples arrived at the lab, they were processed. Semen samples from patients were taken for culture and antibiotic sensitivity testing in specialised, sterile containers that were tightly sealed to prevent contamination. The patient's name, identification number, age, gender, infection site and type, date, and time of collection were all written on the samples. Additionally, each patient was given a questionnaire form, and all information was gathered directly from the patients.

2.3. Culture and identification

The urine obtained after centrifugation was used to inoculate all of the samples onto MacConkey media, mannitol salt, and blood agar. The samples were cultivated using a conventional wire loop with a diameter of 4 mm. For 18 to 24 hours, the plates were incubated at 37°C. After checking the plates for bacterial growth, a loop of semen was injected onto enriched and differential media, among other media, and incubated for 24 hours at 37°C. Following a subsequent culture of the bacterial growth, biochemical assays were used to identify individual colonies.

After first classifying bacterial colonies based on their Gram stain morphology, the isolates were subsequently identified based on their biochemical properties and standard culture. The isolated and pure isolates were kept in nutrient agar slants and cultured for 24 hours at 37°C. At regular intervals, the isolates were subcultured.

Using routine biochemical testing, gram-negative bacteria were discovered [14]. And using the appropriate laboratory tests—catalase, coagulase, and Mannitol test for Staphylococcus aureus—gram-positive bacteria were detected [15].

2.4. Biochemical Characterisation

Among the biochemical tests were assays for sugar fermentation, citrate consumption, urease activity, oxidase, catalase, and indole synthesis. Typical microbiological procedures were used to conduct these tests.

2.5. Antimicrobial susceptibility testing

All isolates were tested for antibiotic susceptibility using the Kirby-Bauer disc diffusion method, in accordance with the National Committee for Clinical Laboratory Standards' (NCCLS) recommendations [16]. On Mueller Hinton agar, standard inocula adjusted to 0.5 McFarland were swabbed and left to soak for two to five minutes. Following that, antibiotic disks were gently pressed into the medium's surface. For 24 hours, Mueller-Hinton agar plates were incubated at 37°C.

The inhibition zones were evaluated and interpreted according to laboratory and clinical criteria after a 24-hour period [17].

Amoxicillin+clavulanic acid 20 µg, Amoxicillin 10 µg, Meropenem 10 µg, Imipenem 10 µg, Ticarcillin + clavulanic

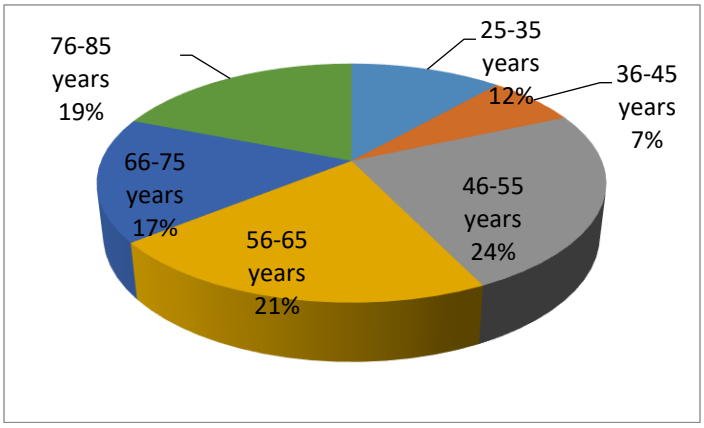
acid 75 µg, Cefoxitin 30 µg, Cefuroxime 30 µg, Cefurotaxime 30 µg, Cefotaxime 5 µg, Aztreonam 30 µg, Amikacin 30 µg, Gentamicin 10 µg, and Ciprofloxacine 5 µg were among the tests conducted on the Gram-negative isolates.

The Gram-positive bacteria were tested against 100 µg of nitrofurantoin, 25 µg of co-trimoxazole, 10 µg of imipenem, 15 µg of erythromycin, 15 µg of pristinamycin, 5 µg of rifampicin, 5 µg of ciprofloxacine, and 30 µg of chloramphenicol. CASFM-EUCAST (Comité d'Antibiogramme de la Société Francaise de Microbiologie/The European Committee on Antimicrobial Susceptibility Testing) 2021 criteria were used to assess the zone of inhibition sizes [18].

3. RESULTS

The current investigation comprised 131 male patients who had been diagnosed with chronic prostatitis and were exhibiting symptoms. Participants ranged in age from 25 to 85 years, with the bulk of them being between the ages of 46 and 55 (24%), and 56 and 65 (21%) Followed by 76-85 years were 19%, 66-75 years were 17%, 25-35 years were 12% and 36-45 years were 7% respectively as shown in the (Figure 1)

Figure 1: Cases with chronic prostate disease according to age group for patients



Investigations were conducted on 131 adult isolates of various strains of prostatitis. According to Table 1, the overall percentages of Gram-positive and Gram-negative isolates were 26/131 (19.84%) and 105/131 (80.15%), respectively.

Table 1: The percentage prevalence of chronic prostate patients caused by different causative agents

Factor	Gram positive (%)	Gram-negative (%)
25-35	2 (13.3 %)	13 (86.7 %)
36-45	1 (11.1 %)	8 (88.9 %)
46-55	9 (28.1 %)	23 (71.9 %)
56-65	6 (21.4 %)	22(78.6 %)
66-75	4 (18.8 %)	18 (81.9 %)
76-85	4 (16 %)	21(84 %)
Total	26 (19.84%)	105 (80.15%)

Tables 5 and 6 demonstrate the different patterns of antibiotic susceptibility displayed by the bacterial isolates.

3.1. Isolated pathogens

Gram-negative bacteria were more common in this study (105/131, or 80.15%) than gram-positive bacteria (26/131, or 19.84%) among the 131 isolates. The most frequently isolated microorganisms, both positive and negative, were Escherichia coli and Staphylococcus aureus.

The most frequent isolates among the gram-positive bacteria were Staphylococcus aureus 19/26 (70.07%), followed by Enterococcus species 7/26 (269.2%) (Table 2).

Table 2: Gram-positive isolate kinds and percentages

Bacterial isolates	Total N=26 (%)
Staphylococcus aureus	19 (73.07 %)
Enterococcus species	7 (26.92 %)

Escherichia coli was the most common gram-negative bacterium, accounting for 44/105 infections (41.90%), followed by Klebsiella pneumoniae (31/105 infections, 29.52%), then Pseudomonas aeruginosa 13/105/(12.38 %), Enterobacter 10/105 infections, 9.52 %) and Proteus mirabilis 7/105 infections, 6.66 %) (Table 3).

Table 3: Gram-negative isolate kinds and percentages

Bacterial isolates	Total N=105 (%)
Escherichia coli	44 (41.90 %)
Klebsiella pneumonia	31 (29.52 %)
Pseudomonas aeruginosa	13 (12.38 %)
Enterobacter	10 (9.52 %)
Proteus mirabilis	7 (6.66 %)

A significant public health concern in recent years has been the steadily rising rates of antibiotic resistance for frequently prescribed antimicrobial drugs as a result of their improper use. Therefore, to reduce the inappropriate use of antibiotics, several local-specific research, including ours, concentrated on tackling the issue of bacterial resistance to antibiotics.

3.3. Antimicrobial susceptibility testing

3.3.1. Gram-positive bacteria

According to Table 4, gram-positive isolates of S. aureus were 100% resistant to erythromycin and pristinamycin, whereas 94.7% (18/19) were resistant to co-trimoxazole, Ciprofloxacine and Chloramphenicol. As for Imipenem and Rifampicine, *S. aureus* isolates showed resistance 89,4% (17/19) for both antibiotics. Regarding *Enterococcus* bacteria, our study showed high resistance 100% (7/7) against co-trimoxazole, Imipenem, Pristinamycin, Rifampicin and Ciprofloxacine. Furthermore, 85,7% (6/7) were resistant to Imipenem. However, the same table also showed that the isolates' sensitivity patterns to various antibiotics varied.

Table 4: Pattern of susceptibility rates (%) for isolated gram-positive bacteria to various antibiotics

Bacteria	sen	NIT	COT	IMP	E	INN	RA	CIP	C
***S. aureus* N=19**	**S**	18 94,7%	0	1 5,3%	0	0	2	1 5,3%	0
	I	1 5,3%	1 5,3%	1 5,3%	0	0	0	0	1 5,3%
	R	0	18 94,7%	17 89,4%	19 100%	19 100%	17 89,4%	18 94,7%	18 94,7%
***Enterococcus species* N=7**	**S**	6 85,7%	0	0	0	0	0	0	1 14,3%
	I	1 14,3%	0	1 14,3%	0	0	0	0	5 71,4%
	R	0	7 100%	6 85,7%	7 100%	7 100%	7 100%	7 100%	1 14,3%

NOTE R Resistant S Sensitive, I Intermediate, NIT Nitrofurantoin, COT co-trimoxazole, IMP Imipenem, E Erthromycin, INN Pristinamycin, RA Rifampicine, CIP Ciprofloxacine, C Chloramphenicol.

3.3.2. Gram-negative bacteria

As shown in Table 5, the bacterial isolates analysed showed patterns of antibiotic resistance to popular antibiotics used to treat chronic prostatitis, according to laboratory testing. The isolates of E. Coli were 100% (44/44) resistant to amoxicillin with clavulanic acid, Amoxicillin and Cefuroxime, Followed by Ciprofloxacine 95, 5% 42/44) 90.9% (40/44) for Nalidixc acide. while the amount of its resistance for other antibiotics was, Ticarcilline + clavulanic acid 88, 6% (39/44), Cefoxitin 59% (26/44), Cefurotaxime 45,5% (20/44), Cefotaxime 22.7% (10/44), Aztreonam 13,64% (6/44). Moreover, *K.pneumonia* showed 100% (31/31) resistance for each Amoxicillin+clavulanic acid, Amoxicillin, Cefoxitin and Cefuroxime. *K.pneumonia* showed a high resistance to Ticarcillin + clavulanic acid, Cefuroxime and Nalidixic acid, with 93, 5% (29/31), 80,6% (25/31), 64,5% (20/31), respectively. In contrast, a low level of resistance was observed to Cefotaxime and Aztreonam were 38,7% (12/31) and Amikacin were 6,5% (2/31). *Pseudomonas aeruginosa* recorded total resistance 100% (13/13) for each *Amoxicillin+clavulanic* acid, Amoxicillin, Cefuroxime, Cefoxitin and Nalidixic acid. On the other hand, *Pseudomonas aeruginosa* showed a high resistance rate was noted with Cefurotaxime 84,6% (11/13) and 76.9% (10/11) for both Cefotaxime and Aztreonam. While a low level of resistance was observed to Ticarcillin + clavulanic acid 53,8% (7/13) and Ciprofloxacine 46,2% (6/13), compared to other antibiotics used. The resistant of *Enterobacter* bacteria to different type of antibiotic was total resistance 100% (10/10) to Amoxicillin+ clavulanic acid, Amoxicillin and Cefuroxime followed by Cefoxitin 80% (8/10), Cefurotaxime 70%(7/10), Cefotaxime 60% (6/10), Aztreonam and Ticarcilline + clavulanic acid were 50% (5/10), Gentamicin 20% (2/10), Nalidixc acide and Ciprofloxacine were10% (1/10), respectively. The antibiotic resistance related to *P.mirabilis* recorded a total resistance of 100% (7/7). For most of the antibiotics used *(*Amoxicillin+clavulanic acid, Amoxicillin, Cefuroxime, Cefotaxime and Ciprofloxacine. While showing a high resistance to (Ticarcillin + clavulanic acid, Cefuroxime and Nalidixic acid) with 85,7% (6/7), followed by Cefoxitin 42,9% (3/7). Resistance levels for Meropenem, Imipenem, Amikacin and Gentamicin were substantially less than those of other antibiotics tested. 0% of E. coli strains, *K.pneumonia, P. aeruginosa, Enterobacter and P.mirabilis* were resistant to Meropenem and Imipenem. Regarding Amikacin antibiotics, of the K. pneumoniae strains that were isolated, 6.5% (2/31) were resistant, but 2.3% (1/44) of E. coli, while the resistance was recorded as 0% for bacteria. *P. aeruginosa, Enterobacter and P.mirabilis* isolates were resistant. Furthermore, of all the bacterial strains isolated, only 20% (2/10) of *Enterobacter* were resistant to Gentamicin, while other bacterial species did not record any resistance 0% against the same antibiotic (Gentamicin).

Table 5: Pattern of susceptibility rates (%) for isolated gram-negative bacteria to various antibiotics.

Bacteria		E. coli N= 44	K. pneumonia N=31	P. aeruginosa N=13	Enterobacter N=10	P. mirabilis N=7
AMC	S	0	0	0	0	0
	I	0	0	0	0	0
	R	44 100%	31 100%	13 100%	10 100%	7 100%
AMX	S	0	0	0	0	0
	I	0	0	0	0	0
	R	44 100%	31 100%	13 100%	10 100%	7 100%
MER	S	44 100%	30 96,8%	13 100%	10 100%	7 100%
	I	0	0	0	0	0
	R	0	1 3,2%	0	0	0
IMP	S	44 100%	31 100%	13 100%	10 100%	7 100%
	I	0	0	0	0	0
	R	0	0	0	0	0
TIM	S	2 4,5%	2 6,5%	5 38,5%	5 50%	0
	I	3 6,8%	0	1 7,7%	0	1 14,3%
	R	39 88,6%	29 93,5%	7 53,8%	5 50%	6 85,7%
FOX	S	17 38,6%	0	0	2 20%	2 28,6%
	I	1 2,3%	0	0	0	2 28,6%
	R	26 59%	31 100%	13 100%	8 80%	3 42,9%
CXM	S	0	0	0	0	0
	I	0	0	0	0	0
	R	44 100%	31 100%	13 100%	10 100%	7 100%
CTX	S	14 31,8%	0	1 7,7%	3 30%	1 14,3%
	I	10 22, 7%	6 19,4%	1 7,7%	0	0
	R	20 45, 5%	25 80,6%	11 84,6%	7 70%	6 85,7%
CTZ	S	34 77.3%	18 58.06%	0	4 40%	0
	I	0	1 3.2%	3 23%	0	0
	R	10 22.7%	12 38,7%	10 76.9%	6 60%	7 100%
ATM	S	38 86.4%	19 61,3%	1 7,7%	5 50%	1 14,3%
	I	0	0	2 15,4%	0	6 85,7
	R	6 13,64%	12 38,7%	10 76,9%	5 50%	0
AMK	S	43 97,7%	29 93,5%	12 92,3%	10 100%	6 85,7
	I	0	0	1 7.7%	0	1 14,3%
	R	1 2,3%	2 6,5%	0	0	0
GEN	S	43 97,8%	31 100%	12 92,3%	8 80%	7 100%
	I	1 2,3%	0	1 7.7%	0	0
	R	0	0	0	2 20%	0
NA	S	4 9,09%	11 35.5%	0	8 80%	1 14,3%
	I	0	0	0	1 10%	0
	R	40 90.9%	20 64,5%	13 100%	1 10%	6 85,7%
CIP	S	0	30 96,8%	6 46,2%	9 90%	0
	I	2 4,5%	0	1 7.7%	0	0
	R	42 95,5%	1 3,2%	6 46,2%	1 10%	7 100%

NOTE S Sensitive, I Intermediate, R Resistant. AMC Amoxicillin+ clavulanic acid, AMX Amoxicillin, MER Meropenem, IMP Imipenem, TIM Ticarcillin + clavulanic acid, FOX Cefoxitin, CXM Cefuroxime, CTX Cefurotaxime, CTZ Cefotaxime, ATM Aztreonam, AMK Amikacin, GEN Gentamicin, NA Nalidixic acid de, CIP Ciprofloxacine.

Ethics approval

All study samples were processed and handled in compliance with the appropriate biological safety and security standards. Before beginning this study, the Ethics Committee of the College of Health and Medical Techniques at Al-Furat Al-Awsat Technical University in Kufa, under the Ministry of Higher Education and Scientific Research in Iraq.

4. DISCUSSION

One of the most prevalent diseases of the male prostate is prostatitis, which is defined as enlargement of the prostate gland. Primarily due to infection. It is believed that around half of all men may suffer symptoms of prostatitis at some point in their lives. [19] The study's findings showed that the patients' ages ranged from 46 to 55 and 56-65 years had a high prevalence of Chronic prostatitis. This is related to hormonal fluctuations, immunological reactions, and among the interrelated factors contributing to the high incidence of chronic prostatitis are psychological factors. In these ages and testosterone levels in these age groups frequently drop, which can affect prostate health and raise the risk of developing chronic prostatitis (Chen *et al.,* 2017) [20]. Graziani et al (2023) conducted the increased incidence of chronic prostatitis (CP) in individuals between the ages of 46 and 55 and 56 and 65 can be linked to several interconnected factors, such as lifestyle choices, inflammatory

reactions, and hormonal changes [21]. Preston and Biddell (2021) discovered the interaction between age-related physiological changes and outside influences that worsen the illness; this group is especially vulnerable [22]. Gram-negative bacteria are the more frequent pathogen that causes chronic prostatitis, even though both gram-positive and gram-negative bacteria can cause prostatitis. Since the gut microbiome is where they naturally reside [23]. This pattern was supported by our investigation, which found that 19.84% of the recovered bacteria were gram-positive and 80.15 were gram-negative. Escherichia coli (41.90%) and Klebsiella pneumonia (29.52%) were the most common species of isolated gram-negative bacteria, making up 71.4% (75/105) of all isolated gram-negative bacteria. Their ability to adhere to the urinary tract epithelium through unique characteristics like flagella and pili may be the cause of their dominance.

In addition to *Klebsiella* and *Escherichia coli* share several virulence factors, most notably polysaccharide capsules that help them adhere to the host and resist the immune system, and lipopolysaccharide (LPS), a major component of the bacterial cell wall and induces a strong immune response, moreover, both bacteria share iron acquisition factors that allow them to utilize available iron in the host's body for their growth, and invasions that aid in tissue penetration and Thus, increase the risk of infection. [24] Our finding aligns with other studies. [25]

In bacterial prostatitis, *E. coli* is the most common infection. [26]. Regarding gram-positive bacteria, the most common isolates were *Staphylococcus aureus*, were the majority of the species that was isolated accounting for 73.07 % of the total isolated gram-positive bacteria. These pathogens have been found in numerous investigations to be the cause of UTI and prostatitis.[27] The incidence and mortality rate due to Staphylococcus aureus are between 20% and 30%. Because it possesses a wide array of virulence factors that contribute to its ability to cause infection and disease, including toxins, enzymes such as proteases and lipases, as well as other mechanisms of immune adaptation, which make it endemic in the human body, and it is highly tissue-destructive. [28]

Higher sensitivity to Meropenem is demonstrated by the tested Gram-negative bacteria, Imipenem, Amikacin and Gentamicin. This is consistent with the work of Misrha *et al.* 2016 [29]. Furthermore, it was observed that all Gram-negative bacteria isolated were total non-effective 100% against to Amoxicillin+ clavulanic acid, Amoxicillin and Cefuroxime because the synthesis of beta-lactamases and changes in bacterial cell permeability are the main causes of Gram-negative bacteria's complete resistance to cefuroxime, amoxicillin, and amoxicillin + clavulanic acid and these elements considerably reduce these antibiotics' effectiveness (Hardefeldt, and Prescott, 2024). [30] These results are similar to those published by Arbab et al in 2021. [31] On the other hand, Gram-negative bacteria recorded high resistance at different rates against Ticarcillin + clavulanic acid, Cefoxitin, Cefurotaxime, Cefotaxime, Aztreonam, Amikacin, Nalidixic acid, and Ciprofloxacine.

Enterococcus species and Staphylococcus aureus were the special Isolated gram-positive bacteria because of the Significant hemolytic activity of *S. aureus* and the ability to form biofilms (Liu *et al,* 2022) [32]. While enterococci are challenging to eradicate in the prostate environment and a propensity to create biofilms (Mendoza-Rodríguez *et al,* 2023) [33], compared to other investigations, this outcome is different [34]. Numerous other bacteria were recovered, including Streptococcus species.

Both isolated Gram-positive bacteria were sensitive only to Nitrofurantoin effectiveness of Nitrofurantoin against uropathogens has been reported in several studies [35]. And co-trimoxazole, Imipenem, Erthromycin, Pristinamycin, Rifampicine, Ciprofloxacine, Chloramphenicol resistance has been reported in several studies [36]. Even though nitrofurantoin has been prescribed extensively for the past few years to treat UTIs, it was still found to be effective against urine isolates in vitro. It is unclear why, after more than 25 years, nitrofurantoin has not developed a resistance mechanism.

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