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Study of the prevalence and antibiotics susceptibility patterns in clinically significant isolates among urine sample in a tertiary care centre

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ABSTRACT

Background: Urinary tract infections (UTIs) are prevalent bacterial infections. Gram-negative bacilli are frequently isolated as the causative pathogens in UTIs, and antibiotics are commonly used to treat them. However, the overuse of antibiotics has led to the development of resistance in some uropathogens.
Aim and Objective: To find the prevalence and antimicrobial susceptibility patterns in clinically significant isolates among urine sample in a tertiary care centre.
Materials and Methods: Fresh mid-stream urine samples were collected aseptically in sterile containers. The samples were inoculated on 5% sheep blood agar and MacConkey agar. The isolates were identified to the species level using biochemical tests/Vitek 2 compact.
Result: A total of 5847 specimens were examined for significant bacteriuria, out of which 1160 showed bacterial growth in urine cultures. The most commonly isolated pathogens were *Escherichia coli* (57.67%) and *Klebsiella pneumoniae* (18.63%). The antibiotics aminoglycosides (84%), carbapenems (79%), and nitrofurantoin (63%) displayed a high rate of sensitivity against these pathogens.
Conclusion: Due to the variability of drug sensitivity among bacterial pathogens over time, regular surveillance and monitoring are essential to provide physicians with updated information for the most effective empirical treatment of UTIs.

1. Introduction

Urinary tract infections (UTIs) are common human microbial diseases that affect lower and upper urinary tract—the kidneys, bladder, urethra, and prostate.[1] After respiratory tract infections, UTIs are the second most common human disease in the community practice.[2,3] UTIs affect people from all age groups including neonates and geriatric age group people.[4] It is also responsible for 30-40% of nosocomial infections. Malnutrition, low socio-economic status with poor hygiene, sexual behaviour, renal disease, catheterization, smoking, benign prostatic hypertrophy (BPH), increasing age, sex, pregnancy, debilitating underlying illnesses and other medical conditions like spinal cord injury, diabetes mellitus, sickle cell disease is some of the predisposing factors responsible for UTIs.[5–7] UTIs based on the site of infection are classified as bladder (cystitis), kidney (pyelonephritis) and urine (bacteriuria).[8]

UTIs based on clinical manifestations whether present or absent, can be classified into symptomatic and asymptomatic. Symptoms of cystitis include tenderness on bladder, urgency, frequency, and dysuria.[9] In cystitis, there are no signs of fever or systemic illness as it is a localized infection.[10] In acute urethral syndrome, frequency, urgency, and dysuria are mostly manifested in sexually active women. There are two routes through which bacteria invade and cause UTI i.e., ascending, and haematogenous

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pathway.[10] In the ascending pathway, first bacteria colonize the vaginal cavity and periurethral area. After that bacteria enters the bladder where they multiply and passes to the kidney through ureters.[11] In less than 5% of cases, infection through the haematogenous route is noted.[12] UTIs can be complicated and uncomplicated. In complicated UTIs, the urinary tract gets compromised due to renal failure, urinary obstruction, pregnancy, renal transplantation, neurological diseases causing urinary retention, indwelling catheters, or other drainage devices[1]. Mostly healthy individuals are affected by uncomplicated UTIs with no neurological or structural urinary tract abnormalities i.e., cystitis and pyelonephritis.[4]

Prevalence of UTIs in male after the neonatal period is relatively lower than females and mostly seen after the age of 60 years due to BPH, in which urine flow is obstructed. Recurrence of infection can be seen in females.[9] There is 60% lifetime risk every woman has in developing infection while male have only 13% risk.[13] In women, postmenopausal woman has higher risk of UTI because of change in vaginal flora with loss of estrogen, incomplete bladder emptying due to bladder or uterine prolapse and lactobacilli loss which results in Gram negative aerobes colonizing periurethral area.[14] If UTIs are not treated on time, it may lead to serious complications such as pyelonephritis with sepsis, recurrent infection, renal damage in young children and preterm birth is also seen in pregnant females.[15] In some cases, recurrent UTIs are also reported within 6-12 months of initial infection. This recurrent UTI may be due to reinfection or due to relapse. In relapse, the same organism is responsible for recurrent UTI, while in reinfection, different organisms are responsible and therefore considered as new infection.[6,8]

E. coli is responsible for about 80-90% of all UTIs.[16–18] Gram positive organisms are less frequent offenders but most seen species causing UTIs are *Enterococcus spp*. and *Staphylococcus spp.*[19] Complicated UTIs are mainly caused by *Pseudomonas spp*., *Klebsiella spp*. and *Enterobacter spp*.[4] Diagnosis is done by the combination of symptoms, growth in urine culture and antibiotic susceptibility testing.[20]

In the treatment of UTIs, antibiotics play a very important role.[21] Some of the powerful drugs like penicillin, third generation cephalosporins ceftazidime, and cefotaxime, fluoroquinolones like ciprofloxacin, aminoglycosides like amikacin and gentamicin have revolutionized the treatment.[21] Treatment with antibiotics is the empirical therapy used for the UTIs but in some cases empirical therapy is started even before the culture and sensitivity report that also with latest antibiotics.[21] Urinary tract infections (UTIs) are typically treated with antimicrobial agents. However, in some cases, patients are given antibiotics before the culture and sensitivity reports.[21] This practice can contribute to the development of antimicrobial resistance against latest antibiotics available.

An estimation of approximately 700,000 people dies annually because of antimicrobial resistance infections.[22] Enterobacterales have developed the resistance against beta lactam antibiotics. E*scherichia coli* and *Klebsiella pneumoniae* have developed resistance against amoxicillin-clavulanic acid, cotrimoxazole or multiple drugs.[23] In India, organisms causing UTIs remain constant but there is change in drug susceptibility pattern according to antibiotic usage.[23] Therefore, to update the information on change in antimicrobial susceptibility pattern, periodic evaluation is necessary.[24]

2. Materials and Methods

2.1. Study design

Prospective study.

2.2. Study setting

Microbiology department, Manipal Hospitals, Bangalore.

2.3. Subjects

Patients with clinically suspected UTI.

2.4. Sample size

Samples were collected for 6 months and data were analysed.

2.5. Study duration

July 2022 to December 2022.

2.6. Inclusion criteria

All the urine samples that were received to the microbiology laboratory were included in the study for the period of 6 months.

2.7. Exclusion criteria

The presence of more than two types of bacteria in a urine sample collected, is regarded as contaminated during collection and hence not included in the study. Additionally, any Candida species present in the urine samples were also excluded from the study.

2.8. Procedure

Midstream clean catch urine samples were collected from outpatients and inpatients. In patients with indwelling catheters, the collection port was disinfected with 70% alcohol, and 5-10 ml of urine was aspirated with a syringe from the sampling port. Semiquantitative method of streaking was done on 5% sheep blood agar and MacConkey agar using calibrated loops, as per standard protocol and followed by 48 hours of aerobic incubation at 37°C. Next day growth was observed, and biochemical tests were done based on characteristics of the colonies. Samples showing growth of Gram-negative organisms with single morphology or up to two types, were considered significant and processed further for identification by biochemical test and susceptibility testing. If no growth was observed, the plates were re-incubated for an additional 24 hours. Samples that grew more than two types of organisms or had evidence of perineal contamination were not included for analysis. For identification of colonies of Gram-negative bacilli, the biochemical media inoculated were mannitol motility agar, triple sugar iron agar (TSI), peptone water for indole production, Christensen's Urea agar, Simmons's Citrate agar, glucose phosphate broth for methyl red (MR), glucose phosphate broth for Voges Proskauer test (VP) and phenylalanine deaminase (PPA) (Table 1). For the identification of Gram-positive cocci, catalase test was performed. If catalase test was positive, then tube coagulase test was performed. If coagulase test was negative, then media inoculated were Streptococcus faecalis broth and Bile esculin agar. The media were incubated overnight at 37°C. When, uropathogen isolated was Streptococcus agalactiae, then Christie–Atkins–Munch-Peterson (CAMP) test was done as confirmatory test.

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method. The guidelines of the Clinical and Laboratory Standards Institute (CLSI)[25] were used for result interpretation and reference. The surface of Muller-Hinton agar plates was inoculated using a sterile cotton swab that had been submerged in a bacterial suspension. The bacterial suspension was prepared by inoculating 4 to 5 pure colonies of bacteria into peptone water and incubating for 2 hours. The bacterial suspension was standardized by matching its turbidity with 0.5 McFarland standard. The surface of the agar plate was swabbed in three directions using the lawn culture technique to ensure complete and even distribution of the bacterial suspension on the entire plate. Antimicrobial disks were applied within 15 minutes of inoculation, with a maximum of 5 disks applied on the agar plate at an appropriate distance (Tables 2 and 3). The plates were then incubated at 37°C for 18 to 24 hours. An inhibition zone formed around the disk showed the presence of antimicrobial activity on the plates. The diameter of the inhibition zones was measured in millimetres using a scale and readings were correlated with the CLSI guidelines.[25]

The isolates were identified to the species level using biochemical reactions / Vitek 2 compact (Figure 3). Vitek system was used to determine the minimum inhibitory concentration (MIC). The cards used were GN, GN405, GN406, GP, and GP628.

Quality control strains used for disk diffusion monitoring were *Escherichia* ATCC 25922, ESBL *Escherichia coli* ATCC 35218, *K. pneumoniae* ATCC 700603, *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923.

2.9. Statistical analysis

The study data was analysed using IBM SPSS software version 26. Descriptive statistics were calculated for variables such as gender and the types of harmful bacteria found in the study population. Tables were created to display the frequency of the different bacteria found and to compare the percentage of antibiotic susceptibility for urinary tract infection.

3. Results

Over a period of six months, a total of 5847 urine samples were collected and analysed. Out of these samples, 78.31% (4579) showed no bacterial growth, 19.84% (1160) showed bacterial growth, and 1.85% (108) showed *Candida species* which were not included in the study (Table 4). Among the positive urine samples, 90.26% (1047) were Gram-negative and 9.74% (113) were Gram-positive. A total of 59.4% (689) samples were from females, while 40.6% (471) were from males (Table 4). The majority of the urinary tract samples obtained in our hospital were from patients who were admitted to the hospital, accounting for 82.67% (959) of the samples (Figure 1). Outpatients accounted for 10.09% (117) of the samples, while the smallest proportion of samples came from patients in the intensive care units, representing only 7.2% (84) of the total (Figure 1). Late adulthood (45 to 74 years) had the highest prevalence of urinary tract infections i.e., 48.88% (567), followed by adults (19 to 44 years) with 27.76% (322), elderly (more than 75 years) with 17.24% (200), and paediatric age group (0 to 18 years) with 6.12% (71) (Table 5). While women typically have a higher risk of urinary tract infections, in the elderly population, the percentage of men with UTIs (10.25%) is higher than the percentage of women with UTIs (6.98%) (Table 5).

The most common organism causing urinary tract infections was *Escherichia coli*, which was responsible for about half of all cases (57.67%). *Klebsiella pneumoniae* was the next most common (18.36%), followed by *Enterococcus species* (7.50%), *Pseudomonas aeruginosa* (5.09%), *Proteus mirabilis* (3.28%), *Citrobacter koseri* (2.33%), and *Staphylococcus saprophyticus* (1.12%) (Table 6). The least frequently occurring bacteria causing urinary tract infections include *Morganella morganii* (0.60%), *Enterobacter cloacae* (0.52%), *Proteus vulgaris* (0.52%), *Staphylococcus aureus* (0.43%), *Streptococcus agalactiae* (0.43%), *Providencia rettgeri* (0.43%), *Acinetobacter species* (0.43%), *Citrobacter freundii* (0.34%), other *coagulase-negative Staphylococcus species* (0.25%),

Table 1: Biochemical reactions for different Gram-negative organisms

Organisms	Oxidase	Mannitol	Motility	TSI	Indole	Urease	Citrate	MR	VP	PPA
Escherichia coli	Negative	Fermented	Motile	A/A with gas	Produced	Hydrolysed	Not Utilized	Positive	Negative	Negative
Klebsiella pneumoniae	Negative	Fermented	Non-Motile	A/A with gas	Not Produced	Hydrolysed	Utilized	Negative	Positive	Negative
Proteus mirabilis	Negative	Not Fermented	Motile	K/A with gas with H2S	Not Produced	Hydrolysed	Utilized/Not Utilized	Positive	Negative	Positive
Pseudomonas spp.	Positive	Not Fermented	Motile	K/K	Not Produced	Not Hydrolysed	Utilized	Negative	Negative	Negative
Citrobacter koseri	Negative	Fermented	Motile	K/A with gas	Produced	Not Hydrolysed	Utilized	Positive	Negative	Negative
Morganella	Negative	Not Fermented	Motile	K/A with gas	Produced	Hydrolysed	Not Utilized	Positive	Negative	Positive
Enterobacter spp.	Negative	Fermented	Motile	A/A with gas	Not Produced	Hydrolysed	Utilized	Negative	Positive	Negative
Proteus vulgaris	Negative	Not Fermented	Motile	K/A with gas and with H2S	Produced	Hydrolysed	Utilized/Not Utilized	Positive	Negative	Positive
Serratia	Negative	Fermented	Motile	K/A with gas	Not Produced	Not Hydrolysed	Not Utilized	Negative	Positive	Negative
Acinetobacter spp.	Negative	Not Fermented	Non-Motile	K/K	Not Produced	Not Hydrolysed	Not Utilized	Negative	Negative	Negative

K = Alkaline
A = Acid

Table 2: Antibiotic panel used for Gram-negative bacteria

Trimethoprim/ Sulfamethoxazole(1.25/23.75 µg)
Nitrofurantoin (300 µg)
Ciprofloxacin (5 µg)
Ofloxacin (5 µg)
Gentamicin (10 µg)
Amikacin (30 µg)
Netilmicin (30 µg)
Tobramycin (10 µg)
Cefuroxime (30 µg)
Cefotaxime (30 µg)
Ceftriaxone (30 µg)
Ceftazidime (30 µg)
Amoxicillin-clavulanate (20/10 µg)
Piperacillin-Tazobactam (100/10 µg)
Cefoperazone – sulbactam (75/30 µg)
Aztreonam (30 µg)
Imipenem (10 µg)
Meropenem (10 µg)

Table 3: Antibiotic panel used for Gram-positive bacteria

Catalase positive (*Staphylococcus*)	**Catalase negative (*Enterococci*)**
Penicillin (10 units)	Penicillin (10 units)
Gentamicin (10 µg)	High level Gentamicin (120 µg)
Trimethoprim/Sulfamethoxazole(1.25/23.75 µg)	Ciprofloxacin (5 µg)
Ciprofloxacin (5 µg)	Nitrofurantoin (200 µg)
Cefoxitin (30 µg)	Vancomycin (30 µg)
Linezolid (30 µg)	Linezolid (30 µg)
Tetracycline (30 µg)	Teicoplanin (30 µg)
Nitrofurantoin (200 µg)	Tetracycline (30 µg)
Vancomycin (MIC)	

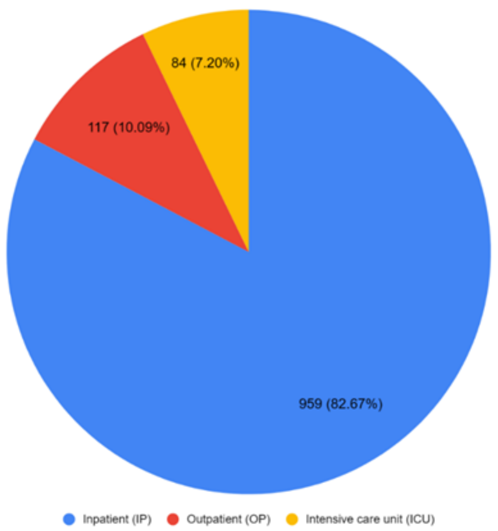

Fig. 1: Distribution of patients in hospital

Enterobacter aerogenes (0.17%), and *Serratia marcescens* (0.17%) (Table 6).

The antimicrobial susceptibility patterns of all uropathogens isolated were analysed, and it was found that Gram-negative organisms showed 84% susceptibility to amikacin, 83.5% to netilmicin, 79.8% to imipenem, 79.6% to meropenem, and 77.8% to cefoperazone/sulbactam (Figure 2).

Among *Enterococcus spp*., linezolid showed the 100% sensitivity rates, followed by vancomycin and teicoplanin which showed the sensitivity rate of 90% each. Four vancomycin resistant *Enterococcus* (VRE) species were also found. *Enterococcus species* displayed low susceptibility rates to tetracycline and ciprofloxacin with sensitivity rates of only 13.8% and 11.8% respectively (Figure 3). For *Staphylococcus species*, linezolid and vancomycin had the sensitivity rate of 100% each, followed by Gentamycin i.e., 95.2% (Figure 4). In contrast, penicillin and ampicillin had the sensitivity rates of 23.80% (Figure 4).

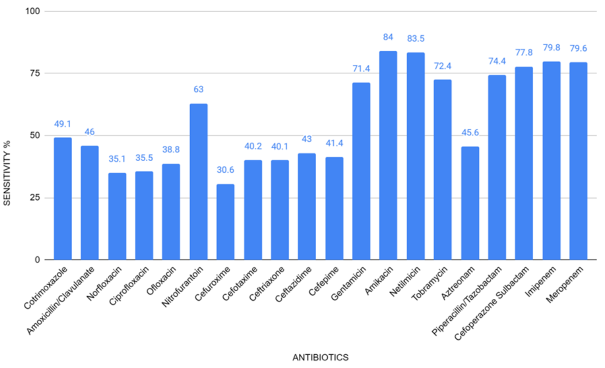

Fig. 2: Percentage susceptibility among Gram negative organisms (N = 1047)

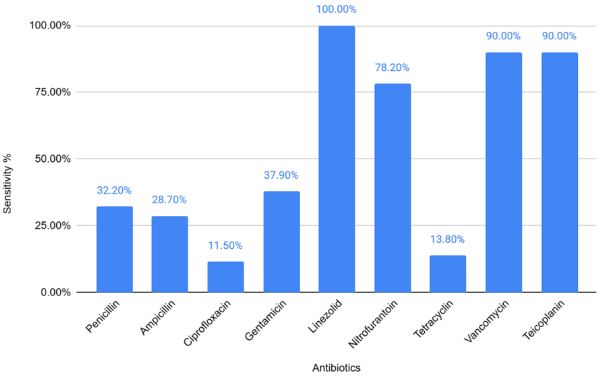

Fig. 3: Percentage susceptibility among *Enterococcus species* (N = 87)

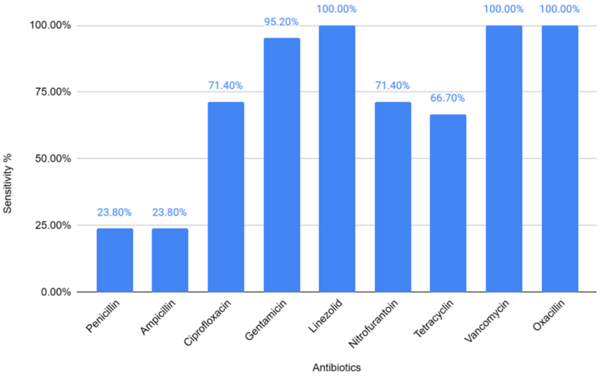

Fig. 4: Percentage susceptibility among *Staphylococcus* species (N = 21)

Table 4: Urinary tract infection's distribution of pathogenic and non-uropathogenic bacteria among tested patients in relation to gender

Characters	Total number (%)
Total urine sample cultured	5847
Samples without bacteria	4579 (78.31%)
Samples with bacteria	1160 (19.84%)
Candida spp.	108 (1.8%)
Gram negative	1047 (90.26%)
Gram positive	113 (9.74%)
Gender	**Total number (%)**
Total females	689 (59.40%)
Total males	471 (40.60%)

Table 5: Age and gender wise distribution among urinary tract infection cases

Age group	Female	Male	Number of isolates %
0 - 18	46 (3.94%)	25 (2.16%)	71 (6.12%)
19 - 44	232 (20%)	90 (7.76%)	322 (27.76%)
45 - 74	330 (28.49%)	237 (20.43%)	567 (48.88%)
>=75	81 (6.98%)	119 (10.25%)	200 (17.24%)
Total	689 (59.40%)	471 (40.60%)	1160 (100%)

Table 6: Prevalence of Gram-positive and Gram-negative bacteria isolated from urine samples

Organisms prevalent in UTI	Number of isolates	Percentage
Escherichia coli	669	57.67
Klebsiella pneumoniae	216	18.63
Enterococcus spp.	87	7.5
Pseudomonas aeruginosa	59	5.09
Proteus mirabilis	38	3.28
Citrobacter koseri	27	2.33
Staphylococcus saprophyticus	16	1.37
Enterobacter spp.	14	1.21
Morganella morganii	7	0.6
Proteus vulgaris	5	0.52
Staphylococcus aureus	5	0.43
Streptococcus agalactiae	5	0.43
Providencia rettgeri	4	0.43
Acinetobacter spp.	3	0.43
Citrobacter freundii	3	0.34
Serratia marcescens	2	0.17
	1160	100.00

4. Discussion

Urinary tract infections are some of the most common bacterial infections and is prevalent worldwide. The treatment of UTIs relies heavily on antimicrobial therapy. However, the overuse of antimicrobial therapy has led to the development of drug-resistant microorganisms. Since microorganisms frequently change their susceptibility patterns, it is essential to continuously monitor antimicrobial susceptibility patterns.

According to our study, out of 5847 samples tested for urinary tract infections (UTIs), 1160 (19.84%) showed growth and 4597 (78.31%) showed no growth. The prevalence rates of UTIs were similar to those found in studies conducted by Pramodh K. et al. (2019)[26] and Siraj et al. (2020),[27] which reported 24.45% and 16.1% positive samples, respectively. However, our study had a lower growth rate than studies conducted by Muktikesh et al. (2013), Pritam et al. (2018), and Vidya et al. (2021), which found a growth rate of 33.96%.[4,21,27]

Our research also found that females exhibited a higher growth rate (59.40%) compared to males (40.60%). These findings align with previous studies conducted by Pritam et al. (2018), Anusuya et al. (2018), Siraj et al. (2020), and Snehashis et al. (2022), where females showed growth rates of 66.78%, 66.8%, 62.90%, and 60%, respectively, while males exhibited growth rates of 33.22%, 33.2%, 37.10%, and 40%.[4,27–29] The higher incidence of UTIs in females could be explained by several factors, such as the shorter length of their urethra and its proximity to the anus, which made it easier for coliforms to colonize. However, in our study, elderly male patients had a higher prevalence of UTIs than elderly female patients. This finding was consistent with the studies conducted by Pritam et al. (2018) and Shuvankar et al. (2020), Adya Chaturvedi et al. (2020) and may be because elderly males were more prone to neurogenic bladder and benign prostatic hyperplasia (BPH) than younger males.[4,9,29]

Based on our study findings, *Escherichia coli* was identified as the most prevalent bacterium, accounting for 57.67% of the analysed samples. This was followed by *Klebsiella pneumoniae* at 18.36% and *Enterococcus spp.* at 7.50%. These findings are consistent with previous research conducted by Uma Ravishankar et al. (2021), which reported *Escherichia coli* at 56.6%, *Klebsiella pneumoniae* at 14.7%, and *Enterococcus spp.* at 11.6%. Similarly, Siraj Ahmad et al. (2021) found *Escherichia coli* at 49.8%, *Klebsiella pneumoniae* at 15.9%, and *Enterococcus spp.* at 14.7%. Dilip Chandrasekhar et al. (2018) also observed *Escherichia coli* at 26.64%, *Klebsiella pneumoniae* at 9.62%, and *Enterococcus spp.* at 5.18%. The results from these studies corroborate our own findings.[15,20,30]

Our study found that amikacin (84%) was the most effective antibiotic against Gram-negative bacteria. This finding aligns with prior research conducted by Dr. Alka Nerurkar et al. (2021) and Muktikesh Dash et al. (2013), which reported sensitivities to amikacin at 82.2% and 94.67%, respectively.[8,31]

In our study, *Enterococcusspecies* were found to be the most frequently identified Gram-positive bacteria, closely followed by *Staphylococcus species*. This was consistent with the findings of the 2020 study by Shuvankar Mukherjee.[9] However, it differed from the 2018 study by Anusuya Devi D et al., which found that *Staphylococcus* species were the most common, followed by *Enterococcus species*.[28] In terms of antibiotic sensitivity, we observed that linezolid demonstrated a 100% effectiveness, and vancomycin showed a 90% effectiveness against Enterococcus species. For Staphylococcus species, vancomycin exhibited a 100% sensitivity, while linezolid showed a 100% sensitivity as well. These results are consistent with a study conducted by Anusuya Devi D et al. (2018), which also reported a 100% sensitivity to linezolid's and vancomycin for both *Enterococcus spp.* and *Staphylococcus spp.*[9]

According to a study by Shuvankar Mukherjee et al. in 2020, the use of fluoroquinolones such as norfloxacin and ciprofloxacin to treat UTIs was no longer effective because more than half of the bacteria causing the infection had developed resistance to these drugs.[9] The extensive use of third-generation cephalosporin to treat various infections, including UTIs, had resulted in almost all uropathogens becoming resistant to it. Although aminoglycoside was still effective against all uropathogens, it was not used as an initial treatment option due to its injectable form. Thus, the only remaining oral option was nitrofurantoin, which had a sensitivity rate of 63% in our study. While carbapenems were highly effective, it should not have been used to treat uncomplicated UTIs because its widespread use may render it useless like fluoroquinolones in the coming decade.

Our study had certain limitations. We have not analysed the risk factors of the patients and our *Staphylococcus* isolates were very few to analyse the susceptibility pattern.

5. Conclusion

Antibiotic resistance has emerged as a significant public health issue, resulting in restricted treatment alternatives, elevated healthcare expenses, and extended hospitalization. It is crucial to continually monitor and investigate the shifting trends in bacterial drug susceptibility pattern, especially in relation to urinary tract infections. This enables treating physicians to remain up to date on the most effective initial treatments for UTIs. Instead of depending on standardized guidelines, the selection of antibiotics for UTI therapy should be determined by periodically understanding the prevalence of particular microorganisms responsible for the infection and assessing their susceptibility to antibiotics in the local region.

6. Source of Funding

None.

7. Conflict of Interest

None.

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