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Original Article

Study of uropathogens in patients with urinary tract infection and their antibiotic sensitivity profile

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ABSTRACT

Urinary tract infections (UTIs), the second most common type, are a health-care problem encountered in medical practice, a major cause of morbidity with a high socio-economic impact. The aim of this study was to identify the most common uropathogens, their susceptibility and resistance to conventional therapies. For this purpose, a total of 280 urine samples were collected from UTI suspected patients of both genders of different age groups and sent for urine culture and antibiotic sensitivity test. The total culture positive cases were 70 (25%). It is more prevalent in female 57 (81.4%) than in male 13 (18.6%). From the Gram-negative bacteria, *Escherichia coli* was the most common identified pathogen (50%), followed by *Klebsiella* spp. (13%), *Acinetobacter* spp. (8%), *Pseudomonas* spp. (6%), and *Enterobacter* spp. (5%). Gram-positive isolates are *Staphylococcus aureus* (10%) and *Streptococcus* spp. (7%). The common urinary pathogens such as *Acinetobacter*, *Streptococcus*, and *Pseudomonas, Escherichia coli* showed high resistance when they were tested against amoxicillin–clavulanic acid, cefixime, ceftazidime, ciprofloxacin, trimethoprim, and nalidixic acid. Amikacin, imipenem, and colistin showed good sensitivity profile throughout all the results. The continuous dynamic of uropathogens in different areas and the increasing resistance to conventional antibiotic therapies is a major contemporary health problem. Therefore, area-specific monitoring studies aimed to gain knowledge about the type of pathogens responsible for UTIs and their resistance patterns may help the clinician to choose the correct empirical treatment.

Keywords: Urinary tract infections, uropathogens, antibiotic sensitivity profile

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INTRODUCTION

Urinary tract infections (UTIs) are a major public health problem representing the second most common infectious disease in community practice and are reported to affect up to 150 million individuals annually worldwide.[1] UTI is among one of the most common infections occurring particularly in women. Nearly 50–60% of all women suffer from an episode of UTI at least once in their lifetime.[2,3] Some studies consider that UTI is the most common cause of morbidity without age or gender distinctions.

UTI is commonly caused by bacteria mostly by Gram-negative bacteria such as *E. coli*, *Proteus* species, *Pseudomonas aeruginosa*, *Acinetobacter* species, *Klebsiella* species, *Enterobacter* species, and *Citrobacter* species. Among Gram-positive bacteria, *Staphylococcus saprophyticus*, *Enterococcus* species, and Coagulase-negative *Staphylococcus* are common predictable spectrum of bacteria which are responsible for causing UTIs.[4,5] Untreated UTI can result in serious complications such as kidney damage, renal scarring, and renal failure.

Patients suffering from a symptomatic UTI are commonly treated with antibiotics; these treatments can result in long-term alteration of the normal micro-biota of the vagina and gastrointestinal tract and in the development of multidrug-resistant microorganisms.[6] The availability of niches that are no longer filled by the altered microbiota can increase the risk of colonization with multidrug-resistant uropathogens.

UTI is one of the most frequent conditions encountered by general practitioners. It is treated often by broad-spectrum

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antibiotics, and treatment is started empirically without performing culture and sensitivity. This inappropriate and non-judicious usage of antibiotics has resulted in the development of worldwide antibiotic resistance in bacteria, leading to the emergence of multi-resistant strains of bacterial pathogens.[7] Importantly, the "golden era" of antibiotics is waning, and the need for rationally designed and alternative treatment is therefore increasing.

In the current scenario, where the antimicrobial resistance pattern is changing very alarmingly and new multidrug-resistant (MDR) bacteria are emerging frequently leading to enhance morbidity and mortality. The antimicrobial susceptibility pattern among bacteria varies from country to country. The main aim of this study was to determine the prevalence of UTI causing pathogens in Bangladesh according to age and sex and their antibiotic susceptibility pattern.

MATERIALS AND METHODS

Study Design

A total of 280 urine samples were collected from patients who were suspected to have UTIs. Samples were collected from indoor and outdoor (both male and female) patients of different age groups having clinical symptoms of microbial infection. This study was undertaken for a period from December 2019 to March 2020. The study was conducted in Dhaka Community Medical College and Hospital (DCMC), Bangladesh.

Collection of Urine Samples

Clean catch midstream urine was collected from each patient using sterile screw capped containers with proper identification number for routine examination and culture sensitivity. All patients were well instructed on how to collect sample aseptically before sample collection to avoid contaminations from urethra.

Bacterial Identification

The urine samples collected were examined microscopically for pus cells and casts. Identification of organisms was done by conventional methods through culturing of samples on different media such as HiCrome UTI Agar, MacConkey Agar, and Blood Agar media at 37°C for 24–48 h, followed by biochemical tests including their distinct colony characteristics.[8]

Antibiotic Sensitivity Testing

Antibiotic susceptibility testing was performed by Kirby–Bauer's disk diffusion method on Muller–Hinton agar in accordance with the standards of the Clinical Laboratory Standards Institute (CLSI, formerly National Committee for Clinical Laboratory Standards) guidelines.[9] Interpretation as "Sensitive" or "Resistant" was done on the basis of the diameters of zones of bacterial growth inhibition as recommended by the disk manufacturer. Antibiotic disks were imipenem (IPM), ceftriaxone (CRO), cefixime (CFM), colistin (CT), nitrofurantoin (F), amikacin (AK), amoxicillin–clavulanic acid (AMC), ciprofloxacin (CIP), nalidixic acid (NA), ceftazidime (CAZ), and trimethoprim (SXT).[10]

RESULTS AND DISCUSSION

Age and Gender Distribution

We approached patients who attended DCMC with their UTI associated complications. We included 280 patients in this study who had severe clinical symptoms. A total of 280 urine samples of both sex and various age groups were collected in a hospital setting and processed in the laboratory. There was marked gender variations is all age groups [Table 1]. Of them, 122 (44%) samples were collected from male patients and the remaining 158 (56%) from female patients. Age distribution of our study cases lies from below 1 year to above 70 years. However, most frequent UTI patients were in the age group between 21 and 30 years.

Culture Positive Cases

We examined 280 urine samples from clinically symptomatic patients. Out of the total samples sent for culture sensitivity test, only 70 (25%) urine samples were culture positive and showing significant growth for UTI [Figure 1], of which 57 (81.4%) were obtained from female patients and 13(18.6%)

Table 1: Gender and age distribution and frequency of study participants

Age (year)	Total	Male (%)	Female (%)
<10	45	25 (55.5)	20 (44.4)
11–20	36	16 (44.4)	20 (55.5)
21–30	58	14 (24.1)	44 (75.8)
31–40	40	15 (37.5)	25 (62.5)
41–50	26	6 (23)	20 (76.9)
51–60	42	28 (66.6)	14 (33.3)
61–70	15	7 (46.6)	8 (53.3)
>71	18	11 (61.1)	7 (38.8)
	280	122 (43.6)	158 (56.4)

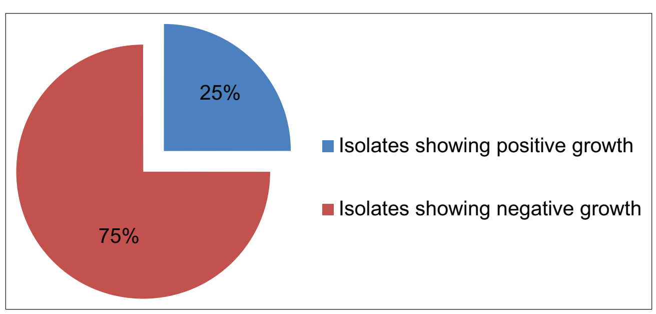

Figure 1: Pattern of culture results (n = 280)

from male patients. Table 2 shows age- and gender-wise distribution for culture positive cases. Most number of positive urine cultures was seen in age group of 21–30 years where 16 (22.8%) participants were culture positive and 15 (93.7%) patients were female.

Gram-negative and Gram-positive Isolates

We have detected 12 Gram-positive UTI pathogens, which was 17.14% of the total pathogen population and 58 (82.86%) Gram-negative pathogens [Figure 2].

The most common Gram-negative and Gram-positive urinary pathogens isolated were *E. coli, Enterobacter* spp., *Streptococcus* spp., *Staphylococcus aureus*, *Klebsiella* spp., *Acinetobacter* spp., and *Pseudomonas* spp. *E. coli* was detected in 35 (50%) of all the positive cultures, followed by *Klebsiella* spp. 9 (12.8%), *S. aureus* 7 (10%), *Acinetobacter* spp. 6 (8.6%), *Streptococcus* spp. 5 (7.1%), *Enterobacter* spp., and *Pseudomonas* spp. 4 (5.7%), respectively [Table 3].

Sensitivity Results

A total of 11 different antibiotics were used to determine the resistance and sensitivity profiles in each bacterial isolation.

Table 2: Frequency of gender and age distribution of culture positive urinary tract infections cases

Age (years)	Female	Male	Total	Percentage (%)
<10	5	5	10	14.3
11–20	7	0	7	10
21–30	15	1	16	22.8
31–40	5	1	6	8.6
41–50	4	1	5	7.1
51–60	11	3	14	20
61–70	6	2	8	11.4
>71	4	0	4	5.7
	57 (81.4%)	13 (18.6%)	70 (25%)	

Table 3: Frequency distribution of uropathogens isolated from patients

S. No.	Isolates (*n*=70)	Total	Percentage (%)
Gram-negative bacteria			
1	*Escherichia coli*	35	50
2	*Klebsiella spp.*	9	12.8
3	*Enterobacter spp.*	4	5.7
4	*Acinetobacter spp.*	6	8.6
5	*Pseudomonas spp.*	4	5.7
Gram-positive bacteria			
6	*Staphylococcus aureus*	7	10
7	*Streptococcus spp.*	5	7.1

The most effective antibiotic for *E. coli* isolates observed was imipenem and ceftriaxone 85.7%, followed by cefixime and colistin (80%), respectively, 77.1% for nitrofurantoin and 74.2% for amikacin. In comparison, high resistance (77.1%) was observed among *E. coli* isolates to AMC, followed by ciprofloxacin (65.7%) and nalidixic acid (57.1%). Rates of resistance to different antibiotics tested against 35 *E. coli* strains isolated from UTIs are given in Figure 3.

One of the striking features of genus *Acinetobacter* is the ability to develop antibiotic resistant extremely rapid in response to challenge with new antibiotics. In the present study, strains were highly resistant (83.3%) to amoxicillin, cefixime, ceftriaxone, ceftazidime, ciprofloxacin, trimethoprim, imipenem, nalidixic acid, and nitrofurantoin. However, colistin (83.3%) and amikacin (66.6%) showed sensitivity against *Acinetobacter* spp [Figure 4].

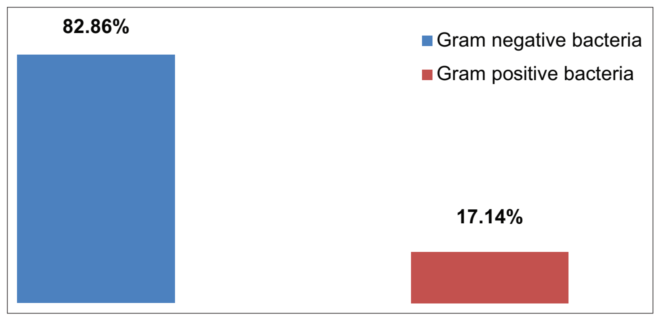

Figure 2: Distribution of Gram-positive and Gram-negative bacteria among urinary tract infections patients

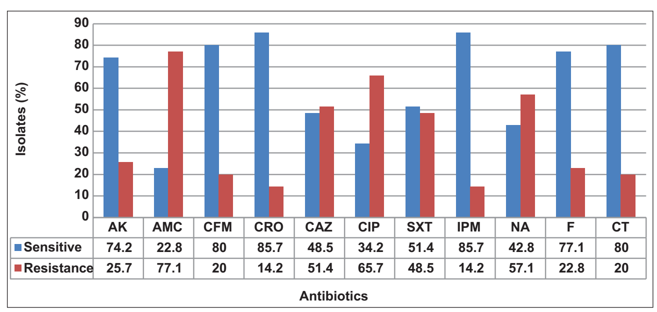

Figure 3: Antibiotic sensitivity and resistance pattern of *Escherichia coli* isolates (n = 35)

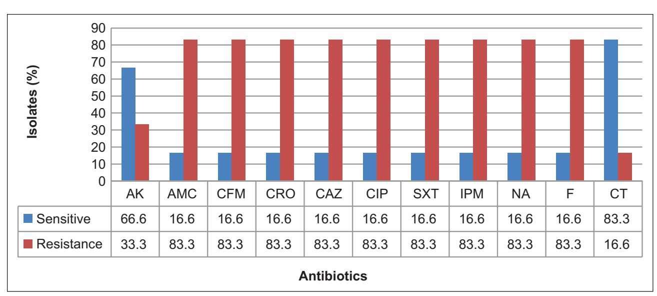

Figure 4: Antibiotic sensitivity pattern of *Acinetobacter* spp. (n = 6)

Klebsiella spp. was the second most frequently cultured uropathogen in this study. High efficacy (88.8%) of imipenem, nalidixic acid, and colistin, followed by ceftazidime and nitrofurantoin (77.7%), was observed against *Klebsiella* isolates. Amikacin and cefixime also showed similar efficacies (66.6%) for *Klebsiella* isolates [Figure 5].

An alarming finding from this study showed a high degree of drug resistance among *Pseudomonas* spp. Our study showed a very high rate of resistance (75%) among *Pseudomonas* isolates to AMC, cefixime, ceftazidime, ciprofloxacin, trimethoprim, nalidixic acid, and nitrofurantoin. High susceptibility (75%) for amikacin, ceftriaxone, imipenem, and colistin was observed among the identified *Pseudomonas* isolates [Figure 6].

Enterobacter species were detected in only 4 (5.7%) of all the positive cultures. Of these four *Enterobacter* cultures, 75% sensitivity was observed to amikacin, cefixime, ciprofloxacin, imipenem, nalidixic acid, and colistin; and 75% resistance to ceftriaxone and ceftazidime was also found [Figure 7].

The overall prevalence of the *S. aureus* isolates was 7 (10%). The majority of the isolates were considerably sensitive to all antibiotics tested. The isolates showed high sensitivity (85.7%) to amikacin. However, high efficacy (71.4%) to AMC, cefixime, ceftriaxone, ciprofloxacin, imipenem, nalidixic acid, nitrofurantoin, and colistin was also observed among the isolates [Figure 8].

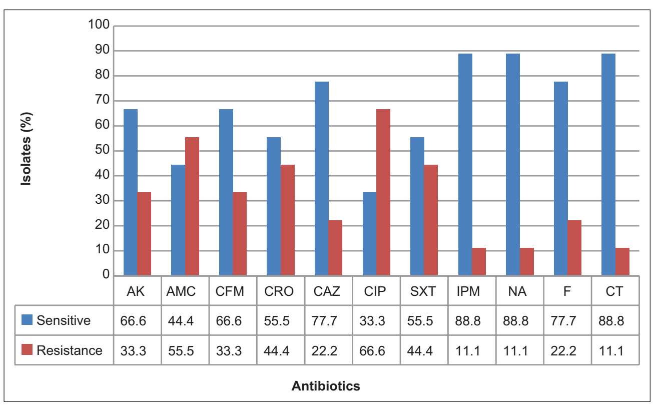

Figure 5: Antibiotic sensitivity pattern of *Klebsiella* isolates ($n = 9$)

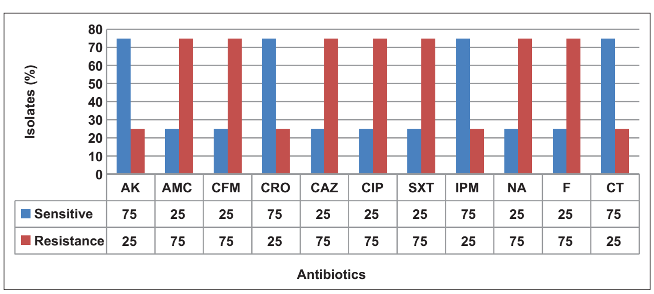

Figure 6: Antibiotic sensitivity pattern of *Pseudomonas* isolates ($n = 4$)

The overall prevalence of *S. aureus* isolates was 5 (7.1%). The *Streptococcus* isolates showed high resistance (80%) to ceftriaxone, ciprofloxacin, and imipenem. However, they were considerably sensitive (80%) to AMC, nitrofurantoin, and colistin [Figure 9].

DISCUSSION

UTIs are a severe public health problem caused by a range of uropathogens. The bacterial culture remains an important test in the diagnosis of UTI, not only because it helps to document infection, but also because it is necessary for determination of the identity of the infecting microorganism(s) and for antimicrobial susceptibility testing. In our study, out of 280 urine samples, 70 (25%) samples were showing significant growth for UTI. Karki *et al.*, 2004; Levitt, 1993; and Obi *et al.*,

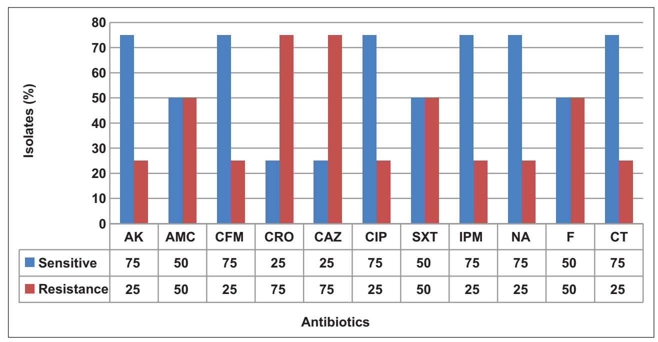

Figure 7: Antibiotic sensitivity pattern of *Enterobacter* isolates ($n = 4$)

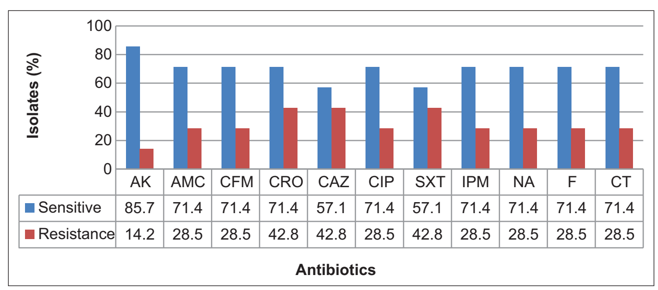

Figure 8: Antibiotic sensitivity pattern of *Staphylococcus aureus* isolates ($n = 7$)

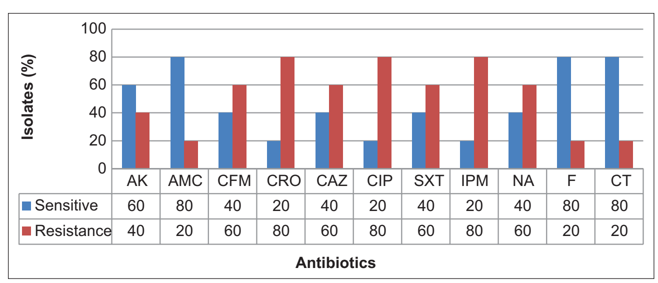

Figure 9: Antibiotic sensitivity pattern of *Streptococcus* isolates ($n = 5$)

1996; also observed such a low rate of growth positivity for UTI.[11-13] The possible cause of low rate of growth positivity might be due to urine samples obtained from patients under treatment, infection due to slow growing organisms or due to those organisms that were not able to grow on the routine media we used.

Most number of positive urine cultures (22.8%) was seen in the age group of 21–30 years and most of the patients were females. This showed that UTI is common in reproductive age group which is comparable to studies done by Subedi and Pudasaini, Banerjee *et al.*, Obiogbolu *et al.*, and Shahina *et al.*[14-17] UTIs occur more often in women than in men, at a ratio of 8:1. Approximately 50–60% of women report at least one UTI in their lifetime and one in three will have at least one symptomatic UTI necessitating antibiotic treatment by age 24.[2,18,19] In our study, the ratio of female patients with UTI was more than the males, 4:1. Therefore, our results showed that the majority of UTIs occurred in women 57 (81.4%) than the males 13 (18.6%), in agreement with the previous studies[20-22] and thereby confirming that adult women have a higher rate of UTI prevalence than men. Higher prevalence of UTI among females is due to various factors that predispose women to UTI.[23] This result was expected, as women are more prone to UTI than males because their urethra is much shorter and closer to the anus than in males; hence, bacteria from the anus can pass easily into the urinary tract. However, this result was inconsistent with the study by Kattel *et al.*[24]

The antibiotic susceptibility of uropathogenic bacteria is known to change with time and is inconsistent in different regions.[25] *E. coli* was the most predominant species isolated in our study population.[26-29] The comparison of rates of *E. coli* resistance to AMC, fluoroquinolones, and trimethoprim determined in different studies performed in Europe and North America since 1990 prompts several remarks. Our study showed a very high rate of resistance (77.1%) among *E. coli* isolates to AMC and high sensitivity (85.7%) was found to imipenem; similar result was also found by Kulkarni *et al.* in 2017 where *E coli* showed 71.90% resistance to AMC and 96.71% sensitivity to imipenem.[30] Higher resistance to AMC also resembles to the study done by Matanovic *et al.* in 2010 where high use of AMC at the University Hospital Osijek (Croatia) contributed to high rates of resistance in *Enterobacteriaceae*, in particular *E. coli* (50%). Thus, to decrease bacterial resistance, AMC use was restricted.[29]

Emergence and spread of *Acinetobacter* species, resistant to most of the available antimicrobial agents, are an area of great concern. Reports of *Acinetobacter* spp. bacteremia are increasing, especially from Asian countries and neighboring countries of Iran such as Iraq, Kuwait, Turkey, and Afghanistan.[31-33] A recent surveillance study from Iran reported that *Acinetobacter* spp. were the most frequently isolated bacteria in the hospital and community-acquired bloodstream infections (32%).[34] The present study revealed high resistance (83.3%) of *Acinetobacter* spp. to different antibiotics such as AMC, cefixime, ceftriaxone, ceftazidime, ciprofloxacin, trimethoprim, imipenem, nalidixic acid, and nitrofurantoin. Kalidas Rit and Rajdeep Saha also stated MDR of *Acinetobacter* spp.;[35] however, imipenem showed high sensitivity (94.8%) which was inconsistent with our findings where high resistance (83.3%) to imipenem was observed.

Several authors around the world have reported the Gram-negative bacteria of *E. coli* and *Klebsiella* spp. being the most frequent organisms causing UTIs.[16,36-39] In the present study, only 13% positive urine cultures demonstrated growth of *Klebsiella* whereas Saha also demonstrated 13.419% positive for *Klebsiella* growth.[40] He demonstrated that *Klebsiella* was highly sensitive to colistin (89.42%) and imipenem (88.94%) which is in agreement with our findings where these two antibiotics showed 88.8% sensitivity.

In our study, percentage of *Pseudomonas* spp. was 5.7% which was close to the percentage reported in a study in Pakistakn as 5.4%[24] and in a European study as 6.9%.[41] High resistance of *Pseudomonas* spp. to different antibiotics was revealed in the present study. According to this study, *P. aeruginosa* in UTI patients can be best treated with imipenem and amikacin with minimum resistance (25%). A similar study conducted by Naeem *et al.* from Pakistan showed 99–100% effectiveness of amikacin and improved therapeutic outcomes with imipenem and piperacillin/tazobactam against *P. aeruginosa*.[42] Muzammil *et al.* also demonstrated similar study where *Pseudomonas* was detected in seven (13.2%) of all the positive cultures, all seven (100%) were sensitive to amikacin, colistin, piperacillin/tazobactam, meropenem, and polymyxin B.[43] This finding is in agreement with our study, as we showed amikacin, colistin, and imipenem sensitivity (75%) in *Pseudomonas* spp. Zúniga-Moya *et al.* isolated *Enterobacter* spp. in 7.8% and observed that the antibiotics to which it presented greater resistance were amoxicillin plus clavulanic acid, cefaclor, and cefadroxil[44] whereas our result showed high resistance (75%) to ceftriaxone, ceftazidime antibiotics and only 50% resistance to AMC, trimethoprim, and nitrofurantoin.

Among Gram-positive bacteria, *S. aureus* was the most common (10%), followed by *Streptococcus* spp. (7.1%). This result correlates with the previous report by Kattel *et al.* in 2008 where he discovered 12.56% *S. aureus* of the total bacterial isolates;[45] however, the number was much higher than what was reported by Ahmed *et al.* (2.2%).[22] *S. aureus* is a potentially harmful human pathogen associated with both nosocomial and community-acquired infections and it is increasingly becoming resistant to most antibiotics. Although Akanbi *et al.* revealed varying susceptibility of *S. aureus* to imipenem (96.7%), levofloxacin (86.7%), chloramphenicol (83.3%), cefoxitin (76.7%), ciprofloxacin (66.7%), gentamycin (63.3%), tetracycline and sulfamethoxazole-trimethoprim (56.7%), and vancomycin and doxycycline (50%).[46] The susceptibility profile of *S. aureus* isolates recovered in that study conforms to our study where we found *S. aureus* is 71.4% sensitive to most of the antibiotics such as AMC, cefixime, ceftriaxone, ciprofloxacin, imipenem, nalidixic acid, nitrofurantoin, and colistin. Iram Shaifali demonstrated high susceptibility patterns to nalidixic acid, clarithromycin, cotrimoxazole, cefixime, cephalexin, and cefaclor (100%), followed by nitrofurantoin (66.66%) among the *Streptococcus* isolates identified were observed.[47] However, we found high susceptibility (80%) patterns to AMC, colistin, and nitrofurantoin, followed by amikacin (60%), nalidixic acid, and cefixime (40%), respectively.

CONCLUSION

Antibiotic sensitivity pattern of uropathogens revealed that amikacin, colistin, and imipenem were the most effective antimicrobials against the strains. However, *Acinetobacter* spp. and *Streptococcus* spp. showed high resistance to imipenem. To successfully treat the patients who are suffering from UTI, it is crucial to accurately identify the causative pathogen. Failure to do so will not only prolong the disease and will render the patient to complications but will also promote negative consequences of bacterial resistance due to a non-judicious use of inappropriate antibiotics. The antimicrobial resistance patterns of the causes of UTI are highly variable and continuous surveillance of trends in resistance patterns of uropathogens is necessary.

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