ANTIBIOTIC RESISTANCE PATTERN OF URINARY ISOLATES IN A TERTIARY CARE HOSPITAL IN BERHAMPORE, WEST BENGAL

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ABSTRACT

BACKGROUND

Urinary Tract Infection (UTI) is one of the commonest infections worldwide and a major public health problem. It may be caused by almost all known bacterial pathogens. A wide range of antibiotics are available for its treatment. In majority of the cases, antibiotic therapy is started empirically without waiting for the report of urine culture. Therefore, it becomes mandatory to determine the bacteriological profile of uropathogens in a particular region and their antimicrobial resistance pattern, so as to ensure the selection of an effective empirical treatment.

MATERIALS AND METHODS

This was a retrospective descriptive study. In this study of one-year duration, 3192 urine samples were processed. Age and gender were recorded from patients' data. Urine culture was performed by conventional methods and the isolates were identified by standard microbiological techniques and conventional biochemical methods. Antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method.

RESULTS

703 samples yielded growth, of which 68.14% were from females and 31.86% were from males. The most commonly isolated pathogen was *Escherichia coli* (35.13%) followed by *Klebsiella spp.* (27.88 %). *Escherichia coli* exhibited lowest resistance to Imipenem (1%) followed by nitrofurantoin (19%). *Klebsiella spp.* exhibited lowest resistance to Imipenem (1%) followed by piperacillin/ tazobactam (27%). The organisms were highly resistant to ticarcillin/ clavulanic acid (82% - 85%). *Pseudomonas spp.* showed highest resistance to ceftazidime (86.7%). They were least resistant to piperacillin/ tazobactam (6.7%) followed by Imipenem (13.3%). *Enterococcus spp.* was least resistant to linezolid (2.2%). They were highly resistant to Penicillin (92.8%), aminoglycosides, macrolides, fluoroquinolones and tetracycline. 14.5% of isolates exhibited vancomycin resistance. *Staphylococcus aureus* isolates showed least resistance to Imipenem (2.6%). 43.6% of the isolates were vancomycin resistant.

CONCLUSION

As the distribution of urinary pathogens and their resistance pattern to antibiotics vary from one region to another, it is necessary to determine the most common uropathogens in a particular area and their resistance pattern so as to develop effective antibiotic policies and design a rational empirical therapy so that the best empirical therapy can be chosen, thus preventing the indiscriminate use of antibiotics and development of antimicrobial resistance.

KEY WORDS

UTI, Antimicrobial Susceptibility Testing, Antimicrobial Resistance Pattern, Uropathogens.

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BACKGROUND

Urinary Tract Infection (UTI) is one of the most common causes of infection worldwide with an estimated 150 million cases per year and the second most common cause of visit to hospitals by patients.[1,2] It is a major public health problem with a high morbidity rate and accounts for the highest healthcare cost among urological diseases.[3] UTI includes a wide spectrum of diseases ranging from mild asymptomatic cystitis to pyelonephritis and septicaemia.[4] The syndrome is commoner in females: approximately 50% of adult women experience at least one episode of UTI during their lifetime, whereas men have a lifetime risk of only 13%.[5,6,7] This is probably because of the shorter length of the urethra in females, which facilitates entry of bacteria into the urinary tract easily.[8] The incidence of uncomplicated UTI in healthy women is 50/ 1000/ year.[9] UTI is most common in diabetic patients and patients with some structural and neurological abnormalities of the urinary tract, which interfere with the urine outflow. Instrumentation in the urinary tract like catheterisation and cystoscopy also predispose to UTI. UTI is also a leading cause of hospital acquired infection.

Almost all known bacterial pathogens have been implicated as possible causative agents of UTI.[10,11] However, the most common isolate in uncomplicated UTI is *Escherichia coli*.[5,6] Other organisms commonly implicated are *Klebsiella spp., Proteus spp., Enterobacter spp., Pseudomonas spp., Staphylococcus aureus,* Coagulase negative Staphylococcus and *Enterococcus spp*.

The introduction of antibiotic therapy has significantly contributed to the management of UTIs. Although, there is a large group of antimicrobial agents available for the treatment of UTIs, none of them can treat all UTIs. Moreover,

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in majority of the cases antibiotic therapy is started empirically without waiting for the report of urine culture from the Microbiology Laboratory. The distribution of urinary pathogens and their resistance pattern to antibiotics vary from one region to another.[12,13] Therefore, it becomes mandatory to determine the bacteriological profile of uropathogens in a particular region and their antimicrobial susceptibility, so as to ensure the selection of an effective empirical treatment.[14] Uropathogens have shown a change in pattern of susceptibility to antibiotics resulting in an increase in resistance to commonly used antibiotics.[15] UTIs are usually treated with broad-spectrum antibiotics, although an antibiotic with a narrow spectrum of activity could have been appropriate due to concerns about infection with resistant organisms. The indiscriminate use of antibiotics has invariably led to development of resistance to many antimicrobial agents, which has become a major problem worldwide in the recent years. The aetiology of UTI and antibiotic resistance pattern of uropathogens have been changing over the past years.[16] Therefore, a knowledge about the pathogens responsible for UTI and their resistance pattern may help clinicians to select the appropriate empirical treatment.[17,14] This may also help to develop antibiotic policies in a health care facility.

We hereby undertook a study to determine the common urinary pathogens in our hospital and their antibiotic resistance pattern.

Aims and Objectives

The study was undertaken to determine the pathogens isolated from urine specimens and their resistance pattern to different antimicrobial agents.

MATERIALS AND METHODS

This is a retrospective descriptive study of one-year duration from May 2017 to April 2018 conducted in the Department of Microbiology in a tertiary care hospital in Berhampore in Murshidabad District of West Bengal. A total of 3192 urine samples received in the department during the study period from symptomatic cases were included in the study. The data concerning the age and gender were collected from patient's records.

Each sample was inoculated on blood agar and MacConkey media with a standard loop and was incubated at 37°C overnight. A growth of ≥ 10^5 colony forming units/mL was considered to be significant. The identification of the bacterial growth was done by Gram staining, standard microbiological techniques and conventional biochemical methods.

Antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method on Mueller-Hinton agar and the interpretations were carried out as per Clinical and Laboratory Standards Institute (CLSI) guidelines.[18,19] The control strains used were *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* 25922. The following antibiotics were tested for gram-negative bacilli: Imipenem (10 mcg), Meropenem (10 mcg), piperacillin/ tazobactam (100/10 mcg), ceftriaxone (30 mcg), cefotaxime (30 mcg), cefixime (5 mcg), cefepime (30 mcg), Cefoperazone (75 mcg), ceftazidime (30 mcg), gentamicin (10 mcg), amikacin (30 mcg), ciprofloxacin (5 mcg), ofloxacin (5 mcg), norfloxacin (10 mcg), ticarcillin/clavulanic acid (75/10 mcg), nitrofurantoin (300 mcg), trimethoprim/sulfamethoxazole (co-trimoxazole) (1.25/23.75 mcg), tetracycline (30 mcg) and moxifloxacin (5 mcg). For gram-positive organisms the following antibiotics were tested: Imipenem (10 mcg), Linezolid (30 mcg), Vancomycin (30 mcg), nitrofurantoin (300 mcg), netilmicin (30 mcg), azithromycin (15 mcg), erythromycin (15 mcg), gentamicin (10 mcg), amikacin (30 mcg), cefotaxime (30 mcg), trimethoprim/sulfamethoxazole (co-trimoxazole) (1.25/23.75 mcg), ciprofloxacin (5 mcg), levofloxacin (5 mcg), penicillin (10U), tetracycline (30 mcg) and cloxacillin (10 mcg) (for *S. aureus*). Dehydrated media were procured from Hi-Media and antibiotic discs from Microexpress. All the analyses were performed using simple counts and percentage method.

RESULTS

Of the total 3192 urine samples cultured, 2101 (65.82%) were from females and 1091 (34.18%) were from males. Out of the total 3192 samples, 703 (22.02%) samples yielded growth [Figure 1].

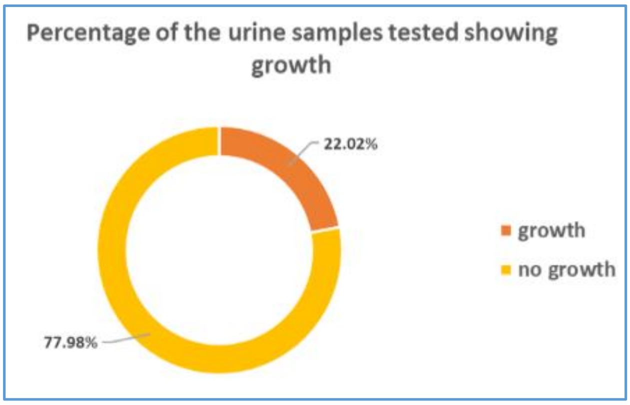

Figure 1. Growth from Urine Samples Tested

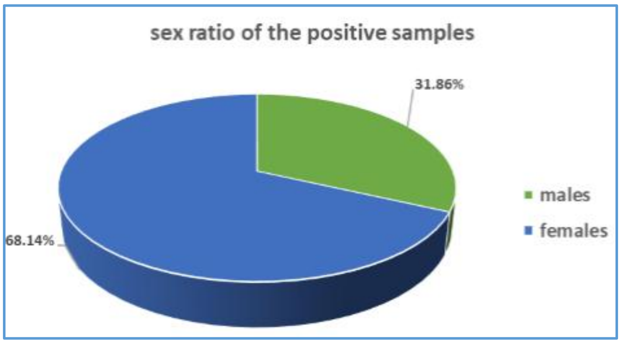

Figure 2. Sex Ratio of the Positive Samples

Among the positive cultures, 479 (68.14%) were from females and 224 (31.86%) were from males [Figure 2].

It was observed that highest number of subjects with positive culture was below 10 years age and the least number were those above 70 years [Table 1].

Age in Years	No.	Percentage
< 10	213	30.30
10-25	170	24.18
26-40	156	22.19
41-55	89	12.66
56-70	58	8.25
> 70	17	2.42

Table 1. Age distribution of Positive Samples

Organism Isolated	No.	Percentage
Escherichia coli	247	35.13
Klebsiella spp.	196	27.88
Citrobacter spp.	6	0.85
Proteus spp.	14	1.99
Pseudomonas spp.	15	2.13
Acinetobacter spp.	3	0.43
Enterococcus spp.	138	19.63
Staphylococcus aureus	78	11.10
CONS	1	0.14
Candida spp.	5	0.71
Total	**703**	**100**

Table 2. The Organisms Isolated from the Positives Cultures

It was observed that the highest number of positive cultures yielded *Escherichia coli* (35.13%) followed by *Klebsiella spp.* (27.88 %). Gram-negative organisms accounted for 481 cases (68.42%), of which 463 (96.26%) were from the Enterobacteriaceae family. Gram-positive organisms accounted for 222 cases (31.58%) [Figure 3].

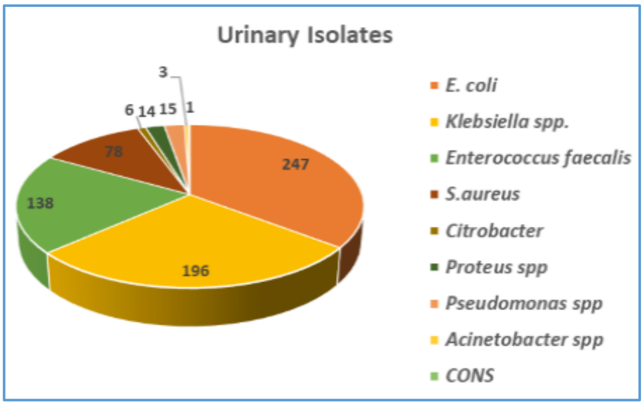

Figure 3. Urinary Isolates from Positive Cultures

Organism	IMP	MRP	PTZ	CTR	CTX	CFM	CPM	CPZ	CAZ	GEN	AK	CIP	OF	NX	TCC	NIT	COT	TE	MXF
E. coli	1	20	30	69.6	62.8	73.7	71.7	83.4	80.6	74	49.4	70.9	57.9	60	82.2	19	72	54.3	85
Klebsiella spp.	1	68.8	27	50.5	56.1	68.8	53.6	36.2	78.6	79.6	45.9	43.4	37.2	45.9	85.2	51.5	54.6	32.1	66.8
Proteus spp.	0	0	21.4	35.7	50	57.1	69.4	45	79.3	64.2	64.2	42.9	32.2	78.6	78.6	42.9	64.2	57.1	57.1
Citrobacter spp.	0	29.6	20	37.2	50	59.6	62.8	42.9	78.6	66.7	50	66.7	44.6	72.7	74.9	20	50	50	64.3
Pseudomonas spp.	13.3	33.3	6.7	45.7	53.3	54.6	60	45.9	86.7	33.3	20	33.3	50	69.4	73.3	28.4	74.9	52.6	69.7
Acinetobacter spp.	0	33.3	33.3	33.3	33.3	33.3	33.3	33.3	66.7	33.3	66.7	33.3	66.7	66.7	100	66.7	100	66.7	66.7

Table 3

Table 3. Antibiotic Resistance Pattern (%) of Gram-Negative Bacilli. Here, IMP: imipenem, MRP: meropenem, PTZ: piperacillin/tazobactam, CTR: ceftriaxone, CTX: cefotaxime, CFM: cefixime, CPM: cefepime, CPZ: Cefoperazone, CAZ: ceftazidime, GEN: gentamicin, AK: amikacin, CIP: ciprofloxacin, OF: ofloxacin, NX: norfloxacin, TCC: ticarcillin/clavulanic acid, NIT: nitrofurantoin, COT: co-trimoxazole, TE: tetracycline, MXF: moxifloxacin.

It was observed that *Escherichia coli* isolates exhibited lowest resistance to Imipenem (1%) followed by nitrofurantoin, meropenem and piperacillin/tazobactam. *Klebsiella spp.* isolates exhibited lowest resistance to Imipenem (1%) followed by piperacillin/tazobactam. The organisms were highly resistant to ticarcillin/clavulanic acid. Both *Escherichia coli* and *Klebsiella spp.* were moderately resistant to the commonly used cephalosporins, fluoroquinolones and gentamycin. *Pseudomonas spp.* showed highest resistance to ceftazidime (86.7%). They were most sensitive to piperacillin/tazobactam followed by Imipenem and Amikacin [Figures 4, 5 and 6].

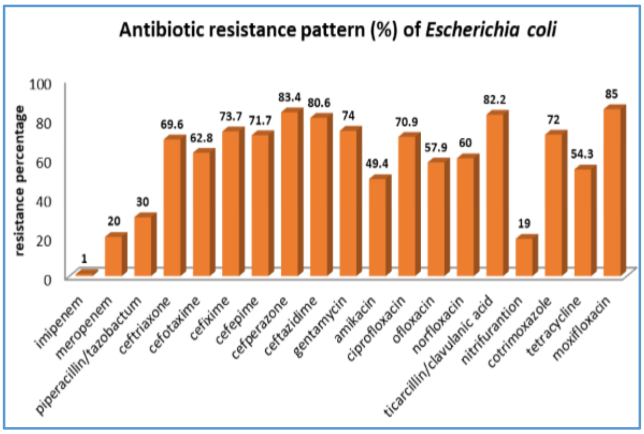

Figure 4. Antibiotic Resistance Pattern (%) of Escherichia coli

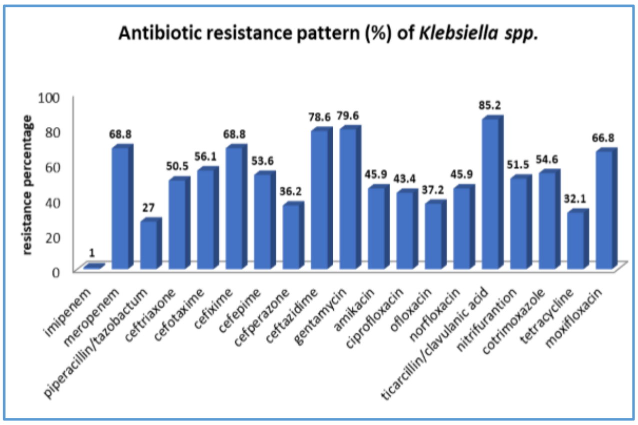

Figure 5. Antibiotic Resistance Pattern (%) of Klebsiella spp.

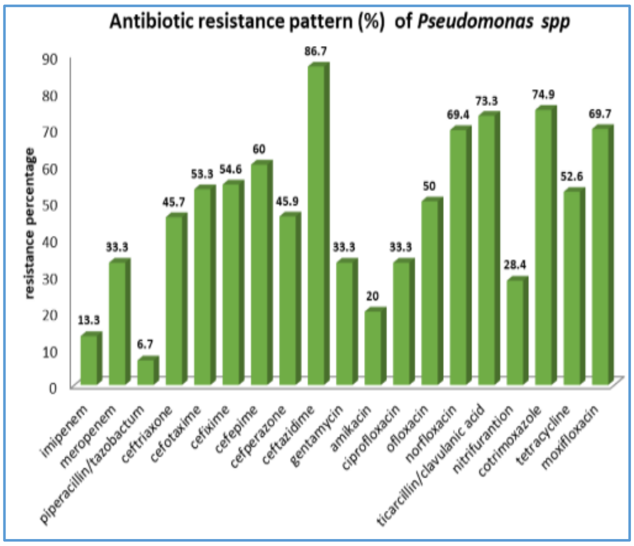

Figure 6. Antibiotic Resistance Pattern (%) of Pseudomonas spp.

Organism	IPM	LZ	VA	NIT	NET	AZM	E	AK	GEN	CTX	COT	CIP	LE	P	TE	COX
Enterococcus spp.	28.2	2.2	14.5	7.2	48.6	88.4	89.9	86.2	86.9	70.3	64.5	84.8	79.7	92.8	83.3	-
S. aureus	2.6	5.1	43.6	11.5	7.7	73	93.6	19.2	59	55.1	76.9	70.5	55.1	86.4	47.4	70.5
CONS	0	100	100	0	100	100	100	0	100	0	0	100	100	100	100	100

Table 4

Table 4. Antibiotic Resistance Pattern (%) of Gram-positive cocci, Here, IMP: imipenem, LZ: Linezolid, VA: Vancomycin, NIT: nitrofurantoin, NET: netilmicin, AZM: azithromycin, E: erythromycin, GEN: gentamicin, AK: amikacin, CTX: cefotaxime, COT: co-trimoxazole, CIP: ciprofloxacin, LE: levofloxacin, P: penicillin, TE: tetracycline, COX: cloxacillin.

Among the Gram-positive organisms *Enterococcus spp.* was least resistant to linezolid (2.2%) followed by nitrofurantoin. They were highly resistant to Penicillin (92.8%), aminoglycosides, macrolides, fluoroquinolones and tetracycline. 14.5% of isolates were resistant to vancomycin. *Staphylococcus aureus* isolates showed least resistance to imipenem (2.6%) followed by linezolid. 43.6% of the isolates exhibited resistance to vancomycin [Figures 7 and 8]. The only CONS isolate was sensitive only to Imipenem, nitrofurantoin, Amikacin, ceftriaxone and cotrimoxazole.

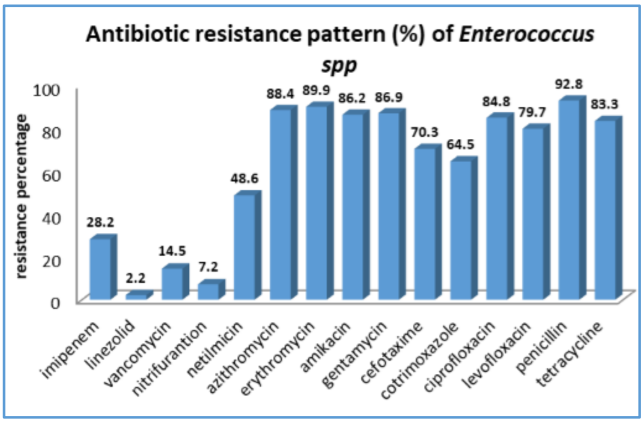

Figure 7. Antibiotic Resistance Pattern (%) of Enterococcus spp.

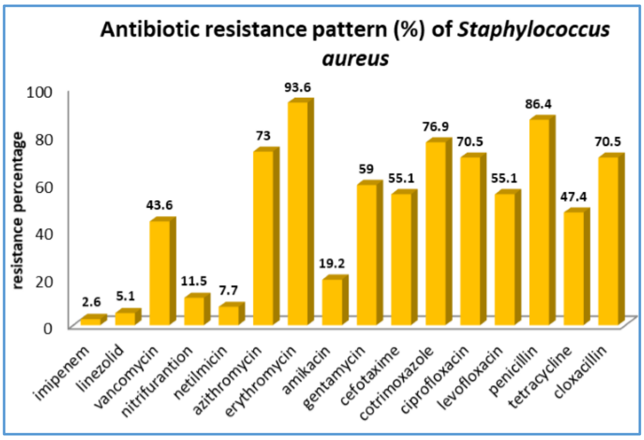

Figure 8. Antibiotic Resistance Pattern (%) of Staphylococcus aureus

DISCUSSION

UTI is one of the commonest syndromes encountered by practitioners. This study was undertaken to determine the common uropathogens from cases of UTI and their antibiotic resistance pattern.

In our study, UTI was reported from 65.82% females and 34.18% males. This was similar to the findings by Sood S et al, who reported UTI from 37.67% males and 62.42% females.[20] Female preponderance was reported from various studies: 57.74% by Kumar A et al, 70.5% by Hasan AS et al and 39.9% females compared to 9.1% males by Semwal AC et al.[21,22,23]

However, Patil S et al reported a slight male preponderance (57%).[24] The female preponderance is probably related to the short length of the female urethra and its proximity to the anal opening facilitating entry of bacteria into the urinary tract easily.[8]

The most commonly isolated organism in our study was *Escherichia coli* (35.13%) followed by *Klebsiella spp.* (27.88%). This is in accordance with studies by Kumar A et al and Hasan AS et al, where *Escherichia coli* accounted for 37.41% and 50.7% of isolates followed by *Klebsiella spp.* accounting for 32.79% and 27.6% respectively.[21,22] Similar results were also reported by Ranjbar R et al, Amin M et al, Tankhiwale SS et al and Somashekara SC et al.[25-28] On the contrary, Chan RK et al found *Klebsiella spp.* (25%) to be more prevalent than *Escherichia coli* (17.7%).[29]

Among the Gram-positive organisms, the commonest isolate in our study was *Enterococcus spp.* (19.63%) followed by *Staphylococcus aureus* (11.10%), whereas Hasan AS et al and Semwal AC et al and reported more *Staphylococcus aureus* than *Enterococcus spp.* in their studies.[22,23] In the present study, only one Coagulase-negative Staphylococcus (CONS), was isolated. The differences in the frequency of isolation of uropathogens in different studies from different geographic areas reflects the influence of various demographic factors.

In our study, the members of the Enterobacteriaceae family showed least resistance to Imipenem (1% for both *Escherichia coli* and *Klebsiella spp.).* Imipenem was also reported as the least resistant antibiotic by Kumar A et al (0-1.76%), Somashekara SC et al (14.7%) and Manjunath GN et al (7.5%).[21,28,30] The organisms were highly sensitive to meropenem, piperacillin/tazobactam and nitrofurantoin except for the *Klebsiella spp.,* which showed an unusually high resistance to meropenem (68.8%) for unknown reasons. This is in concordance with studies by Kumar A et al and Semwal AC et al.[21,23] Similar low resistance to nitrofurantoin was reported by Sood S et al and Patil S et al (15%).[20,24] This may be related to the low usage of Imipenem, meropenem and piperacillin/ tazobactam in the community. As for nitrofurantoin, this antibiotic has a narrow spectrum and hence limited indication and is highly concentrated in the urine, thereby retaining its activity in spite of many years of usage, especially against *Escherichia coli.*[1] The organisms showed a moderate-to-high resistance to cephalosporins, aminoglycosides, fluoroquinolones, tetracycline, cotrimoxazole and ticarcillin/clavulanic acid. This may be due to the indiscriminate use of these antibiotics in the community for empirical treatment of UTI.

The Gram-positive organisms isolated in our study exhibited high resistance to penicillin (*Enterococcus spp.* 92.8% and *Staphylococcus aureus* 86.4%). Similar results were obtained by Hasan AS et al,(22) who reported 98.8% and 65.6% resistance to penicillin for *Enterococcus spp.* and *Staphylococcus aureus* respectively. *Enterococcus spp.* were least resistant to linezolid followed by nitrofurantoin. They were highly resistant to aminoglycosides, macrolides and fluoroquinolones as also reported by Hasan AS et al.[22] Vancomycin resistance was reported in 14.5% of isolates as compared to 1.82% reported by Sood S et al.[20] 5.1% of *Staphylococcus aureus* exhibited resistance to linezolid compared to the 9.1% reported by Hasan AS et al.[22] Vancomycin resistance was shown by 43.6% of *Staphylococcus aureus* isolates similar to the study by Kumar A et al (43.87%).[21] In our study, imipenem resistance was reported in 28.2% of *Enterococcus spp.* and 2.6% of *Staphylococcus aureus* in contrast to 8.2% and 14% reported by Somashekara SC et al respectively.[28] The rising resistance to vancomycin is a matter of great concern, as it is one of the few antibiotics available for treatment of resistant cases of *Staphylococcus aureus* and also *Enterococcus spp.*

CONCLUSION

Our study together with other studies from different geographic areas, highlight the importance of performing Antibiotic Susceptibility Test (AST) to determine the most effective antibiotics for the isolated uropathogens. Since the results of AST are not available to the practitioners immediately, clinicians have to depend on empirical choice of antibiotics. The distribution of urinary pathogens and their resistance pattern to antibiotics vary from one region to another. Therefore, it is necessary to determine the most common urinary isolates in a particular area and their local resistance pattern, so as to develop effective antibiotic policies and design a rational empirical therapy based on all the data of AST report, which would be somewhat customised to each geographic area. This will be helpful in choosing the best antibiotics for empirical therapy and avoiding the indiscriminate use of antimicrobial agents, which leads to selection of resistant mutants and emergence of drug resistance. Antibiotic resistance may be prevented by rotation of antibiotics, which is guided by a good hospital antibiotic policy. Imipenem still remains one of the most effective antibiotics for the treatment of infection caused by both Gram-positive and Gram-negative organisms.

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