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Research article

A retrospective study of *anti-microbial* susceptibility pattern among bacterial strain isolated from cases of urinary tract infection

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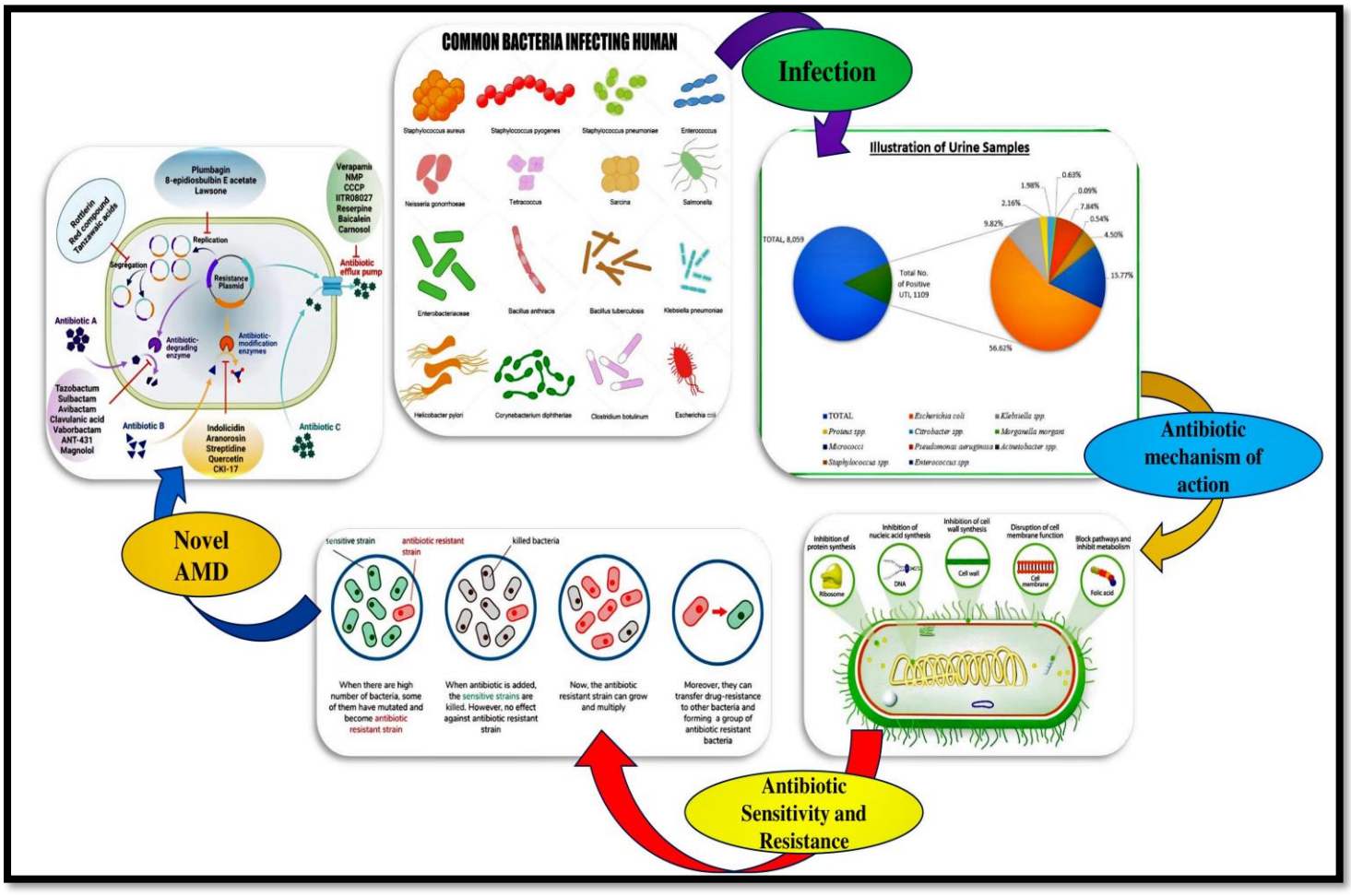
ABSTRACT



Urinary Tract Infections (UTI) are common among the individuals and most prevalent bacterial infections seen in clinical practice. It has been observed that nosocomial infection was most common in many hospitals. The study findings shows the causative agents for UTI's were antibiotics and they exhibit poor patterns of antimicrobial sensitivity. The Institute of Medical Science, Banaras Hindu University (IMS-BHU), which serves a large number of partially treated or maltreated patients, may have various etiological agents and sensitivity patterns. To investigate the range of bacterial infections causing UTI among patients, as well as their antibiotic sensitivity pattern. This retrospective investigation was carried out from January 2020 to December 2020 at the Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

Clean catch midstream urine samples were collected from all suspected UTI patients. The urine samples were cultured and tested for antibiotic susceptibility in accordance with normal standards. A total of 8,059 urine samples were tested for urine culture and sensitivity. A total of 1,109 samples were found to be positive for bacterial Infections. To facilitate analysis, positive isolates (n = 1,109) were further classified as Enterobacteriales (n = 791), NLF oxidase-positive (*P. aeruginosa*; n = 87), NF (*Acinetobacter*; n = 6), *Enterococcus* (n = 175), and others. Patients with UTI who present to a tertiary hospital are found to have several bacterial infections. As the antimicrobials recommended for these isolates differ as per the CLSI guidelines, it is essential to determine antimicrobial sensitivity of these isolates is very crucial.

Keywords: Antimicrobial susceptibility pattern, Bacterial isolates, Gram - Gram-negative bacteria, Urinary tract infection, Antibiotic susceptibility.

INTRODUCTION

Urinary tract infections (UTIs) are some of the most common bacterial infections, affecting 150 million people each year worldwide [1, 2]. Urinary tract infection (UTI) is described as bacteriuria with urinary symptoms. It is very common infection by the bacteria in clinical practice particularly in developing countries with a high rate of morbidity and financial cost [3]. Some of the key factors predisposing to urinary tract infection have been attributed to poor personal hygiene and urinary tract abnormalities [3, 4]. The various type causative agents were identified till now for UTI's and it was varied from case to case and place to place to exhibit their susceptibility and pattern of resistance against organisms or different microbial pathogens [5].

UTI's are clinically classified inti uncomplicated and complicated types. Uncomplicated UTI's generally occur in individuals who are otherwise and have no structural or functional abnormalities of the urinary tract. These are further divided into lower UTI's (such as cystis) and upper UTI's (such as pyelonephritis). Risk factors for cystis include being female, a history of previous UTI's, sexual activity, vaginal infections, diabetes, obesity, and genetic predisposition.

In contrast, complicated UTI's are associated with conditions that impair the urinary tract or the host's immune defences. These includes urinary obstructions, retention due to neurological disorders, immunosuppression, chronic kidney diseases, renal transplantation, pregnancy, the presence of foreign bodies like kidney stones, indwelling catheters, or other drainage urinary devices [6].

The most common pathogenic organisms of UTI are Enterobacteriales *(Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Citrobacter* spp.,), *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Staphylococcus* spp., and *Enterococcus* spp., [5, 6, 7]. The bacterial infection prevalence was asymptomatic and exhibit as bacteriuria and it is estimated from the range of 2% to 10% globally as per findings of recent research statistics. But in Indian context it higher from 3% to 24% and noted their prevalence as both asymptomatic bacteriuria and symptomatic infection [6]. UTIs are caused by both Gram-negative and Gram-positive bacteria, as well as by certain fungi. The common causative agent was *Escherichia coli* and it was uncomplicated and sometime complicated UTIs to exhibit as an uro-pathogenic in nature (UPEC) [14]. Apart from this, many other causative agents were involved it for complicated and uncomplicated UTIs, and they act as Uropathogenic stratins such as *Escherichia coli* (UPEC) is followed in prevalence by *Enterococcus* spp., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus* spp., *Proteus* spp., *Citrobacter* spp., *Morganella morganii*, *Acinetobacter* spp. and *Micrococci* [1,4, 7].

The emergence of drug resistance among the uro-pathogens isolated has posed a big challenge in dealing with urinary tract infections. The misuse or overuse of any antibiotics may cause adverse effects and it is poor defence of patient and compliance become more worsen to further aggravate and exhibit chronic problem. The emergence of resistance to such drugs is a natural biological phenomenon. The empirical treatment and management of UTI have made the matter worse. The observation of local infection and their susceptibility may support effectively in treatment significantly. Hence the basis for antimicrobial agent selection should be based upon the expected resistance pattern of that geographic area [8, 9, and 10].

Based on the above observation, current study was planned to address the prevalence of the infective disease, identification causative agents, and their antibiotic sensitivity when identified UTI's frequent issues to screen and isolation of the pathogenic bacteria.

Objectives

To study the prevalence of uro-pathogens from Urine samples and its sensitivity to the commonly used antibiotics at BHU - IMS Hospital, Varanasi, India.

MATERIAL AND METHOD

Material

Urine Sample, Universal Container, Nichrome wire loop, CLED agar (Cystine–lactose–electrolyte-deficient agar), Mac Conkey Agar, Blood Agar, Muller Hilton Agar, Swab Stick, Mc Farland Standard 0.5, Normal Saline, Pipette, Pipette tip, Petri plates, Bunsen Burner, Antibiotic Discs, Forceps.

Methods

This study was an observational study carried out at the Microbiology Department of the Institute of Medical Science, Banaras Hindu University, Varanasi during the period between January 2020 to December 2020. The study designed to include all type of patients who came for treatments for the hospital with symptoms and signs of UTI's. All the patients Urine sample were collected for the analysis in a wide-mouthed sterile container. Contaminated samples and non-sterile samples were separated during the study and discarded in bio hazardous bags.

Collection of Samples

The urine is collected in a wide-mouthed universal container from patients. A midstream specimen is the most ideal for processing.

Inoculum Preparation

Suspension of usually log phase growth cells of bacteria. Plate count is done making serial dilution first.

Source of Bacteria

The indirect source can be a cultured plate from pure culture and the direct source can be a pathological specimen, e.g., urine sample.

Isolation and Identification of Organisms

Isolation of uro-pathogens was performed by using a calibrated nichrome wire loop of 0.01 mm diameter and were plated on CLED agar (Cystine–lactose–electrolyte-deficient agar) and incubated aerobically at 37ºC for 24 hrs. Selected colonies from the cultures were examined and counted for the data. A growth of >=105 CFU/ml was highly significant bacterial population and exhibit for the causative agent of UTIs. These cultures were examined using standard microbiological techniques for characterization.

Choosing the appropriate Antibiotics

The first line of sensitivity is the drugs that are available in most hospitals and for which routine testing should be carried out for every strain. The second line of sensitivity is the drugs that are tested only when the causative organism is resistant against to primary source of drugs, or at the special request of the physicians.

Antibiotic Susceptibility Testing

In this testing of the bacterial isolates was performed by Kirby Bauer disc diffusion method and the interpretation was done according to Clinical and Laboratory Standards Institute (CLSI) guidelines. All bacterial isolates were tested for their antibiotic sensitivity pattern against the following panel of antibiotics: Ampicillin (3), Cefazolin (6), Gentamicin (12), Ofloxacin (of), Co-trimoxazole (22), Nitrofurantoin (23), Cefotaxime (9), Amikacin (14), Piperacillin – tazobactam (PTZ), Cefepime (Cp), Imipenem (27), Ertapenem (Erta), Meropenem (Me), Piperacillin (pip), Ceftazidime (11), Aztreonam (AZT), Ampicillin sulbactam (Amsu), Ciprofloxacin (18), Levofloxacin (Lv), Penicillin (1), Cefoxitin (Cxt), Vancomycin (25), Linezolid (26), Netilmicin (15), Moxifloxacin (Mox), H.S. Gentamicin (28).

All antibiotics discs were obtained from (Hi-Media Labs Mumbai India). Plating of the suspension was done on Mueller Hinton agar plates by lawn method and then incubated at 37ºC for 24 hrs. All the disks were placed it for 20 mm and only changed for Mueller Hinton agar medium inoculated with 0.5 McFarland suspension of the tested bacterial isolate. Plates were incubated at 37 degrees Celsius for 24 hrs.

Table 1: Sensitivity Reporting Chart of Urine Sample

Organism	First line of Sensitivity	Second Line of Sensitivity
Lactose fermenters and Non-lactose fermenters (Oxidase-negative)	Ampicillin (3), Cefazolin (6), Gentamicin (12), Ofloxacin (of), Co-trimoxazole(22), Nitrofurantoin (23)	Cefotaxime (9), Amikacin (14), Piperacillin-tazobactam (PTZ), Ertapenem (Erta), Cefepime (Cp), Imipenem (27), Meropenem (Me)
Non-lactose fermenter (Oxidase-positive)	Piperacillin (pip), Ceftazidime (11), Gentamicin (12), Amikacin (14), Ofloxacin (of)	Piperacillin tazobactam (PTZ), Aztreonam (AZT), Cefepime (Cp), Imipenem (27), Meropenem (Me)
Acinetobacter spp.	Ampicillin-sulbactam(AMS), Ceftazidime(11), Gentamicin(12), Ciprofloxacin(18), Levofloxacin (Lv), Imipenem(27), Meropenem(Me)	Cefotaxime (9), Amikacin(14), Cotrimoxazole(22), Cefepime(Cp), Piperacillin-tazobactam(PTZ)
Staphylococcus spp.	Penicillin (1), Cefoxitin (Cxt), Cotrimoxazole (22), Nitrofurantoin (23)	Vancomycin (25), Linezolid (26), Ciprofloxacin (18), Netilmicin (15), Moxifloxacin (Mox), Gentamicin (12)
Enterococcus spp.	Ampicillin (3), Ciprofloxacin (18), Nitrofurantoin (23), Vancomycin (25), Linezolid (26), H.S. Gentamicin (28)	

RESULTS

During the study period (January 2020 to December 2020), out of 8,059 samples of urine a total of 1,109 samples were shown positive and considered as urinary tract infection. (As shown in the

pie chart).

For the ease of analysis, positive isolates (Uropathogens; n = 1109) were further categorized into isolates obtained by Enterobacteriales (*E. coli*, *Klebsiella* spp., *Proteus* spp., *Citrobacter* spp., *Morganella morganii*, *Micrococci*. n = 791), by NLF oxidase-positive (*P. aeruginosa*; n = 87), by NF (*Acinetobacter*; n = 6), by *Staphylococcus* (n = 50) and *Enterococcus* (n = 175). *Escherichia coli* was the most frequently isolated urinary pathogen in all categories (56.62%). *Enterococcus* spp. was the second on the list, (15.77%) followed by *Klebsiella* spp., (9.82%), *Pseudomonas aeruginosa* (7.84%), *Staphylococcus* spp., (4.50%), *Proteus* spp., 2.16%), *Citrobacter* spp., (1.98%), *Morganella morganii* (0.63%), *Acinetobacter* spp., (0.54%) and *Micrococci* (0.09%), as shown in Table 2.

In this study, it is shown that the antibiotic sensitivity pattern of Enterobacteriales is different from other isolates. Figure 1 showed that Nitrofurantoin shows the highest sensitivity in the First line of sensitivity pattern, followed by Gentamicin, Co-trimoxazole, Ofloxacin, Cefazolin, and then Ampicillin. Ampicillin showed the least sensitivity pattern among all patients, whereas in the Second line of sensitivity it is shown that Imipenem shows the highest sensitivity followed by Ertapenem, Meropenem Amikacin, Piperacillin tazobactam, Cefepime, and Cefotaxime showed the least sensitivity pattern that means it is most resistance among all patients, as shown in Figure 2.

In Non-Lactose Fermenter oxidase-positive (NLF oxidase +ve) it is shown that in the First line of sensitivity Piperacillin shows the highest sensitivity among all patients followed by Ceftazidime, Amikacin, Gentamicin, Ofloxacin as shown in Figure 3, whereas, in the second line of sensitivity it is shown that the Piperacillin tazobactam shown the highest sensitivity followed by Aztreonam, Imipenem, Cefepime and Meropenem showed the least sensitivity among all patients, as shown in Figure 4.

Table 2: Distribution of most common uropathogens (%) in various categories

Uropathogens		N = x	%
Enterobacteriales (Lactose Fermenter and Non-Lactose Fermenter, oxidase-negative)	*Escherichia coli*	628	56.62%
	Klebsiella spp.	109	9.82%
	Proteus spp.	24	2.16%
	Citrobacter spp.	22	1.98%
	Morganella morgani	7	0.63%
	Micrococci	1	0.09%
Non-Lactose Fermenter (Oxidase-positive)	*Pseudomonas aeruginosa*	87	7.84%
Non Fermenter	*Acinetobacter spp.*	6	0.54%
Staphylococcus spp.		50	4.50%
Enterococcus spp.		175	15.77%

Figure 1: First Line of Sensitivity Pattern of Enterobacteriales (LF and NLF oxidase negative)

	Ampicillin	Cefazolin	Gentamicin	Ofloxacin	Co-tri moxazole	Nitrofurantoin
Susceptible	66	135	463	140	294	628
Resistance	725	653	299	649	489	134
Intermediate	0	3	29	2	8	29

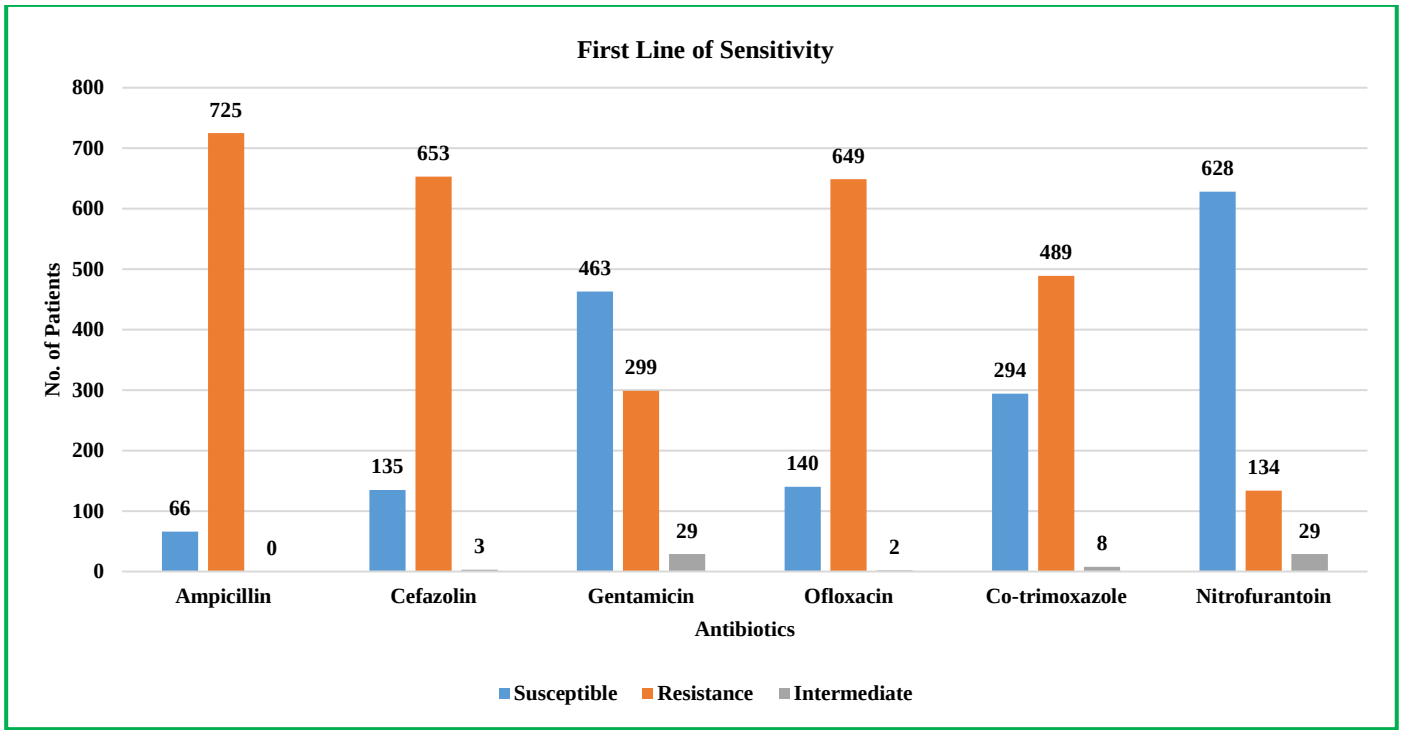


Figure 2: Second Line of Sensitivity Pattern of Enterobacteriales (LF and NLF oxidase negative)

	Cefotaxime	Amikacin	Piperacillin tazobactam	Cefepime	Imipenem	Ertapenem	Meropenem
Susceptible	25	123	116	67	162	135	129
Resistance	183	81	81	141	7	69	74
Intermediate	0	4	11	0	39	4	4

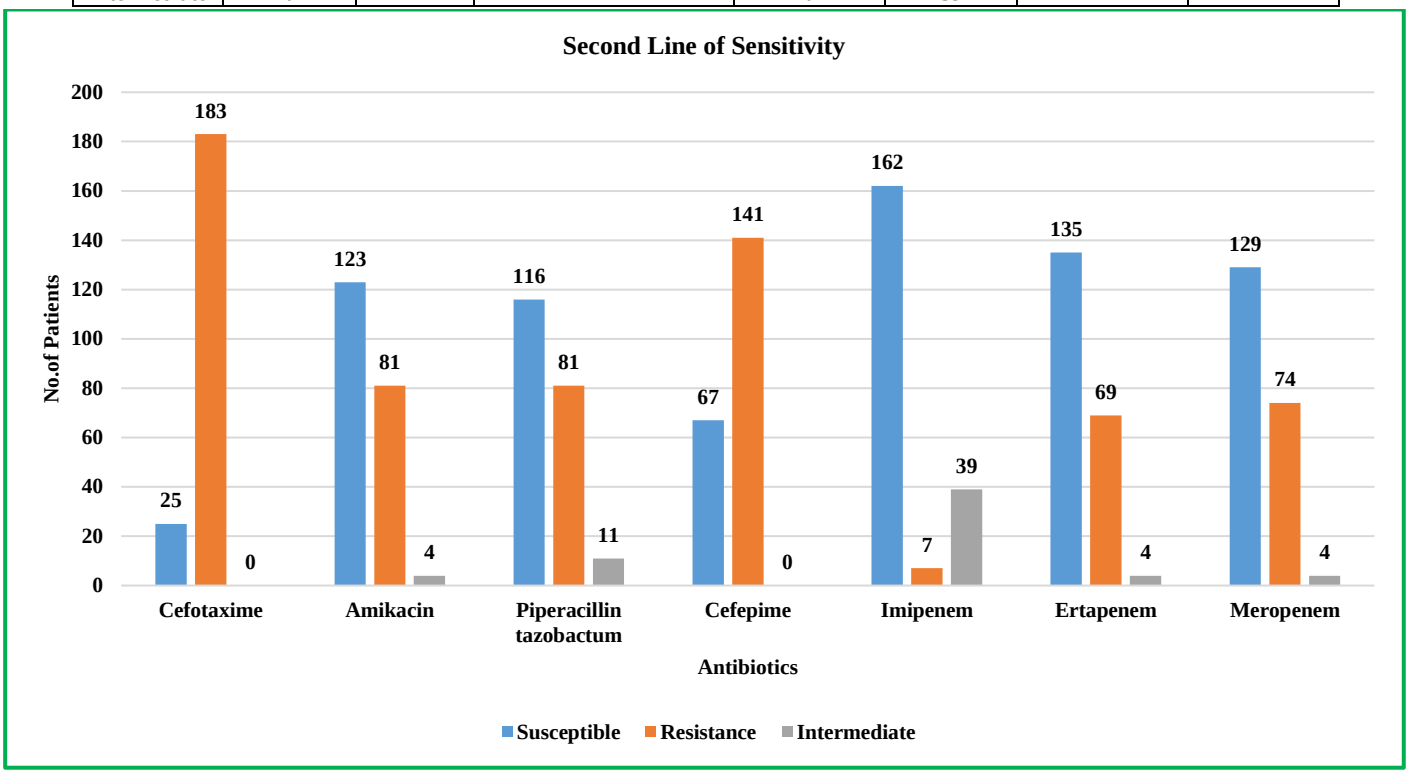


Figure 3: First line of Sensitivity Pattern of NLF oxidase positive *Pseudomonas aeruginosa*

	Pip	Ceftazidime	Gentamicin	Amikacin	Ofloxacin
Susceptible	47	45	36	38	21
Resistance	28	39	50	46	62
Intermediate	10	2	0	1	2

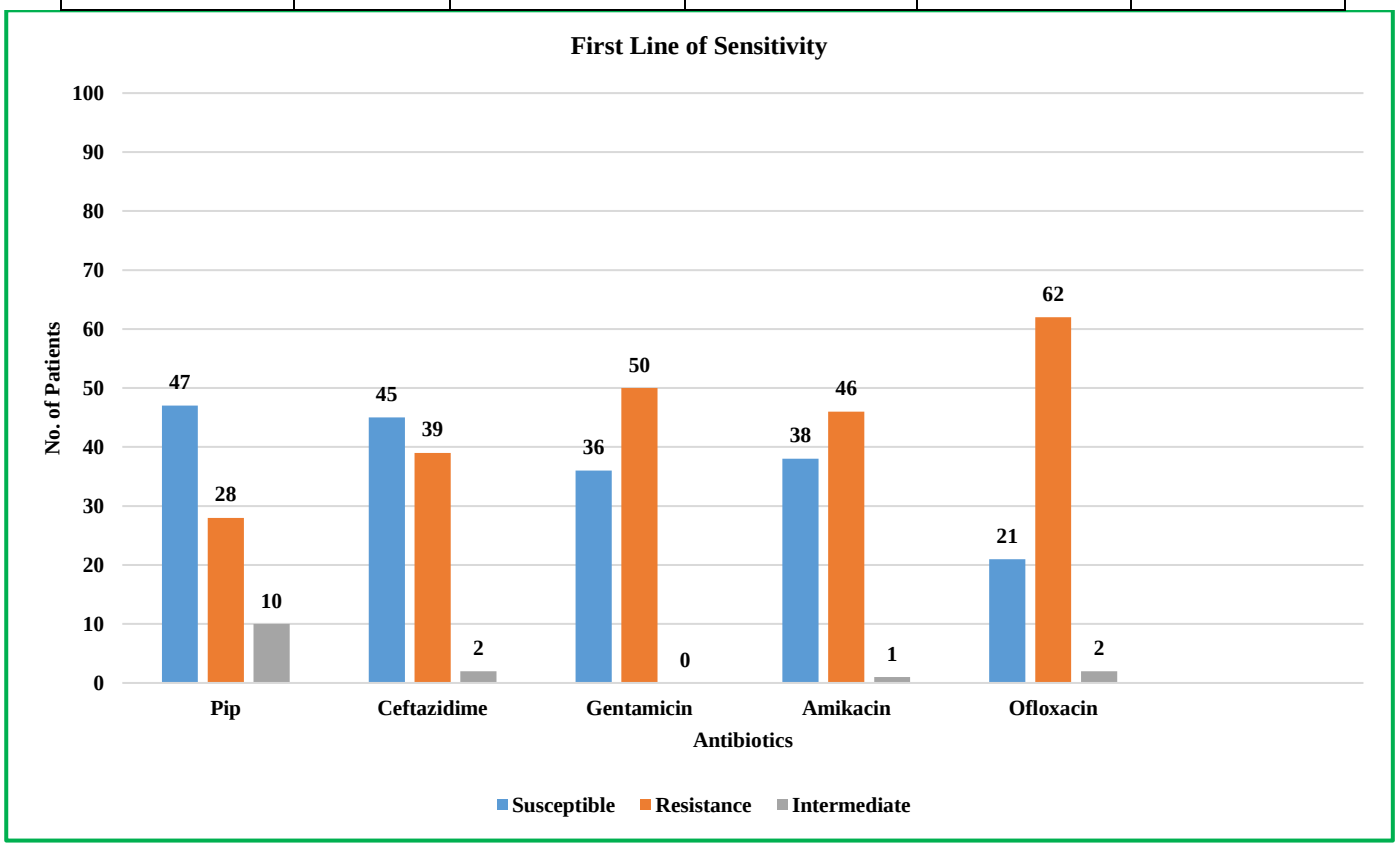


Figure 4: Second line of Sensitivity Pattern of NLF oxidase positive *Pseudomonas aeruginosa*

	PTZ	Aztreonam	Cefepime	Imipenem	Meropenem
Susceptible	17	14	12	14	6
Resistance	6	9	16	11	23
Intermediate	6	6	1	4	0

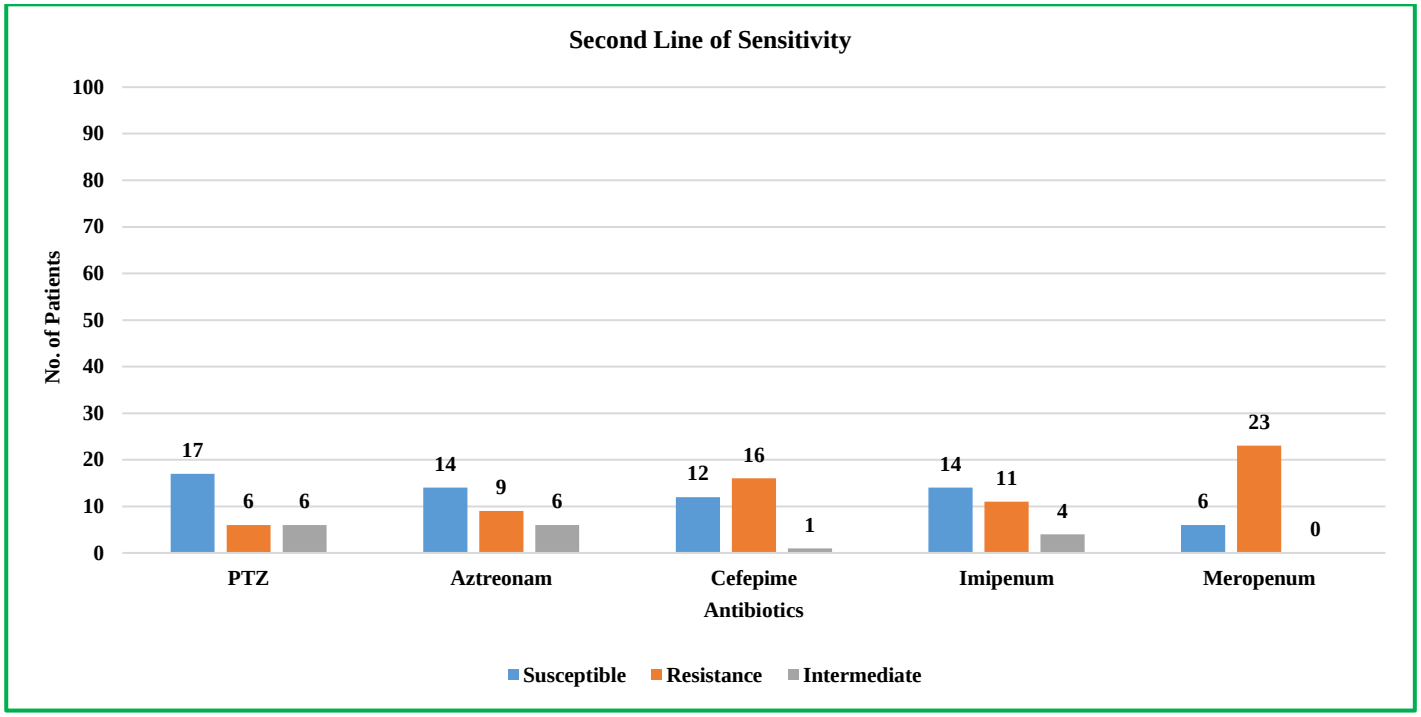


Figure 5: First Line of Sensitivity Pattern of NF *Acinetobacter spp.*

	Ampicillin-sulbactam (Amsu)	Ceftazidime	Gentamicin	Ciprofloxacin	Levofloxacin	Imipenem	Meropenem
Susceptible	1	2	5	1	1	3	2
Resistance	4	4	1	5	4	2	4
Intermediate	1	0	0	0	1	1	0

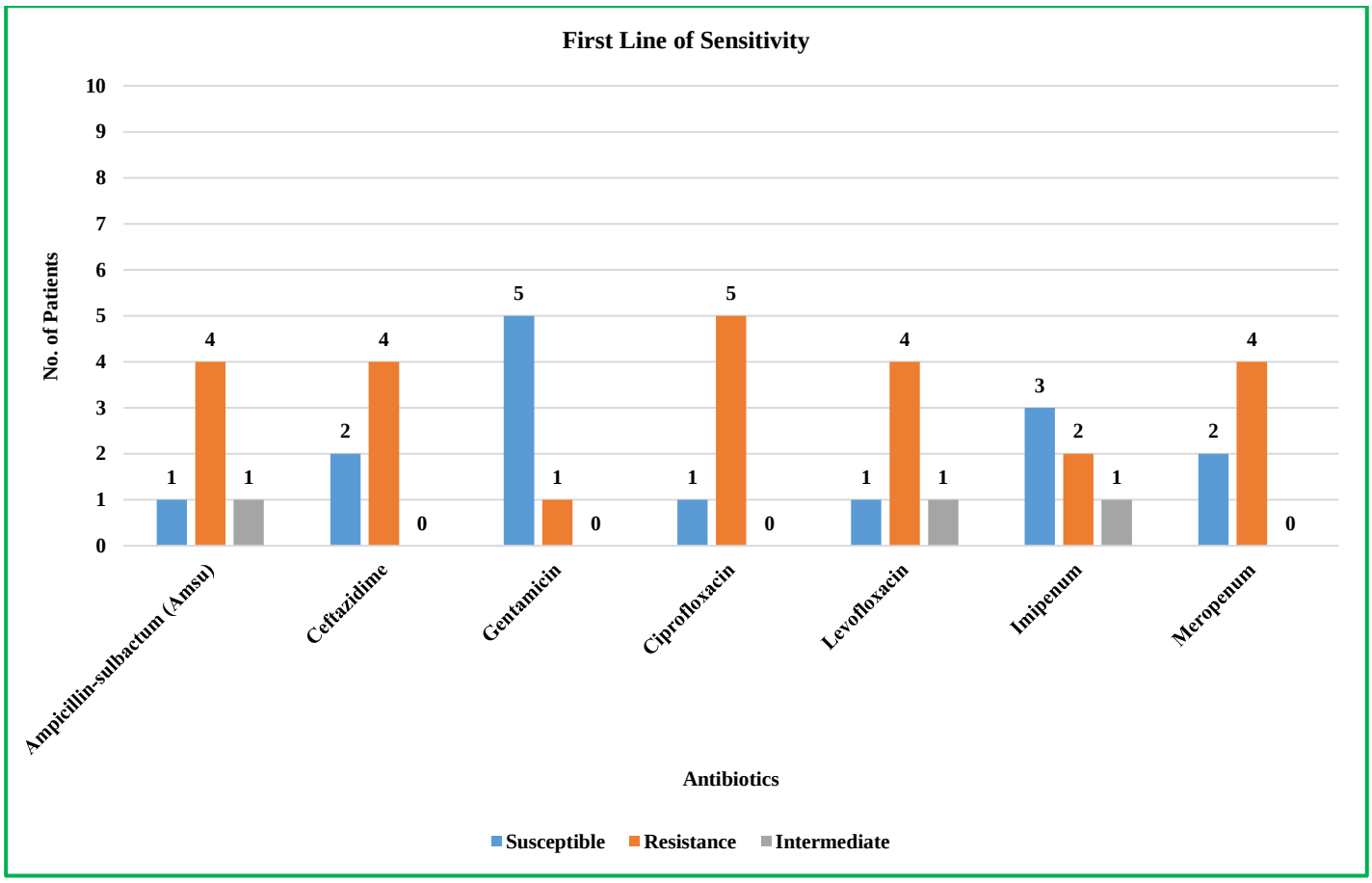


Figure 6: Second Line of Sensitivity Pattern of NF *Acinetobacter spp*

	Cefotaxime	Amikacin	Cotrimoxazole	Cefepime	Piperacillin - tazobactam (Ptz)
Susceptible	1	1	3	1	2
Resistance	4	4	2	4	3

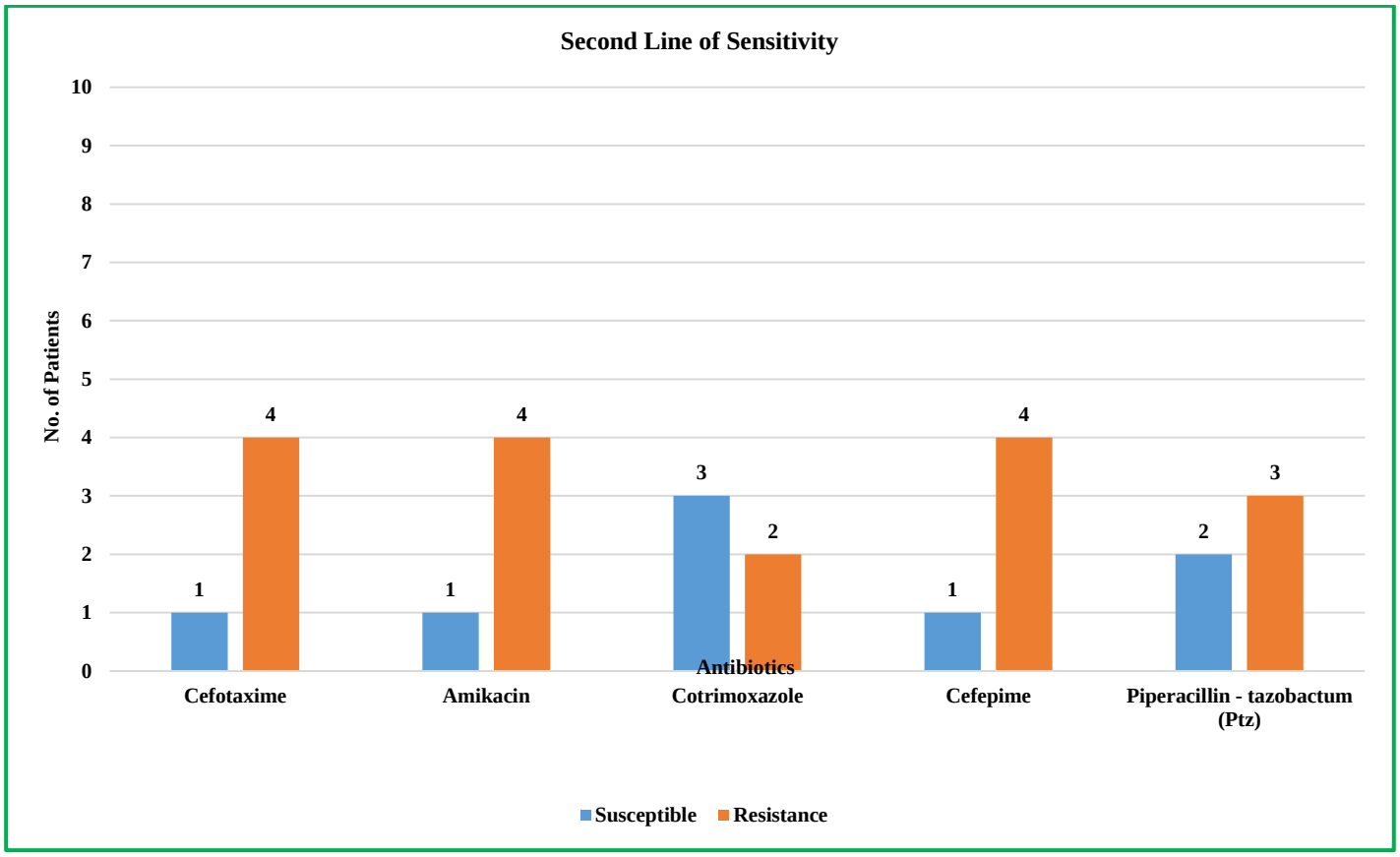


Figure 7: First Line of Sensitivity Pattern of *Staphylococcus spp.*

	Penicillin	Cefoxitin	Oxacillin	Cotrimoxazole	Nitrofurantoin
Susceptible	0	20	20	24	47
Resistance	50	30	30	23	3
Intermediate	0	0	0	3	0

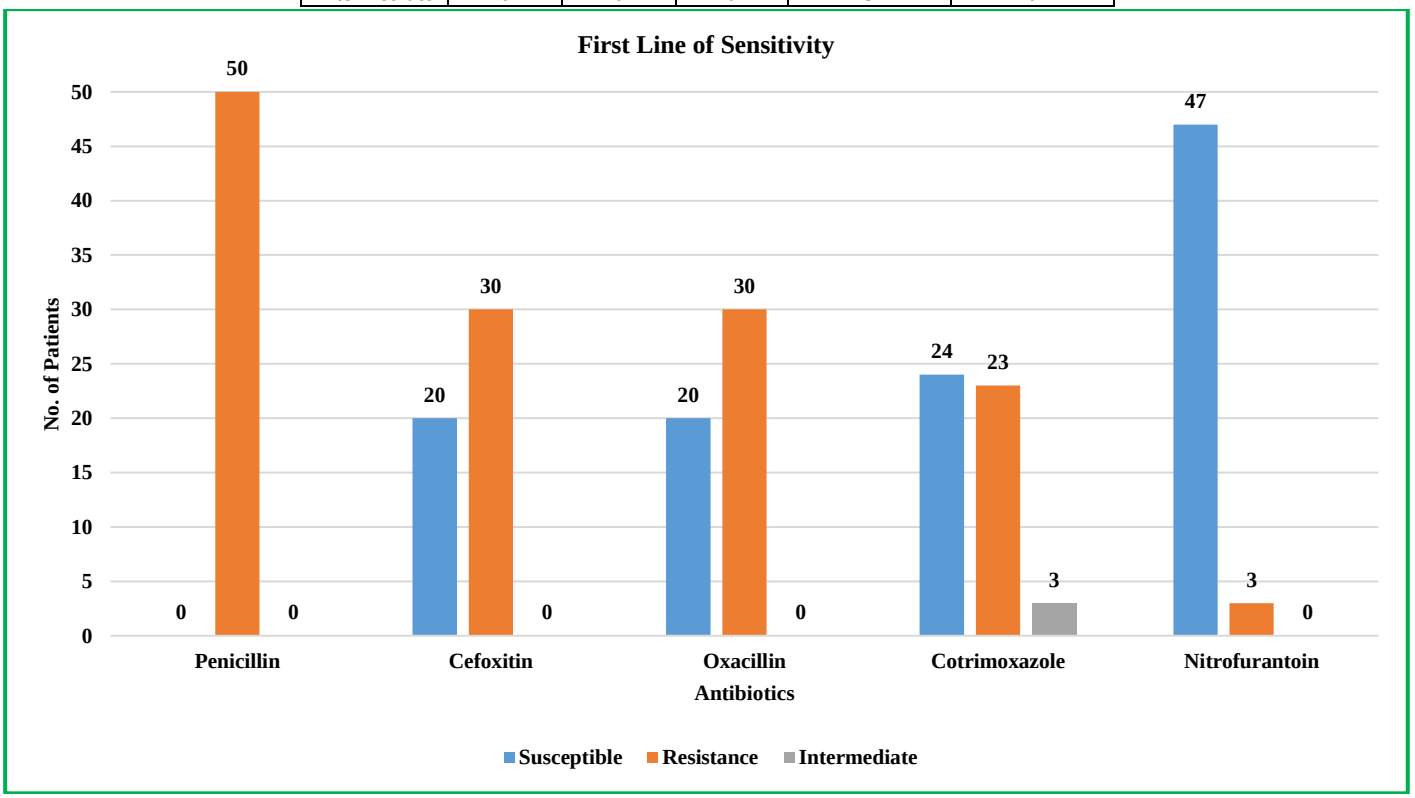


Figure 8: Second Line of Sensitivity Pattern of *Staphylococcus spp.*

	Vancomycin	Linezolid	Ciprofloxacin	Netilmicin	Moxifloxacin	Gentamicin
Susceptible	7	7	5	7	7	4
Resistance	0	0	2	0	0	3

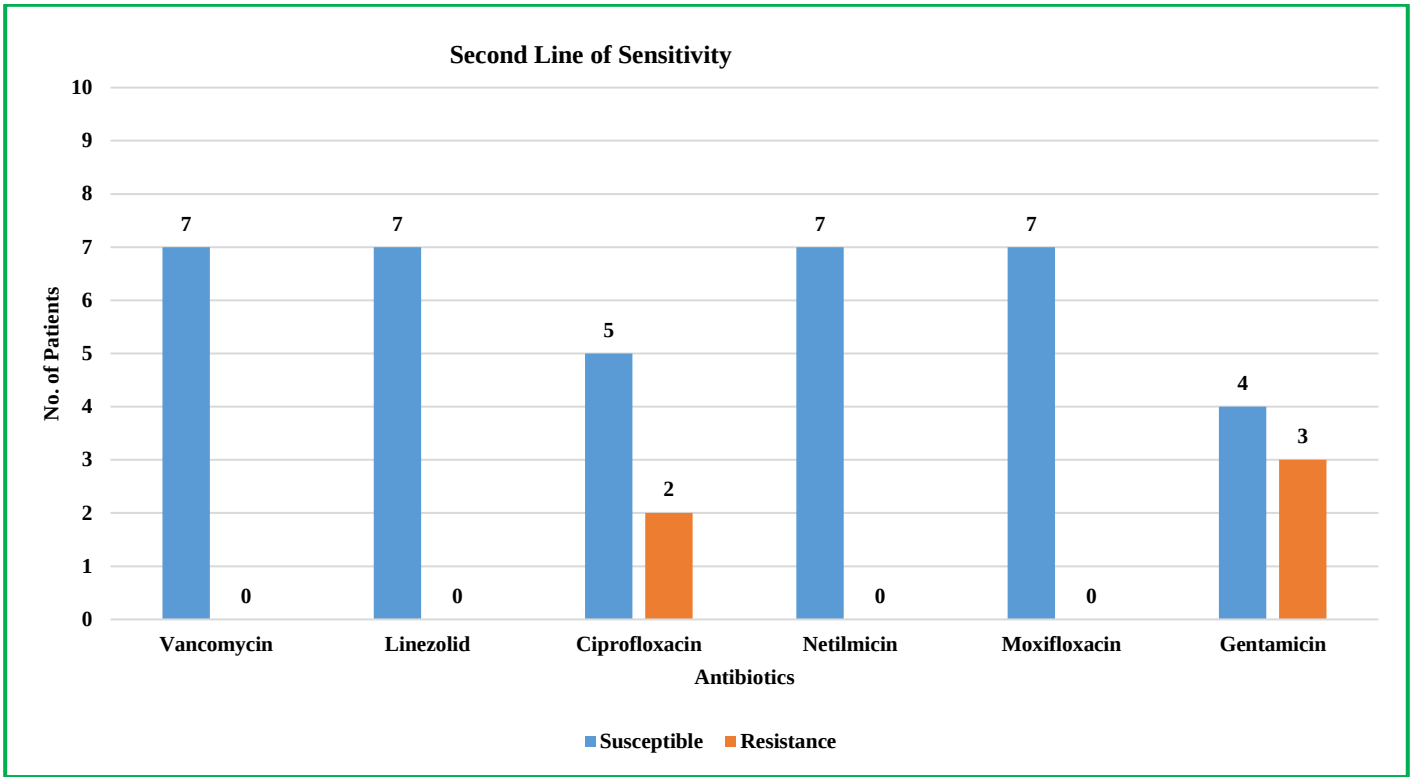
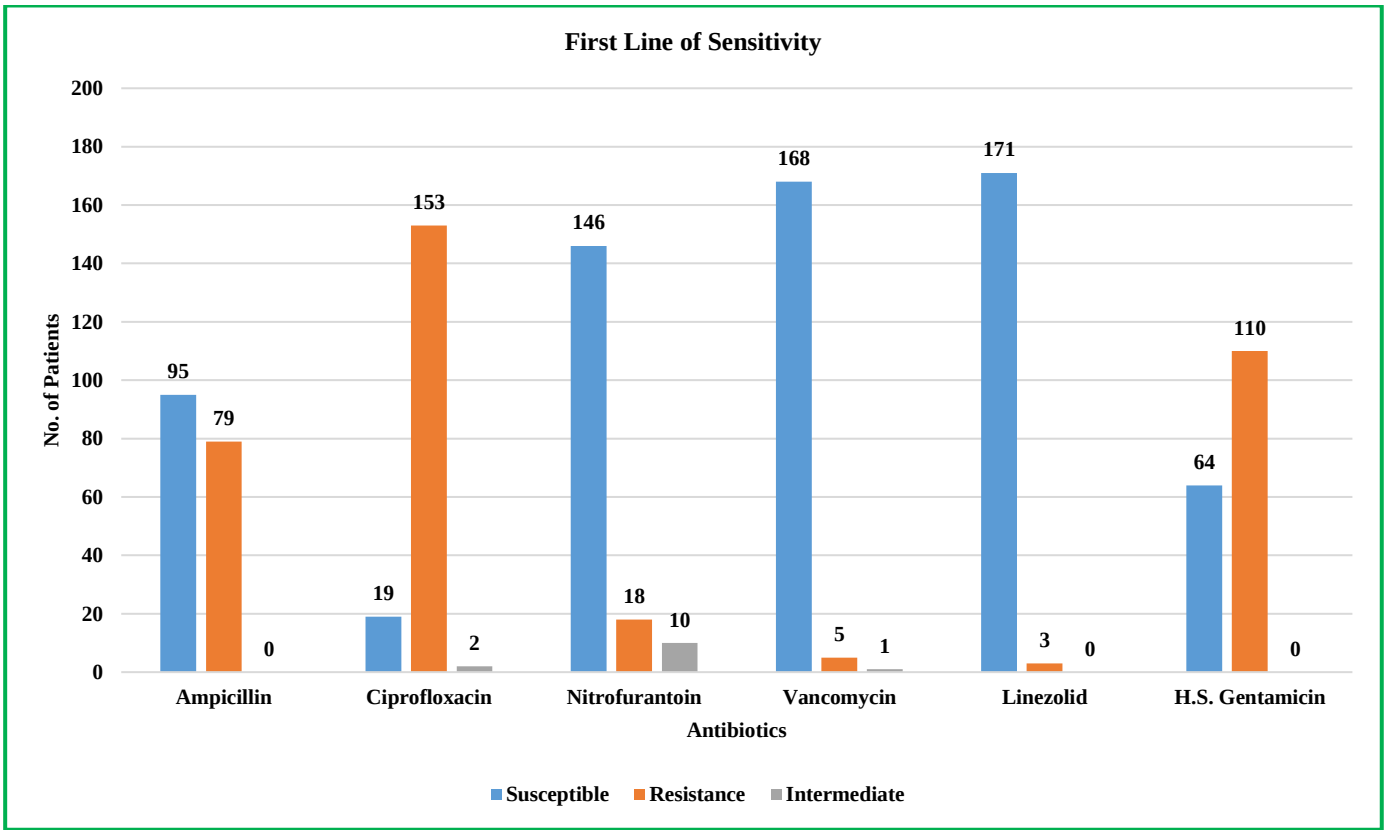


Figure 9: Sensitivity Pattern of *Enterococcus spp.*

	Ampicillin	Ciprofloxacin	Nitrofurantoin	Vancomycin	Linezolid	H.S. Gentamicin
Susceptible	95	19	146	168	171	64
Resistance	79	153	18	5	3	110
Intermediate	0	2	10	1	0	0



In Non-Fermenter (NF) it is shown that in the First line of sensitivity Gentamicin shows the highest sensitivity among all patients followed by Imipenem, Ceftazidime, Meropenem, Piperacillin sulbactam, Ciprofloxacin, and Levofloxacin, whereas in the second line of sensitivity it is showed that Co-trimoxazole shoed the highest sensitivity followed by Piperacillin tazobactam, Cefotaxime, Amikacin, Cefepime among all patients as shown in Figure 5 and Figure 6.

In *Staphylococcus* spp., it is shown that in the First line of sensitivity Nitrofurantoin shows the highest sensitivity among all patients followed by Co-trimoxazole, Cefoxitin, and Oxacillin, whereas in the second line of sensitivity it is showed that Vancomycin showed the highest sensitivity followed by Linezolid, Netilmicin, Ciprofloxacin, and Gentamicin showed the least sensitivity among all patients as shown in Figure 7 and Figure 8.

In *Enterococcus* spp., it is shown that in the First line of sensitivity Linezolid shows the highest sensitivity among all patients followed by Vancomycin, Nitrofurantoin, Ampicillin, H.S. Gentamicin, and Ciprofloxacin showed the least sensitivity among all patients as shown in Figure 9.

DISCUSSION

The Institute of Medical Sciences, Banaras Hindu University (IMS-BHU), is a leading healthcare institution in North India. It serves not only the local population of Varanasi but also patients from surrounding regions. A significant number of these patients arrive after receiving inappropriate or incomplete antibiotic treatments elsewhere, leading to a high incidence of partially treated or mismanaged cases.

This study was undertaken to assess the current antimicrobial sensitivity patterns of uropathogens isolated from patients referred to IMS-BHU. The most frequently isolated organism was *Escherichia coli* (56.62%). However, the prevalence of *Enterococcus* spp. (15.77%), *Klebsiella* spp. (9.82%), *Pseudomonas aeruginosa* (7.84%), *Staphylococcus* spp. (4.50%), *Proteus* spp. (2.16%), *Citrobacter* spp. (1.98%), *Morganella morganii* (0.63%), *Acinetobacter* spp. (0.54%), and *Micrococci* (0.09%) was notably higher compared to other studies. This suggests a possible shift in the uropathogen profile, with *Enterococcus* and *Klebsiella* spp. increasingly replacing *E. coli* as dominant pathogens.

Antibiotic sensitivity testing revealed that:

Nitrofurantoin and *Imipenem* were most effective against *Enterobacteriales*.

Piperacillin and *Piperacillin-Tazobactam* were effective against *P. aeruginosa*.

Gentamicin and *Co-trimoxazole* showed efficacy against *Acinetobacter* spp.

Nitrofurantoin and *Vancomycin* were effective against *Staphylococcus* spp.

Linezolid was the most effective against *Enterococcus* spp.

Notably, *Nitrofurantoin*, a long-standing oral antibiotic, demonstrated high efficacy, with sensitivity observed in 70–75% of outpatient isolates. Its low resistance rate globally (0–5.4%) is attributed to its localized action within the urinary tract, making it a cost-effective and reliable first-line treatment for UTIs [11, 12].

The growing resistance of uropathogens to commonly used antibiotics is a pressing public health issue in India. According to the Infectious Diseases Society of America (IDSA) 2011 guidelines, empirical use of an antibiotic is discouraged if resistance exceeds 20%. Alarmingly, most antibiotics evaluated in this study surpass this threshold, rendering current empirical treatment guidelines inadequate for this population.

This highlights the urgent need for large-scale surveillance studies and a revision of national treatment protocols to ensure effective management of UTIs.

CONCLUSION

There is a critical need for continuous surveillance of microbial culture and antimicrobial susceptibility patterns across diverse healthcare settings in India. Such monitoring is essential to detect shifts in pathogen prevalence and resistance trends, particularly in the context of rising multidrug-resistant (MDR) organisms. Establishing robust antimicrobial stewardship programs (ASPs) at institutional and regional levels can facilitate evidence-based prescribing practices and curb the misuse of antibiotics.

Furthermore, public health initiatives should prioritize community education campaigns aimed at improving awareness regarding the importance of adherence to prescribed antimicrobial regimens. These programs should emphasize the consequences of incomplete or inappropriate antibiotic use, which contribute significantly to the emergence and dissemination of resistant strains.

To inform local treatment guidelines and optimize empirical therapy, region-specific epidemiological studies are imperative. These studies should focus on characterizing the resistance profiles of prevalent uropathogens and other clinically significant bacteria. The integration of such data into national surveillance networks will support the development of dynamic, context-specific antimicrobial policies and enhance the overall effectiveness of infection control strategies.

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Conflict of interests

The authors declare no conflicts of interest.

Data Availability

There are no additional data, the data presented in the methods of this manuscript are presented in the paper.

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