Research Article

Study Of Antimicrobial Resistance And Antibiotic Sensitivity Patterns Among Urinary Isolates With Esbl Producers

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Abstract

Background: Urinary tract infections (UTIs) are among the most common bacterial infections worldwide. The emergence of Extended Spectrum Beta-Lactamase (ESBL)-producing organisms has become a major challenge due to their multidrug-resistant nature and limited treatment options.

Objective: To determine the prevalence of ESBL-producing urinary isolates and evaluate their antimicrobial susceptibility patterns among patients with urinary tract infections.

Materials and Methods: A cross-sectional study was conducted from March 2023 to May 2024. A total of 230 urine samples were collected from outpatient and inpatient departments of Kidney Stone Hospital, Prayagraj. Samples were processed using standard microbiological methods. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method according to CLSI guidelines. ESBL production was confirmed using the Phenotypic Confirmatory Disc Diffusion Test.

Results: Among 230 urine samples, 197 showed significant bacterial growth. Gram-negative bacilli constituted 95.4% of isolates. Escherichia coli was the predominant pathogen followed by Citrobacter spp. and Klebsiella spp. High susceptibility was observed towards amikacin, nitrofurantoin, cefoperazone-sulbactam and meropenem. ESBL production was detected in 54.4% (56/103) of E. coli and 55% (11/20)of Klebsiella spp. isolates. ESBL-producing organisms exhibited significantly higher resistance to commonly used antibiotics compared to non-ESBL producers.

Conclusion: The study demonstrated a high prevalence of ESBL-producing urinary pathogens with increased resistance to commonly used antibiotics. Amikacin, nitrofurantoin, cefoperazone-sulbactam, and meropenem showed the highest antimicrobial activity. Regular surveillance of antimicrobial susceptibility patterns is important to support appropriate antibiotic therapy.

Keywords: Urinary tract infection, ESBL, antimicrobial resistance, E. coli, antibiotic susceptibility.

1. Introduction

Urinary tract infection (UTI) is one of the most common bacterial infections affecting millions of individuals annually. It is a major cause of morbidity in both community and hospital settings. Women are more frequently affected due to anatomical predisposition, while elderly individuals and catheterized patients are at increased risk of complicated infections.

Escherichia coli remains the leading cause of UTI worldwide, accounting for approximately 70–90% of community-acquired infections. Other commonly isolated organisms include Klebsiella spp., Proteus spp., Citrobacter spp., Enterobacter spp., Pseudomonas aeruginosa and Acinetobacter spp..

The increasing emergence of antimicrobial resistance among urinary pathogens has become a major public health concern. One of the most important mechanisms of resistance is the production of Extended Spectrum Beta-Lactamases (ESBLs). These enzymes hydrolyze extended-spectrum cephalosporins and monobactams, rendering these antibiotics ineffective.

ESBL-producing organisms are frequently associated with resistance to multiple antimicrobial classes, including fluoroquinolones, aminoglycosides and sulfonamides. Consequently, infections caused by ESBL-producing bacteria are associated with prolonged hospitalization, increased healthcare costs and poor clinical outcomes.

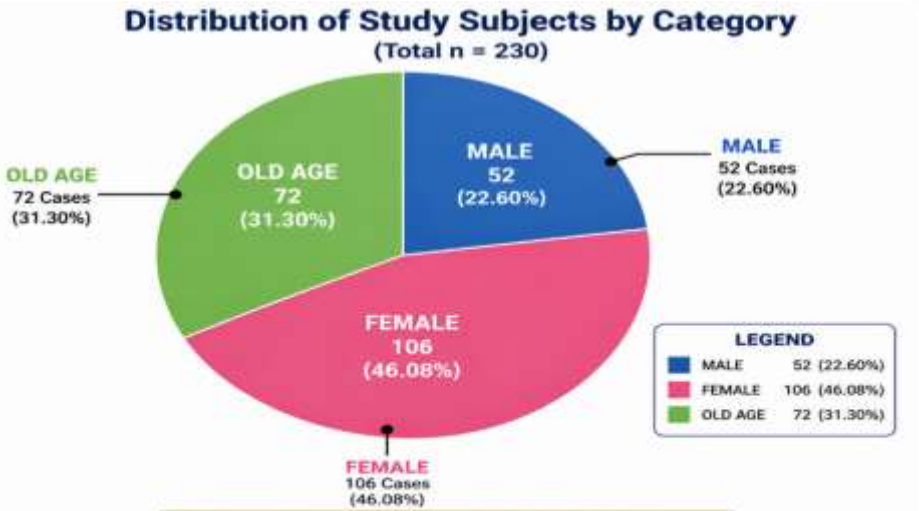
The present study was undertaken to identify urinary pathogens, evaluate their antimicrobial susceptibility patterns and determine the prevalence of ESBL-producing isolates among patients with urinary tract infections.

2. Materials And Methods

A study was conducted in the Department of Microbiology, Kidney Stone Hospital, Prayagraj from March 2023 to May 2024.

Study Population

A total of 230 urine samples were collected from patients clinically suspected of urinary tract infections attending outpatient and inpatient departments of Kidney Stone Hospital, Prayagraj. Sample Collection and Processing Clean-catch midstream urine specimens were collected aseptically in sterile containers and transported immediately to the microbiology laboratory.



DETAILS OF STUDY SUBJECTS

CATEGORIES	OPD NO. (%)	IPD NO. (%)	TOTAL NO. (%)
MALE	32 (13.91%)	20 (08.69%)	52 (22.60%)
FEMALE	36 (15.65%)	70 (30.43%)	106 (46.08%)
OLDAGE	22 (09.56%)	50 (21.73%)	72 (31.30%)
TOTAL	90 (39.14%)	140 (60.86%)	230 (100%)

Microscopy and Culture

For microscopic analysis, 5 mL of urine was transferred into a sterile centrifuge tube and centrifuged at 3000 rpm for 5 minutes. Following centrifugation, the supernatant was discarded, and a drop of the sediment was placed on a clean glass slide and covered with a coverslip. The preparation was examined under a light microscope using 10× and 40× objectives. Samples showing more than five pus cells per high-power field (HPF) were considered eligible for further microbiological investigation.

Culture Technique

Urine specimens containing >5 pus cells/HPF were cultured on Blood agar, MacConkey agar, and CLED agar using a calibrated inoculating loop delivering 0.001 mL of urine. The inoculated plates were incubated aerobically at 37°C for 24 hours. After incubation, culture plates were examined for bacterial growth and the presence of potential urinary pathogens.

Identification of Isolates

Preliminary identification of isolates was based on colony morphology, pigmentation, and growth characteristics observed on , MacConkey agar, and CLED agar. Definitive identification was performed using standard biochemical and microbiological tests, including Gram staining, lactose fermentation, oxidase activity, citrate utilization, motility, indole production, urease activity, pigment production, and Triple Sugar Iron (TSI) reactions.

Gram-positive organisms were further characterized using catalase, coagulase, test. Prior to organism identification, colony counts were determined using a calibrated wire loop to assess significant bacteriuria. (0.001ml) method. The number of colonies grown were counted and interpreted as CFU/ml of urine by multiplying the colonies grown by 1000. Colony counts ≥ 10^5 CFU/mL (100,000 CFU/mL) were taken as significant bacteriuria

Antimicrobial Susceptibility Testing

Susceptibility testing was performed using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar following CLSI recommendations.

The antimicrobial susceptibility patterns of bacterial isolates were evaluated using different antibiotic disc according to the bacterial species identified.

Members of the Enterobacteriaceae family were tested against Amoxicillin-Clavulanic acid (AMC), Co-trimoxazole (SXT), Ciprofloxacin (CIP), Ceftriaxone (CRO), Cefotaxime (CTX), Ceftazidime (CAZ), Nalidixic acid (NA), Nitrofurantoin (F), Cefuroxime, Gentamicin, Azithromycin, and Imipenem (IPM).
For Pseudomonas spp., susceptibility testing was performed using Amoxicillin-Clavulanic acid (AMC), Ciprofloxacin (CIP), Ceftriaxone (CRO), Cefotaxime (CTX), Ceftazidime (CAZ), Nitrofurantoin (F), Nalidixic acid (NA), Gentamicin, Azithromycin, and Imipenem (IPM).

Detection of ESBL
Initial screening for ESBL-producing isolates was performed using Cefotaxime (30 µg) and Ceftazidime (30 µg) discs by the Kirby–Bauer method. Zone diameters were interpreted according to CLSI criteria.
Isolates producing inhibition zones of less than 21 mm with Ceftazidime and less than 26 mm with Cefotaxime were considered potential ESBL producers and were selected for further confirmation.

Detection and interpretation of ESBL production
ESBL production was confirmed using the double disc synergy test.
After incubation, the plates were examined for any change in the zone of inhibition around the antibiotic discs. ESBL positive isolates A clear increase in the zone of inhibition of cefotaxime towards the amoxicillin,clavulanic acid disc was considered positive. This produces shows a characteristic keyhole appearance, indicating ESBL production. This occurs due to inhibition of the ESBL enzyme by clavulanic acid, which enhances the activity of the antibiotic.

Number Of Organisms Producing Esbl

ORGANISM	IPD	OPD
Escherichia coli*	28	28
Klebsiella spp.*	5	6
Citrobacter spp.	4	2
Enterobacter spp.	0	2
TOTAL	**37**	**38**

3. Results

Among 230 urine samples processed, 197 yielded significant bacterial growth. Females constituted the majority of UTI cases. Gram-negative bacilli accounted for 188 isolates (95.4%), while Gram-positive cocci accounted for only 9 isolates (4.6%).
Escherichia coli was the most common pathogen isolated (52.3%), followed by Citrobacter spp. (15.2%) , Klebsiella spp. (10.2%).and (22.3%) consists of the other organisms (like - Pseudomonas spp. Proteus spp. Enterococcus spp. Staphylococcus aureus)
Amikacin demonstrated the highest sensitivity among Gram-negative isolates, followed by cefoperazone-sulbactam and nitrofurantoin. Resistance to ampicillin, co-trimoxazole was markedly high.
ESBL production was detected in 56 of 103 E. coli isolates and 11 of 20 Klebsiella isolates. ESBL-producing isolates showed significantly higher resistance to the antimicrobial agents tested than non-ESBL-producing isolates.

NOTE:-A total of 230 clinically suspected cases of urinary tract infection (UTI) were enrolled in the study, comprising both in-patients (IPD) and out-patients (OPD). Among these, 11 urine samples showed growth of Candida spp. and 22 samples were grossly contaminated; therefore, these samples were excluded from further analysis. Thus, 197 urine samples exhibiting significant and/or doubtful significant bacteriuria were considered eligible for the study. Of these 197 bacterial isolates, 188 (95.4%) were Gram-negative bacilli and 9 (4.6%) were Gram-positive cocci. The 188 Gram-negative isolates comprised Escherichia coli (103), Klebsiella spp. (20), Citrobacter spp. (30), Enterobacter spp. (15), Acinetobacter spp. (9), Pseudomonas spp. (6), and Proteus spp. (5). The 9 Gram-positive isolates comprised Enterococcus spp. (6) and Staphylococcus aureus (3). For the present analysis, the study was specifically focused on E. coli and Klebsiella spp., which together accounted for 123 isolates (103 E. coli and 20 Klebsiella spp.).
Among the Gram-positive cocci, Enterococcus spp. accounted for 6 (4.5%) isolates, comprising 1 isolate from IPD and 5 isolates from OPD patients, whereas Staphylococcus aureus was isolated in 3 (1.5%) cases.
The distribution of these isolates between IPD and OPD patients is presented in the following table.

Distribution Of Bacterial Agents In Ipd & Opd Of Uti (N=197)

ORGANISM	IPD No.of cases (%)	OPD No.of cases (%)
Gram-negative bacilli		
Escherichia coli*	46 (51.1)	57 (53.3)
Klebsiella spp.*	9 (10)	11 (10.3)
Citrobacter spp.	14 (15.6)	16 (14.9)
Enterobacter spp.	11 (12.2)	4 (3.7)
Acinetobacter spp.	1 (1.1)	8 (7.4)
Pseudomonas spp.	4 (4.4)	2 (1.9)
Proteus spp.	3 (3.3)	2 (1.9)
Gram-positive cocci		
Enterococcus spp.	1 (1.1)	5 (4.7)
Staphylococcus aureus	1 (1.1)	2 (1.9)
TOTAL	**90 (100)**	**107 (100)**

Comparison Of Percentage Resistance To Various Antimicrobial Agents Between Esbl Producers And Esbl Non-Producers.

ANTIBIOTIC	E.coli N=103		Klebsiella spp. N=20	
	ESBL producers N=56 No. of cases (% Resistance)	ESBL non-producers N=47 No. of cases (% Resistance)	ESBL producers N=11 No. of cases (% Resistance)	ESBL non-producers N=9 No. of cases (% Resistance)
Amikacin	8 (14.3)	14 (29.8)	1 (9.1)	0
Gentamicin	43 (76.8)	21 (44.7)	7 (63.6)	4 (44.4)
Nalidixic acid	56 (100)	33 (70.2)	11 (100)	3 (33.3)
Norfloxacin	55 (98.2)	29 (61.7)	10 (90.9)	4 (44.4)
Levofloxacin	55 (98.2)	30 (63.8)	10 (90.9)	4 (44.4)
Chloramphenicol	24 (42.9)	15 (31.9)	1 (9.1)	0
Co-trimoxazole	52 (92.9)	32 (68.1)	11 (100)	4 (44.4)
Nitrofurantoin	12 (21.4)	9 (19.2)	1 (9.1)	5 (55.6)
Ampicillin	56 (100)	33 (70.2)	11 (100)	5 (55.6)
Cefaclor	54 (96.4)	20 (42.6)	11 (100)	3 (33.3)
Cefuroxime	55 (98.2)	19 (40.4)	11 (100)	4 (44.4)
Cefotaxime	54 (96.4)	14 (29.8)	8 (72.7)	2 (22.2)
Amoxicillin-clavulanic acid	28 (50)	16 (34)	6 (54.6)	2 (22.2)
Cefoperazone-Sulbactam	3 (5.4)	11 (23.4)	3 (27.3)	1 (11.1)
Meropenem	0	0	0	0

Elaborates the comparative analysis of ESBL producing and non-producing E.coli and Klebsiella spp. for resistance to various antimicrobial agents. It was found that ESBL producers (both E.coli and Klebsiella spp.) were much more resistant to almost all the antibiotics than their non-ESBL producing counterparts except amikacin. Amikacin was found more resistant (29.8%) in non-ESBL producing E.coli than ESBL producing E.coli (14.3%).

ESBL producing Klebsiella spp. was found to be much less resistant to chloramphenicol and nitrofurantoin (9.1% each) in comparison to ESBL producing E.coli which showed 42.9% and 21.4% resistance for the above drugs respectively.

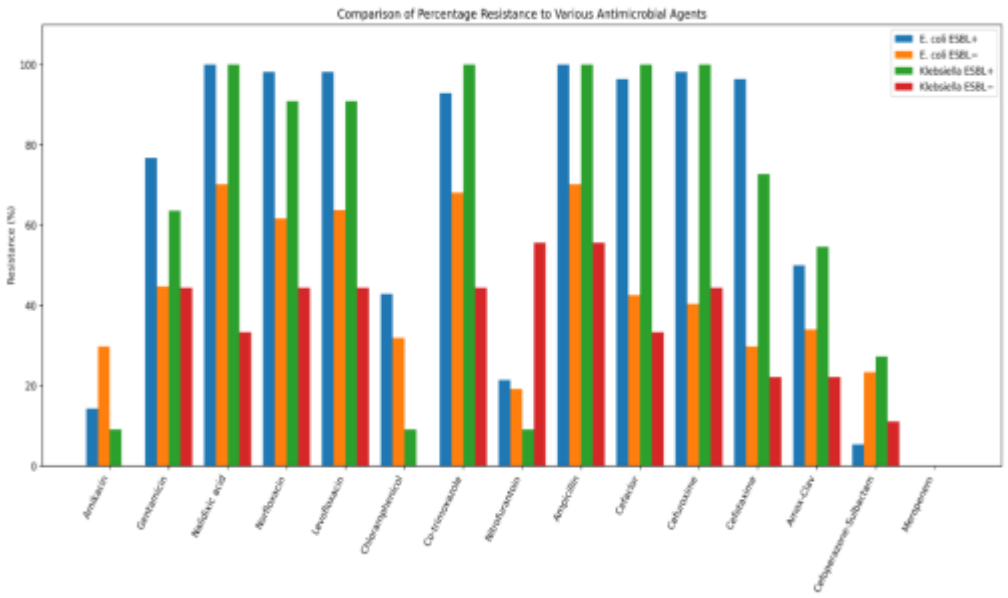
None of the isolates, regardless of ESBL production, was resistant to meropenem.

Comparison Of Percentage Resistance To Various Antimicrobial Agents Between Esbl Producing And Esbl Non-Producing E.Coli And Klebsiella Among Indoor And Outdoor Patients

ANTIBIOTIC	IPD N=68		OPD N=55	
	ESBL producers N=33 No. of cases (% Resistance)	ESBL non-producers N=35 No. of cases (% Resistance)	ESBL producers N=34 No. of cases (% Resistance)	ESBL non-producers N=21 No. of cases (% Resistance)
Amikacin	4 (12.1)	3 (8.6)	5 (14.7)	11 (52.4)
Gentamicin	25 (75.8)	12 (34.3)	25 (73.5)	13 (61.9)
Nalidixic acid	33 (100)	22 (62.9)	34 (100)	14 (66.7)
Norfloxacin	33 (100)	19 (54.3)	32 (94.1)	14 (66.7)
Levofloxacin	32 (96.9)	19 (54.3)	33 (97.1)	15 (71.4)
Chloramphenicol	11 (33.3)	5 (14.3)	14 (41.2)	10 (47.6)
Co-trimoxazole	32 (96.9)	21 (60)	31 (91.2)	15 (71.4)
Nitrofurantoin	7 (21.2)	4 (11.4)	6 (17.7)	10 (47.6)
Ampicillin	33 (100)	22 (62.9)	34 (100)	16 (76.2)
Cefaclor	32 (96.9)	10 (28.6)	33 (97.1)	13 (61.9)
Cefuroxime	32 (96.9)	10 (28.6)	34 (100)	13 (61.9)
Cefotaxime	32 (96.9)	6 (17.1)	30 (88.2)	10 (47.6)
Amoxicillin-clavulanic acid	19 (57.6)	9 (25.7)	15 (44.1)	9 (42.9)
Cefoperazone-Sulbactam	2 (6.1)	6 (17.1)	4 (11.8)	6 (28.6)
Meropenem	0	0	0	0

showing the comparison of percentage resistance to various antimicrobial agents between ESBL producers and ESBL non-producers among indoor and outdoor patients revealed that the ESBL producing isolates were much more resistant to co-trimoxazole and beta lactams than the ESBL non-producing isolates in both the patient groups.

Neither ESBL producers nor ESBL non-producers showed any resistance in both OPD or admitted cases to meropenem. Amongst OPD ESBL producers nalidixic acid, norfloxacin and ampicillin showed 100% resistance, followed by levofloxacin, co-trimoxazole, cefaclor, cefuroxime and cefotaxime more than 96% resistance.



4. Discussion

The predominance of E. coli observed in this study is consistent with previous reports worldwide. The high prevalence of ESBL-producing E. coli and Klebsiella spp. highlights the growing burden of antimicrobial resistance among uropathogens.

The poor susceptibility of isolates to ampicillin, co-trimoxazole and fluoroquinolones indicates that these agents may no longer be suitable for empirical treatment in the study region. Nitrofurantoin and amikacin demonstrated excellent activity against most urinary isolates and may serve as useful therapeutic options.

The complete susceptibility of ESBL-producing isolates to meropenem confirms the continued effectiveness of carbapenems for severe multidrug-resistant infections. However, indiscriminate use of carbapenems should be avoided to prevent the emergence of carbapenem-resistant organisms.

5. Conclusion

The present study demonstrated that Escherichia coli was the predominant uropathogen causing urinary tract infections, followed by Citrobacter spp. and Klebsiella spp. A high prevalence of ESBL-producing isolates was observed, indicating the increasing burden of antimicrobial resistance among urinary pathogens. ESBL-producing isolates exhibited significantly higher resistance to commonly used antibiotics than non-ESBL producers. Among the antimicrobial agents tested, Meropenem, Amikacin, Nitrofurantoin, and Cefoperazone-Sulbactam showed the highest effectiveness against urinary isolates. These findings emphasize the importance of routine urine culture, antimicrobial susceptibility testing, and continuous surveillance of resistance patterns to guide appropriate antibiotic therapy and support antimicrobial stewardship.

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