



The Prevalence of Multidrug-Resistant Isolates of *Escherichia coli* and *Klebsiella pneumoniae* at a Tertiary Care Hospital in Chitwan District, Nepal

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ABSTRACT

Background: The increasing Antimicrobial Resistance (AMR) among *Escherichia coli* and *Klebsiella pneumoniae* is a growing global health concern. This study aims to evaluate the antimicrobial susceptibility patterns of these bacteria isolated at a tertiary care hospital in Nepal.

Methods: This study included 629 non-duplicate *Enterobacteriaceae* isolates, collected from various clinical specimens at Chitwan Medical College Teaching Hospital, Nepal, between February and November 2020. The Kirby-Bauer disk diffusion method was used to assess their AMR.

Results: Among the 629 *Enterobacteriaceae* isolates, *E. coli* was the most common (69.8%), followed by *Klebsiella pneumoniae* (19.9%). *E. coli* showed the highest resistance to penicillins (95.0%), followed by macrolides (70.0%), folate pathway inhibitors (63.6%), cephalosporins (62.6%), fluoroquinolone (61.6%), β -lactamase inhibitors (53.8%), carbapenems (30.8%), and aminoglycosides (21.4%). *K. pneumoniae* also had the highest resistance to penicillins (88.9%), followed by nitrofurans (67.7%), cephalosporins (67.2%), β -lactamase inhibitors (53.3%), folate pathway inhibitors (45.8%), carbapenems (20.8%) and aminoglycosides (19.7%). Polymyxins were the most effective antibiotics against both pathogens. Multidrug resistance was observed in 73.6% of *E. coli* and 69.6% of *K. pneumoniae*.

Conclusion: This study underscores the high level of drug resistance in Nepal, highlighting the urgent need for better infection control.

Keywords: Drug-resistance; *E. coli*; *K. pneumoniae*; Nepal

INTRODUCTION

Escherichia coli and *Klebsiella pneumoniae* are important gram-negative bacteria from the family *Enterobacteriaceae* that normally reside in the human gastrointestinal tract. However, they can cause a variety of infections, including Urinary Tract Infection (UTI), diarrhea, septicemia, pneumonia, peritonitis, meningitis, and infection related to medical devices [1,2].

The misuse of antibiotics both in hospitals and communities has led to the emergence of antimicrobial resistance worldwide. Infections with antimicrobial-resistant bacteria are often serious

and are associated with prolonged hospital stays and increased mortality rates [3]. The spread of drug-resistant microbes has become a global health crisis, especially in countries with limited resources, leading to decreased effectiveness of antimicrobial agents, increased illness and death rates, and increased healthcare costs [3,4].

Beta-lactamases are the enzymes that breakdown β -lactam antibiotics, and their production is a major factor contributing to β -lactam resistance in gram-negative bacteria, including those in the *Enterobacteriaceae* family. Among the group of β -lactamases, Extended-Spectrum B-Lactamases (ESBLs) confer

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resistance to many commonly used antibiotics, such as expanded-spectrum (or third generation) cephalosporins, monobactams and penicillins, but not carbapenems. ESBL-producing bacteria often develop resistance to additional antibiotics, such as fluoroquinolones, aminoglycosides, and sulfonamides [5,6].

ESBLs are commonly found in *Klebsiella* spp. and *E. coli*, as well as in other *Enterobacteriaceae*, including *Enterobacter* spp., *Proteus* spp., *Citrobacter* spp., *Morganella* spp., *Providencia* spp., *Salmonella* spp., and *Serratia* spp. with increasing reports of multidrug-resistant ESBL producing *E. coli* and *K. pneumoniae*, clinicians should be aware of potential treatment failures for serious infections caused by these bacteria [5-7]. Carbapenems, though used as the last resort treatment, have faced increasing resistance due to the production of carbapenemases, which belong to Ambler classes A, B, and D [1].

Assessing the antibiotic resistance of bacterial pathogens from both healthcare facilities and communities is necessary to understand their prevalence and develop effective infection control strategies. In Nepal, antibiotic resistance has become a significant concern. Nevertheless, studies on the drug resistance profile of *Enterobacteriaceae* isolated in Nepal are limited. This study aims to evaluate the antimicrobial susceptibility patterns of *Escherichia coli* and *Klebsiella pneumoniae* from clinical samples at Chitwan Medical College Teaching Hospital (CMCTH), a 750 bed tertiary care hospital in Nepal. The information from this study will be useful for designing better antimicrobial resistance control policies in the country.

MATERIALS AND METHODS

Isolates

This laboratory-based cross-sectional study was conducted at CMCTH from February to November 2020. We isolated a total of 629 non-duplicate *Enterobacteriaceae* isolates from various clinical samples, including urine (n=470), sputum (n=55), pus (n=50), endotracheal aspirate (n=31) and blood (n=23) samples. Those samples were obtained from both outpatients (OPD; n=378) and inpatients (n=251) across various hospital units, such as general medicine (n=81), gynecology and obstetrics (n=54), surgery (n=43), ICU (n=30), pediatric (n=27), orthopedic (n=11), cardiology (n=3) and psychiatric (n=2) units. Standard laboratory techniques [8] were used for the isolation and identification of *Enterobacteriaceae*.

Antimicrobial Susceptibility Test (AST)

AST was performed *via* the Kirby-Bauer disc diffusion method on Mueller-Hinton agar plates according to the guidelines of the Clinical Laboratory Standards Institute (CLSI). The antibiotics tested included carbapenems (imipenem (10 µg) and

meropenem (10 µg)), aminoglycosides (amikacin (30 µg)), cephalosporins (ceftriaxone (30 µg), cefixime (5 µg), cefpodoxime (30 µg), cefoperazone/sulbactam (30 µg), cefoperazone (30 µg), cefotaxime (30 µg), penicillin (ampicillin (10 µg)), penicillins with β-lactamase inhibitors (ampicillin-sulbactam (10/10 µg), amoxicillin-clavulanic acid (10 µg)), antipseudomonal penicillins with β-lactamase inhibitors (piperacillin-tazobactam (100/10 µg)), fluoroquinolones (ciprofloxacin (5 µg), ofloxacin (5 µg)), macrolides (azithromycin (10 µg), folate pathway inhibitors (trimethoprim-sulfamethoxazole (25 µg)), nitrofurantoin (300 µg), polymyxin (colistin (10 µg)) and tigecycline (15 µg)). The results were interpreted according to CLSI guidelines [9].

Multidrug Resistant (MDR) isolates were identified based on criteria from the joint initiative of the European Centre for Disease Prevention and Control and the Centers for Disease Control and Prevention. MDR is defined as non-susceptibility to at least one agent in three or more antimicrobial categories.

RESULTS

Distribution of *Enterobacteriaceae* by demographics, specimens and hospital units

In this study, a total of 629 *Enterobacteriaceae* were recovered from various clinical specimens at CMCTH. The most common species identified were *E. coli* (n=439, 69.8%), followed by *Klebsiella pneumoniae* (n=125, 19.9%), *K. oxytoca* (n=24, 3.8%), *Enterobacter* spp. (n=25, 3.9%), *Proteus mirabilis* (n=10, 1.6%), *Providencia* spp. (n=3, 0.48%), *Salmonella paratyphi* (n=2, 0.32%), and *Citrobacter* spp. (n=1, 0.16%). Among these *Enterobacteriaceae* isolates, 470 (74.7%) were recovered from urine, 55 (8.7%) from sputum, 50 (7.9%) from pus, 31 (4.9%) from ET aspirate, and 23 (3.7%) from blood (Table 1).

The *Enterobacteriaceae* isolates were distributed across age groups as follows: 285 (45.3%) from patients aged 15–50 years, 208 (33.1%) from patients over 50 years, and 136 (15.9%) from those under 15 years. The cohort included 380 (60.4%) females and 249 (39.6%) males (Table 1).

Among the *Enterobacteriaceae* isolates, 378 (60.1%) were from OPD patients, whereas 251 (39.9%) were from inpatients admitted to different units, including the ICU (n=30; 4.8%), general medicine (n=81; 12.9%), gynecology and obstetrics (n=54; 8.6%), surgery (n=43; 6.8%), pediatrics (n=27; 4.3%), orthopedic (n=11; 1.8%), cardiology (n=3, 0.5%), and psychiatric (n=2; 0.3%) (Table 1).

Table 1: Distribution of *Enterobacteriaceae* (n=629) according to demographic characteristics, sample types and hospital units from February to November 2020.

Variables (Number)	Distribution of <i>Enterobacteriaceae</i> isolates No. (%)						
	<i>E. coli</i> (n=439)	<i>K. pneumoniae</i> (n=125)	<i>K. oxytoca</i> (n=24)	<i>Enterobacter</i> spps. (n=25)	<i>Proteus mirabilis</i> (n=10)	Others* (n= 6)	
Gender	Male (249)	161 (64.7)	58 (23.3)	13 (5.2)	10 (4.0)	4 (1.6)	3 (1.2)
	Female (380)	278 (73.2)	67 (17.6)	11 (2.9)	15 (4.0)	6 (1.6)	3 (0.8)
Age group	<15 years (136)	93 (68.4)	26 (19.1)	4 (2.9)	10 (7.4)	0 (0.0)	3 (2.2)
	15–50 years (285)	211 (74.0)	47 (16.5)	8 (2.8)	10 (3.5)	7 (2.5)	2 (0.7)
	> 50 years (208)	135 (64.9)	52 (25.0)	12 (5.8)	5 (2.4)	3 (1.4)	1 (0.4)
Specimens	Urine (470)	372 (79.2)	70 (14.9)	11 (2.3)	7 (1.5)	8 (1.7)	2 (0.4)
	Blood (23)	5 (22.0)	8 (34.8)	0 (0.0)	8 (34.8)	0 (0.0)	2 (8.7)
	Sputum (55)	19 (34.6)	21 (38.2)	7(12.7)	6 (10.9)	2 (3.6)	0 (0.0)
	ET aspirate (31)	11 (35.5)	16 (51.6)	2 (6.5)	2 (6.5)	0 (0.0)	0 (0.0)
	Pus (50)	32 (64.0)	10 (20.0)	4 (8.0)	2 (4.0)	2 (4.0)	1 (2.0)
Hospital units	ICU (30)	9 (30.0)	13 (43.3)	2 (6.7)	5 (16.7)	0 (0.0)	1 (3.3)
	General medicine (81)	55 (67.9)	18 (22.2)	6 (7.4)	2 (2.5)	0 (0.0)	0 (0.0)
	Gynecology and obstetrics (54)	42 (77.8)	10 (18.5)	0 (0.0)	0 (0.0)	1 (1.9)	1 (1.9)
	Pediatrics (27)	18 (66.7)	5 (18.5)	1 (3.7)	4 (14.8)	0 (0.0)	0 (0.0)
	Surgery (43)	29 (67.4)	12 (27.9)	1 (2.3)	1 (2.3)	0 (0.0)	0 (0.0)
	Orthopedics (11)	8 (73.0)	2 (18.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (9.1)
	Psychiatric (2)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Cardiology (3)	2 (75.0)	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
OPD (378)	274 (72.5)	65 (17.2)	14 (3.7)	13 (3.4)	9 (2.4)	3(0.8)	

Note: Other included *Providencia* spp. (n=3), *S. paratyphi* A (n=2) and *Citrobacter* spp. (n=1).

Antimicrobial resistance

The antibiotic resistance patterns of *Enterobacteriaceae* strains isolated from various clinical specimens are detailed in Table 2. *E. coli* showed the highest resistance to ampicillin and cefoperazone (95.0% each), followed by amoxicillin-clavulanic acid (79.7%), cefoperazone-sulbactam (78.5%), ciprofloxacin (77.2%), cefixime (70.0%), azithromycin (70.0%), cefpodoxime (67.9%), trimethoprim-sulfamethoxazole (63.6%), cefotaxime

(62.7%), ofloxacin (55.3%), ceftriaxone (58.4%), and levofloxacin (55.0%). Moderate resistance of *E. coli* was observed for nitrofurantoin (18.3%), amikacin (20.2%), meropenem (29.5%) and imipenem (41.1%). Tigecycline and polymyxin had the lowest resistance rates, at 1.2% and 3.5%, respectively.

Table 2: Resistance rates of *E. coli* and *K. pneumoniae* to drugs of different antimicrobial categories.

Antimicrobial categories	Drugs	<i>E. coli</i> (n = 439)		<i>K. pneumoniae</i> n=(125)	
		No. of tested isolates	No. (%) of resistance	No. tested isolates	No. (%) of resistance
Carbapenems	MEM	383	113 (29.5)	101	17 (16.8)
	IMP	56	23 (41.1)	25	10 (40.0)
Aminoglycosides	AMK	425	86 (20.2)	122	24 (19.7)
Cephalosporins	CTX	324	203 (62.7)	107	60 (56.1)
	CRO	435	254 (58.4)	125	71 (56.8)
	CPD	28	19 (67.9)	10	6 (60.0)
	CPZ/SBT	65	51 (78.5)	21	14 (66.7)
	CFM	77	54 (70.0)	26	17 (65.4)
	CPZ	20	19 (95.0)	4	4 (100.0)
Quinolones/ Fluroquinolones	CIP	123	95 (77.2)	45	19 (42.2)
	LUX	233	128 (55.0)	56	15 (26.8)
	OFX	85	47 (55.3)	23	6 (26.1)
Penicillins	AMP	21	20 (95.0)	9	8 (88.9)
p lactamase inhibitors	AMC	128	102 (79.7)	37	33 (89.2)
	SAM	123	59 (48.0)	29	11 (37.9)
	TZP	385	164 (42.6)	113	53 (46.9)
Macrolides	AZM	30	21 (70.0)	37	26 (70.3)
Polymaxin	PMB	86	3 (3.5)	29	0 (0.0)
	CST	165	0 (0.0)	36	0 (0.0)
Glycylcyclines	TGC	164	2 (1.2)	34	4 (11.8)
Folate pathway inhibitors	SXT	409	260 (63.6)	120	55 (45.8)
Nitrofurans	F	349	64 (18.3)	62	42 (67.7)

Note: AMC: Amoxicillin-Clavulanic acid, AMK: Amikacin, AMP: Ampicillin, AZM: Azithromycin, CFM: Cefixime, CRO: Ceftriaxone, CPD: Cefpodoxime, CPZ/SBT: Cefoperazone/Sulbactam, CPZ: Cefoperzone, CIP: Ciprofloxacin, CST: Colistin, CTX: Cefotaxime, IMP: Imipenem, LVX: Levofloxacin, MEM: Meropenem, F: Nitrofurantoin, OFX: Ofloxacin, TZP: Piperacillin-Tazobactem, SXT: Trimethoprim-Sulfamethoxazole, PMB: Polymyxin B, SAM: Ampicillin-Sulbactam, TGC: Tigecycline

Among the *K. pneumoniae* isolates, the highest resistance rate was observed for cefoperazone (100.0%), followed by amoxicillin-clavulanic acid (89.2%), ampicillin (88.9%), azithromycin (70.3%), nitrofurantoin (67.7%), cefoperazone/sulbactam (66.7%), cefixime (65.4%), cefpodoxime (60.0%), ceftriaxone (56.8%), cefotaxime (56.1%), piperacillin-tazobactam

(46.9%), and trimethoprim-sulfamethoxazole (45.8%). Moderate resistance was observed for levofloxacin (26.8%), ofloxacin (26.1%), and ciprofloxacin (42.2%). Resistance to imipenem, amikacin and meropenem was observed in 40.0%, 19.8%, and 16.3% of the *K. pneumoniae* isolates, respectively. Tigecycline had

the lowest resistance rate, at 11.8%. Neither *E. coli* nor *K. pneumoniae* isolates were resistant to colistin.

Resistance rates for different antibiotic classes

Penicillins had the highest level of resistance, with 95.0% in *E. coli* and 88.9% in *K. pneumoniae*. Cephalosporins were the second most resistant antibiotic, with 62.6% resistance in *E. coli* and 67.2% resistance in *K. pneumoniae*.

Compared with *K. pneumoniae*, *E. coli* presented higher resistance rates to the following antibiotic classes: Macrolides (70.0% vs. 27.3%), folate pathway inhibitors (63.6% vs. 45.8%), fluoroquinolone (61.6% vs. 34.4%), and carbapenems (30.8% vs.

20.8%). However, both bacteria presented similar resistance rates to aminoglycosides, with 21.4% for *E. coli* and 19.7% for *K. pneumoniae*. Glycylcyline had the lowest resistance rates for both *E. coli* (1.2%) and *K. pneumoniae* (11.8%) (Table 3).

Multidrug resistance

Regarding multidrug resistance, 459 of the 629 (70.1%) *Enterobacteriaceae* strains were found to be multidrug resistant. Among the *E. coli* and *K. pneumoniae* isolates, 323 (73.6%) and 87 (69.6%), respectively, exhibited MDR phenotypes (Table 3).

Table 3: Resistance rates (%) of *E. coli* and *K. pneumoniae* isolates against different antimicrobial categories.

Antimicrobial categories	<i>E. coli</i>	<i>K. pneumoniae</i>
Penicillins	95	88.9
Macrolides	70	27.3
Folate pathway inhibitors	63.6	45.8
Cephalosporins	62.6	67.2
Fluroquinolone	61.6	34.4
B-lactamase inhibitors	53.8	53.3
Carbapenems	30.8	20.8
Aminoglycosides	21.4	19.7
Nitrofurans	18.3	67.7
Glycylcylines	1.2	11.8
Polymaxin	0.6	0
MDR	73.6	69.6

DISCUSSION

The emergence of drug-resistant *Enterobacteriaceae*, especially *E. coli* and *K. pneumoniae*, is a significant global public health concern. Our study revealed high rates of multidrug resistance in *E. coli* (73.6%) and *K. pneumoniae* (69.6%) at a tertiary care hospital in the Chitwan district in Nepal. Similar previous studies conducted in different regions of Nepal reported high multidrug resistance rates, ranging from 38.2 to 95.52% for *E. coli* and 25–100% for *K. pneumoniae*.

In our study, we observed resistance rates of more than 50% in *E. coli* for the following six major antibiotic classes: Penicillins (95.0%), macrolides (70.0%), folate pathway inhibitors (63.6%), cephalosporins (62.6%), fluoroquinolones (61.6%), and β -lactamase inhibitors (53.8%). However, these resistance rates were lower than those reported by Baral et al. from Nepal, who reported higher rates for quinolones (ciprofloxacin and ofloxacin, both 92.6%), penicillins (amoxicillin, 94.1%), cotrimoxazole (86.8%), and cephalosporins (cefotaxime, 79.4% and cefixime, 77.9%). In our study, *K. pneumoniae* exhibited the greatest resistance to penicillins (88.9%) and cephalosporins (67.2%), which is consistent with results from a previous study conducted in India.

Carbapenem is a highly effective antibiotic for treating infections caused by MDR gram-negative bacteria, but the emergence of resistance to this antibiotic among

Enterobacteriaceae threatens future treatment options. In our study, we observed a high rate of carbapenem resistance in both *E. coli* (30.8%) and *K. pneumoniae* (20.8%), which aligns with recent findings from neighboring countries such as India and Bangladesh. In contrast, a study from India reported much greater carbapenem resistance in *K. pneumoniae* (53.9%) than in *E. coli* (15.6%). This variation could be due to several factors, such as differences in pathogen prevalence, sampling methods and areas, study designs, and local drug prescription practices and usages.

Colistin, tigecycline, and aminoglycosides are essential for treating carbapenem-resistant infections, yet resistance to these antibiotics is increasingly reported globally. Our study revealed high rates of aminoglycoside resistance in *E. coli* (21.4%) and *K. pneumoniae* (19.7%), highlighting the urgent need for stringent and ongoing monitoring of antibiotic resistance in Nepal. In our study, colistin and tigecycline were the most effective agents against both *E. coli* and *K. pneumoniae*, which is consistent with the findings of a previous study in Taiwan.

The primary factors contributing to the high rates of antimicrobial resistance in our study may include patients' prolonged hospital stays, inappropriate and excessive prescriptions of antibiotics in hospitals, and prior antibiotic therapy. In Nepal, approximately 30% of individuals who visit healthcare facilities have received prior antibiotic treatment. Moreover, antibiotics are readily available without prescription

at drugstores and community pharmacies, often at subtherapeutic doses. This can contribute to the emergence of multidrug resistance in the community, which may then spread to the environment and animals, continuing the cycle of resistance.

The present study revealed a high rate of UTIs caused by MDR pathogens in children and elderly individuals. This could be due to children not being well trained with toilets, which increases the likelihood of ascending infections from the fecal flora. In elderly people, factors such as altered immunity, exposure to nosocomial pathogens, and comorbidities increase the risk of infection. Additionally, the study revealed a greater incidence of UTIs in female patients than in male patients, suggesting that women are more susceptible to UTIs because of the shorter length of the female urethra and frequent colonization of the perineum by enteric pathogens.

CONCLUSION

The high rate of MDR isolation among hospitalized patients in this study indicates that the hospital serves as a reservoir for resistance. The widespread use of antibiotics in various wards, particularly in critical care units, imposes selection pressure and promotes the development of MDR pathogens.

This study has some limitations. First, only clinical samples from patients in the Chitwan district were investigated, which may not accurately reflect the broader drug resistance scenario of Nepal. Furthermore, the study presents phenotypic results but not the molecular characteristics of the isolated bacteria. Investigating these molecular aspects could offer more comprehensive insights into drug resistance in the country.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval was obtained from the Chitwan Medical College Institutional Review Committee before starting the study. All methods were conducted in accordance with relevant guidelines and regulations. Informed consent was obtained from all patients whose clinical samples were used in this study.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this article and its supplementary information file.

COMPETING INTERESTS

The authors declare that they have no conflicting interests.

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No funding was received for this study.

AUTHORS' CONTRIBUTIONS

A.P. designed the study, conducted data analysis and interpretation, and wrote the main manuscript. S.S. performed lab procedures, data collection and analysis. B.N. assisted with data analysis and reviewed the manuscript. All authors read and approved the final version of the manuscript.

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