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ORIGINAL ARTICLE

PHENOTYPIC INSIGHTS INTO BETA-LACTAMASE-MEDIATED MULTIDRUG RESISTANCE IN ESCHERICHIA COLI CLINICAL ISOLATES

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ABSTRACT

Introduction: The global rise of multidrug-resistant (MDR) bacteria is largely attributed to the production of β-lactamases, including extended-spectrum β-lactamases (ESBLs), metallo-β-lactamases (MBLs), and AmpC β-lactamases. This study aimed to evaluate the phenotypic characteristics of β-lactamase-producing MDR *Escherichia coli* isolates and assess their antibiotic resistance profiles.

Method: A cross-sectional study was conducted over six months (November 2021–April 2022) at Manmohan Memorial Teaching Hospital, Kathmandu. Clinical samples were processed using standard microbiological techniques to isolate *E. coli*. The Kirby-Bauer disk diffusion method was used for antimicrobial susceptibility testing. Phenotypic detection of ESBL, MBL, and AmpC β-lactamases was performed using the combined disk method.

Results: Out of 127 E. coli isolates, 55 (43.3%) were identified as MDR. Among these, 26 (47.3%) expressed β-lactamase activity: AmpC (34.54%), MBL (7.27%), and ESBL (5.45%). No co-existence of β-lactamases was observed. High resistance was noted against amoxicillin (100%), cefixime (90.9%), and cotrimoxazole (85.5%), whereas amikacin (90.9%) and meropenem (89.0%) showed strong effectiveness.

Conclusion: AmpC β-lactamase was the predominant resistance mechanism among MDR *E. coli* isolates. Early detection of β-lactamase production is crucial for effective infection control and to curb the spread of MDR pathogens.

Key words: ***MDR E. coli, ESBL, MBL, AmpC***

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INTRODUCTION

Escherichia coli (E. coli) is a clinically significant Gram-negative bacterium that is commonly isolated from human infections and plays a major role in community- and hospital-acquired diseases, including urinary tract infections, bloodstream infections, and gastroenteritis[1]. Over the past few decades, *E. coli* has exhibited a concerning trend toward antimicrobial resistance (AMR), especially multidrug resistance (MDR), which severely limits treatment options[2,3]. The widespread use and misuse of antibiotics have driven the global emergence and dissemination of *E. coli* strains with MDR phenotypes[4,5,6]. These strains are often resistant to multiple classes of antibiotics, including commonly prescribed agents like erythromycin, amoxicillin, and tetracycline[7,8]. In particular, β-lactamase-mediated resistance mechanisms such as extended-spectrum β-lactamases (ESBLs), carbapenemases, and metallo-β-lactamases-have rendered even broad-spectrum β-lactam antibiotics increasingly ineffective[9]. As a result, clinical treatment becomes more difficult, often leading to prolonged hospital stays, increased treatment costs, and higher mortality rates[10].

Antimicrobial resistance has emerged as one of the most pressing public health threats of the 21st century, currently responsible for an estimated 700,000 deaths each year. Alarmingly, this figure is projected to rise to 10 million deaths annually by 2050, with a staggering global economic burden estimated to reach US$100 trillion[11]. In low-resource settings like Nepal, where the irrational and excessive use of antibiotics is prevalent, the impact of AMR is escalating at an alarming pace[12].

A study by Baral et al. (2019) highlighted this growing crisis, revealing a high prevalence of multidrug-resistant *Escherichia coli* isolates. Among these, 25.7% were identified as producers of extended-spectrum β-lactamases (ESBLs), while 11.8% produced metallo-β-lactamases (MBLs)-enzymes that confer resistance to some of the most potent antibiotics[13]. This rise in MDR *E. coli* strains poses a serious threat to public health, particularly as effective therapeutic options become increasingly limited.

Despite increased awareness and efforts to combat AMR, β-lactamase-mediated resistance remains poorly characterized in many clinical settings. The phenotypic expression of such resistance mechanisms in *E. coli* clinical isolates poses diagnostic and therapeutic challenges. A deeper understanding of the phenotypic patterns of β-lactamase production among these isolates is essential to inform targeted antimicrobial therapy and guide infection control strategies.

In Nepal, the growing incidence of MDR *E. coli*, combined with limited local data on phenotypic resistance profiles, underscores the urgent need for surveillance and research. This study seeks to investigate phenotypic traits associated with β-lactamase-mediated resistance in *E. coli* isolates from clinical specimens, offering insight into resistance patterns that may help shape empirical treatment guidelines and containment measures. By identifying the prevalence and resistance patterns, this research aims to contribute to the global effort to manage MDR bacterial infections effectively.

METHODS

A laboratory-based cross-sectional study was conducted among patients visiting Manmohan Memorial Teaching Hospital, Kathmandu, Nepal. Clinical samples-including pus, sputum, and urine that met the criteria recommended by the American Society for Microbiology (ASM) were included for further processing and analysis; those that did not meet the criteria were excluded.

Isolation and Identification of *E. coli*

Clinical specimens (pus, sputum, and urine) were inoculated on MacConkey Agar, Nutrient Agar, and Blood Agar using standard aseptic techniques. The inoculated plates were incubated aerobically at 37°C for 18–24 hours. Suspected colonies showing pink, lactose-fermenting colonies on MacConkey Agar were selected for further identification. Presumptive identification of *E. coli* was performed based on colony morphology, Gram staining (revealing Gram-negative,

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rod-shaped bacteria), and a series of biochemical tests, including indole production, methyl red test, Voges-Proskauer test, citrate utilization, urease test, and triple sugar iron (TSI) agar test. Isolates positive for indole and methyl red, but negative for Voges-Proskauer and citrate tests, with characteristic acid/acid (A/A) reaction on TSI without H2S production, were confirmed as *E. coli* [14,15].

Identification of MDR *E. coli*

Antibiotic susceptibility testing (AST) of E. coli isolates was carried out using the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar (MHA), following Clinical and Laboratory Standards Institute (CLSI) guidelines[16]. A bacterial suspension was prepared and adjusted to a 0.5 McFarland turbidity standard, and uniformly swabbed onto MHA plates. Commercially available antibiotic discs were placed aseptically onto the inoculated surface and incubated at 37°C for 18–24 hours.

The following antibiotics were tested: Amoxicillin (25µg), Oxacillin (5µg), Ceftazidime (30µg), Cefotaxime (30µg), Cefixime (5µg), Cefepime(30µg), Imipenem (10µg), Meropenem (10µg), Tetracycline (30µg), Gentamicin(30µg), Amikacin (30µg), Ofloxacin (5µg), Ciprofloxicin (5µg), Levofloxacin (5µg), Ceftazidime + Clavulanic acid (20µg+10µg). Zones of inhibition were measured and interpreted as sensitive, intermediate, or resistant according to CLSI 2010 breakpoints17. E. coli isolates were classified as multidrug-resistant (MDR) if they exhibited resistance to at least one agent in three or more antimicrobial categories, as defined by Magiorakos et al., 2012[17].

Detection of Extended Spectrum Beta Lactamase (ESBL)

All *Escherichia coli* isolates identified as multidrug-resistant (MDR) were initially screened for ESBL production using the Cefotaxime (30 µg) disk diffusion method on Mueller-Hinton Agar (MHA). A bacterial suspension equivalent to 0.5 McFarland standard was uniformly inoculated on MHA plates. The plates were incubated at 37°C for 18–24 hours, and zones of inhibition were measured. Isolates exhibiting reduced susceptibility to third-generation cephalosporins (e.g., cefotaxime) were considered potential ESBL producers, and results were interpreted according to CLSI guidelines[18].

Confirmation of ESBL production was performed using the combined disk method. Two antibiotic disks were placed 20 mm apart (center to center) on an MHA plate previously inoculated with the test organism:

- Ceftazidime (30 µg)
- Ceftazidime + Clavulanic Acid (30 µg: 20 µg + 10 µg)

After incubation at 37°C for 18–24 hours, the zone diameters were measured. An increase in zone diameter of ≥5 mm with the combination disk (ceftazidime + clavulanic acid) compared to the ceftazidime disk alone was considered positive for ESBL production, indicating inhibition of beta-lactamase activity by clavulanic acid[19].

Detection of Metallo Beta Lactamases (MBL)

Isolates that showed non-susceptibility to Imipenem were considered presumptive MBL producers. Confirmation of MBL production was performed using the combined disk method. In this method, two discs were placed 20 mm apart on Mueller-Hinton Agar (MHA) plates inoculated with the test organism:

- Imipenem (IPM, 10 µg) disc
- Imipenem (10 µg) disc + 10 µL of 0.5 M EDTA (a chelating agent)

After incubating the plates at 37°C for 18–24 hours, the zones of inhibition (ZOI) around both discs were measured. A ≥5 mm increase in the ZOI for the Imipenem disc containing EDTA compared to the Imipenem disc alone was considered confirmatory for MBL production, indicating the ability of the EDTA to enhance the activity of Imipenem by inhibiting the metallo-β-lactamase enzyme[19,20].

Detection of *Amp*C β-lactamase

Isolates that showed non-susceptibility to Cefoxitin (30 µg) were considered presumptive AmpC β-lactamase producers. To confirm AmpC production, the Boronic Acid Disk Test method was used.

In this method, a 30 µg Cefoxitin disc was placed on an inoculated Mueller-Hinton Agar (MHA) plate. A second 30 µg Cefoxitin disc was placed 20 mm away, with 20 µL of 15 µg/mL phenylboronic acid (PBA) solution applied to the disc. After 18–24 hours of incubation at 37°C, the zones of inhibition (ZOI) were measured.

A ≥5 mm increase in the ZOI around the cefoxitin disc with phenylboronic acid, compared to the cefoxitin disc alone, confirmed AmpC β-lactamase production. This increase in inhibition is due to the ability of phenylboronic acid to inhibit AmpC β-lactamase activity, which results in enhanced susceptibility to cefoxitin[19,21].

Ethical consideration

Ethical approval for this study was obtained from the Institutional Review Committee (IRC) of the Manmohan Memorial Institute of Health Sciences (MMIHS), Kathmandu, Nepal, under approval number MMIHS-IRC 631/2078. Prior to participation, the purpose and objectives of the study were clearly explained to each participant. Informed written consent was then obtained from all participants, ensuring their voluntary participation in the study. All procedures followed ethical guidelines and ensured the confidentiality and privacy of patient data.

Data Analysis

Each sample was assigned a unique identification number to ensure confidentiality. The findings were recorded manually and subsequently entered into a secure database for analysis. Data were analyzed using SPSS version 20 (IBM, Armonk, NY). The results were interpreted based on frequency distribution percentages and mean values. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Distribution of MDR *E. coli*

A total of 127 *Escherichia coli* isolates were obtained from various clinical samples. Of these, 55 (43.3%) isolates were identified as multidrug-resistant (MDR), while 72 (56.7%) were non-multidrug-resistant (Non-MDR). The distribution of MDR and Non-MDR *E. coli* across different clinical samples is shown in the table 1. The Chi-square test indicated no statistically significant difference in the distribution of MDR and Non-MDR *E. coli* across different clinical samples.

Table 1: Distribution of MDR and Non-MDR E. coli in Different Clinical Samples

Clinical samples	MDR *E. coli* (%)	Non-MDR *E. coli* (%)	Total Number of *E. coli* (%)	*p-Value*
Urine	50 (43.1)	66 (46.9)	116 (91.3)	0.874
Pus	4 (50.0)	4 (50.0)	8 (6.3)	
Sputum	1 (33.3)	2 (66.7)	3 (2.4)	
Total	55 (43.3)	72 (56.7)	127 (100.0)	

Distribution and Phenotypic Characterization of Beta lactamases producing MDR *E. coli*

Out of the 55 multidrug-resistant (MDR) *E. coli* isolates, 47.3% (n = 26) exhibited beta-lactamase activity. Notably, no MDR isolates were found to coexist with multiple types of beta-lactamases. The phenotypic characterization of beta-lactamase-producing MDR *E. coli* isolates revealed that 34.5% (n = 19) were AmpC β-lactamase producers, followed by 7.3% (n = 4) producing metallo-β-lactamases (MBL), and 5.5% (n = 3) producing extended-spectrum β-lactamases (ESBL). The distribution of these phenotypes across various clinical samples is summarized in Table 2.

Table 2: Phenotypic Characterization of MDR *E. coli* (n = 55)

Clinical samples	ESBL(%)	MBL(%)	AmpC(%)	Total MDR *E. coli*(%)
Urine	03 (5.5)	04 (7.3)	16 (29.0)	23 (41.8)
Pus	00 (0.0)	00 (0.0)	03 (5.5)	03 (5.5)
Sputum	00 (0.0)	00 (0.0)	00 (0.0)	00 (0.0)
Total	03 (5.5)	04 (7.3)	19 (34.5)	26 (47.3)

Antibiogram of MDR *E. coli* isolates (n=55)

Amoxicillin showed the highest resistance (100%) among MDR *E. coli* isolates, followed by Cefixime (90.9%) and Cefotaxime (76.4%). In contrast, Polymyxin B and Colistin sulphate demonstrated 100% sensitivity. Notably, high resistance was also observed to fluoroquinolones-Ciprofloxacin (67.3%), Levofloxacin (65.5%), and Ofloxacin (67.3%)-highlighting the widespread prevalence of multidrug resistance (Table 3).

Table 3: Antibiogram of MDR *E. coli* isolates (n=55)

Antibiotics	Sensitive (%)	Resistant (%)
Amoxycillin	00 (00)	55 (100)
Ceftazidime	16 (29.1)	39 (70.9)
Cefotaxime	13 (23.6)	42 (76.4)
Cefixime	05 (9.1)	50 (90.9)
Aztreonam	35 (63.6)	20 (36.5)
Imipenem	47 (85.5)	08 (14.5)
Meropenem	49 (89.0)	06 (11.0)
Tetracycline	15 (27.3)	40 (72.7)
Gentamycin	40 (72.7)	15 (27.3)
Amikacin	50 (90.9)	05 (9.1)
Ofloxacin	18 (32.7)	37 (67.3)
Levofloxacin	19 (34.5)	36 (65.5)
Ciprofloxacin	18 (32.7)	37 (67.3)
Cotrimoxazole	08(14.5)	47 (85.5)
Cefepime	20 (36.4)	35 (63.6)
Ceftazidime /Clavulanate	17 (31.0)	38 (69.0)

DISCUSSION

This study highlights the growing prevalence of multidrug-resistant *Escherichia coli* in clinical settings. Out of 127 *E. coli* isolates, 43.3% were identified as MDR, which is consistent with findings from similar studies reporting MDR prevalence between 30% to 50% in clinical isolates[22,23,24].The distribution of MDR and non-MDR isolates across clinical samples (urine, pus, and sputum) did not show a statistically significant association, suggesting a widespread emergence of resistance regardless of infection site. Similar studies by Baral SK et al. reported a higher prevalence of multidrug-resistant *E. coli* compared to our findings. In one study, 50.3% of *E. coli* isolates were identified as MDR, many of which also expressed key virulence factors such as hemolysin and biofilm production, especially in extra-intestinal samples[25]. Another study revealed that over 59% of *E. coli* isolates from various clinical specimens showed resistance to multiple antibiotics, including cephalosporins and fluoroquinolones, further confirming the alarming rise in MDR strains in clinical practice[13].

Among the MDR isolates, 47.3% were confirmed to produce β-lactamase enzymes, with AmpC being the most prevalent (34.5%), followed by MBL (7.3%) and ESBL (5.5%). These findings align with previous reports that highlight the increasing occurrence of AmpC producers in nosocomial infections[26,27]. But higher distribution of ESBL producers (25.70%) and MBL producers (11.80%) was reported by Baral et al. in MDR *E. coli* clinical isolates[13]. Notably, no co-production of multiple β-lactamases was observed, which contrasts with studies reporting simultaneous presence of ESBL and MBL or AmpC enzymes in some clinical isolates[28]. The variation may be attributed to differences in regional antibiotic use practices and diagnostic approaches.

The antibiogram revealed alarmingly high resistance to commonly used antibiotics, with Amoxicillin showing 100% resistance, followed by Cefixime (90.9%) and Cefotaxime (76.4%), indicating reduced efficacy of β-lactam antibiotics. These trends are supported by other studies which show similar resistance patterns to penicillins and cephalosporins among MDR *E. coli* strains[29,30]. Conversely, Polymyxin B and Colistin sulphate exhibited 100% sensitivity, reaffirming their role as last-resort drugs against MDR pathogens[31].

A notable resistance was also observed against fluoroquinolones, including Ciprofloxacin (67.3%), Levofloxacin (65.5%), and Ofloxacin (67.3%). These resistance rates are consistent with other regional studies and reflect the overuse and empirical administration of fluoroquinolones in clinical practice[32,33]. Compared to earlier research, our findings differ slightly. For example, Parajuli et al. (2017) and Pandit et al. (2020) reported different prevalence and resistance profiles, possibly due to differences in hospital settings, patient demographics, and sample collection periods[34,35]. These discrepancies emphasize the need for continuous surveillance and local antibiotic stewardship to guide empirical therapy.

CONCLUSION

Overall, the distribution patterns and resistance mechanisms identified in this study underscore the urgent need for robust antimicrobial surveillance and stewardship programs. The predominance of AmpC β-lactamase-producing *E. coli* further highlights the limitations of empiric therapy with third-generation cephalosporins and reinforces the necessity of implementing routine β-lactamase phenotypic screening in clinical laboratories to guide appropriate and effective antimicrobial therapy.

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CONFLICT OF INTEREST

Authors declared, there is no conflict of interest.