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Research Article

CARBAPENEM-RESISTANT AND METALLO- β -LACTAMASES PRODUCING ENTEROBACTERALES FROM CLINICAL SPECIMENS AT MAHAKALI PROVINCIAL HOSPITAL, FAR WESTERN NEPAL

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ABSTRACT

The emergence of Carbapenem-Resistant Enterobacterales (CRE) is a global health threat, including Far Western, Nepal. This study investigated the prevalence of carbapenemase and metallo-β-lactamase (MBL) production among enterobacterales in this underrepresented region. A hospital-based cross-sectional study was conducted at Mahakali Provincial Hospital from November 2024 to February 2026**.** In total, 385 enterobacterales isolates were screened out. Phenotypic carbapenem resistance was determined by the disc diffusion method, while carbapenemase and MBL production were confirmed using the modified Carbapenem Inactivation Method (mCIM) and EDTA-modified CIM (eCIM). Multidrug resistance (MDR) was observed in 55.32% (213/385) of isolates. Phenotypic carbapenem non-susceptibility was detected in 5.71% (22/385), whereas 8.57% (33/385) were confirmed as carbapenemase producers by mCIM. MBL production was identified in 3.12% (12/385) of isolates. Statistical analysis revealed a significant association between MDR and all carbapenem resistance markers with all CRE and 91.67% of MBL producers exhibiting the MDR phenotype. This study identifies a significant reservoir of carbapenem resistant bacterial isolates from clinical samples in Far-Western Nepal. Mandatory integration of low-cost phenotypic screening like mCIM is essential for effective antimicrobial stewardship in resource-limited provincial settings.

Keywords: Carbapenem resistance, mCIM, eCIM, Enterobacterales, Far-Western Nepal

INTRODUCTION

The rapid emergence and global dissemination of Carbapenem-Resistant Enterobacterales (CRE) represents a critical threat to modern medicine, significantly compromising the efficacy of carbapenems, which are often considered the ultimate therapeutic option for multi-drug resistant (MDR) Gram-negative pathogens (Nordmann & Poirel, 2019). Although the formation of extended spectrum β-lactamase (ESBL) activity has traditionally been the main cause of resistance in the Enterobacteriaceae family, the escalating prevalence of carbapenemase-producing strains has shifted the clinical landscape towards higher mortality rates and limited therapeutic options (Acharya et al., 2024). The synthesis of Metallo-β-Lactamase (MBL) is one of the several mechanisms of carbapenem resistance that is particularly concerning. MBLs are characterized by their ability to hydrolyze almost all β-lactams, including carbapenems, while remaining inhibited by metal chelators like EDTA. In South Asia, the high prevalence of New Delhi MBL (NDM) variants has made the management of hospital-acquired infections increasingly difficult (Parajuli et al., 2017).

Geographically, Sudurpashchim Province in Far-Western Nepal remains significantly underrepresented in national antimicrobial resistance (AMR) surveillance data, as most research has traditionally focused on tertiary centers in Kathmandu valley. However, the unique position of Kanchanpur, characterized by high population mobility and an open border with India, creates a potential corridor for the "to-and-fro" transmission of resistant clones. Despite the clinical significance of these pathogens, phenotypic screening for

carbapenemase production using methods like the modified Carbapenem Inactivation Method (mCIM) and the EDTA-mCIM (eCIM) is rarely performed in provincial laboratories. This study aims to determine the prevalence of carbapenemase and MBL production among enterobacterales at Mahakali Provincial Hospital. By evaluating the association between MDR status and these specific enzymatic markers in an under-researched region, this study aims to provide essential data to inform regional antibiotic stewardship and infection control strategies.

MATERIALS AND METHODS

Study site and duration

A cross-sectional study was conducted at Mahakali Provincial Hospital, Kanchanpur, Nepal, from November 2024 to February 2026 among 385 enterobacterales isolated from 1485 different clinical specimens. A total of 1485 clinical samples were collected, and 385 enterobacteriace were analyzed.

Specimen collection and processing

The clinical samples were collected by trained medical personnel at Mahakali Provincial Hospital. The blood, pus, wound swabs, throat swabs, tissue, and urine samples were processed in the hospital's microbiology laboratory for bacterial culture. Standard microbiological techniques were used to isolate and identify the bacteria (Forbes et al., 2007).

Detection of carbapenem resistance

Antibiotic susceptibility testing (AST) was performed to detect carbapenem resistance using the modified Kirby-Bauer method, following CLSI guidelines. Briefly, the inoculum was adjusted to a turbidity equivalent to that of 0.5 McFarland standard. A sterile swab was dipped and rolled into the suspension firmly against the side of the tube. Then, it was swabbed across the entire surface of the sterile Mueller Hinton Agar (MHA) plates (CLSI, 2024).

Carbapenem antibiotic discs, including imipenem disc (10 µg), meropenem disc (10 µg), and ertapenem disc (10 µg), manufactured by Hi Media, India were placed onto the inoculated plates. Plates were incubated aerobically at 35 ± 2°C for 16-18 hours. After an overnight incubation, the diameter of the zone of inhibition was measured in millimeters and classified as susceptible, intermediate, or resistant according to the CLSI guidelines. Isolates showing resistance to at least one carbapenem agent were considered as carbapenem-resistant. Isolates categorized as intermediate or resistant were regarded as carbapenem non-susceptible and were further subjected to carbapenemase detection (CLSI, 2024).

Detection of carbapenemase activity by modified carbapenem inactivation method (mCIM) A loopful of overnight culture of enterobacterales from agar plates was emulsified in 2 mL trypticase soy broth (TSB) in a tube. An antibiotic disc containing 10 µg meropenem was immersed into a TSB tube. It was incubated at 37 °C for four hours. Then, the disc was removed from the tube and it was kept on a sterile MHA plate containing a lawn culture of 0.5 McFarland suspension of *Escherichia coli* ATCC 25922. Plate was incubated for 37ºCfor 24 hours. After incubation, if the test bacterial isolate produced carbapenemase, the meropenem was inactivated and yielded an inhibition zone diameter between 6-15 mm, it was interpreted as positive for carbapenemase production, whereas a non-carbapenemase isolates did not inactivate the meropenem and diameter of inhibition zone increases 16-18 mm was considered as indeterminate and ≥19 mm as negative (CLSI, 2024).

Detection of metallo-β-lactamase (MBL) production

Isolates positive for carbapenemase production by mCIM were further tested for MBL production using the EDTA-mCIM. Briefly, a loopful of test organism was emulsified in sterile 2 mL TSB containing 5mM EDTA followed by the addition of a meropenem (10 µg) disc. The suspension was then incubated at 35 ± 2°C for 4 hours. This antibiotic disc was taken out and kept on the MHA petriplate, which was spread with culture of 0.5 McFarland suspension of *Escherichia coli* ATCC 25922. Incubation was done at 37°C overnight. The zone diameters of inhibition were recorded using a scale. An increase in the diameter of the zone of inhibition ≥5 mm in the eCIM test compared to mCIM was interpreted as indicative of MBL production, whereas an increase of ≤4 mm indicated the absence of MBL activity (CLSI, 2024).

Quality control

Quality control was ensured using *E. coli* ATCC 25922 as the indicator strain for the mCIM and eCIM testing following CLSI guidelines.

Statistical analysis

SPSS (Statistical Package for the Social Sciences) version 24.0 was used to analyze the data after being coded and categorized using Microsoft Excel 2010. Data were presented in numbers and percentages within the table and figure for descriptive analysis. The Chi-square test was used to evaluate the association between variables and Fisher's exact test in case of tables having cell values less than 5, and a p-value of less than 0.05 was deemed statistically significant.

RESULTS

Out of 1485 clinical specimens processed for culture, 385 (25.9%) yielded isolates belonging to the enterobacterales.

Carbapenem resistance and carbapenemase production

Phenotypic carbapenem non-susceptibility (CRE) was detected in 22 (5.71%) of the 385 enterobacterales isolates. The highest proportion of CRE was observed in *K. pneumoniae* (10.94%, 7/64), followed and *E. coli* (4.75%, 14/295). Upon further testing with the mCIM, 33 (8.57%) isolates were confirmed as carbapenemase producers. Notably, several isolates that were not categorized as resistant by disc diffusion, including *Citrobacter* spp. (66.67%, 2/3) and *Serratia* spp. (33.33%, 1/3), tested positive for carbapenemase production by mCIM (Table 1).

Metallo-β-Lactamase (MBL) production

MBL production was confirmed in 12 (3.12%) of the total enterobacterales isolates. Among the MBL producers, *E. coli* was the most common, followed by *P. mirabilis*. Single cases of MBL production were noted in *K. pneumoniae*, *K. oxytoca*, and *Citrobacter* species (Table 1).

Table 1. Carbapenemase and MBL production among Enterobacterales

Organism	MDR n (%)	Carbapenem Resistant (CRE) n (%)	Carbapenemase positive (mCIM) n (%)	MBL positive (eCIM) n (%)
E. coli (n=295)	162 (54.92)	14 (4.75)	17 (5.76)	7 (2.37)
K. pneumoniae (n=64)	34 (53.13)	7 (10.94)	10 (15.36)	1 (1.56)
P. mirabilis (n=10)	7 (70.00)	1 (10.00)	3 (30.00)	2 (20)
Morganella spp. (n=5)	1 (20.00)	0	0	0
Citrobacter spp. (n=3)	2 (66.67)	0	2 (66.67)	1 (33.33)
Serratia spp. (n=3)	3 (100.00)	0	1 (33.33)	0
K. oxytoca (n=2)	2 (100.00)	0	0	1 (50.00)
Salmonella spp. (n=3)	2 (66.67)	0	0	0
Total (n=385)	**213 (55.32)**	**22 (5.71)**	**33 (8.57)**	**12 (3.12)**

Association of MBL with MDR

The prevalence of MDR among the Enterobacterales was 55.32% (213/385). There was a significant association between MDR and all the tested carbapenem resistance markers. Specifically, all 22 CRE isolates were also classified as MDR, showing a strong statistical correlation between CRE status and MDR ($p<0.001$). Similarly, the presence of carbapenemase production by mCIM was significantly higher in the MDR group compared to non-MDR isolates ($p<0.001$). Furthermore, MBL production was observed in the MDR population, with 91.67% (11/12) of confirmed MBL producers being MDR, highlighting the severely limited therapeutic options available for managing these infections (Table 2).

Phenotypic Characters	MDR (n=213) n (%)	Non-MDR (n=172) n (%)	Total (n=385) n (%)	Fisher's Exact Test (p-value)
Carbapenem-Resistant Enterobacterales (CRE)	22 (10.33)	0	22 (5.71)	0.000
Carbapenemase positive (mCIM)	29 (13.62)	4 (2.33)	33 (8.57)	0.000
MBL positive (eCIM)	11 (5.16)	1 (0.58)	12 (3.12)	0.010

Table 2. Distribution of phenotypes of carbapenem resistance

DISCUSSION

This study provides a critical evaluation of the AMR landscape in Far-Western region frequently underrepresented in the national surveillance data. The observed prevalence of MDR among Enterobacterales (55.32%) aligns with the trends reported in major urban centers of Nepal (Acharya et al., 2017; Devkota et al., 2018; Dhungana et al., 2019). However, the specific identification of carbapenemase and MBL-producing isolates at Mahakali Provincial Hospital highlights a significant resistance burden in Far-Western Nepal. A notable observation was the discordance between phenotypic carbapenem resistance (5.71% by disc diffusion) and confirmed carbapenemase production (8.57% via mCIM). This finding suggests a reservoir of isolates that harbor carbapenemase enzymes but do not yet meet the full clinical resistance breakpoints. Such isolates may exhibit low-level phenotypic expression, common in certain NDM or OXA-48 variants, potentially leading to errors in the standard susceptibility testing where a carbapenemase producer is misidentified as intermediate or susceptible (Tamma & Simner, 2018). This discrepancy emphasizes that mCIM is a more sensitive indicator of underlying resistance mechanisms than zone diameter alone, especially in species such as *Citrobacter* spp. and *Serratia* spp.

In this study, the prevalence of MBL was 3.12%, higher than the 1.3% reported by Mishra et al. (2012). However, the higher prevalence was reported as 5.8% by Thapa et al. (2017). These fluctuating prevalence rates highlight the considerable regional and methodological variability across Nepal. Furthermore, the near-total overlap between MBL production and the MDR phenotype confirms these enzymes are typically carried on multi-resistance plasmids, severely limiting therapeutic options in the provincial settings.

The study has certain limitations. Firstly, due to resource constraints in a provincial hospital setting, molecular characterization was not performed to identify specific carbapenemase genotypes. Secondly, being a hospital-based cross-sectional study, the results may not fully represent the broader community-level resistance in Sudurpashchim Province. Despite these limitations, CLSI-recommended phenotypic methods like mCIM and eCIM provide high diagnostic accuracy and serve as a feasible model for AMR surveillance in resource-limited laboratories.

CONCLUSION

This study identifies a significant reservoir of carbapenem resistant enterobacterales in Far Western Nepal. Mandatory integration of low-cost phenotypic screening like mCIM is essential for effective antimicrobial stewardship in resource limited provincial settings.

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AUTHORS CONTRIBUTION

Conceptualization: SS, DRB; Methodology: MS; Formal analysis: MS; Supervision SS, DRB; Validation: SS, DRB; Writing-Original draft: MS; Writing-review and editing: SS, DRB

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

ETHICAL STATEMENT

Nepal Health Research Council (NHRC) granted ethical approval (826/2024). Specimens collected as part of routine clinical care were included after anonymizing each sample prior to analysis.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available from the corresponding author, upon reasonable request.

SUPPLEMENTARY INFORMATION

None

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