e-ISSN 2371-770X

Modern Medical Laboratory Journal

DOI:10.30699/mmlj17.7.1.12

Detection of Metallo-beta-lactamase Producers among Clinical Isolates of Enterobacterales in a Tertiary Care Centre, Nepal

Bijaya Dhakal[1,*], Junu Richhinbung Rai[1,2], Shristi Raut[1,2], Hari Prasad Kattel[2], Sujata Thapa[1], Marichman Bhul[1], Naresh Bahadur Khadka[2], Sangita Sharma[1,2]

1. *Department of Microbiology, Maharajgunj Medical Campus, Institute of Medicine, Kathmandu, Nepal.*
2. *Department of Microbiology, Tribhuvan University Teaching Hospital, Institute of Medicine, Kathmandu, Nepal.*

KEYWORDS

Carbapenemase;

eCIM;

Enterobacterales;

mCIM;

MDR;

Phenotypic detection of

MBL

Article Info

Received 2024/10/25;

Accepted 2024/12/20;

Published Online 2024

ABSTRACT

Background and Objective: Increased reports of metallo-beta-lactamase (MBL)-producing isolates of *Enterobacterales* indicate that there are limited therapeutic options available for the treatment of infections caused by these organisms. To the best of our knowledge, there have been only a limited number of studies on MBL-producing Enterobacterales in Nepal. Therefore, this study was conducted to determine the current distribution of MBL-producing isolates in our setting.

Methods: This was a cross-sectional study conducted over a period of six months (from August 2023 to January 2024) at the Department of Microbiology, TU Teaching Hospital, Nepal. A total of 243 clinical isolates of Enterobacterales from various clinical specimens were subjected to the Modified Carbapenem Inactivation Method (mCIM) and the EDTA-Modified Carbapenem Inactivation Method (eCIM) for the detection of MBL-producing isolates, according to the standard methodology outlined by the latest Clinical and Laboratory Standards Institute (CLSI) guidelines.

Result: A total of 111 (45.70%) isolates were identified as potential carbapenemase producers, based on their resistance or intermediate susceptibility to carbapenems (Meropenem and/or Imipenem) and/or resistance to cephalosporin subclass III (Ceftazidime). Among the total isolates of Enterobacterales, 52 (21.40%) were confirmed as carbapenemase producers by the mCIM method, 45 (18.50%) were confirmed as MBL producers by the eCIM method, while the remaining 7 (2.90%) were identified as serine-carbapenemase producers. Multidrug resistance (MDR) was observed in 151 (62.10%) of the total isolates, and all MBL-producing isolates were also MDR.

Conclusion: This study found a higher percentage of MBL-producing Enterobacterales isolates, along with a high prevalence of multidrug resistance. Regular surveillance and stringent infection control policies are essential to minimize the spread of resistant strains.

Corresponding Information: *Bijaya Dhakal, Maharajgunj Medical Campus, Institute of Medicine, Maharajgunj, Kathmandu, Nepal, Email: dhakalb538@gmail.com.*

Abbreviations

AMR: Antimicrobial Resistance; ASM: American Society of Microbiology; ATCC: American type culture collection; BA: Blood Agar; CA: Chocolate Agar; CAZ: Ceftazidime; CLSI: Clinical and Laboratory Standards Institute; CRE: Carbapenem-resistant Enterobacterales; eCIM: EDTA- Modified Carbapenem Inactivation Method; EDTA: Ethylene Diamine Tetraacetic Acid ESBL: Extended Spectrum β lactamase; GIM: German Imipenemase; HAI: Hospital Acquired Infection; ICU: Intensive Care Unit; IMP: Imipenemase; IOM: Institute Of Medicine; IPM: Imipenem; IRC: Institutional Review Committee; MA: MacConkey Agar; MBL: Metallo Beta Lactamase; mCIM: Modified Carbapenem Inactivation Method; MDR: Multiple Drug Resistant; MDRE: Multidrug-Resistant Enterobacterales; MHA: Mueller Hinton Agar; MHT: Modified Hodge Test; MIC: Minimum inhibitory concentration; MRP: Meropenem; PDR: Pan Drug Resistant; QC: Quality control; SBL: Serine Beta Lactamase; SIM: Seoul Imipenemase; SPM: Sao Paulo MBL; SPSS: Statistical Package for Social Sciences; TSB: Tryptic Soy Broth; TUTH: Tribhuvan University Teaching Hospital; VIM: Verona Integron encoded Metallobetalactamase; XDR: Extensively Drug Resistant; ZOI: Zone Of Inhibition.

Introduction

Infectious disease burden and antimicrobial resistance (AMR) are serious global health problems, particularly in developing countries like Nepal (1,2). The spread of multidrug-resistant *Enterobacterales* (MDRE) is a major cause of both hospital and community-acquired infections, which are associated with high morbidity, mortality, and rising healthcare costs (3-5). Beta-lactams and fluoroquinolones are the primary therapeutic options for treating infections caused by these microorganisms, but the widespread presence of beta-lactamases, which are responsible for beta-lactam resistance, results in the ineffectiveness of these antimicrobials (5,6). Metallo-beta-lactamase (MBL) activity has emerged as one of the most feared resistance mechanisms due to its ability to hydrolyze a broad range of beta-lactams in the presence of zinc ions, including penicillins, cephalosporins, cephamycins, and carbapenems, but not monobactams (e.g., aztreonam) in vitro (7,8). Ethylene diamine tetra acetic acid (EDTA) and other chelating compounds of divalent cations are universal inhibitors of MBLs (9). Inappropriate and unnecessary use of beta-lactam drugs is one of the major causes of bacterial resistance (2,10). Since these resistance genes are often carried by plasmids, they facilitate the spread of resistance (2,11). Furthermore, conventional beta-lactamase inactivators, such as clavulanate, sulbactam, and tazobactam, are ineffective against MBL-producing bacteria. Recently, MBLs have emerged at an increasing rate among the members of Enterobacterales, and clinical data regarding their incidence in these organisms, particularly in Nepal, remain surprisingly scarce. Therefore, this study was conducted to determine the incidence of MBL production and its co-occurrence with multidrug resistance (MDR) among Enterobacterales isolates (12,14,15). Despite the high accuracy and reliability of PCR, its accessibility is often limited to reference laboratories, and this research is part of a thesis with limited funding. Thus, phenotypic methods such as mCIM (Modified Carbapenem Inactivation Method) combined with EDTA-modified carbapenem inactivation method (eCIM), which have high sensitivity and specificity and are recommended by the recent clinical and laboratory standards institute (CLSI) guidelines, were selected over molecular methods. Furthermore, if feasible, AMR gene detection in multiple healthcare centers would be a valuable approach to assess the AMR status in various geographical areas of Nepal (12,13).

Materials and Methods

A laboratory-based, cross-sectional analytical study was conducted at the Bacteriology Laboratory, Department of Microbiology, Tribhuvan University Teaching Hospital (TUTH), Institute of Medicine, Kathmandu, from August 2023 to January 2024. This study was ethically approved by the Institutional Review Board of the Institute of Medicine (Approval No: 77 (6-11) E2 080/081) and by the Head of the Department of Microbiology, Institute of Medicine. The data were analyzed using the Statistical Package for Social Sciences (SPSS), version 16.0. The study excluded repeated samples from patients with identical isolates.

Screening test

Clinical isolates of Enterobacterales obtained from routine culture and susceptibility testing in the laboratory were included in the study. Isolates that were resistant to at least one agent in three or more antibiotic categories were classified as multi-drug resistant Enterobacterales. Screening for carbapenemase production was conducted by identifying isolates resistant or intermediately susceptible to carbapenems, i.e., a Zone of Inhibition (ZOI) ≤ 22 mm for Meropenem (MRP) and/or Imipenem (IPM), and/or resistant to third-generation cephalosporins (Ceftazidime (CAZ) with ZOI <18 mm) (12,16).

Confirmation of carbapenemase producers by mCIM test

A 1 µL loopful of the test strain was suspended in 2 mL of tryptic soy broth (TSB), with an antibiotic disc (MRP) added, and incubated at 37°C for about 4 hours (± 15 minutes). A bacterial suspension of the meropenem-susceptible indicator strain (*Escherichia coli* ATCC 25922), which had a similar density to the 0.5 McFarland turbidity standard, was inoculated onto an MHA plate. The susceptible or resistant pattern of the pre-incubated antibiotic disc was tested by removing the disc using a 10 µL inoculation loop.

If the test strain produced carbapenemases, the zone of inhibition of the susceptible indicator strain would be decreased compared to the control with the untreated antibiotic disc (4, 12). This test has been reported to accurately identify carbapenemases but does not distinguish metallo-beta-lactamases from serine-carbapenemases. Therefore, further modification of the mCIM test by including EDTA (the eCIM test) was performed to accurately identify metallo-beta-lactamases (12).

Confirmation of MBL producers by eCIM test

The test strains were additionally incubated with 20 µL of 0.5 M EDTA, along with the antibiotic disc, in 2 mL of tryptic soy broth (TSB). After incubation, the subsequent procedure was the same as for mCIM. An eCIM result was interpreted only when the mCIM test was positive for the test strain. When there was a ≥5 mm increase in the zone diameter for eCIM compared to that for mCIM, the test isolate was considered an MBL-producing isolate (12, 17).

Data analysis and Statistical tests

After obtaining the results, the analysis was conducted on the data using Microsoft Excel and SPSS version 16, with interpretation based on frequency distribution and percentage. A Chi-square test was used to determine the significant association between MBL producers and MDR isolates, MBL-producing isolates and gender, and wherever applicable, with a p-value of <0.05 regarded as statistically significant.

Results

Distribution of Organisms among different clinical specimens

A total of 243 Enterobacterales isolates were obtained in the bacteriology laboratory, department of microbiology, TUTH, from various clinical specimens received for culture and susceptibility testing from August 2023 to November 2023. Among the eight different bacteria isolated, *E. coli* (57.20%) was the most predominant organism, followed by *Klebsiella pneumoniae* (20.20%), *Citrobacter freundii* (18.10%), *Klebsiella oxytoca* (1.60%), *Citrobacter koseri* (1.20%), *Klebsiella aerogenes* (0.80%), *Morganella morganii* (0.40%), and *Proteus vulgaris* (0.40%).

The highest growth of Enterobacterales was found in urine (52.70%), followed by sputum (16.90%), swabs (12.30%), body fluids (7.80%), pus (4.10%), blood (3.70%), tissue (1.20%), and tracheal aspirate (1.20%).

MBL production among clinical isolates of Enterobacterales

Among the 243 clinical isolates of *Enterobacterales*, 62 (25.50%) were resistant to both meropenem and ceftazidime, 49 (20.20%) were resistant to ceftazidime but not to meropenem, and 111 (45.70%) were suspected to be potential MBL-producing isolates. Fifty-two (21.40%) of the isolates were confirmed as carbapenemase producers by the mCIM method, of which 45 (18.50%) were confirmed as MBL producers by the eCIM method, and 7 (2.90%) were found to be serine carbapenemase producers, being negative on the eCIM test.

Distribution of MBL producing clinical isolates of Enterobacterales

The highest proportion of MBL-producing clinical isolates of *Enterobacterales* was found in *C. koseri* (66.70%), followed by *C. freundii* (29.50%), as shown in Table 1. However, among different clinical specimens, most MBL-producing clinical isolates of *Enterobacterales* were from tracheal aspirates (100.00%), followed by tissue (66.70%), which was found to be statistically significant (p-value < 0.05), as shown in Table 2.

Table 1.Distribution of MBL among clinical isolates of Enterobacterales (N=243)

Organisms	Total isolate	MBL isolate	%	p-value
C. koseri	3	2	66.7	
C. freundii	44	13	29.5	
K. pneumoniae	49	10	20.4	
E. coli	139	20	14.4	0.112
K. oxytoca	4	0	0.0	
K. aerogenes	2	0	0.0	
M. morganii	1	0	0.0	
P. vulgaris	1	0	0.0	
Total	**243**	**45**	**18.5**	

Table 2. Distribution of MBL producing Enterobacterales among different clinical specimens (N=243).

Specimen	Total isolate	MBL isolate	%	p-value
Tracheal Aspirate	3	3	100.0	
Tissue	3	2	66.7	
Sputum	41	13	31.7	
Swab	30	8	26.7	0.00
Body Fluid	19	5	26.3	
Blood	9	1	11.1	
Urine	128	13	10.2	
Pus	10	0	0.0	
Total	**243**	**45**	**18.5**	

MDR isolates among the clinical isolates of Enterobacterales

Out of the 243 clinical isolates of *Enterobacterales*, 151 (62.10%) were MDR isolates. The majority of MDR organisms were *K. oxytoca* (75.00%), followed by *C. freundii* (70.50%), as shown in Table 3.

Table 3. MDR isolates among the clinical isolates of Enterobacterales (N=243)

Enterobacterales	Total isolate	MDR isolate	%	p-value
K. oxytoca	4	3	75.0	
C. freundii	44	31	70.5	
C. koseri	3	2	66.7	
E. coli	139	87	62.6	
K. pneumoniae	49	27	55.1	0.534
K. aerogenes	2	1	50.0	
M. morganii	1	0	0.0	
P. vulgaris	1	0	0.0	
Total	**243**	**151**	**62.1**	

Association between MDR and MBL production among clinical isolates of Enterobacterales

Among the 243 clinical isolates of Enterobacterales, the majority were found to be MDR (151, i.e., 62.10%). Among these MDR isolates, 45 (29.80%) were also MBL producers. Among the 92 (37.90%) non-MDR isolates, none were MBL-producing isolates. This indicates that all MBL-producing isolates were MDR, but not all MDR isolates were MBL producers. The association was found to be statistically significant (p-value < 0.05).

Gender and age wise distribution of MBL producing clinical isolates of Enterobacterales

There were 45 MBL-producing clinical isolates of Enterobacterales, and their distribution according to gender and age is shown in Figures 1 and 2.

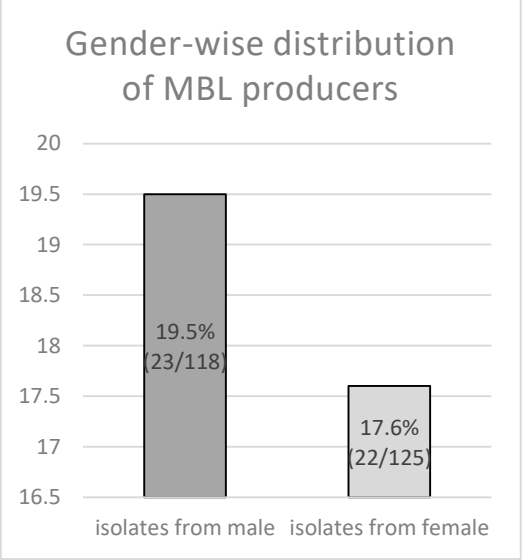

Figure 1. Gender wise distribution of MBL producing clinical isolates of Enterobacterales.

Distribution of MBL among MDR clinical isolates of Enterobacterales

The distribution of MBL among MDR clinical isolates of Enterobacterales is shown in Table 4.

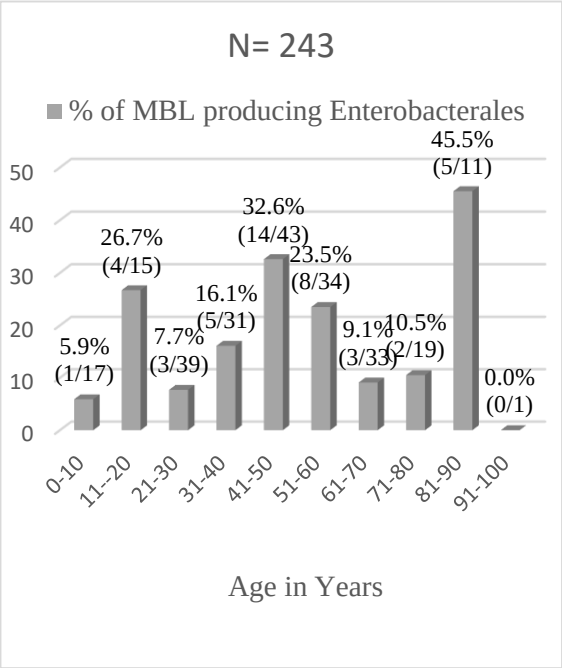

Figure 2.Distribution of MBL with age.

Table 4. Distribution of MDR and MBL production among clinical isolates of Enterobacterales (N=243).

Enterobacterales isolated	No. of MDR isolate	No. of MBL producers among MDR isolates (%)	p-value
C. koseri (3)	2	2 (100.00%)	
C. freundii (44)	31	13 (41.90%)	
K. pneumoniae (49)	27	10 (37.00%)	
E. coli (139)	87	20 (23.00%)	0.00
K. oxytoca (4)	3	0 (0.00%)	
K. aerogenes (2)	1	0 (0.00%)	
M. morganii (1)	0	0 (0.00%)	
P. vulgaris (1)	0	0 (0.00%)	
Total (243)	151	45 (29.80%)	

Discussion

Carbapenems, which have long been considered the last resort for treating critically ill patients with a variety of infections caused by MDR gram-negative pathogens, were effective against Enterobacterales. However, in the last two decades, the incidence of carbapenem resistance in Enterobacterales has increased rapidly around various parts of the world, mainly due to MBLs (13,18).

Out of 243 non-duplicate clinical isolates of Enterobacterales, 111 (45.70%) isolates were suspected to be potential carbapenemase producers, but only 45 (18.50%) were found to be MBL producers, while 7 (2.90%) were serine carbapenemase producers. This is similar to the study by Wadekar *et al* (5). in India in 2013, which reported an MBL production prevalence of 18.00% among Enterobacteriaceae. An earlier study in Nepal by Mishra et al. (16) in 2008, Thapa *et al.* (19) in 2014, and Shrestha *et al.* (7) in 2018 reported lower incidences of MBL-producing Enterobacteriaceae, with rates of 0.00% and 6.63%, respectively, among gram-negative pathogens. Similarly, a study by Paudel *et al.* (20) in 2020 on the rate of extensively drug-resistant K. pneumoniae found that 20% were serine carbapenemase producers, while 20% were MBL producers among cardiac patients. The highest prevalence of MBL-producing Enterobacteriaceae was reported in two different studies in India, by Vamsi *et al.* (21) in 2021 (91.50%) and Kadel *et al.* (22) in 2023 (87.75%).

Among these 243 *Enterobacterales*, the highest number of MBL producers were *C. koseri* (66.70%), followed by *C. freundii* (29.50%) and *K. pneumoniae* (20.40%). This finding differs from the study conducted by Shrestha *et al.* (7), which showed maximum MBL activity in E. coli (38.00%), followed by *Pseudomonas* (31.00%), and *K. pneumoniae* (19.00%) among gram-negative pathogens. However, a similar study conducted in India by Vamsi *et al.* (21) in 2021 reported maximum MBL production in *Klebsiella* spp. (94.20%), followed by *E. coli* (80.30%) among gram-negative pathogens. This disparity could be due to the different geographical areas of study.

In this study, the highest prevalence rate of MBL producers was observed in tracheal aspirates (100.00%), followed by tissue samples (66.70%). This finding is similar to the results reported by Vamsi *et al.* (21) in 2021, which showed that the highest number of MBL producers were found in endotracheal secretions (31.30%), followed by blood (22.10%). In contrast, a study conducted by Thapa *et al*. (19) in 2017 in Nepal reported that the highest number of MBL producers were recovered from urine (33.30%) and pus (33.30%). This variance could be due to differences in the year of study and the study population.

In the present study, 151 (62.10%) of the total isolates were MDR isolates of *Enterobacterales*, which closely matches the findings of Mishra *et al.* (16), who reported a prevalence rate of MDR *Enterobacteriaceae* at 68.90%. Another study conducted by Thapa *et al.* showed a prevalence rate of MDR isolates at 57.50%, with most of them being *E. coli* (56.80%). Similarly, a study conducted by Salvia *et al.* (19) in 2022 in India showed a prevalence rate of MBL-producing *Enterobacterales* at 76.00%, whereas a higher prevalence rate was reported in 2019 in Saudi Arabia by Bandy *et al.* (23) at 81.00%. This high resistance and subsequent prevalence rate of MDR could be due to the free availability of antimicrobials without a doctor's prescription from pharmacies and self-medication, which is a common practice in developing countries like Nepal (23, 24).

The majority of MDR organisms in this study were *Klebsiella oxytoca* (75.00%), followed by *Citrobacter freundii* (70.50%). This finding slightly differs from the results of Salvia *et al.* (2) and Bandy *et al*. (23), who reported the majority of MDR isolates as *Escherichia coli* and *Klebsiella pneumoniae*, with prevalence rates of 74.91% and 73.86%, respectively. These two organisms were found in nearly equal proportions. Hospitalization and prior inappropriate use of antimicrobials are common risk factors associated with infections caused by MDR pathogens (25).

Among the 151 MDR isolates identified in this study, 45 (29.80%) were found to be MBL-producing isolates. A similar study conducted by Mishra *et al*. (16) showed that, among 448 Gram-negative bacterial isolates, non-fermentative bacterial isolates were more likely to be MDR (77.80%) compared to *Enterobacteriaceae* isolates (68.90%). Furthermore, only non-fermentative bacterial isolates were MBL producers, as determined by the Combination Disk (CD) and Double Disk Synergy Test (DDST) methods. In contrast, no *Enterobacteriaceae* isolates were found to be MBL producers.16 Similarly, another study by Salvia *et al.* reported that among 304 MDR *Enterobacteriaceae* isolates, 39 (12.80%) were found to be MBL producers by the Combination Disk (CD) method, and 44 (14.80%) were identified as MBL producers by the CarbaNP test.

All (100.00%) MBL-producing isolates were MDR, but not all MDR isolates were MBL producers. This finding aligns with the studies conducted by Bora *et al.* and Thapa *et al*. (26,19). This observation serves as a critical alert regarding the clinical management of infected individuals, as it highlights the narrowing of therapeutic options available to medical professionals when treating infections caused by these pathogens (19).

In the present study, a slightly higher prevalence rate of MBL-producing isolates was observed in clinical specimens from male patients (51.10%) compared to female patients (48.90%). This finding is in agreement with the results reported by Vamsi *et al*. (21) in 2021, which showed a prevalence of 60.30% in male patients and 39.60% in female patients. However, a study conducted by Kumar *et al.* (27) in 2015 in India reported a higher prevalence rate of MBL producers among female patients with *E. coli* and *Klebsiella* spp.

The highest prevalence rate of MBL-producing *Enterobacterales* was observed among patients aged 81-90 years (45.50%), followed by those aged 41-50 years (32.60%) in this study. However, in the study conducted by Vamsi *et al.* (21) in 2021, the majority of MBL-producing isolates were obtained from patients aged 0-9 years (54.30%), followed by those aged 40-49 years (13.30%). Similarly, a study conducted by Yaffee *et al.* (28) in 2015 in the U.S. showed a higher prevalence rate of VIM-producing carbapenem-resistant *Enterobacterales* (CRE) in the neonatal intensive care unit (NICU) compared to the adult ICU. However, the first VIM-producing *Enterobacterales* identified in the U.S. were in an adult patient. This disparity in findings may be attributed to the inappropriate use of antibiotics by adult patients, along with the fact that they are often immunocompromised.

Limitations of the study

- The study was limited to clinical specimens received exclusively in the Department of Microbiology at TUTH, so it may not represent the entire population.
- Identification of MBL-producing Enterobacterales was conducted phenotypically, not genotypically.

- The time allocated for the study was insufficient for more detailed research.
- Only Enterobacterales was included as the study population.

Conclusion

The higher prevalence rates of MDR and MBL-producing Enterobacterales are a serious concern in developing countries such as Nepal. To limit the spread of these MBL producers among *Enterobacterales*, which pose a significant therapeutic and epidemiological threat, early detection in clinical microbiological laboratories and effective infection control practices are essential defenses against these organisms. If carbapenem is found to be ineffective in vivo, or if an Enterobacterales isolate is identified as resistant to ceftazidime and/or meropenem, it should be suspected to potentially produce MBL. In the absence of molecular detection techniques, the modified carbapenem inactivation method (mCIM) and the extended carbapenem inactivation method (eCIM) offer sensible phenotypic alternatives for the detection of MBL production and can be routinely implemented. Further studies at the molecular level to elucidate the genetic makeup of the genes responsible for resistance, as well as to identify the different classes of MBL, would be beneficial in understanding the underlying causes of the MDR patterns.

Declaration

Acknowledgements

We acknowledge the patients from whose clinical specimen the bacterial isolates were obtained in this study.

Funding
Not applicable.

Conflicts of interest/Competing interests
The authors declare no conflict of interest.

Authors' contributions

BD, JRR, SS, SR were responsible for study design and supervision of work. BD, MB, ST, HPK and NBK contributed to laboratory work and data analysis. BD and JRR contributed to writing and manuscript preparation. All authors have read and approved the final manuscript.

Ethical Consideration:

This study was conducted after obtaining approval from the Head of Department of Microbiology, Institute of Medicine and from IRC (77 (6-11) E2 080/081).

References

1. Thapa Shrestha U, Shrestha S, Adhikari N, Rijal KR, Shrestha B, Adhikari B, et al. Plasmid profiling and occurrence of β-lactamase enzymes in multidrug-resistant uropathogenic Escherichia coli in Kathmandu, Nepal. Infection and drug resistance. 2020 Jun 23:1905-17.

2. Salvia T, Dolma KG, Dhakal OP, Khandelwal B, Singh LS. Phenotypic Detection of ESBL, AmpC, MBL, and Their Co-occurrence among MDR Enterobacteriaceae Isolates. Journal of Laboratory Physicians. 2022 Apr 20;14(03):329-35.

3. Lin Q, Wang Y, Yu J, Li S, Zhang Y, Wang H et al. Bacterial characteristics of carbapenem-resistant Enterobacteriaceae (CRE) colonized strains and their correlation with subsequent infection. BMC infectious diseases. 2021 Dec;21:1-2.

4. Noster J, Thelen P, Hamprecht A. Detection of multidrug-resistant Enterobacterales—From ESBLs to carbapenemases. Antibiotics. 2021 Sep 21;10(9):1140.

5. Wadekar MD, Anuradha K, Venkatesha D. Phenotypic detection of ESBL and MBL in clinical isolates of Enterobacteriaceae. Int J Curr Res Aca Rev. 2013;1(3):89-95.

6. Studentova V, Papagiannitsis CC, Izdebski R, Pfeifer Y, Chudackova E, Bergerova T, et al. Detection of OXA-48-type carbapenemase-producing Enterobacteriaceae in diagnostic laboratories can be enhanced by addition of bicarbonates to cultivation media or reaction buffers. Folia microbiologica. 2015 Mar;60:119-29.

7. Shrestha A, Acharya J, Amatya J, Paudyal R, Rijal N. Detection of beta-lactamases (ESBL and MBL) producing gram-negative pathogens in national public health laboratory of Nepal. International Journal of Microbiology. 2022 Oct 6;2022.

8. Ramanath K, Sharma MK, Rajput MS, Negi S, Bhate V. An approach of phenotypic detection of metallo-beta lactamase producing Pseudomonas aeruginosa: A study in Madhya Pradesh. Medical Journal of Dr. DY Patil University. 2023 Jun 2.

9. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-β-lactamases: the quiet before the storm?. Clinical microbiology reviews. 2005 Apr;18(2):306-25.

10. Tang SS, Chee E, Teo JQ, Chlebicki MP, Kwa AL. Incidence of a subsequent carbapenem-resistant Enterobacteriaceae infection after previous colonisation or infection: a prospective cohort study. International journal of antimicrobial agents. 2021 Jun 1;57(6):106340.

11. Gurung S, Kafle S, Dhungel B, Adhikari N, Thapa Shrestha U, Adhikari B, et al. Detection of OXA-48 gene in carbapenem-resistant Escherichia coli and Klebsiella pneumoniae from urine samples. Infection and Drug Resistance. 2020 Jul 14:2311-21.

12. Wayne, PA. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 33rd informational supplement.:CLSI.2023; M100-S33.

13. Tsai YM, Wang S, Chiu HC, Kao CY, Wen LL. Combination of modified carbapenem inactivation method (mCIM) and EDTA-CIM (eCIM) for phenotypic detection of carbapenemase-producing Enterobacteriaceae. BMC microbiology. 2020 Dec;20(1):1-7.

14. Walsh TR. Emerging carbapenemases: a global perspective. International journal of antimicrobial agents. 2010 Nov 1;36:S8-14.

15. Bora A, Sanjana R, Jha BK, Narayan Mahaseth S, Pokharel K. Incidence of metallo-beta-lactamase producing clinical isolates of Escherichia coli and Klebsiella pneumoniae in central Nepal. BMC research notes. 2014 Dec;7(1):1-7.

16. Mishra SK, Acharya J, Kattel HP, Koirala J, Rijal BP, Pokhrel BM. Metallo-beta-lactamase producing gram-negative bacterial isolates. Journal of Nepal Health Research Council. 2012 Dec;10(3).

17. Sfeir MM, Hayden JA, Fauntleroy KA, Mazur C, Johnson JK, Simner PJ, et al. EDTA-modified carbapenem inactivation method: a phenotypic method for detecting metallo-β-lactamase-producing Enterobacteriaceae. Journal of clinical microbiology. 2019 May;57(5):10-128.

18. Lee YL, Chen HM, Hii M, Hsueh PR. Carbapenemase-producing Enterobacterales infections: Recent advances in diagnosis and treatment. International Journal of Antimicrobial Agents. 2022 Feb 1;59(2):106528.

19. Thapa P, Bhandari D, Shrestha D, Parajuli H, Chaudhary P, Amatya J, et al. A hospital based surveillance of metallo-beta-lactamase producing gram negative bacteria in Nepal by imipenem-EDTA disk method. BMC research notes. 2017 Dec;10(1):1-6.

20. Paudel S, Shah PK, Adhikari P, KC SS, Shrestha UT. Higher Rate of Extreme Drug-resistant Klebsiella pneumoniae Infections among Cardiac Patients. Journal of Nepal Health Research Council. 2022 Dec; 19(04):661-668.

21. Vamsi KS, Moorthy SR, Murali TS, Hemiliamma M, Reddy Y, Reddy B, et al. Phenotypic Methods for the Detection of Metallo-Beta-Lactamase Production by Gram-negative Bacterial Isolates from Hospitalized Patients in A Tertiary Care Hospital in India. Journal of Pure & Applied Microbiology. 2021 Dec 1;15(4).

22. Kadel S, Bilolikar AK, Eswaran SP, M K, Fatima R, Sahu S. Detection of carbapenemase production in gram negative bacilli in medical and surgical intensive care unit patients in a tertiary care hospital. J Med Sci Res. 2023;11(4):301-9.

23. Bandy A, Tantry B. ESBL activity, MDR, and carbapenem resistance among predominant Enterobacterales isolated in 2019. Antibiotics. 2021 Jun 19;10(6):744.

24. Pathak P, Jaishi N, Yadav BK, Shah PK. Prevalence of extended spectrum beta lactamases (ESBL) and metallo beta lactamases (MBL) mediated resistance in gram negative bacterial pathogens. Tribhuvan University Journal of Microbiology. 2017;4:49-54.

25. Nepal K, Pant ND, Neupane B, Belbase A, Baidhya R, Shrestha RK, et al. Extended spectrum beta-lactamase and metallo beta-lactamase production among Escherichia coli and Klebsiella pneumoniae isolated from different clinical samples in a tertiary care hospital in Kathmandu, Nepal. Annals of clinical microbiology and antimicrobials. 2017 Dec;16(1):1-7.

26. Bora A, Sanjana R, Jha BK, Narayan Mahaseth S, Pokharel K. Incidence of metallo-beta-lactamase producing clinical isolates of Escherichia coli and Klebsiella pneumoniae in central Nepal. BMC research notes. 2014 Dec;7(1):1-7.

27. Kumar M, Dutta R, Saxena S, Singhal S. Risk factor analysis in clinical isolates of ESBL and MBL (including NDM-1) producing Escherichia coli and Klebsiella species in a tertiary care hospital. Journal of Clinical and Diagnostic Research: JCDR. 2015 Nov;9(11):DC08.

28. Yaffee AQ. Notes from the Field: Verona integron-encoded metallo-beta-lactamase–producing carbapenem-resistant Enterobacteriaceae in a neonatal and adult intensive care unit—Kentucky, 2015. MMWR. Morbidity and mortality weekly report. 2016;65.

How to cite this article: Bijaya Dhakal, Junu Richhinbung Rai, Shristi Raut, Hari Prasad Kattel, Sujata Thapa, Marichman Bhul, Naresh Bahadur Khadka, Sangita Sharma. Detection of Metallo-beta-lactamase Producers among Clinical Isolates of Enterobacterales in a Tertiary Care Centre, Nepal. Mod Med Lab J. 2024;7(1): 12-20.