Journal of Medical Microbiology and Infectious Diseases

JoMMID
ISSN: 2345-5349
eISSN: 2345-5330

Status of Metallo-β-Lactamase-Producing Organisms in Clinical Samples

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ARTICLE INFO

Original Article

Keywords: Carbapenem resistance, eCIM, Enterobacteriaceae, Metallo-beta-lactamases (MBLs), *Pseudomonas aeruginosa*

Received: 03 Sep. 2024
Received in revised form: 07 Apr. 2025
Accepted: 11 Apr. 2025
DOI: 10.61186/JoMMID.13.1.30

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ABSTRACT

Introduction: Carbapenem resistance, due to the production of carbapenemase enzymes in various bacteria, is responsible for numerous outbreaks and is significantly associated with healthcare-associated infections. Metallo-β-lactamases (MBLs) are carbapenemases that hydrolyze all β-lactam antibiotics except monobactams. The most prominent bacteria exhibiting this resistance mechanism include members of the Enterobacteriaceae family and *Pseudomonas aeruginosa*. Therefore, this study aims to assess the prevalence of MBL production among Gram-negative bacteria at Kirtipur Hospital. **Methods**: This study was conducted at Kirtipur Hospital from June 26, 2022, to September 28, 2022. Organisms were isolated and identified from clinical samples including urine, blood, wound swabs, sputum, tissues, pus, catheter tips, and other body fluids, following standard laboratory protocols. Carbapenemase production was detected using the modified Carbapenem Inactivation Method (mCIM), with metallo-β-lactamase (MBL) production confirmed by the EDTA-modified Carbapenem Inactivation Method (eCIM) test. Results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines, M100, 31st ed., 2021. **Results**: From 1988 clinical samples, 388 Gram-negative bacteria were isolated, with *Escherichia coli*, *Klebsiella pneumoniae*, and *P. aeruginosa* being the predominant species. Antibiotic susceptibility testing revealed that Amikacin was the most effective against Enterobacteriaceae, exhibiting a susceptibility rate of 84.05%. In contrast, Cefepime was the most effective against non-Enterobacteriaceae Gram-negative bacteria with susceptibility rate of 60%. Screening identified 23.40% (84/359) of isolates as potential carbapenemase producers, with 15.32% (55/359) confirmed as carbapenemase producers via mCIM. Of these, 56.36% (31/55) were MBL producers, representing 8.63% (31/359) of all screened isolates. Among confirmed carbapenemase producers, *P. aeruginosa* exhibited the highest MBL production rate at 77.78% (7/9 isolates), followed by *K. pneumoniae* at 73.68% (14/19), *E. coli* at 41.18% (7/17), *C. koseri* at 33.33% (2/6), and *P. mirabilis* at 25% (1/4). **Conclusion**: This study underscores the significant threat posed by MBL-producing *E. coli*, *K. pneumoniae*, and *P. aeruginosa* in this healthcare setting. Therefore, implementing routine screening for MBL-producing organisms in diagnostic laboratories is crucial for controlling the spread among hospital patients and guiding effective antibiotic therapy.

INTRODUCTION

The rapid spread of antibiotic resistance has become a critical global health challenge, accounting for an estimated 4.95 million deaths annually as of 2019 [1]. Projections suggest this could rise to 10 million annual deaths by 2050 if unaddressed [2]. Of particular concern is the rapid spread of carbapenem resistance among Gram-negative bacteria, especially in members of the Enterobacteriaceae family, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* complex [3]. Carbapenem resistance can arise from three main mechanisms: the

[DOI: 10.61186/JoMMID.13.1.30]

expression of carbapenemase enzymes, alterations in bacterial permeability due to loss of porins or overexpression of efflux pumps, and modifications of the target site via genetic mutations or post-translational changes. Among these, the activity of carbapenemases is the predominant cause of resistance, as these enzymes effectively hydrolyze carbapenems [4]. Furthermore, bacteria carrying carbapenemase enzymes on mobile genetic elements often harbor genes conferring resistance to aminoglycosides, fluoroquinolones, trimethoprim, sulfonamides, rifampicin, and chloramphenicol. The co-occurrence of these resistance genes with carbapenemases significantly limits treatment options, thereby contributing to high mortality rates [5].

Metallo-β-lactamases (MBLs), which rely on zinc for their activity, are especially concerning because they can hydrolyze all currently available β-lactam antibiotics except for monobactams like aztreonam. Unlike serine-based β-lactamases, which are inhibited by compounds such as clavulanic acid, sulbactam, tazobactam, or avibactam, there are currently no clinically effective inhibitors for MBLs [6]. The prevalence of MBLs shows considerable global variability, influenced by socio-demographic factors, geographic location, and patient characteristics. The first reported MBL, IMP-1, was identified in *P. aeruginosa* in Japan in 1988 [7]. Since this initial report, MBLs have been detected worldwide, with varying frequencies among Enterobacterales and *A. baumannii* [8].

The mortality rate associated with MBL-positive bacteria is markedly higher than with MBL-negative strains. Specifically, bacteremia caused by MBL-producing *P. aeruginosa* and Enterobacterales has mortality rates ranging from 23% to 42.5% [9, 10]. Studies in Nepal have reported MBL production rates ranging from 14% to 31% among Gram-negative pathogens, particularly *Pseudomonas* spp. and Enterobacterales, highlighting a growing public health concern [11]. A 2022 meta-analysis reported a reduced MBL prevalence of 14% among *P. aeruginosa* isolates in Nepal [12]. Despite these insights, comprehensive data on the overall prevalence of MBLs across all bacterial species in Nepal remains scarce.

Given the rising infection and mortality rates, along with escalating healthcare costs, there is an urgent need for the surveillance of MBLs in various clinical samples. The modified Carbapenem Inactivation Method (mCIM) followed by the EDTA-modified Carbapenem Inactivation Method (eCIM) represents an effective strategy for MBL detection. Considering that many MBL-producing isolates are confirmed as agents causing nosocomial infections, it is crucial to curb their spread and to enforce antimicrobial stewardship policies, particularly in low-income countries like Nepal. Thus, this study aims to assess the prevalence of MBLs in diverse clinical specimens and examine their antimicrobial resistance profiles.

METHODS

This cross-sectional study was conducted over a three-month period from June 26, 2022, to September 28, 2022, at the Microbiology Department of Kirtipur Hospital, Kathmandu. Ethical approval was granted by the Institutional Review Committee (IRC) of Nobel College, Sinamangal, Nepal (IRC number: 079/080/090). Written informed consent was obtained from all participating patients; for minors, consent was provided by their guardians or attendants. The study examined variables including age, sex, patient status (inpatient or outpatient), sample types, and antimicrobial susceptibility testing (AST) patterns. The study population included all consenting patients whose clinical samples were submitted for microbiological analysis. Samples including urine, blood, wound swabs, sputum, tissue, pus, catheter tips, and other body fluids were collected according to standard protocols [13, 14]. Sample rejection criteria included unlabeled samples, non-midstream urine, dried blood samples, and swabs not pre-moistened with transport medium. Rejected samples were excluded from the final analysis (n=1988).

Culturing of bacterial pathogens. All samples except urine and blood were inoculated onto MacConkey and Blood agar and incubated aerobically at 37°C for 24 h. Urine samples were processed by inoculating a 1 µL aliquot onto CLED agar for semi-quantitative bacterial estimation to confirm bacteriuria. Blood samples were introduced into Brain Heart Infusion (BHI) broth (1:10 dilution), followed by a 24-h incubation period, during which they were monitored manually for signs of microbial growth such as turbidity or hemolysis. If there was no sign of growth, further incubation was done for up to 96 hours before reporting no growth. Upon detection of growth, these samples were subcultured onto Blood agar and MacConkey agar. Bacterial identification was furthered through standard phenotypic methods, including Gram staining, catalase and oxidase tests, and a series of biochemical assays (IMViC, TSI, Urease) [15].

Antibiotic susceptibility test (AST). The antibiotic susceptibility test was conducted using the Kirby Bauer Disc diffusion method. The antibiotics used in this study were beta-lactams: Amoxicillin (10mcg), Cefepime (30mcg), Ceftazidime (30mcg), Ceftriaxone (30mcg), Imipenem (10mcg), Meropenem (10mcg); beta-lactamase inhibitors: Piperacillin-tazobactam (100/10mcg); fluoroquinolones: Ciprofloxacin (5mcg), Levofloxacin (5mcg), Norfloxacin (10mcg); aminoglycosides: Amikacin (30mcg), Gentamicin (10mcg); tetracyclines: Doxycycline (30mcg), Tigecycline (15mcg); and others: Cotrimoxazole (25mcg), Nitrofurantoin (300mcg). For the screening of carbapenemase production in Enterobacterales, Ertapenem (10mcg) was used, whereas Meropenem

[DOI: 10.61186/JoMMID.13.1.30]

(10mcg) and Imipenem (10mcg) were applied for *Pseudomonas* spp., acknowledging that Ertapenem resistance is intrinsic in this genus. To detect carbapenemase in suspected isolates, the mCIM was applied, and the eCIM was used to distinguish metallo-β-lactamases (MBL) from serine β-lactamases [16].

Statistical analysis. Data were analyzed using descriptive statistics (frequencies, percentages) to summarize sample characteristics, pathogen distribution, and antibiotic susceptibility patterns. Analyses were performed using SPSS version 23.

RESULTS

Patient and sample characteristics. From June 26 to September 28, 2022, the Microbiology Laboratory at Kirtipur Hospital, Kirtipur, Nepal, processed 1988 clinical samples for culture and susceptibility testing. Patient demographics including age, sex, and the origin of samples are detailed in Table 1. The predominant sample types were urine, blood, wound swabs, and sputum; additional samples included catheter tips, tissue, pus, stool, samples from the Eustachian tube, umbilical vein tips, pleural fluid, throat swabs, high vaginal swabs, femoral tips, central venous catheter tips (CVP tips), labial swabs, Foley tips, eye swabs, bone, and semen. Out of these, 1454 samples originated from the outpatient department (OPD), and 534 were from the inpatient department (IPD).

Table 1. Patient and sample characteristics

Category	Number	Percentage (%)
Patient status		
Outpatients	1454	73.14
Inpatients	534	26.86
Sex		
Male	747	37.58
Female	1241	62.42
Age group		
0-15	410	20.62
16-30	710	35.71
31-45	422	21.23
46 and above	446	22.43
Clinical sample		
Urine	1065	53.57
Blood	509	25.60
Wound swab	170	8.55
Sputum	86	4.32
Catheter tips	32	1.61
Others	126	6.34

Distribution of bacterial isolates. From the total of 1988 clinical samples, 459 (23.09%) yielded positive bacterial cultures. Of these, 388 isolates (84.53%) were identified as Gram-negative bacteria, and 71 isolates (15.47%) as Gram-positive. Among the Gram-negative bacteria, 300 isolates (77.32%) belonged to the Enterobacteriaceae family, with *E. coli* being predominant, accounting for 188 isolates (48.45%). The distribution of Gram-negative isolates by clinical specimen type is detailed in Table 2.

Table 2. Distribution of Gram-negative bacterial isolates by clinical sample type

Bacteria	Urine	Blood	Wound swab	Sputum	Catheter tips	Others	Number (%)
E. coli	156	2	12	-	7	11	188 (48.45%)
K. pneumoniae	37	6	6	8	6	8	71 (18.30%)
P. aeruginosa	5	2	18	3	5	10	43 (11.08%)
A. baumannii complex	2	4	8	2	1	2	19 (4.90%)
Citrobacter koseri	4	1	7	1	-	6	19 (4.90%)
P. mirabilis	4	-	7	-	-	1	12 (3.10%)
Acinetobacter lwoffii	-	1	5	-	1	3	10 (2.58%)
Klebsiella oxytoca	5	1	2	-	1	-	9 (2.32%)
Salmonella Typhi	-	5	-	-	-	-	5 (1.29%)
Citrobacter freundii	1	-	1	-	-	2	4 (1.03%)
Enterobacter cloacae	1	-	-	-	-	2	3 (0.77%)
Proteus vulgaris	2	-	1	-	-	-	3 (0.77%)
Klebsiella aerogenes	-	1	-	-	-	-	1 (0.26%)
Providencia spp.	-	-	-	-	-	1	1 (0.26%)
Total	**217**	**23**	**67**	**14**	**21**	**46**	**388**

Antibiotic susceptibility testing. Antibiotics for susceptibility testing were chosen according to the type of clinical sample. All Gram-negative isolates were initially screened with first-line antibiotics. Isolates demonstrating resistance to these were subsequently tested against second-line antibiotics. The susceptibility profiles for

[DOI: 10.61186/JoMMID.13.1.30]

isolates from the Enterobacteriaceae family and those from non-Enterobacteriaceae are detailed in Tables 3 and 4, respectively.

Table 3. Antibiotic susceptibility profiles of Enterobacteriaceae family

Antibiotics	*E. coli*	*K. pneumoniae*	*C. koseri*	*C. freundii*	*E. cloacae*	*S.* Typhi	*K. aerogenes*	*K. oxytoca*	Total (%)
Amikacin (AK)									
Susceptible (S)	174	49	14	4	1	-	1	8	251(85.08)
Resistant (R)	14	22	5	0	2	-	0	1	44 (14.91)
Amoxicillin (AMX)									
Susceptible (S)	61	-	-	-	-	5	-	-	66 (34.20)
Resistant (R)	127	-	-	-	-	0	-	-	127 (65.80)
Ceftazidime (CAZ)									
Susceptible (S)	89	32	8	3	0	5	1	4	142 (47.33)
Resistant (R)	99	39	11	1	3	0	0	5	158 (52.67)
Ciprofloxacin (CIP)									
Susceptible (S)	125	46	11	4	2	2	1	7	198 (66)
Resistant (R)	63	25	8	0	1	3	0	2	102 (34)
Cotrimoxazole (COT)									
Susceptible (S)	130	43	13	4	1	5	1	7	204(68)
Resistant (R)	58	28	6	0	2	0	0	2	96 (32)
Ceftriaxone (CTR)									
Susceptible (S)	101	27	6	2	1	5	1	6	149 (49.66)
Resistant (R)	87	44	13	2	2	0	0	3	151 (50.34)
Levofloxacin (LE)									
Susceptible (S)	124	49	13	4	2	4	1	7	204 (68)
Resistant (R)	64	22	6	0	1	1	0	2	96(32)
Nitrofurantoin (NIT)									
Susceptible (S)	137	7	2	1	1	-	-	3	151(74.02)
Resistant (R)	19	30	2	0	0	-	-	2	53 (25.98)
Norfoxacin (NX)									
Susceptible (S)	102	31	3	1	1	-	-	5	143 (70.1)
Resistant (R)	54	6	1	0	0	-	-	0	61 (29.9)
Doxycycline (DO)									
Susceptible (S)	14	11	-	-	-	-	-	-	25 (8.33)
Resistant (R)	34	26	-	-	-	-	-	-	60 (20)
Piperacillin-tazobactam (PTZ)									
Susceptible (S)	35	20	-	-	-	-	-	-	55 (18.33)
Resistant (R)	13	17	-	-	-	-	-	-	30 (10)
Tigecycline (TGC)									
Susceptible (S)	15	7	-	-	-	-	-	-	22 (7.33)
Resistant (R)	33	30	-	-	-	-	-	-	63 (21)

Note: Antibiotics not tested because of bacteria are intrinsically resistant to that antibiotics or not clinically relevant from that particular samples.

Carbapenemase test. Screening for carbapenemase production was performed using Ertapenem (10mcg) for Enterobacterales, and both Meropenem (10mcg) and Imipenem (10mcg) for *P. aeruginosa*. Carbapenemase screening was not applied to *Acinetobacter* spp. due to the lack of CLSI endorsement for the mCIM and eCIM tests. Of 388 Gram-negative isolates, 359 Gram-negative isolates were screened, 84 (23.40%) were positive for potential carbapenemase producer. Subsequent confirmation by the mCIM test identified 55 isolates (15.32%) as true carbapenemase producers, as detailed in Table 5.

Of the 55 carbapenemase-producing organisms, 31 (56.36%) tested positive in the eCIM assay, indicating they were MBL producers, while 24 isolates were identified as serine carbapenemase producers. Within the Enterobacterales and *Pseudomonas aeruginosa*, MBL production was identified in 8.63% of the cases. The highest positivity of MBL producers was seen in *K. pneumoniae* with 19.71% (14/71) isolates followed by *P. aeruginosa* 16.28% (7/43). The rest data are shown in Table 6 and MBL producing *K. pneumoniae* is shown in Figure 1.

[DOI: 10.61186/JoMMID.13.1.30]

Table 4. Antibiotic susceptibility profiles of non-Enterobacteriaceae isolates

Antibiotics	*P. aeruginosa*	*A. baumannii* complex	*P. mirabilis*	*A. lwoffii*	*P. vulgaris*	*Providencia* spp.	Total (%)
Amikacin (AK)							
Susceptible (S)	25	6	9	2	2	1	45 (51.13)
Resistant (R)	18	13	3	8	1	0	43 (48.87)
Amoxicillin (AMX)							
Susceptible (S)	-	-	7	-	-	0	7 (53.85)
Resistant (R)	-	-	5	-	-	1	6 (46.15)
Ceftazidime (CAZ)							
Susceptible (S)	16	0	8	1	-	0	25 (29.41)
Resistant (R)	27	19	4	9	-	1	60 (70.59)
Ciprofloxacin (CIP)							
Susceptible (S)	25	6	6	2	3	1	43 (48.86)
Resistant (R)	18	13	6	8	0	0	45 (51.14)
Cotrimoxazole (COT)							
Susceptible (S)	-	6	9	4	0	1	20 (44.44)
Resistant (R)	-	13	3	6	3	0	25 (55.56)
Cefepime (CPM)							
Susceptible (S)	27	7	11	5	-	1	51 (60)
Resistant (R)	16	12	1	5	-	0	34 (40)
Ceftriaxone (CTR)							
Susceptible (S)	-	1	7	1	-	1	10 (23.81)
Resistant (R)	-	18	5	9	-	0	32 (76.19)
Gentamicin (GEN)							
Susceptible (S)	26	9	9	2	3	1	50 (56.82)
Resistant (R)	17	10	3	8	0	0	38 (43.18)
Levofloxacin (LE)							
Susceptible (S)	26	7	6	2	2	1	44 (50)
Resistant (R)	17	12	6	8	1	0	44(50)
Doxycycline (DO)							
Susceptible (S)	3	8	-	1	-	-	12 (17.61)
Resistant (R)	40	9	-	7	-	-	56 (82.35)
Imipenem (I)							
Susceptible (S)	27	4	-	0	-	-	31 (45.59)
Resistant (R)	16	13	-	8	-	-	37 (54.41)
Meropenem (MRP)							
Susceptible (S)	30	4	-	0	-	-	34(50)
Resistant (R)	13	13	-	8	-	-	34 (50)
Piperacillin-Tazobactam (PTZ)							
Susceptible (S)	32	4	-	0	3	-	39 (54.93)
Resistant (R)	11	13	-	8	-	-	32 (45.07)
Tigecycline (TGC)							
Susceptible (S)	-	2	-	0	-	-	2 (8.33)
Resistant (R)	-	15	-	8	-	-	23 (91.67)

Note: Antibiotics not tested because of bacteria are intrinsically resistant to that antibiotics or not clinically relevant from that particular samples

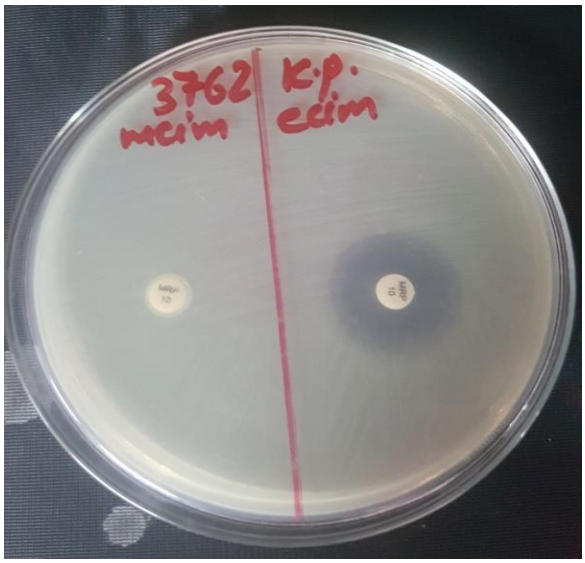

Fig. 1. Detection of Carbapenemase producer (mCIM test) and MBL producer (eCIM test)

[DOI: 10.61186/JoMMID.13.1.30]

Table 5. Distribution and confirmation of carbapenemase-producing bacteria by mCIM test

Organism	Total isolates	Screening test positive (%)	Carbapenemase producer (%)	Non-producer	Intermediate
E. coli	188	29	17 (9.04%)	7	5
K. pneumoniae	71	27	19 (26.76%)	7	1
P. aeruginosa	43	16	9 (20.93%)	2	5
C. koseri	19	8	6 (31.57%)	2	0
P. mirabilis	12	4	4 (33.33%)	0	0
Other Gram-negative isolates	26	0	-	-	-
Total	**359**	**84 (23.40%)**	**55 (15.32%)**	**18**	**11**

Table 6. Prevalence of MBL and serine carbapenemase production among various bacterial isolates

Organism	Total isolates	mCIM positive (%)	MBL producers (%)	Serine carbapenemase producers (%)
E. coli	188	17	7 (3.72%)	10 (5.32%)
K. pneumoniae	71	19	14 (19.71%)	5 (7.04%)
P. aeruginosa	43	9	7 (16.28%)	2 (4.65%)
C. koseri	19	6	2 (10.52%)	4 (21.05%)
P. mirabilis	12	4	1 (8.33%)	3 (25%)
Other Gram-negative isolates	26	0	-	-
Total	**359**	**55 (15.32%)**	**31 (8.63%)**	**24 (6.68%)**

DISCUSSION

The β-lactam antibiotics are generally regarded as the safest and most effective therapeutic options due to their broad spectrum of activity and low toxicity profile. However, the overuse and misuse of β-lactam antibiotics have contributed to the emergence and spread of antibiotic resistance worldwide, posing a significant clinical challenge. In cases where resistance to other β-lactams develops, carbapenem antibiotics are frequently employed as a treatment of last resort due to their potency and broader spectrum against resistant strains. Multiple studies have documented the global dissemination of carbapenem-resistant strains, underscoring an urgent need for enhanced antimicrobial stewardship and infection control measures. Metallo-β-lactamases (MBLs) represent a significant clinical concern for multiple reasons: they can hydrolyze virtually all β-lactam antibiotics, there are currently no clinically effective inhibitors for MBLs, new variants are emerging rapidly, their genes are highly transferable among bacteria, and they are prevalent in both clinical settings and the broader environment [6]. These MBL variants are commonly detected in major pathogenic bacteria, including *Pseudomonas* spp., *Acinetobacter* spp., and various species within the Enterobacteriaceae family, with particular emphasis on *E. coli* and *K. pneumoniae*.

A total of 1988 clinical samples were analyzed, consisting of 534 from the IPD and 1454 from the OPD. Most patients were aged 16-30 years (35.71%), possibly due to higher healthcare-seeking behavior or demographic representation in the study population. From the samples, 459 microorganisms were isolated, representing an isolation rate of 23.09%. Of these, 388 (84.53%) were Gram-negative, and 71 (15.47%) were Gram-positive. Among the 14 distinct Gram-negative species isolated, *E. coli* was predominant with 188 isolates (48.45%), followed by *K. pneumoniae* with 71 isolates (18.30%). *K. aerogenes* and *Providencia* spp. were among the least frequently isolated. Our findings regarding the predominance of *E. coli* and *K. pneumoniae* are consistent with those reported in a previous study from Kathmandu [17]. Furthermore, another study at a tertiary care center in Kathmandu, spanning from August 2017 to January 2018, reported a comparable distribution of microbial isolates, although with variations in prevalence rates, potentially due to differences in sample sizes [18]. The high prevalence of *E. coli* (48.45%) and *K. pneumoniae* (18.30%) may be associated with the predominance of urine samples (53.57%), as these organisms are frequent causes of urinary tract infections.

Antibiotic susceptibility testing of the Enterobacteriaceae isolates revealed Amikacin as the most effective, with a susceptibility rate of 85.08% (251 out of 295 isolates). This was followed by Cotrimoxazole and Levofloxacin, both showing a susceptibility rate of 68% (204 out of 300 isolates each). Among urinary isolates, Nitrofurantoin demonstrated superior efficacy over Norfloxacin. These results are comparable to those from a study conducted at Manmohan Memorial Medical College and Teaching Hospital [18]. *Salmonella Typhi* (1.29%, n=5) was isolated exclusively from blood, consistent with typhoid fever cases, and was included to reflect the full spectrum of Gram-negative isolates. *S. Typhi* isolates showed no resistance to Amoxicillin and Cotrimoxazole. In individual cases, Amikacin was typically the primary antibiotic of choice, with the secondary antibiotic varying according to the bacterial species. For instance, Cotrimoxazole was the secondary choice for *E. coli* and *Citrobacter koseri*, whereas Ciprofloxacin served as the secondary option for *K. pneumoniae* and *E. cloacae*. Additionally, second-line antibiotics, notably Piperacillin-tazobactam, exhibited significant efficacy, with low resistance rates observed in *K. pneumoniae* and *E. coli* isolates.

Cefepime and Gentamicin were confirmed as the first and second drugs of choice for Gram-negative non-Enterobacteriaceae, showing susceptibility rates of 60% (51 out of 85 isolates) and 56.82% (50 out of 88 isolates),

[DOI: 10.61186/JoMMID.13.1.30]

respectively. These results differ from those reported by Pariyar *et al*. (2023) [18], where different susceptibility patterns were noted. In the case of *Pseudomonas* spp., Piperacillin-tazobactam and Meropenem were identified as the most effective, with susceptibility rates of 74.42% and 69.77%, respectively. Comparative data from another tertiary care center in Nepal revealed a 70% susceptibility to Piperacillin-tazobactam, while Meropenem showed no resistance [17]. Additionally, a longitudinal study in Nepal highlighted variable resistance rates for *P. aeruginosa*, with resistance to Piperacillin-tazobactam ranging between 17-41% and to Meropenem between 35-61% [19]. Most antibiotics tested against the *A. baumannii* complex showed high levels of resistance. However, Gentamicin and Cefepime exhibited the highest susceptibility rates among those tested, at 47.36% (9/19 isolates) and 36.84% (7/19 isolates), respectively. These results align with those from a study conducted at Nepal Mediciti Hospital [20]. A different hospital-based study in Kathmandu also found comparable susceptibility rates for Cefepime, whereas the rates for Gentamicin were notably lower [21].

Antibiotic resistance in Gram-negative bacteria represents a major concern in healthcare settings. The production of various β-lactamase enzymes confers resistance to a range of β-lactam antibiotics, including third-generation cephalosporins and monobactams. Carbapenems resist many β-lactamase enzymes, positioning them as drugs of last resort for treating severe infections caused by Gram-negative bacilli. Over the last decade, the rise of carbapenem-resistant Gram-negative bacteria, especially Enterobacterales, *P. aeruginosa*, and *Acinetobacter* spp., has emerged as a significant threat. Among the various carbapenemases, MBLs are among the most prevalent in these pathogens. Screening tests utilizing an ertapenem disk for Enterobacterales and imipenem or meropenem disks for *P. aeruginosa* identified 84 isolates (23.40%) as positive for carbapenemase production. Following CLSI guidelines, the mCIM identified that 15.32% of the isolates (55 out of 359) were positive for carbapenemase production. Among these, *K. pneumoniae* was the most prevalent, accounting for 5.29% (19/359) of all samples, followed by *E. coli* at 4.73% (17/359). Among the isolates tested, *P. mirabilis* exhibited the highest frequency of carbapenemase production at approximately 33.3% (4 out of 12), whereas *E. coli* showed the lowest frequency at 9.05% (17 out of 188).

Few studies have explored the mCIM in *P. mirabilis*; one such study reported a carbapenemase frequency of approximately 66.7% (4 out of 6) [22]. The frequency of carbapenemase-producing *E. coli* in our study differs from that reported by Ko *et al*. (2023), who found a frequency of only 3.97% [23]. The frequencies of carbapenemase production in other clinically significant bacteria were as follows: *K. pneumoniae* at 26.76% (19/71), *P. aeruginosa* at 20.93% (9/43), and *C. koseri* at 31.57% (6/19). Apart from *E. coli*, the frequency of carbapenemase-producing *K. pneumoniae* in our study does not concur with findings by Zhao *et al*. (2021), which documented an increase from 5.65% to 9.90% over four years [24]. The discrepancies in carbapenemase prevalence between *E. coli* and *K. pneumoniae* across two studies could be attributed to differences in sample size, study period, and geographical location, among other factors. However, our findings regarding the incidence of carbapenemase in *P. aeruginosa* align with a study from Iran, which reported a prevalence of 23.7% [25].

According to CLSI guidelines, isolates positive for mCIM were subjected to the eCIM test. Out of 55 isolates tested, 31 were eCIM positive, indicating that MBL producers comprised 8.63% of the isolates potential to produce carbapenemase. Of the carbapenemase-producing isolates, 56.36% (31 out of 55) were identified as MBL producers, and the remaining were presumed to be serine β-lactamase producers. The highest frequency of MBL producers was observed in *K. pneumoniae* at 19.71%, while *E. coli* showed the lowest at 3.72%. *P. aeruginosa*, another significant pathogen in healthcare-associated infections, accounted for 16.28% of the MBL cases. Among the carbapenemase-producing isolates, *P. aeruginosa* exhibited the highest MBL positivity rate at 77.77% (7/9). This was followed by *K. pneumoniae* at 73.68% (14/19), *E. coli* at 41.18% (7/17), *C. koseri* at 33.33% (2/6), and *P. mirabilis* at 25% (1/4).

In summary, the overall prevalence of MBL producers in our study was similar to that found in a study from Nepal, which reported an MBL prevalence of 8.12%. However, the prevalence rates for individual bacterial species, with the exception of *E. coli*, differed from those reported in the Nepalese study [11]. Our test results for *E. coli* were in line with those of Ko and Kulkarni *et al*. (2022), who reported prevalence rates of 41.93% and 40.67%, respectively [23, 26]. In contrast, while our study found a positivity rate for *K. pneumoniae* MBL producers to be different, other studies have reported rates around 50%: Kulkarni *et al*. (2023) observed 43.66% [26], Zhao *et al*. (2021) reported 34.04% [24], and Chauhan *et al*. (2015) found 52.94% [27]. The observed differences in statistical values may be attributed to variations in sample volume, sample origin and procedural differences like study conducted by Zhao and Ko utilized the mCIM and eCIM protocols, while the others employed the Combined Disc Test [23, 24, 26, 27] .

In conclusion, the finding of MBL enzymes in *E. coli*, *K. pneumoniae*, and *Pseudomonas* spp. highlights the significant therapeutic challenge and raises serious concerns for infection control management. This study's findings, derived from a single tertiary hospital, may not be generalizable to broader settings in Nepal or beyond. Therefore, it is imperative to implement regular screening for MBL-producing organisms in different diagnostic laboratories following CLSI guideline to minimize the-

[DOI: 10.61186/JoMMID.13.1.30]

dissemination of such strains among hospital patients and to enhance antibiotic therapy management.

ACKNOWLEDGEMENT

We thank the microbiology staff at Kirtipur Hospital and the Institutional Review Committee at Nobel College for their technical support and feedback, which were instrumental to this study.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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[DOI: 10.61186/JoMMID.13.1.30]

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Cite this article:
Amatya NM, Shrestha S, Magar PT, Dhaubhadel R, Tamang S, Panta S. Status of Metallo-β-Lactamase-Producing Organisms in Clinical Samples. J Med Microbiol Infect Dis, 2025; 13 (1): 30-38. DOI: 10.61186/JoMMID.13.1.30.

[DOI: 10.61186/JoMMID.13.1.30]