

Original Research Article

Metallo-beta-lactamase-producing isolates of *Escherichia coli* and *Klebsiella pneumoniae* from clinical and non-clinical sources in Enugu, Nigeria: A threat to public health

Commented [U1]: Remove hyphen

ABSTRACT

Background: A potential threat to public health is the rapidly spreading *Enterobacteriaceae*, especially *Escherichia coli* and *Klebsiella pneumoniae* which produce metallo-beta-lactamases (MBL). This study evaluated the prevalence of metallo-beta-lactamase (MBL) from clinical and non-clinical sources in Enugu Metropolis. **Methodology:** The study was conducted in the Microbiology Laboratory of the University of Nigeria Teaching Hospital, Ituku-Ozalla between October 2020 and July 2021. A total of 150 isolates including 85 and 65 isolates of *Escherichia coli* and *Klebsiella pneumoniae* respectively was recovered. Standard microbiology procedures were used to identify and characterize the isolates. Antimicrobial susceptibility was done using the Kirby-Bauer disc diffusion technique. Phenotypic detection of Metallo-beta-lactamase production was determined using Combined Disk Tests. **Results:** Imipenem resistance was detected in 22(25.9%) isolates of *E. coli* and 18 (27.7%) isolates of *K. pneumoniae*. Of the 22 strains of *E. coli* that were imipenem resistant, 8(9.4%) and 14(16.5%) were found to be MBL producers and non-MBL respectively. Of the 18 strains of *Klebsiella pneumoniae* that were imipenem resistant, 10 (15.4%) were MBL producers. The highest prevalence of MBL was recovered from urine sources in both *E. coli* and *K. pneumoniae*. All MBL-producing isolates were multidrug resistant. **Conclusion:** The overall prevalence of MBL in this study was 12.0%. Public health is at risk due to the occurrence of metallo-beta-lactamase. Antimicrobial stewardship and the implementation of infection control strategies are required to halt the spread of these resistant bacteria in the environment. The use of antibiotics should be with utmost prudence.

Commented [U2]: enterobacteriaceae

Commented [U3]: space

Commented [U4]: what of non MBL producers

Keywords: Antibiotic resistance, Carbapenemase, Metallo-beta-lactamase, phenotypic detection, *Escherichia coli*, *Klebsiella pneumoniae*

1. INTRODUCTION

Global health is challenged and threatened by the rise and spread of life-threatening infections caused by bacteria resistant to antibiotics^[1]. Gram-negative bacteria such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and Enterobacteriaceae, which is the most common in humans, are of special interest due to their increased resistance^[2]. The main challenge facing the healthcare sectors in developing countries like Nigeria is the rise and spread of multidrug-resistant (MDR) bacteria, especially *Escherichia coli* and *Klebsiella pneumoniae* harboring resistance genes. Accurate susceptibility testing, cautious use of currently available antibiotics and measurement of the prevalence of multidrug-resistant bacteria are necessary to reduce the potential risks associated with infections caused by multi-drug-resistant strains^[3]. As a last resort, carbapenems like imipenem, meropenem, and ertapenem are used to treat infections caused by bacteria that are resistant to many antibiotics^[4]. The primary reason for their exceptional antibacterial activity is their ability to swiftly penetrate gram-negative bacteria membrane and their resistance to most naturally occurring and acquired beta-lactamases, such as cephalosporinases and extended-spectrum beta-lactamases^[5]. However, bacteria have developed defense mechanisms against these carbapenems through adaptation. This resistance is associated either with the relationship of the over-expression of extended-spectrum β -lactamases with the efflux pump and impermeability or to the expression of β -lactamases hydrolyzing carbapenems, known as carbapenemases^[6]. Selection pressure has, as predicted, led to the development of carbapenem resistance in bacterial pathogens^[7, 8]. Though the situation in many countries is yet unknown, the prevalence of carbapenemases, which are typically located on mobile genetic elements, has the potential to be universal. Often, carbapenemase-producing bacterial isolates exhibit multiple antibiotic resistances^[7]. Carbapenemases fall under three of the four classes of β -lactamases in Ambler's classification (A, B, C, and D). Metallo-lactamases (MBLs) are a group of carbapenemases that are especially important to healthcare because of their ability to spread over the globe and the resulting limited number of therapeutic options that are accessible^[9]. Zinc or another heavy metal is needed for the catalysis of these class B carbapenemases. MBLs have a wide range of substrates and are capable of catalyzing the hydrolysis of nearly all beta-lactam antibiotics, including carbapenems, except monobactams like aztreonam^[7]. Furthermore, they are blocked by chelating agents such as ethylene-diamine tetra-acetic acid (EDTA)^[3]. One further issue with strains that produce carbapenemase is that they are resistant to all of the strongest antibiotics, which means that colonization with them carries a significant risk of mortality^[10]. The distribution of different carbapenemases produced by Gram-negative bacteria varies geographically^[11]. Standard antimicrobial susceptibility testing can be combined with early identification of particular carbapenem resistance mechanisms in resource-constrained settings by employing simple phenotypic methods. Test results can provide information that can help with the timely deployment of appropriate antibiotics, preventing and containing the spread of bacteria that are resistant to several drugs. Based on detection, suitable therapies may be selected^[11].

Commented [U5]: *Acinetobacter baumannii* and enterobacteriaceae

Given the unmatched range of activity and resistance to therapeutic serine beta-lactamase inhibitors, MBLs are now considered a health risk and an important cause of the growing rates of morbidity and mortality. The gold standard for MBL detection and classification is still PCR-based genotyping; nevertheless, diagnostic laboratories still need to perform culture-based phenotypic testing to quickly identify MBL activity [12]. The MBL enzyme can be identified phenotypically in pathogenic bacteria. The recognized techniques are the Modified Hodge Test (MHT), Double-Disc Synergy Test (DDST), Combined Disc Diffusion Test with Imipenem and EDTA (CDDT), and MBL E-Test [13, 14]. Since there isn't much information on MBL prevalence in Enugu, this study was carried out to determine how common MBLs are in isolates that are resistant to imipenem (carbapenem) from hospitals and other environmental sources in the city of Enugu. To prevent the spread of these infections, determine the best course of treatment, and put infection control measures in place, it is essential to identify MBL-producing organisms as early as possible.

2. MATERIALS AND METHODS

2.1 Study Design

The study was carried out from October 2020 to July 2021 at the University of Nigeria Teaching Hospital's Microbiology Laboratory in Ituku-Ozalla, Enugu.

2.2 Study Area

2.2 Collection of sample

2.2 Bacterial Isolates Isolation of bacteria

The work was done in the Microbiology Laboratory at the University of Nigeria Teaching Hospital in Ituku-Ozalla. The study included 65 *Klebsiella pneumoniae* isolates and 85 *Escherichia coli* isolates that were recovered from 600 isolates from different sources. Non-clinical isolates were recovered from water, tiger nut milk, soya milk, and zobo drink, whereas clinical isolates were recovered from wound swabs, urine, sputum, feces, and high vaginal swabs. Standard laboratory protocols for microbiology were employed to identify the isolates. All isolated organisms were brought to the laboratory and maintained in nutrient agar slants. The isolates were reactivated on MacConkey agar (Central Drug House (P) Ltd, and incubated for 24 hours at 37°C on various agar plates to obtain a pure culture.

2.3 Identification of Bacteria Isolates

Conventional microbiological techniques, such as colony morphology, Gram stain, biochemical testing, and the API 20E confirming procedure, were used to identify the isolates [15].

2.4 Antimicrobial Susceptibility Test

In compliance with the Clinical Laboratory Standard Institute guidelines, the antibiotic susceptibility of each isolate was assessed using the Kirby Bauer disk diffusion method on Muller-Hinton agar (HiMedia Laboratories India) [16]. The antimicrobial used were Imipenem (10µg), cefoxitin (30µg), ofloxacin (5 µg), ciprofloxacin (5 µg), gentamicin (10 µg), amoxicillin/clavulanate (20/10 µg), nitrofurantoin (50 µg), ceftazidime (30 µg), cefixime (30 µg), cefotaxime (30 µg), and cefuroxime (30 µg). Mueller-Hinton agar plates were inoculated with a standardized suspension of each isolate comparable to 0.5 McFarland turbidity standards, and the antibiotics disc was aseptically placed on the agar plates. The plates were then incubated at 37°C for 18 to 24 hours. Following the 2015 CLSI recommendations, the Inhibition Zone Diameter (IZD) was measured and recorded after incubation [17].

2.5 Screening for carbapenemase production

MBL-producing *E. coli* and *K. pneumoniae* resistant to imipenem were tested for MBL production. Isolates with reduced susceptibility to imipenem with an inhibition zone diameter of ≤ 21 mm were used as cut-off values according to CLSI guidelines [16].

Commented [U6]: Brief explanation of the study area

Commented [U7]: Brief explanation of how the samples were collected.

Commented [U8]: consider changing to this title
Briefly explain how the organisms were isolated from clinical and non clinical samples as well as methods used.

Formatted: Font: Bold

Commented [U9]: Take to Results

Commented [U10]: Take to Identification of Bacteria Isolates

2.6 Detection of metallo-beta-lactamase production

Combined Disc Diffusion Test (CDDT)

MBL production was phenotypically detected using the Combined Disc Diffusion Test as per Franklin *et al.* with slight modifications^[18]. Two 10µg imipenem discs (one impregnated with 10µl of 750µg EDTA Sigma Chemicals, St. Louis, MO) were placed at a distance of 25mm apart on the Mueller Hinton (MH) agar medium inoculated with test organism standardized with 0.5 McFarland standards. After 24 hours of incubation at 37°C, the zones of inhibition around imipenem and imipenem + EDTA discs were compared. MBL production was deemed to have occurred when the zone diameter around the imipenem + EDTA discs increased by more than 4mm when compared to the imipenem alone. Before being added to the antibiotics disk(s), EDTA was tested on the isolate(s) alone to be sure it wouldn't suppress the test organism and result in a false positive ^[3].

2.7 Statistical Analysis

All statistical analyses were performed using SPSS for Windows version 22 (SPSS, Chicago, IL, USA). Descriptive statistics were used to describe categorical variables (frequencies and percentages). The Chi-square (X^2) test (at 95% confidence intervals) was used to compare the antimicrobial profile of bacterial isolates as well as the proportions of MBL producers between hospital and environmental isolates. P-value ≤ 0.05 is considered statistically significant.

3. RESULTS

The distribution of MBL and non-MBL-producing isolates based on the source of the isolate is shown in Table 1. Out of the 85 isolates of *E. coli* recovered, 22 (25.9%) had reduced susceptibility to imipenem. Of these 22 isolates 8 (9.4%) were MBL producers while 14 (16.5%) isolates were non-MBL producers. The highest prevalence of MBL producers was recovered from Urine and environmental samples 3(37.5%) followed by stool and HVS 1(12.5%) each. Statistically, there was no significant difference between MBL production and the source of the isolate. The overall prevalence of MBL was 9.4% (8/85).

Commented [U11]: Italicize the name of microorganism

Table1: Distribution of MBL and non-MBL producing *E. coli* Isolates based on sample source

Source of Isolate	Number of Isolate	No/ % Resistant to Imipenem	MBL Producers	Non-MBL Producers
Urine	34(40.0)	7 (31.8)	3(37.5)	4 (28.6)
HVS	20 (23.5)	4 (18.2)	1(12.5)	3 (21.4)
Stool	13 (15.3)	5 (22.7)	1(12.5)	4(28.6)
Non-clinical	18 (21.2)	6 (27.3)	3 (37.5)	3 (21.4)
Total	85	22(25.9)	8 (9.4)	14 (16.5)
P= 0.920	X²= 3.86			

The distribution of MBL and non-MBL-producing isolates of *K. pneumoniae* according to sources of isolates is shown in Table 2. Of the 18 (27.7%) isolates of *K. pneumoniae* that were resistant to imipenem, 10 (15.4%) were MBL producers while 8 (12.3%) were non-MBL producers. The highest prevalence of MBL was recovered from urine 4(40%), followed by sputum 3 (30%) and the least was from wound 1 (10%). There was no significant difference between MBL producers and the source of the isolates, P= 0.981.

Commented [U12]: Italisize

Commented [U13]: The same

Table 2: Distribution of MBL and non-MBL producing *K. pneumoniae* isolates based on sample source

Source of Isolate	Number of Isolate	No/% Resistant to Imipenem	MBL Producers	Non-MBL Producers
Urine	35 (53.8)	7(38.9)	4 (40.0)	3 (37.5)
Sputum	12 (19.4)	5 (27.8)	3 (30.0)	2 (25.0)
HVS	6 (9.2)	1 (5.5)	0 (0.0)	1 (12.5)
Wound	7 (10.8)	2 (11.1)	1(10.0)	1 (12.5)
Non-clinical	5 (7.7)	3 (16.7)	2(20.0)	1 (12.5)
Total	65	18 (27.7)	10 (15.4)	8 (12.3)
P = 0.981	X² = 3.74			

Commented [U14]:

Commented [U15]: 18.5

Commented [U16]: 5.6

Table 3 is the in vitro antibacterial susceptibility and resistant profile of MBL and Non-MBL producing *E. coli*. MBL-producing isolates showed higher resistance in comparison to non-MBL producers with an average resistance prevalence of 76.3% as against 60.8%. MBL-producing isolates showed complete (100%) resistance to cefixime, ceftazidime, cefuroxime, cefotaxime, and augmentin. The most potent antibiotic was nitrofurantoin in both MBL and non-MBL producers 87.5% and 78.5% respectively.

Table 3: In Vitro Antibacterial profile of MBL and Non-MBL isolates of *E. coli* (n=85)

Antibiotics	MBL Producers (n=8)		Non-MBL Producers (n= 14)		%
	No/% Susceptible	No/ % Resistant	No/% Susceptible	No/ Resistant	
Cefixime	0 (0.0)	8 (100)	0 (0.0)	14 (100)	
Ceftazidime	0 (0.0)	8 (100)	6 (42.9)	8 (57.1)	
Cefuroxime	0 (0.0)	8 (100)	0 (0.0)	14 (100)	
Cefotaxime	0 (0.0)	8 (100)	6 (42.9)	8 (57.1)	
Cefoxitin	5 (62.5)	3 (37.7)	8 (57.1)	6 (42.9)	
Ofloxacin	1 (12.5)	7 (85.5)	9 (64.3)	5 (35.7)	
Augmentin	0 (0.0)	8 (100)	0 (0.0)	14 (100)	

Ciprofloxacin	2 (25.0)	6 (75.0)	2 (14.3)	12 (85.7)
Gentamicin	2 (25.0)	6 (75.0)	6 (42.9)	8 (57.1)
Nitrofurantoin	7 (87.5)	1 (12.5)	11 (78.8)	3 (21.4)

Commented [U17]: 78.6

Table 4 shows the in vitro antibacterial profile of MBL and non-MBL isolates of *K. pneumoniae*. MBL producers were 100% resistant to cefixime, ceftazidime, augmentin, and ofloxacin, 80% and 70% for cefotaxime and ciprofloxacin respectively. There was a high susceptibility to aminoglycoside gentamicin 80% and ceftazidime 70%. Of the 8 isolates that were non-MBL, there was high resistance of isolates to most of the antibiotics, 100% resistance to augmentin, 87.5 % resistance to cefixime, cefuroxime, cefotaxime, and ofloxacin. There was high susceptibility of non-MBL isolates to ciprofloxacin 87.5%, nitrofurantoin and gentamicin 75%, and ceftazidime 62.5%.

Table 4: In Vitro Antibacterial profile of MBL and Non-MBL isolates of *K. pneumoniae* (n=65)

Antibiotics	MBL Producers (n= 10)		Non-MBL Producers (n= 8)	
	No/% Susceptible	No/ % Resistant	No/% Susceptible	No/ % Resistant
Cefixime	0 (0.0)	10 (100)	1(12.5)	7 (87.5)
Ceftazidime	0 (0.0)	10 (100)	0 (0.0)	8 (57.1)
Cefuroxime	0 (0.0)	10 (100)	1(12.5)	7 (87.5)
Cefotaxime	2 (20.0)	8 (80.0)	1(12.5)	7 (87.5)
Cefoxitin	7 (70.0)	3 (30.0)	5 (62.5)	3 (37.5)
Ofloxacin	0 (0.0)	8 (100.0)	1(12.5)	7 (87.5)
Augmentin	0 (0.0)	10 (100)	0 (0.0)	8 (100)
Ciprofloxacin	3(30.0)	7 (70.0)	7 (87.5)	1 (12.5)
Gentamicin	8 (80.0)	2 (20.0)	6 (75.0)	2 (25.0)
Nitrofurantoin	4 (40.0)	6 (60.0)	6 (75.0)	2 (25.0)

Commented [U18]: 100

Commented [U19]: 80

DISCUSSION

Global public health is seriously threatened by the rapidly evolving and spreading *Enterobacteriaceae* that produce MBL, especially *K. pneumoniae* and *E. coli*^[19]. MBLs are beta-lactamase enzymes that hydrolyze carbapenems, such as imipenem, making them useless as therapeutics^[7]. In our study, 26.7% (40/150) of the total isolates of *E. coli* and *K. pneumoniae* were found to be resistant to imipenem. Many researchers have reported the same. Kumarasamy et al., in their research on the emergence of a new antibiotic resistance mechanism in India, Pakistan, and the United Kingdom reported a prevalence of 24% while Panchal et al., reported 28% in India^[20, 21]. However, Javed et al., recorded lower prevalence of 11.81% in Pakistan^[12]. Imipenem resistance was also found to be higher in *K. pneumoniae* than in *E. coli* 27.7% and 25.9% respectively, many researchers had reported the same^[19, 22, 23]. The extensive use of carbapenems in the treatment of severe infections brought on by organisms that produce extended-spectrum beta-lactamases (ESBLs) has been a major factor in the rise of carbapenemases^[24]. The two main problems associated with carbapenemase production are their compromise to the effectiveness of antibiotics used as a last resort infection treatment and their ability to spread to different bacteria globally^[7].

Commented [U20]: enterobacteriaceae

Commented [U21]: italicize and add year

Commented [U22]: the same

Commented [U23]: the same

This study showed the prevalence of MBL in *K. pneumoniae* and *E. coli* to be 15.4%, and 9.4% respectively giving an overall prevalence of 12%. Mittal et al., and Oberoi et al. reported 23.37% and 22.72% respectively in India^[25, 26] and Shanhandeh et al., recorded 23.1% in Iran^[27]. Bora et al., reported a

prevalence of 18.98% and 21.08% in *E. coli* and *K. pneumoniae* respectively in Nepal^[19]. However, research purportedly carried out in several nations revealed that the rates of MBL generation for *K. pneumoniae* and *E. coli* range from 33–36% and 13.4–61.5%, respectively^[28]. This study demonstrates that there are wide regional variations. These differences might be due to variations in sample size, detection technique, and isolate sources^[3].

In our study, MBL production was found to be higher in *K. pneumoniae* than in *E. coli*. Similar findings have been reported by many authors^[19, 22, 29]. Higher resistance in *K. pneumoniae* may be due to alterations in cellular permeability caused by modifications in the AcrAB-TolC and KpnEF efflux pump systems, as well as the loss of the putative porin KpnO^[30].

In this study, the highest rate of MBL-producing isolates was recovered from urine isolates 38.9% (7/18). This agrees with the 32.35% reported in India^[25] but does not align with what was reported by Singh et al which recorded the highest prevalence of 62.5% in ET secretion^[32] and Kamble et al recorded the highest in pus at 42.55%^[31]. Bora et al recorded the highest MBL-producing isolates from blood^[19]. Our higher prevalence in urine could be due to the large number of urine isolates included in our study. In addition *E. coli* and *K. pneumoniae* are the common cause of urinary tract infection.

There is evidence linking carbapenemase producers to numerous additional non-beta-lactam resistance factors that contribute to MDR^[6]. The antibacterial profile showed that MBL producers displayed resistance to nearly all the commonly used antibiotics and this raises serious concerns. Many other investigations have found that MBL-positive isolates generally resist even carbapenems, employed as a last resort for treating MDR Gram-negative bacteria infections^[33]. There was complete resistance of MBL-producing isolates to cefixime, ceftazidime, cefuroxime, cefotaxime, and Augmentin. There was a high resistance of isolates to fluoroquinolones. According to our findings, all MBL-producing isolates are multidrug-resistant (MDR). This is consistent with the work of Bora et al.^[19]. It has been reported that the prevalence of drug-resistant bacteria varies not only between nations but also between healthcare facilities therein. This variation may be partially attributed to variations in local prescribing practices of antibiotics and the efficacy of infection control programs among various healthcare facilities. Hospitalization and prior antibiotic use are also common risk factors linked to infection by multidrug-resistant bacteria^[28]. These findings suggest that patients may be at risk because there are few available treatment options^[19].

5. Conclusion

Our research revealed that a sizable percentage of *K. pneumoniae* and *E. coli* produced MBL. It was found that every isolate that produced MBL was multidrug resistant. Consequently, it is increasingly necessary for diagnostic microbiology laboratories to routinely identify isolates of *K. pneumoniae* and *E. coli* that produce MBL. The combined disc test provides a reasonable alternative for phenotypic detection of MBL production in the absence of molecular detection and can be done in laboratories regularly. Routine surveillance is also necessary to develop appropriate empirical antibiotic therapy and control the spread of MBL-producing bacteria in our environment. This necessitates introduction of novel antibiotics with fresh mechanisms of action to stop the spread of MDR bacteria. Setting up an efficient infection control program is also crucial.

ETHICAL APPROVAL

Commented [U24]: Also compare with the work of Eze and Ayo-Loto 2021. Citation: Eze E.M Ayo-Loto F.J. Prevalence of Extended Spectrum Beta-Lactamase producing enterobacteriaceae isolated from Clinical samples in Ilorin Metropolis, Kwara state Nigeria. J. Microbiol Biotechnol 2021, 6(2):000195.

Not applicable

CONSENT

Not applicable

REFERENCES

1. Khalifa SM, Abd El-Aziz AM, Hassan R., Abdelmegeed ES. β -Lactam Resistance Associated with β -Lactamase Production and Porin Alteration in Clinical Isolates of *E.coli* & *K. pneumoniae*. *PLoS ONE* 2021;16(5):e0251594. <https://doi.org/10.1371/journal.pone.0251594>
2. Tamma PD, Simner PJ. Phenotypic Detection of Carbapenemase-Producing Organisms from Clinical Isolates. *Journal of Clinical Microbiology* 2018;56: e01140
3. Ejikeugwu C, Nworie O, Saki M. Metallo- β -lactamase & AmpC Genes in *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* isolates from abattoir and poultry origin in Nigeria. *BMC Microbiology* 2021; 21:124.
4. Durrmeyer X, Cohen R. Utilisation des carbapénèmes en pédiatrie. *Archives de pédiatrie* 2010; 17, S163-S170. [https://doi.org/10.1016/S0929-693X\(10\)70919-2](https://doi.org/10.1016/S0929-693X(10)70919-2)
5. Nordmann P, Carrer A. Les carbapénèmes des entérobactéries. *Archives de Pédiatrie* 2010; 17: S154-S162. [https://doi.org/10.1016/S0929-693X\(10\)70918-017](https://doi.org/10.1016/S0929-693X(10)70918-017)
6. Nordmann P, Dortet L, Poirel L. Carbapenem Resistance in Enterobacteriaceae: Here Is the Storm! *Trends in Molecular Medicine* 2012; 18: 263-272. <https://doi.org/10.1016/j.molmed.2012.03.003>
7. Halat DH, Moubareck CA. The Current Burden of Carbapenemases: Review of Significant Properties and Dissemination among Gram-Negative Bacteria. *Antibiotics* 2020;(9): 186; doi:10.3390/antibiotics904018
8. Bush K. Past and Present Perspectives on β -Lactamases. *Antimicrob. Agents Chemother.* 2018; 62(10):e01076-18. Doi: 10.1128/AAC.01076-18.
9. Ugwu MC, Shariff M, Nnajide, CM, Beri K, Okezie M, Iroha IR, Esimone CO. Phenotypic and Molecular Characterization of β -Lactamases among Enterobacterial Uropathogens in Southeastern Nigeria. *Canadian Journal of Infectious Diseases and Medical Microbiology* 2020;9(2): 1-9
10. Landelle C, Dautzenberg M, Wekesa A, Gniadkowski M, Antoniadou A, Giamarellou H. et al. The Association between Colonization with Carbapenemase-Producing Enterobacteriaceae and Overall ICU Mortality. *Critical Care Medicine* 2015; 43: 1170-1177. <https://doi.org/10.1097/CCM.0000000000001028>
11. Gupta V, Singh M, Datta P, Goel S, Singh S, Prasad K, Chander J. Detection of various Beta-Lactamases in *Escherichia coli* and *Klebsiella* spp: A study from Tertiary Care Centre of North India. *Indian J. Med Microbiol* 2020;38: 390-396

12. Javed H, Ejaz H, Zafar A, Rathore AW, Haq I. Metallo-beta-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae*: A rising threat for hospitalized children. *Journal of Pakistan Medical Association* 2016; 66(9) :1068-1071
13. Sachdeva R, Sharma B, Sharma R. Evaluation of Different Phenotypic Tests for Detection of Metallo- β -lactamases in Imipenem-Resistant *Pseudomonas aeruginosa*. *Journal of laboratory physicians* 2017; 9: 249.
14. Fazlul MK, Nazmul M.H, Najnin A, Munira B. Detection of Metallo- β -Lactamase Encoding Genes among Clinical Isolates of *Escherichia coli* in a Tertiary Care Hospital, Malaysia. *Journal of International Pharmaceutical Research*, 2019; 11(1): 1-8. <https://doi.org/10.31838/ijpr/2019.11.01.031>
15. Baron EJ, Peterson LR, Finegold SM. Baily and Scott's Diagnostic Microbiology 9th Edition; Mosby Inc. St Louis Baltimore; 1994: 362-385
16. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-fifth Informational Supplement M100-S21. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.
17. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-first Informational Supplement M100-S25. Wayne, PA: Clinical and Laboratory Standards Institute; 2015
18. Franklin C, Liolios L, Peleg AY. Phenotypic detection of carbapenem-susceptible metallo- β -lactamase-producing gram-negative bacilli in the clinical laboratory. *Journal of clinical microbiology* 2006; 44(9): 3139-3144.
19. Bora A, Sanjana R, Jha BK, Mahaseth SN, Pokharel K. Incidence of Metallo-Beta-lactamase Producing Clinical Isolates of *Escherichia Coli* and *Klebsiella pneumoniae* in Central Nepal. *BMC Research Notes* 2014; 7:557.
20. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R et al . Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: A molecular, biological, and epidemiological study. *Lancet Infect Dis*. 2010; 10 (9):597-602. doi: 10.1016/S1473-3099(10)70143-2
21. Panchal CA, Oza SS, Mehta SJ. Comparison of four phenotypic methods for detection of metallo- β -lactamase-producing gram-negative bacteria in rural teaching hospital. *J. Lab Physicians* 2017;9:81-3
22. Kulkarni SS, Mulay MV. Phenotypic detection of metallo-beta-lactamase production in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* in a tertiary care hospital. *MGM J. Med Sci* 2022;9:149-153
23. Agarwal N, Chauhan S, Sing P, Sharma VK. Phenotypic detection of carbapenem resistance and metallo-beta-lactamase among Enterobacteriaceae from clinical samples in tertiary care hospital. *Int. J. Res Med Sci* 2019;7:2373-6
24. Kanj SS, Kanafani ZA. Current concepts in antimicrobial therapy against resistant gram-negative organisms: Extended-spectrum beta-lactamase-producing, Enterobacteriaceae, carbapenem-resistant Enterobacteriaceae, and multidrug-resistant *Pseudomonas aeruginosa*. *Mayo Clin. Proc.* 2011; 86:250-25
25. Mittal S, Mamoria VP, Singla A, Bacchani D, Chaudhary P. Phenotypic Detection of Metallo- β -lactamase in Carbapenem Resistant Clinical Isolates of *Klebsiella pneumoniae ssp. pneumoniae* at a Tertiary Care Hospital in Jaipur. *Journal of Medical Science and Clinical Research* 2020; 8 (3): 703-708
26. Oberoi L, Singh N, Sharma P, Agarwal A. ESBL, MBL and Ampc β Lactamases Producing Superbugs – Havoc in the Intensive Care Units of Punjab India. *Journal of Clinical and Diagnostic Research*. 2013 Jan; 7(1): 70-73.

27. Shahandeh Z, Sadighian F, Rekabpou KB. Phenotypic study of Extended-spectrum beta-lactamase, AmpC and Carbapenemase among *E.coli* clinical isolates in affiliated hospitals of Babol University of Medical Sciences. *Int J Health Syst Disaster Manage* 2015; 3:74-78.
28. Nepal K, Pant ND, Neupane B1, Belbase A, Baidhya R, Shrestha RK, Lekhak B, Bhatta DR, Jha B. Extended spectrum beta-lactamase and metallo beta-lactamase production among *Escherichia coli* and *Klebsiellapneumoniae* isolated from different clinical samples in a tertiary care hospital in Kathmandu, Nepal. *Ann Clin MicrobiolAntimicrob* 2017;16: 62. Doi 10:1186/s12941-017-0236-7
29. Adhikary M, Chakrabarty PS. Evaluation of metallo-beta-lactamase enzyme among gram-negative isolates from patients admitted in a tertiary care hospital, India. *J Dental Med Sci* 2017;16:95-100.
30. Andrade LN, Vitali L, Gaspar GG, Bellissimo-Rodrigues F, Martinez R, Darini AL. Expansion and evolution of a virulent, extensively drug-resistant (polymixin B-resistant), QnrSI-, CTX-M-2-, and KPC-2-producing *Klebsiella pneumoniae* ST11 international high-risk clone. *J. Clin Microbiol.* 2014; 52:2530-2535
31. Kamble D. Phenotypic detection of ESBL and MBL in Gram Negative bacilli isolated from clinical specimens. *Int J Med Res Rev* 2015; 3(8):866-870.
32. Singh M, Kakati B, Agarwal RK, Kotwal A. Detection of *Klebsiella pneumoniae* carbapenemases (KPCs) among ESBL / MBL producing clinical isolates of *Klebsiella pneumoniae*. *International Journal of current Microbiology and Applied Sciences.* ISSN: 2319-7706 Volume 4 Number 4 (2015) pp. 726-731
33. 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. doi.org/10.1155/2022/5474388