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Isolation of Metallo Beta Lactamases from Clinical Sample (MBL)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The rapid spread of acquired metallo-beta-lactamases (MBLs) among major Gram-negative pathogens is an emerging threat and a matter of particular concern worldwide. Carbapenems are among the few useful antibiotics against gram negative bacteria particularly those with extended spectrum beta-lactamase. Resistant to carbapenem is mediated by loss of outer membrane proteins and production of beta lactamase that is capable of hydrolyzing carbapenems.During this study period, 80 different clinical samples were collected from various hospitals of Raipur. All clinical samples were processed according to standard microbiological methods. Isolated GNRs were subjected to susceptibility testing against antibiotics by disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Carbapenem-resistant isolates were subjected to the detection of MBL production by different methods. Out of 80 samples, 28 (35%) samples showed significant growth. MBL production was observed in 20 samples.

Keywords: MBL; ESBLs; carbapenem-resistant gram-negative; CDT; DDST.

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1. INTRODUCTION

New Delhi Metallo-beta-lactamase-1 (NDM-1) is an enzyme that makes bacterial resistant to a broad range of beta-latam antibiotics. These include the antibiotics of the carbapenem family, which are a mainstay for the treatment of antibiotic-resistant bacterial infections [1]. The gene for NDM-1 is one member of a large gene family that encodes beta-lactamase enzyme called carbapenemase. Unlike class A, C and D beta-lactamases, NDM-1 has zinc ions at its active site, and it can hydrolyse all beta-lactam antimicrobials except for monobactam [2,3]. Moreover, most NDM-1-positive bacteria are resistant to a wide variety of other antimicrobial classes. Bacteria that produce carbapenemases are often referred to in the news media as "superbugs" because infections caused by them are difficult to treat. NDM was first reported in the literature in 2009 in *Klebsiella pneumoniae* isolate and was referred to as NDM-1 [4]. It was later detected in bacteria in India, Pakistan, the United Kingdom, the United States, Canada,and Japan. The most common bacteria that make this enzyme are Gram-negative such as *Escherichia coli* and *Klebsiella pneumoniae*, but the gene for NDM-1 can spread from one strain of bacteria to another by horizontal gene transfer [5]. Carbapenem are a class of beta-lactam antibiotics that are capable of killing most bacteria by inhibiting the synthesis of one of their cell wall layers. The carbapenems were developed to overcome antibiotic resistance mediated by bacterial beta-lactamase enzymes. However, the bla_{NDM-1} gene produces NDM-1, which is a carbapenemases beta-lactamase - an enzyme that hydrolyzes and inactivates these carbapenem antibiotics. These enzymes possess the characteristic hallmarkof being universally inhibited by EDTA as well as other chelating agents of divalent cations, a quintessential feature of MBLs that correlates with their mechanistic function [6].

2. MATERIALS AND METHODS

2.1 Sample Collection and Handling

Total 80 different clinical samples were collected over a period of 90 days from different hospitals of Raipur, Chhattisgarh. The samples were collected irrespective of age and sex. The samples were processed and isolates were characterized following standard laboratory procedures [7].

2.1.1 Isolation and characterization

For isolation of organisms all the clinical samples were inoculated first on Mac-conkey's agar media and incubated at 37 for 24 hours. After incubation, culture characteristics of isolates were identified based on various biochemical characterizations as per standard microbiological techniques [7].

2.1.2 Antibiotic susceptibility testing

The isolates were tested for the antimicrobial susceptibilities by disc diffusion technique on Muller-Hinton agar [8]. The turbidity of inoculums suspension was adjusted to 0.5 Macfarland's standard. Then this suspension was inoculated onto Muller-Hinton agar plate by lawn culture. After that antibiotic discs were placed using sterile forceps and pressed gently to confirm proper contact with medium [9]. The plates were then incubated at 37 for 24 hrs. The zone of inhibition was measured and interpreted as per CLSI guidline CLSI 2008 [10].

2.2 Detection of MBLs

2.2.1 Modified hodge test

All the carbapenem resistant strains were subjected to MHT for detection of carbapenemases. The suspension of ATCC *E. coli* was prepared in comparison to 0.5 McFarland standard in 5 mL of sterile saline using the direct colony suspension. A 4.5 mL of sterile saline was pipetted out into a sterile tube. Then 0.5 mL of the ATCC *E. coli* suspension was added to 4.5 mL of saline to make a 1 : 10 dilution. Diluted ATCC *E. coli* was inoculated to a Mueller Hinton (MH) plate containing 70 µg/mL of zinc sulphate and then streaked over the entire plate with the help of sterile cotton swab. A 10 µg meropenem susceptibility disk was placed in the centre of the MH plate. Then the test organism, positive control (*Klebsiella pneumoniae* ATCC) and negative control (*Klebsiella pneumoniae* ATCC), was streaked in a straight line from the edge of the meropenem disk to the edge of the plate and then incubated at 35ºC ± 2ºC in an ambient air for 16–20 hours. Carbapenemase production was detected by the appearance of the enhanced ATCC *E. coli* growth along the test organism that revealed a clover-leaf-like indentation which indicated a positive test.

2.2.2 Imipenem disc diffusion method

Imipenem disk diffusion method was employed as a screening test to select suspected MBL-PA strains showing resistance to imipenem which were further confirmed by imipenem-EDTA combined disk method and imipenem- EDTA double disk synergy test. 0.5M EDTA (Hi-Media, Mumbai, India) was prepared with distilled water and sterilized by autoclaving. Imipenem disks were supplemented with EDTA by dispensing 10µl of this solution to each imipenem disk.

2.2.3 Imipenem EDTA combine disk method

Imipenem-EDTA combined disk method (CDT) was performed as described by Yong et al. [11]. A lawn culture of test isolates was prepared. After allowing it to dry for five minutes, two imipenem discs, one with 0.5 M EDTA and the other a plain imipenem disc, were placed on the surface of agar plates approximately 30mm apart. The plates were incubated overnight at 37°C. An increase in zone diameter of ≥ 7mm around imipenem+EDTA disk in comparison to imipenem disk alone indicated production of MBLs.

2.2.4 Imipenem-EDTA double disk synergy test (DDST)

Imipenem-EDTA double disk synergy test (DDST) was performed. as described by Lee et al. [11]. Test organisms were inoculated on to plates with Mueller Hinton agar as recommended by CLSI. An imipenem (10µg) disk was placed 20mm centre to centre from a blank disk containing 10µL of 0.5 M EDTA (750µg). Enhancement of the zone of inhibition in the area between imipenem and EDTA disk in comparison with the zone of inhibition on the far side of the drug was interpreted as a positive result for MBL production.

3. RESULTS

During this study period, 80 different clinical samples were collected out of which 28 (35%) samples showed significant growth. The gram stain and series of biochemical test was performed for the identification of the isolates. They were identified as *Escherichia Coli* (n=13), *Klebsiella pneumoniae* (n=6), *Pseudomonas aeruginosa* (n=9). Metallo beta lactam production was observed in 20 isolates (71%) *Escherichia Coli* (n=7), *Klebsiella pneumoniae* (n=5), *Pseudomonas aeruginosa* (n=8). Out of 28 clinical isolates Imipenem resistance was seen in 19 isolates (*E. coli*-7, Pseudomonas-8 & Klebsiella-5), Meropenem resistance was seen in 10 isolates (*E. coli*-2, Pseudomonas-6 & Klebsiella-2), Ertapenem resistance was seen in 6 isolates (*E. coli*-3, Pseudomonas-3 & Klebsiella-0), Dorepenem resistance was seen in 11 isoaltes (*E. coli*-4, Pseudomonas-4 & Klebsiella-3), while faropenem resistance was seen in only 1 isolate of Pseudomonas (Fig. 1).

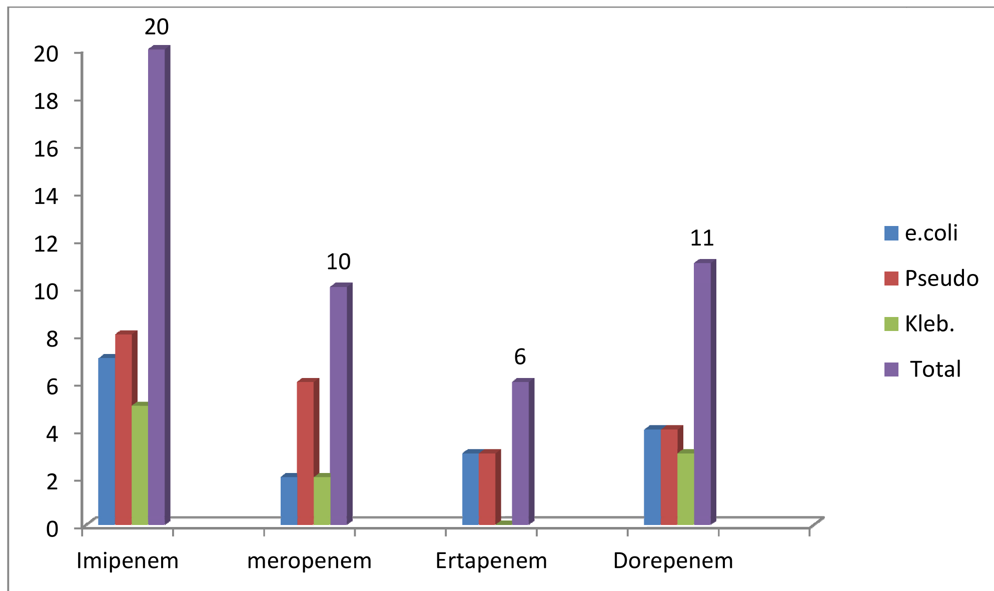

Fig. 1. Graph showing organisms resistant to carbapenems

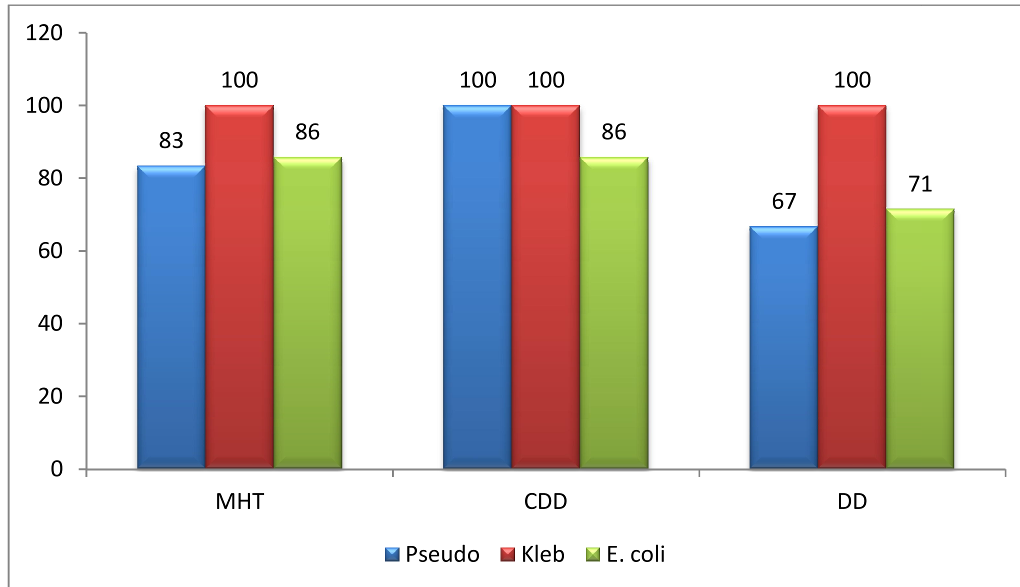

Fig. 2. Graph showing the sensitivity of different method for detection of Metallobetalactams

4. DISCUSSION

Frequency of carbapenem resistance was observed in a study conducted by Hussein et al. [12] in which (58.26%) isolates were resistant to both meropenem and imipenem. The meropenem resistant strains were screened for carbapenemase production by MHT and 83.3% were identified as carbapenemase producers [12,13] reported that 71% of the isolates were carbapenemase producers by the MHT, which are in agreement with the current study especially in regards to imipenem [13]. This was also in concordance with the results which were reported by Lee et al. [14] in Korea, where 73% of the isolates were found to be carbapenemase positive by the MHT [14] .The imipenem resistant isolates were further screened for MBL production. 95.4% were positive by CDT and 77.2% were positive by DDST. Similar study conducted by Pandya et al. [15] showed that 96.30% of isolates were MBL positive by CDT and 81.4% were positive by DDST [15]. The results of present study also correlate with the results of study conducted by Irfan et al. [16] at Aga Khan University, Karachi, in which 96.6% of the carbapenem resistant strains were MBL producers by CDT [15]. Similar results were obtained with the study conducted by Noori et al. [17], in which 86.8% of isolates were identified as MBL producers by CDT [15].

Begum et al. (2013) performed a study at Quaid-i-Azam, University Islamabad, in which 42.85% infections caused by MDR organism. Islahi et al. [18] also observed a high percentage of nosocomial infections more in males (76.0%) than females (23.9%) [18]. Similar results were seen in a study performed by Peymani et al. (2011) in which 72% were male and 28% were female patients [19].

The most frequent site of infection was urinary tract infections (21.2%) followed by blood stream infections (18.2%) and respiratory infections (13.6%). The results were different from other studies which reported that respiratory tract infections were the common site of infection. Noori et al. [17] found the highest percentage of MBL producing *E. coli* in respiratory tract specimens (52.8%) followed by urine (26.9%) and blood (7.4%) [17].

Current study depicts that all the MBL producers possess very high resistance to all antimicrobials (beta-lactams, aminoglycosides, and fluoroquinolones) from 71.2% to 100% and also revealed 83.3% resistance to piperacillin/ tazobactam. All MBL producing. *E. coli* 18.2%to sulbactam/ cefoperazone. The present study showed that colistinsulphate and sulbactam/ cefoperazone were the most effective drugs for the treatment of MDR organism.

5. CONCLUSION

The present study demonstrates the presence of high level of multiantibiotic resistance among carbapenem resistantisolates from tertiary care patients as assessed in 2016 in Raipur. Comparison of multiple phenotypic assays for the detection of metallo-beta-lactamases in bacteria indicates that the combined disk test provides the highest rate of positive tests.

CONSENT AND ETHICAL APPROVAL

As per international standard or university standard guideline patients consent and ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kumarasamy KK, Toleman MA, Walsh TR. 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.
2. Queenan AM, Bush K. Carbapenemases: The versatile beta-lactamases. Clin Microbiol Rev. 2007;20(3):440–458.
3. Cornaglia G, Giamarellou H, Rossolini GM. Metallo-beta-lactamases: A last frontier for beta-lactams? Lancet Infect Dis. 2011;11(5):381-93.
4. Yong D, Toleman MA, Giske CG. Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob Agents Chemother. 2009; 53(12):5046–5054
5. Hudson Corey, Zachary W Bent, Meagher RJ, Williams KP. Resistant determinants and mobile genetic elements of an NDM-1-encoding *Klebsiella pneumonia* strains. PloS one. 2014;9(6):99209.
6. Rasmussen BA, Bush K. Carbapenemhydrolyzing β-lactamases. Antimicrob. Agents Chemother. 1997;41: 223-232.
7. Bergey's Manual of Systematic Bacteriology. Book Review Int. J. of Syst. Bact.1985;408.
8. Prescott LM, Harley JP, Klein DA. Classification of prokaryotes according to the second edition of Bargey's manual of systematic bacteriology in microbiology, fifth ed.Publisher Mc Graw Hill, New York. 2003;22-26.
9. Anwar M, Ejaz H, Zafar A, Hamid H. Phenotypic detection of metallo-beta-lactamases in carbapenem resistant Acinetobacter baumannii isolated from pediatric patients in Pakistan. Journal of Pathogens; 2016.
10. Clinical and laboratory standards institute (CLSI),performance Standards for antimicrobial susceptibility testing; eighteenth informational supplement. Screening and confirmatory tests for ESBLs in *Klebsiella pneumonia*, *K. oxytoca, Escherichia coli* and *Proteus mirabills*. CLSI. 2008;100-18;28(1):162-63.
11. Lee KY, Chong HB, Shin YA Kim, Yong D, Yum JH. Modified HODGE and EDTA-disk synergy tests to screen metallo-beta-lactamase-producting strsins of *pseudomonas* and Acinetobacter spices. Clin. Microbio. Infect. 2001;7:88-91.
12. Hussain HN, Al Mathkhury JF, Sabbah AM. Imipenem resistant Acinetobacter baumanni isolated from patients and hospital environment in Baghdad. Iraqi Journal of Science. 2013;54(4):803-812.
13. Kumar AV, Pillai VS, Dinesh, K, Karim S. The phenotypic detection of carbapenemase in meropenem resistant acineto bacterrcalcoaceticus- baumannii complex in a tertiary care hospital in South India. Journal of Clinical and Diagnostic Research. 2001;5(2):223-22.
14. Lee K, Lim YS, Yong, JH Yum, Chong Y. Evaluation of the Hodge test and the Inimenem- EDTA double-disk synergy test for differentiating metallo-beta-lactamase producing isolates of Pseudomonas spp. and Acinetobacter spp. Journal of Clinical Microbiology. 2003;41(10):4623-4629.
15. Pandya PN, Prajapati BS, Mehta JS, Kikani MK, Joshi JP. Evaluation of various methods for detection of MBL production in Gram negative bacilli, International Journal Biological and Medical and Medical Research. 2011;2(3):775-777.
16. Irfan S, Zafa A, Guhar D, Ahsan T, Hasan R. Metallo beta-lactamase-producing clinical isolates of *Acinetobacter species*

and *Pseudomonas aeruginosa* from insentive careunit patients of a tertiary care hospital. Indian Journal of Medical Microbiology. 2008;26(3):243-245.

17. Noori M, Karimi A, Fallah F. High prevalence of metallo-beta-lactamase producing Acinetobacter baumanni isolated from two hospitas of Tehran, Iran, Archives of Pediatric Infectious Disease. 2014;2(3):15439.
18. Islahi S, Ahmad F, Khareet V. Prevalence and resistance pattern of *Acinetobacter* spices in hospitalized patients in a tertiary care centre, Journal of Evolution of Medical and Dental Science. 2014;3(17): 4629-4635.
19. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: A clinical update. Clinical Microbiology. 2005;18(4): 657-86.

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