ORIGINAL ARTICLE

Study of the prevalence of metallo-β-lactamase-producing enterobacteriaceae from a tertiary care hospital in Mumbai

Pravin K. Nair, Michelle S. Vaz

Department of Microbiology, Holy Spirit Hospital, Mumbai, Maharashtra, India

ABSTRACT

Background and Objectives: The increasing prevalence of metallo-β-lactamase (MBL)-producing enterobacteriaceae in many geographical regions and their potential for rapid dissemination has become a serious threat to public health. Thus, the present study was conducted to analyze the prevalence of MBL-producing enterobacteriaceae in a tertiary care hospital in Mumbai. **Materials and Methods:** The study was carried out in the Microbiology Department of a 330-bedded tertiary care hospital over a 5-month period, using clinical samples. Bacterial isolates were identified using the Vitek 2 compact system. All enterobacteriaceae isolates were checked for MBL production by the MBL E Test using MBL Ezy MIC™ Strips (HiMedia); and for carbapenem resistance using the Vitek 2 compact system. **Results:** The prevalence rate for MBL-producing enterobacteriaceae from the study was found to be 7.56%, of which the majority of isolates was detected in urine samples (76.92%). Although most of the MBL isolates were detected in samples from the wards (53.85%), a noteworthy number of isolates was also detected from the outpatient department (OPD;15.38%). **Conclusion:** Thus, the study shows a significant rate of MBL production among enterobacteriaceae isolated from hospitalized and OPD patients. This emphasizes the urgent need for antimicrobial stewardship at the hospital and community level.

Key words: Antimicrobial Stewardship, carbapenem resistance, metallo-β-lactamase (MBL)

INTRODUCTION

The emergence of carbapenem-resistant enterobacteriaceae (CRE) recently, has become a serious threat to public health.[1] Carbapenem resistance is mostly due to the production of carbapenemases, which can be either serine β-lactamases or metallo-β-lactamases (MBLs). MBLs are capable of hydrolyzing carbapenems, and most cephalosporins and penicillin. They resist hydrolysis by clavulanate, but are inhibited by chelating agents such as ethylenediaminetetraacetic acid (EDTA).[2,3]

MBL producers are currently a significant risk worldwide due to the high mortality, potential dissemination rates, and limited treatment options associated with these organisms.[4] Thus, the present study was conducted to evaluate the prevalence of MBL-producing enterobacteriaceae in a tertiary care hospital.

Access this article online

Quick Response Code:

Website: www.jacmjournal.org

DOI: 10.4103/0972-1282.144705

MATERIALS AND METHODS

This study was carried out in the Microbiology Department of a tertiary care hospital in Mumbai, Maharashtra, India over a 5-month period.

Isolation and Identification of Bacterial Isolates

All isolates obtained were from clinical samples collected from patients hospitalized in the wards, the Intensive Care Unit (ICU), the Cardiac Care Unit (CCU), the Neonatal Intensive Care Unit (NICU), the Pediatric Intensive Care Unit (PICU), as well as from Outpatient Department (OPD). Samples included blood, urine, endotracheal secretions, sputum, pus, and wound samples; all of which were processed as per standard microbiology protocol.[5] The identification of all clinical isolates was done using the Vitek 2 compact system (bioMérieux, France). All identified enterobacteriaceae isolates were then subjected to antibiotic susceptibility testing by the Vitek 2 compact system. Isolates that showed resistance to one or all of the following carbapenems — Meropenem, Imipenem, and Ertapenem; as well as resistance to Ceftriaxone,

Address for correspondence: Dr. Pravin K. Nair, E-mail: drpravinnair@gmail.com

Cefotaxime, and Ceftazidime were considered to be CRE. The resistance breakpoints used for detecting carbapenem resistance among enterobacteriaceae was ≥4 μg/ml for Imipenem and Meropenem, and ≥2 μg/ml for Ertapenem. The resistance breakpoints used for Ceftriaxone and Cefotaxime was ≥64 μg/ml and for Ceftazidime was ≥32 μg/ml. All resistance breakpoints were according to Clinical and Laboratory Standard Institute (CLSI) guidelines (document M100-S22).[6,7]

Detection of MBL producers

All identified enterobacteriaceae were then tested for MBL production by the MBL E test. The test was performed using Meropenem with and without EDTA Ezy MIC™ Strips manufactured by HiMedia. Results were recorded and interpreted as per manufacturer's instructions after overnight incubation at 37°C.[8]

Interpretation Criteria for MBL E test [Table 1]

RESULTS

A total of 546 bacterial isolates were obtained from the clinical samples tested over 5 months, of which 172 were enterobacteriaceae. Of these 172 enterobacteriaceae, 17 isolates were found to be carbapenem resistant as they fulfilled the above mentioned criteria.

The CRE isolates obtained are given in [Table 2].

Detection of MBL producers

All enterobacteriaceae isolates were then tested for MBL production by the MBL E test. Results obtained are given in [Table 3].

Thus, the prevalence of MBL-producing enterobacteriaceae in the hospital was found to be 13 out of 172 (7.56%). Out of these 13 MBL-producing enterobacteriaceae, one *Enterobacter cloacae* isolate obtained from an ICU patient's endotracheal secretion was not found to be carbapenem resistant.

The location-wise distribution of the enterobacteriaceae that tested positive for MBL production is given in, and the sample-wise distribution is given in [Table 2].

DISCUSSION

MBL carbapenemases are currently the most significant threat when compared to other carbapenemases. This is because the MBL coding genes are present in many unrelated bacterial species spread across the world, especially the Indian subcontinent.[9] MBL producers have the ability to colonize the human gut and spread through the faeco-oral route within the community.[1] Thus, the spread of MBL producers is deeply disconcerting in a country such as India with a reservoir of more than 1.4 billion people.[9] The increasing prevalence of MBL-producing Gram-negative bacilli in many geographical regions

Table 1: Interpretation criteria for MBL E test

Report	Formula (MIC in μ/ml) from E strip	Interpretative criteria
MBL positive strain	Meropenem (MRP) MIC ≥ 8 (MRP+EDTA) MIC	When the ratio of the MIC value obtained for MRP: the value of MRP + EDTA is more than or equal to 8 Or If zone is observed on the side coated with MRP + EDTA and no zone is observed on the opposite side
MBL negative strain	MRP MIC ≤ 8 (MRP + EDTA) MIC	When the ratio of the value obtained for MRP: the value of MRP + EDTA is less than or equal to 8
MBL (nonconclusive)	MRP/(MRP + EDTA) = >256/ > 64 Or MRP/(MRP + EDTA) = < 4/ < 1	When no zone of inhibition is obtained on either side Or if complete inhibition is obtained on both sides of the strip

*As per Ezy MIC™ kit insert. MBL: Metallo-β-lactamase; MIC: Minimum inhibitory concentration; EDTA: Ethylenediaminetetraacetic acid

Table 2: Strain and sample distribution of CRE isolates

	ICU/CCU		Ward		NICU/PICU		OPD		Total
	Klebsiella spp	Enterobacter spp	*E.coli*	Klebsiella spp	*E.coli*	Klebsiella spp	*E.coli*	Klebsiella spp	
Urine	1	0	2	5	1	0	3	0	12
Pus	0	0	0	2	0	0	0	0	2
Sputum	1	0	0	0	0	0	0	0	1
ET Sec	1	0	0	0	0	0	0	0	1
Blood	0	1	0	0	0	0	0	0	1
Total	3	1	2	7	1	0	3	0	17

CRE: Carbapenem-resistant enterobacteriaceae; ICU: Intensive care unit; CCU: Cardiac care unit; NICU: Neonatal intensive care unit; PICU: Pediatric intensive care unit; OPD: Outpatient department; ET Sec: Endotracheal secretions

Table 3: MBL-producing enterobacteriaceae

Organism	Total number of MBL producers
E.coli	5
Klebsiella spp.	7
Enterobacter spp.	1
Total	13

MBL = Metallo-β-lactamase

and their potential for rapid dissemination, makes it essential to detect MBL-producing isolates by simple and rapid phenotypic methods. Polymerase chain reaction (PCR) analysis is considered as the gold standard for the detection of MBL producers, but it is not suitable for daily testing in clinical laboratories due to the cost constraints.[8,10]

From our study, the prevalence of MBL-producing enterobacteriaceae in the hospital was found to be 7.56%; majority of which were obtained from urine samples (76.92%). Although most MBL isolates were obtained from patients admitted in the hospital wards (53.85%), two MBL producers were also isolated from OPD patient samples (15.38%). This suggests that some MBL isolates in the study may have been community acquired.

Out of 13 MBL-producing enterobacteriaceae isolates, one was not found to be carbapenem resistant. This is because carbapenemase production may not necessarily confer significant resistance to carbapenems among enterobacteriaceae. Thus, although an enterobacteriaceae isolate may be carbapenemase producing, it may have relatively low MICs to carbapenems, and hence not be considered as a CRE.[11] Such organisms pose additional risks since they carry hidden MBL genes that may spread unnoticed, leading to infection control problems.[10]

Although several cases of MBL-producing Gram-negative bacilli have been reported the world over.[3,12] studies on the prevalence of MBL-producing enterobacteriaceae specifically are not very extensive. From our study, MBL production was found to be 7.56%, which is intermediate compared to other similar studies from India. Deshmukh *et al.*, (2011) reported a prevalence rate of 1.25% for MBL-producing enterobacteriaceae isolated from a tertiary care hospital, while Shevade and Agrawal (2013) reported a prevalence rate as high as 10%.[13,14] In order to establish a clearer perspective into the current epidemiology of MBL production in the hospital setup, active surveillance of MBL producers is required.

MBLs have recently emerged as one of the most worrisome resistance mechanisms among enterobacteriaceae.[15] Current prevention strategies involve the rapid identification of patients colonized or infected with MBL producers, followed by the implementation of contact precautions. Patients who are colonized or infected with MBL producers often enter into multiple types of healthcare institutions during their illnesses. Therefore, it is necessary to have a broader, multi-institutional or regional approach to prevention, particularly in regions where MBLs are just beginning to be recognized.[16]

Our study had two major limitations: Our sample size was small and we were unable to use molecular techniques to confirm MBL production in isolates obtained.

CONCLUSION

This study shows high rates of MBL production among enterobacteriaceae isolated from hospitalized and OPD patients, and thus further emphasizes the need for proper detection and control of MBL dissemination within the hospital and community. Prevention will need concerted action from microbiologists, clinicians, facility administrators, and public health officials.

REFERENCES

1. Deshpande P, Rodrigues C, Shetty A, Kapadia F, Hedge A, Soman R. New Delhi Metallo-beta lactamase (NDM-1) in enterobacteriaceae: Treatment options with Carbapenems Compromised. J Assoc Physicians India 2010;58:147-9.
2. Datta P, Gupta V, Garg S, Chander J. Phenotypic method for differentiation of carbapenemases in enterobacteriaceae: Study from north India. Indian J Pathol Microbiol 2012;55:357-60.
3. Queenan AM, Bush K. Carbapenemases: The Versatile beta-Lactamases. Clin Microbiol Rev 2007;20:440-58.
4. Omair M, Usman J, Kaleem F, Hassan A, Khalid A, Fahim Q. Evaluation of combined disc method for the detection of metallo-β-lactamase producing Gram negative bacilli. Malays J Microbiol 2012;8:21-5.
5. Konemann DW, Allen SH, Janda MW, Schreckenberger PC, Winn Jr WC. In: Colour Atlas and Textbook of Diagnostic Microbiology. 6th ed. Philadelphia: Lippincott Williams and Wilkins;1997.
6. Centers for Disease Control and Prevention (CDC). Guidance for Control of Carbapenem-resistant enterobacteriaceae (CRE). CRE Toolkit 2012;1-29.
7. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: 22th informational supplement. CLSI publication 2012;M100-S22. Clinical and Laboratory Standard Institute, Wayne, Pennsylvania.
8. Galani I, Rekatsina PD, Hatzaki D, Plachouras D, Souli M, Giamarellou H. Evaluation of different laboratory tests for the detection of metallo-beta-lactamase production in enterobacteriaceae. J Antimicrob Chemother 2008;61:548-53.
9. Nordmann P, Naas T, Poirel L. Global Spread of Carbapenemase-producing enterobacteriaceae. Emerg Infect Dis 2011;17:1791-8.
10. Renu G, Rajeev T, Smita S. The existence of metallo beta lactamases in carbapenem susceptible gram negative bacilli: A cause for concern. J Clin Diagn Res 2010;4:2679-84.

11. Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL. Carbapenemases. *Klebsiella pneumoniae* and other enterobacteriaceae: An evolving crisis of global dimensions. Clin Microbiol Rev 2012;25:682-96.
12. Gupta N, Limbago BM, Patel JB, Kallen AJ. Carbapenem resistant enterobacteriaceae: Epidemiology and prevention. Clin Infect Dis 2011;53:60-7.
13. Deshmukh DG, Damle AS, Bajaj JK, Bhakre JB, Patwardhan NS. Metallo-β-lactamase-producing clinical isolates from patients of a tertiary care hospital. J Lab Physicians 2011;3:93-7.
14. Shevade S, Agrawal G. Study of drug resistance in enterobacteriaceae isolates in a tertiary care hospital. Natl J Integrated Res Med 2013;4:113-7.
15. Toleman AM, Biedenbach D, Benett D, Jones NR, Walsh RT. Genetic Characterization of a novel metallo-β-lactamase gene, blaIMP-13, harboured by a novel Tn-*5051*-type transposon disseminating carbapenemases gene in Europe: Report from the SENETRY. J Antimicrob Chemother 2003;52:583-90.
16. Centers for Disease Control and Prevention. Vital Signs: Carbapenem-Resistant enterobacteriaceae. Morb Mortal Wkly Rep 2013;62:165-70.

How to cite this article: Nair PK, Vaz MS. Study of the prevalence of metallo-β-lactamase-producing enterobacteriaceae from a tertiary care hospital in Mumbai. J Acad Clin Microbiol 2014;16:53-6.

Source of Support: Nil. **Conflict of Interest:** None declared.