Research Article

Surveillance of Metallo-β-Lactamase Producers in Gram-Negative Bacteria from Ear Discharges in a Teaching Hospital

Saumya Ranjan Das,*[1] Sibanarayan Rath[2], Rabindra N Padhy[2]

[*1]Dept of Ear, nose and throat, IMS and SUM Hospital, Siksha 'O' Anusandhan University, K-8, Kalinga Nagar, Bhubaneswar, Odisha, India.

[2]Central Research Laboratory, Institute of Medical Sciences and SUM Hospital, Siksha 'O' Anusandhan University, Bhubaneswar, Odisha, India.

***Corresponding author's E-mail:** saumya.ranjandas3@gmail.com

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ABSTRACT

Prevalence of Gram-negative bacteria resistant to imipenem and to assess metallo-β-lactamase (MBL) producers among the imipenem resistant isolates are described. There were 630 isolates in total, with 211 isolates of *Acinetobacter baumannii*, 23 isolates of *Citrobacter freundii*, 22 isolates of *Klebsiella oxytoca*,63 isolates of *Escherichia coli*, 24 isolates of *Proteus mirabilis* and 289 isolates of *Pseudomonas aeruginosa*;of 630 bacterial isolates, 59 were MBL positive.

Keywords: Gram-negative bacteria, metallo-β-lactamase, imipenem resistant.

INTRODUCTION

Metallo-β-lactamases (MBLs) are a diverse set of enzymes with one or two zinc atoms that catalyze the hydrolysis of a broad range of β-lactam drugs including carbapenems, by an altered mechanism.[1] In addition, there are currently no clinically available inhibitors to block metallo-β-lactamase action.[2] In clinical settings, proliferation of MBL producing gram negative bacteria will pose a serious global problem in future. MBL's have been identified from clinical isolates worldwide with increasing frequency for over the past few years.[3]

Surveillance of MBL producer's identification and reporting will aid infection control practitioners in preventing the spread of these multi drug resistant isolates. It will also help in therapeutic guidance for confirmed infections. MBL can hydrolyze β-lactams from all classes except monobactams. Higher mortality has been reported in patients infected with the imipenem resistant strains. The need for early recognition of MBL producing strains, rigorous infection control measures and restricted clinical use of broad spectrum of β-lactams, including carbapenems is mandatory. The present study was conducted to evaluate, prevalence of gram negative isolates for the resistance to imipenem and to confirm the production of MBL in the imipenem resistant isolates.

METHODOLOGY

Bacterial isolation and biochemical identification

The study was conducted on pus samples of ear discharges from patients, attending OPD of Ear, nose and throat (ENT) department, from January 2013 to December 2014. A total of 630 pus samples were cultured in agar media (HiMedia, Mumbai). The 6 GN bacteria, *Acinetobacter baumannii*, *Citrobacter freundii*, *Escherichia coli*, *Klebsiella oxytoca, Proteus mirabilis* and *Pseudomonas aeruginosa* were isolated and identified with standard strains.[4]

Detection of MBL strains

The MBLs production was detected by the ceftazidime + EDTA and the imipenem + EDTA double disc synergy test. Isolated bacteria were considered to be MBL producers, if an increase in the inhibition zone-size of the β-lactam+EDTA disk was ≥ 5 mm.[1] A disc of augmentin was flanked by discs of two 3GC antibiotics, ceftazidime 30 and cefotaxime 30 mg, at a distance of 30 mm apart on a on an Mueller-Hinton agar plate.

RESULTS

Six types of pathogenic GN bacteria were identified from the total of 630bacterial isolates over 24 months (Table 1). There were 211 isolates of *A. baumannii*; 23 isolates of *C. freundii*; 22 isolates of *K. oxytoca;* 63 isolates of *E. coli*; 24 isolates of *P. mirabilis;* and 289 isolates of *P. aeruginosa*. Of 630bacterial isolates, 59 were MBL positive.

Of isolated 298 imipenem resistant strains, 59 were detected producing MBL, by using imipenem-EDTA Disc method. MBL producing 59 isolates were in numbers as follows: 34, *P. aeruginosa*; 22, *A. baumannii*; 2, *E. coli;* and 1, *K. oxytoca*. Furthermore, 143 of the total imipenem resistant *P. aeruginosa* isolates produced MBL (Table 2).

Table 1: Bacteria isolated from 630 pus samples during 2 years from ear discharge samples of ENT-OPD or community around the hospital.

Isolated bacterium	MTCC number	Jan-June 2013	July-Dec 2013	Jan-June 2014	July-Dec 2014	Total isolates
Acinetobacter baumannii	1425	37	49	56	69	211
Citrobacter freundii	1658	4	8	6	5	23
Escherichia coli	443	11	12	17	21	61
Klebsiella oxytoca	2275	2	6	5	5	22
Proteus mirabilis	NA	3	7	4	10	24
Pseudomonas aeruginosa	1688	65	67	76	81	289
Total		118	149	164	199	630

Note: MTCC, Microbial Type Culture Collections; OPD, outpatients department; NA, Not applicable. 630 strains were isolated from the total 870 ear discharge samples.

Table 2: Growth of imipenem resistant and MBL positive isolates.

Bacteria	Total isolates	Imipenem resistant Isolates	MBL positive isolates
A. Baumannii	211	124	22
C. freundii	23	-	-
E. coli	61	19	2
K. oxytoca	22	12	1
P. mirabilis	24	-	-
P. aeruginosa	289	143	34
Total	630	298	59

Note: - no growth.

DISCUSSION

In the present study, 59 of 298 (20.41%) isolates of MBL strains of 6 cited GN bacterial strains is an alarmingly large number of intractable isolates, as imagined from the recent report from this hospital.[5] Of several reasons, the use of a large number of broad spectrum antibiotics could be the pertinent cause of emergence of MBL producing strains. From Scotland, a 13% prevalence of MBL producing *P. aeruginosa* amongst all carbapenem resistant strains was recorded.[6] The present figures are figuratively more than the Scotland study. However, 62.5% MBL producing *P. aeruginosa* strains were isolated in Brazilian hospitals.[7] As in the recent case described from this hospital patients selected in Scotland and Brazil hospitals were critically ill, having multiple infections and were with rigorous regimen or rather numbers of antibiotics against MBL producing *P. aeruginosa*.[5,7,8] In the present study, higher rate (34/59 MBL strains or 87.5%) MBL producing *P. Aeruginosa* was isolated.[9] *E .coli* and *K. oxytoca* were too detected as MBL producers. In India, the presence of MBL producers in other GN bacteria are not reported much. A heedful attention on MBLs is required as those have spread from the non-fermenting, saprophytic GN bacilli (*P. aeruginosa* and *Burkholderi acepacia,* for example) to GN enteric bacilli (*E. coli, Klebsiella* and *Enterobacter*, mainly). This would lead to more critical scenario in future. Herein, pus specimens yielded such a large number of MBL producers. This signifies that the transmission could have been person to person in community level. A significant presence of MBL producers with *P. aeruginosa* and *A. baumannii* have been noted in pus specimen, in the present study.

It was found that the community around have enough MBL producing isolates of *P. aeruginosa* to the highest followed by *A. baumannii*, *E. coli* and *K. oxytoca*. And, it was reported from Australia that MBL genes from *P. aeruginosa* and *Acinetobacter* sp. were spreading to species of Enterobacteriaceae family.[10]

Moreover, MBLs have recently emerged as one of the most gruesome resistant pathogens. The concerned genes are carried on highly mobile genetic elements, which cause dissemination among other GN bacteria.[11] The control of these multidrug resistant bacteria is difficult clinically. Extended surveys of human infections with MBL producers have not been done, worldwide.

CONCLUSION

Of isolated 298 imipenem resistant isolates, 59 were detected producing MBL, as follows: 34, *P. aeruginosa*; 22, *A. baumannii*; 2, *E. coli;* and 1, *K. oxytoca*; 143 of the total imipenem resistant *P. aeruginosa* isolates 34 were produced MBL.

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