SindhUniv. Res. Jour. (Sci. Ser.) Vol. 51 (01) 71-74 (2019)

http://doi.org/10.26692/sujo/2019..01.13

SINDH UNIVERSITY RESEARCH JOURNAL (SCIENCE SERIES)

Phenotypic Detection of Metallo-β-lactamasesin Imipenem-resistant Bacteria at Hyderabad, Pakistan

A. A. PATOLI, B. B. PATOLI[++], S. JABEEN, B. SHAIKH

Institute of Microbiology, University of Sindh, Jamshoro, Pakistan

Received 16th November 2018 and Revised 12th February 2019

Abstract: The current study was aimed to evaluate the occurrence and prevalence of metal based β-lactamase producing Gram-negative bacterial pathogens from various clinical specimens. A total of eighty four (84)Gram-negative clinical isolatesfrom pus, blood, urine and peritoneal fluids were processed for the production of Metallo-β-Lactamases (MBL)using Combined Disk Diffusion Test.About 26.1% were found to produce metallo-β-lactamases. Among the various categories of clinicalspecimens the maximum percentage of MBL was produced by blood isolates (35%) followed by pus (30%) and urine (9%) isolates. Comparatively higher percentage (26.6%) of MBL producing isolates were recovered from the specimens of female patients than male (25.6%) patients. Odds Ratio [OR] was calculated to be 0.95 [CI (95%) = 0.36-2.52]. Statistically non-significant differences (p-value < 0.05) for MBL productions were seen between the isolates recovered from male and female patients, suggesting MBL production to be an independent phenomenon of gender effect.

Keywords: Gram-negative bacteria, metallo-β-lactamase, Imipenem , Hyderabad.

1. INTRODUCTION

β-lactam antibiotics are one the most diverse group of the antibiotics, preferentially employed for the treatment of infections caused by both gram positive and gram negative bacteria. These antibiotics cease the growth of bacteria by inhibiting their cell wall synthesis(Kong, *et al.*,2010). Resistance to β-lactam antibiotics is now a common trait for several pathogenic bacteria which is often mediated by theβ-lactamase enzyme that cleaves the β-lactam ring of antibiotic (Kong, *et al.*, 2010) (Wilke, *et al.*, 2005) (Bush, *et al.*, 1995)rendering it ineffective. Penicillin, cephalosporins, carbapenems, and monobactams are among the most clinically important β-lactam antibiotics. Two classification systems forβ–lactamases are currently defined. The Classificationbased on their sequence homology divides the β -lactamase into four classes (A-D),(Bush, *et al.*, 1995) while a functional classification based on the phenotypic characteristics separates the β-lactamases in three broad groups comprising various sub-groups(Bush, *et al.*, 1995) (Bush, 1989). Amongst these, Group3 enzymes are known as metallo-β-lactamases (MBLs), since they require the presence of metal Zinc, for their activity(Xu, *et al.*,2006)(Sabath, and Abraham, 1966). The presence of metal sequesters like, EDTA, inactivates the activity of MBLs. To date, the emergence of MBL genes are a major cause of spread of multi-drug resistance in various pathogenic gram negative bacteria, including *Klebsiella pneumonia*, *Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa* of the Enterobacteriaceae family (Wilke, *et al.*,2005) (Bush, *et al.*,1995) (Bush, *et al.*,1995) (Bush, 1989). (Senda, *et al.*,1996) MBLs is virtually resistant to the inhibitors of β-lactamase such as clavulanic acid or sulbactam and their broad-spectrum activity can inactivate several β-lactam antibiotics excluding monobactams.(Drawz, and Bonomo, 2010) (Palzkill, 2013) Encoded by *blaKPC*, *blaNDM*-1, *blaIMP*, and *blaVIM* genes in various GNBthe MBL can be chromosomal and plasmid mediated (Yotsuji, *et al.*,1983).

Only a very few studies from Pakistan have reported the occurrence of MBL(Khan, *et al.*,2016), however data from the second largest city of province (Hyderabad) Sindh has never been analyzed. In the current study we aim to evaluate the MBL in various Gram Negative Bacteria (GNB) isolated from clinical specimens.

2. MATERIALS AND METHODS

Bacterial Cultures: The gram negative bacterial pathogens (Isolated from Blood, Pus, Urine andPeritoneal Fluid) used during the current study were collected from Diagnostic and Research Laboratory LUMHS. The cultures were obtained irrespective of gender, ethnicity and age of the patients. The cultures were brought to the Laboratory of Molecular Microbiology and Genetics at the Institute of Microbiology University ofSindh, Jamshoro within one hour. These were then sub-cultured on MacConkey's agar medium.All the clinical isolates were tested for the

[++]Corresponding AuthorBushra Bano Patoli. *E.mail*.bushrapatoli@gmail.com Tel : +92 (0) 3113005252
E.mail=atifpatoli@gmail.com,Shagufta.jabeen@usindh.edu.pk, bakhtawarshaikhusindh@gmail.com

production of metallo-β-lactamases using Combined Disk Test (CDT) as described in(Anwar, *et al.*,2016). The analysis of the data was performed based on the host related risk factors for the prevalence ofMBL producers. In order to measure the association between MBL production and gender, the Odds Ratios (OR) and 95% Confidence Interval (CI) were calculated manually and using an online statistics calculator where applicable. The *p*-values were calculated using Fisher's Exact test employing 2x2 contingency table. The duration of the study was from August 2018 to December 2018.

Determination of Metallo-β -Lactamase:

For the determination of MBL, CDT was used as described in(Anwar, *et al.*, 2016). Briefly, overnight cultures of the test organisms were diluted to OD_{600} = 0.5 to achieve the *McFarlands* standard. Using a sterile cotton swab the diluted culture was inoculated on Muller Hinton Agar and spread evenly. Two commercially available 10ug imipenem antibiotic discs (Oxoid) were placed on the agar surface. A gentle pressure was applied on the discs to get flat contact with the agar surface. 4 μL of 0.5M EDTA solution was placed on to one of them. The plates were incubated at 37°C for 24 hours. The restoration of imipenem antibacterial activity around the disc containing EDTA solution is considered as a positive MBL test (Yong, *et al.*, 2002).

3. RESULTS

Eighty four (84) gram negative bacterial pathogens were processed for the production of MBL during the current study. Comparatively, 46.4% (n=39) of the cultures were from male patients while 53.6% (n=45) were from female patients. Over all 26.1% (n=22) of the clinical isolates were found to express MBL, while 73.9% (n=62) were non MBL producers. Categorically, from blood specimen 14 (35%) isolates were found to produce MBL, while from pus and urine specimens 6 (30%) and 2 (09%) isolates were MBL producers respectively **(Fig. 1).** Out of 39 samples belonging to male category 10 (25.6%) were MBL producers while 12 (26.6%) out of 45 samples belonging to female category were determined to be MBL producers (Figure 2).To probe if the differences were significant enough for gender to affect the MBL production in clinical isolates we performed statistical analysis. The OR was calculated to be 0.95 with (95%) CI = [0.36-2.52]. The Fisher's Exact test employing 2x2 contingency table was applied to calculate the *p*-value. The *p*-value was calculated to be 1.000 suggesting an impartial effect of gender on the production of MBL in clinical isolates **(Fig. 2).**

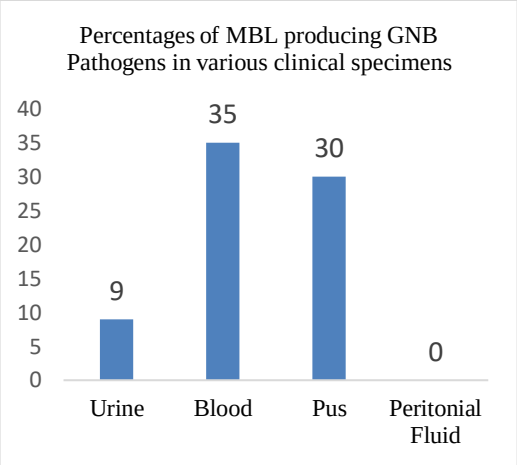

Fig. 1 Bar diagram displaying the relative percentages of MBL producing Gram Negative Bacterial Pathogens in various clinical specimens. MBL = Metallo-β-Lactamase, GNB = Gram Negative Bacteria.

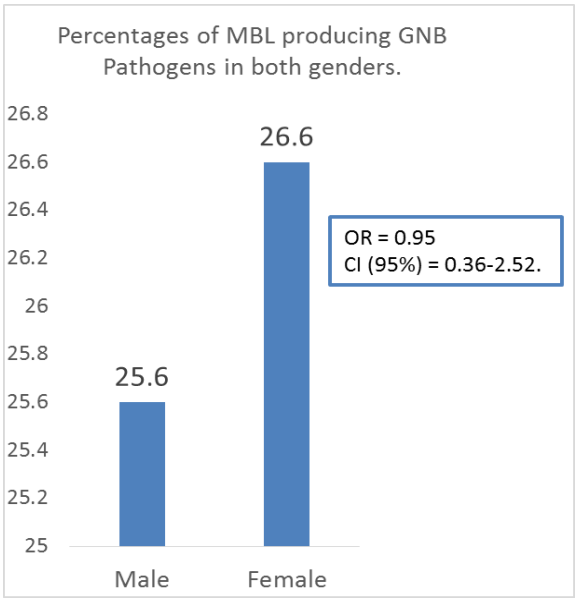

Fig. 2 Bar diagram displaying the relative percentages of MBL producing Gram Negative Bacterial Pathogens isolated from both genders. MBL = Metallo-β-Lactamase, GNB = Gram-Negative Bacteria, OR = Odds Ratio, CI = Confidence Interval.

The data were also analyzed to evaluate the MBL production for each bacterial species included in the current study. Table 1displays the absolute values of MBL producing Gram-negative bacteria isolated from both genders. Among various bacterial species the highest percentage (71.4%) of MBL producing strains were from *Enterobacter species* followed respectively by *Burkholderiacapacia* (66.6%) and *K. pneumonia* (50%). The percentage of MBLproducing strains

among *E. coli* and *S. typhi* was 19.35% and 16% respectively, whereas none of the *P. aeruginosa*was found to produce the MBL **(Fig. 3).**

Table 2A compound table displaying absolute and relative percentages for Metallo-β-Lactamase producing and non-producing Gram-negative clinical isolates from both genders.

Gender	MBL [Producer]	MBL [Non-producer]	Total
Over all			
Male	*10*	*29*	*39*
Female	*12*	*33*	*45*
Total	*22*	*62*	*84*
E. coli			
Male	*3*	*14*	*17*
Female	*3*	*11*	*14*
Total	*6*	*25*	*31*
S. typhi			
Male	*3*	*9*	*12*
Female	*1*	*12*	*13*
Total	*4*	*21*	*25*
P. aeruginosa			
Male	*0*	*4*	*4*
Female	*0*	*4*	*4*
Total	*0*	*8*	*8*
Enterobacter species			
Male	*4*	*1*	*5*
Female	*1*	*1*	*2*
Total	*5*	*2*	*7*
Burkholderiacapacia			
Male	*0*	*1*	*1*
Female	*4*	*1*	*5*
Total	*4*	*2*	*6*
K. pneumonia			
Male	*0*	*0*	*0*
Female	*3*	*3*	*6*
Total	*3*	*3*	*6*
Proteus			
Male	*0*	*0*	*0*
Female	*0*	*1*	*1*
Total	*0*	*1*	*1*

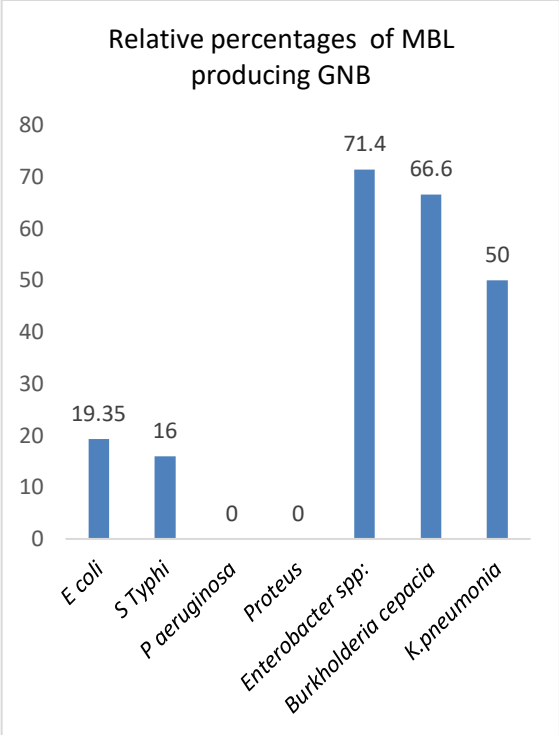

Fig. 3 Bar diagram displaying the relative percentages of MBL producing Gram Negative Bacterial Pathogens. MBL = Metalo-Beta-Lactamase, GNB= Gram Negative Bacteria

4. DISCUSSION

Metallo-β-lactamases (MBLs) are a group of β-lactamases that are usually produced in combination with other β-lactamases in clinical isolates. They are structurally unique, by their requirement for zinc ion at active site. Functionally they are distinguished primarily by their ability to hydrolyze carbapenems. The MBLs are not inhibited by Beta-lactamase inhibitors such as Clavulanate or Tazobactam (14).In the current study we analyzed various clinical GNB for the production of MBLs. The overall prevalence of MBLs among these was found to be 26.6%. Using Polymerase Chain Reaction for the amplification of MBL specific gene in *K. pneumonia*, (Khan *et al* 2016) reported 66.6% MBL producers in Karachi, the current study however reports slightly decreased percentage (50%) among *K. pneumonia.* They also reported about 31% of MBL producers among the *E. coli*, while in the current study 19.35 % of *E. coli* isolates were MBL producers. In the current study we have also evaluated the percentages of MBL production in GNB recovered from various clinical specimens. The highest (35%) percentage of MBL producers was among the isolates that were recovered from blood specimens.

5. CONCLUSION

MBL is found widely disseminated among various gram negative pathogenic species at Hyderabad. The possible occurrence of MBL encoding genes on transferable genetic elements can pose a serious threat to the indigenous population. This study, though preliminary in this domain can serve to boot up more studies in future.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

The author declares that there is no conflict of interest.

ACKNOWLEDGMENT:

We would like to thank Prof. Dr. Ikram-u-Din Ujan, In-charge Diagnostic and Research laboratory LUMHS for provision of the bacterial cultures.

REFERENCES:

Anwar, M., H. Ejaz,A. Zafar, and H. Hamid, (2016) Phenotypic Detection of Metallo-Beta-Lactamases in Carbapenem Resistant Acinetobacter baumannii Isolated from Pediatric Patients in Pakistan. *Journal of Pathogens* 6Pp

Bush, K., G. A. Jacoby, and A. A. Medeiros, (1995) A functional classification scheme for beta-lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother*39, 1211-1233

Bush, K. (1989) Characterization of beta-lactamases. *Antimicrob Agents Chemother*33, 259-263

Bush, K., and G. A. Jacoby, (2010) Updated functional classification of beta-lactamases. *Antimicrob Agents Chemother*54, 969-976

Drawz, S. M., andR. A. Bonomo, (2010) Three decades of beta-lactamase inhibitors. *Clin Microbiol Rev*23, 160-201

Kong, K. F., L. Schneper, and K. Mathee, (2010) Beta-lactam antibiotics: from antibiosis to resistance and bacteriology. *Apmis*118, 1-36

Khan, E., S. B. A. IrfanSultan, A. Nasir, and R. Hasan, (2016) Dissemination and spread of New Delhi Metallo-beta-lactamase-1 Superbugs in hospital settings. *JPMA. The Journal of the Pakistan Medical Association*66, 999-1004

Palzkill, T. (2013) Metallo-β-lactamase structure and function. *Ann N Y Acad Sci*1277, 91-104

Sabath, L. D., and E. P. Abraham, (1966) Zinc as a cofactor for cephalosporinase from Bacillus cereus 569. *Biochem J*98, 11c-13c

Senda, K.,Y. Arakawa,S.Ichiyama,K. Nakashima, H.Ito, S.Ohsuka, K.Shimokata,N.Kato, and M. Ohta, (1996) PCR detection of metallo-beta-lactamase gene (blaIMP) in gram-negative rods resistant to broad-spectrum beta-lactams. *J Clin Microbiol*34, 2909-2913

Wilke, M. S., A. L. Lovering, and N. C. Strynadka, (2005) Beta-lactam antibiotic resistance: a current structural perspective. *Curr Opin Microbiol*8, 525-533

Xu, D.,D. Xie, and H. Guo, (2006) Catalytic mechanism of class B2 metallo-beta-lactamase. *J Biol Chem*281, 8740-8747

Yotsuji, A., S. Minami, M. Inoue, and S. Mitsuhashi, (1983) Properties of novel beta-lactamase produced by Bacteroides fragilis. *Antimicrob Agents Chemother*24, 925-929

Yong, D., K. Lee, J. H. Yum, H. B. Shin, G. M. Rossolini, and Y. Chong, (2002) Imipenem-EDTA disk method for differentiation of metallo-beta-lactamase-producing clinical isolates of Pseudomonas spp. and Acinetobacter spp. *J Clin Microbiol*40, 3798-3801