Original Article

ID: JCDR/2012/4562:2434

Microbiology Section

The Prevalence of Metallo β-Lactamases in the Clinical Isolates of *Pseudomonas aeruginosa* in a Tertiary Care Hospital: An Alarming Threat

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ABSTRACT

Introduction: *Pseudomonas aeruginosa* is most frequently responsible for nosocomial infections. The current isolates of *P. aeruginosa* are often multi-drug resistant.

Objective: Thus, the present study was done to find out the prevalence of metallo-β–lactamases (MBL) in the clinical isolates of *P. aeruginosa.*

Methods: A total 310 clinical isolates of *Pseudomonas* were identified and 200 clinical isolates were selected for the study, based on their resistance to at least the 2, third generation cephalosporins.

Results: 29(9.35%) isolates of *P. aeruginosa* had a zone size of <28mm for imipenem , 27 were positive for MBL by the EDTA double -disc synergy test (EDTA DDST) and all 29 were positive by the modified Hodge test. The Minimum Inhibitory Concentrations (MICs) were determined by the agar dilution method and 26 isolates out of 29 were resistant, 2 were intermediate isolates and 1 was susceptible as per the Clinical and Laboratory Standards Institute (CLSI) breakpoints. Out of the 29 MBL producing isolates, 25 were sensitive to both polymyxin B and colistin, 3 isolates were resistant to polymyxin B but sensitive to colistin and 1 was resistant to polymyxin B and colistin both, by the disc diffusion method. The sensitivity of the MBL producing strains to polymyxin B was found to be 86% and that of colistin was found to be 96.55% in our study.

Conclusion: The early detection of the MBL carrying organisms, together with the judicious use of antibiotics, help in extending the longevity of the carbapenems, the last resort antibiotic . MBLs are the major threats for the 21st century, which pertain to the bacterial drug resistance.

Key Words: *P. aeruginosa*, MDR, MBL, DDST

INTRODUCTION

Pseudomonas aeruginosa is a troublesome opportunistic pathogen which is most frequently responsible for nosocomial infections. The spectrum of the infections ranges from superficial skin infections to fulminant sepsis. It possesses an intrinsic resistance to many antibiotics and it has an ability to develop resistance through mutations in different chromosomal loci or through the horizontal acquisition of resistant genes which are carried on plasmids, transposons or integrons [1].

Carbapenems are resistant to hydrolysis by most of the β-lactamases (ESBLs and AmpC beta lactamases) and they are often used as antibiotics of the last resort in infections which are caused by multi-drug resistant gram negative bacilli [2]. Carbapenem resistance has been observed frequently in *P. aeruginosa* [3]. The carbapenem hydrolyzing β-lactamases are called as metallo β-lactamases (MBL) and they belong to the Bush and Jacoby group 3 classification of β-lactamases. They require the divalent cations of zinc as co-factors for their enzyme activity and they are inhibited by chelating agents like $CuCl_3$, $FeCl_3$, EDTA, sodium mercaptoacetic acid (SMA), 2 mercapto-propionic acid (2MPA), 2 mercaptoethanol (2ME) in vitro [4].

P. aeruginosa which produces MBL was first reported from Japan in 1991 [5] and since then, its incidence has been reported from various parts of the world, which include Asia [2], Europe [6,7,8] Australia [9,10] south America [11] and north America [10,12].

The current isolates of *P. aeruginosa* are often multi-drug resistant (MDR) [2]. The infections which are caused by such bacteria are believed to result in high mortality as well as high healthcare costs and a prolonged hospitalization and so, a regular monitoring of the incidence of the β-lactamase producing organisms has become the need of the time [13].

MATERIALS AND METHODS

This study was carried out in the Department of Microbiology in a tertiary care hospital from December 2008 to November 2010. Some criteria were applied for the isolation of the clinically significant *P. aeruginosa* isolates as per the CDC/NHSN surveillance definition of the health care–associated infections and the criteria for specific types of infections in the acute care setting [14].

The identification of *P.aeruginosa* was done as per the standard of biochemical tests [15]. The third generation cephalosporin resistant *P. aeruginosa* which displayed a reduced susceptibility or resistance to imipenem by the disc diffusion method were selected for further studies (the zone size for imipenem was <28mm), since MBL was responsible for the carbapenem resistance. These strains were further screened for the MBL production by the double disc synergy test (DDST) which used EDTA and by the modified Hodge test. The minimum inhibitory concentration of imipenem was obtained by the agar dilution method as was recommended by clinical laboratory standard institute (CLSI) guidelines [16].

The Double Disc Synergy Test with EDTA [17]

The test strains were adjusted to McFarland's 0.5 standard and a lawn culture was put up onto Mueller Hinton agar plates. 2 discs of 10 µg imipenem were placed on plate at 15mm distance. To one of the imipenem discs, 10µl of 0.5 M EDTA was added. This disc contained 1900 µg of EDTA. After an overnight incubation at 37ºC, the MBL-positive isolates could be well demarcated from the MBL-negative isolates on the basis of the criterion of more than 7 mm of increase of the inhibition zone with the disks to which EDTA was added .

The Modified Hodge Test [18]

The indicator organism, *Escherichia coli ATCC 25922*, at a turbidity of 0.5 McFarland's standard, was swab inoculated onto the surface of a Mueller Hinton agar plate. The test strain was heavily streaked from the centre of the plate to its periphery and a 10µg imipenem disc was placed at the centre. The plate was incubated overnight. The presence of a distorted inhibition zone was interpreted as a positive result for the carbapenem hydrolysis screening.

All these MBL producing strains were tested for their MICs against imipenem by the agar dilution method and they were then tested for their susceptibility to polymyxin B (300 units) and colistin (10 µg) by using commercially available disks (Hi Media), by the Kirby Bauer disc diffusion method [19].

RESULTS

A total of 310 isolates of *P. aeruginosa* were isolated, of which 200 isolates were resistant to th 3rd generation cephalosporins (ceftazidime 30µg/ cefotaxime 30µg).

Of these isolates, 29 had a zone size of <28mm for imipenem , out of which 27 were positive for MBL by the DDST with EDTA test and all 29 were positive by the modified Hodge test, as has been shown in [Table/Fig-2]

One isolate was found to be susceptible to imipenem on the basis of its MIC, but it was also found to be positive for MBL by both the methods, thus indicating that MBL producers could show susceptibility to imipenem.

All these MBL producing isolates were tested for their susceptibility to polymyxin B and colistin by the disc diffusion method.

DISCUSSION

The prevalence of MBLs in *P. aeruginosa* was reported to be 10-30% among various clinical samples which were tested across the country [20]. In our study, the frequency of the MBL producing *P. aeruginosa* was highest in urine i.e., 3.8%, followed by 2.2% in pus, 1.9% in respiratory specimens, 0.6% in blood and 0.3% in other samples as shown in [Table/Fig-1]. Similarly, K Lee et al. (2009) [2], Ami Varaiya et al. (2008) [20], I. Aibinu et al. (2007) [21] had mentioned similar incidences of the MBL producing *P. aeruginosa* in various samples. Various studies had reported varying results regarding the comparative outcome of the DDST and the modified Hodge tests, but there no clear reason was found for such a variation amongst the 2 tests.

In our experience, out of the 29 imipenem resistant isolates, all the 29 were found to be positive for metallo beta lactamases by the modified Hodge test and 27 were found to be positive by the double disc synergy test. As per our study, the modified Hodge test had detected 2 additional MBL producers as compared to the DDST test. Although (comparative statistical analysis) the p values of these two tests were not significant (<0.05), any of these 2 tests could be used for screening MBLS. However, the modified Hodge test is easy to perform, it doesn't require any inhibitors and it is a cheaper test in comparison to DDST. In this study, 26 of the 29 MBL producing *P. aeruginosa* was resistant to imipenem, 2 were intermediately resistant and 1 isolate was sensitive as per breakpoints as shown in [Table/Fig-3].

Beta lactamases	Urine (n=73)	Respiratory Specimens (50)	Pus (44)	Blood (19)	Other samples (14)
MBL	12 (3.8%)	6 (1.9%)	7 (2.2%)	2 (0.6%)	2 (0.3%)

[Table/Fig-1]: Distribution of beta lactamases according to samples (n=200)

Total no of isolates	DDST with EDTA	HODGE TEST
29	27(93.10%)	29(100%)

[Table/Fig-2]: Results of Hodge & DDST with EDTA among 29 isolates which were imipenem resistant

MIC in µg/ml	No of isolates
64	19
32	3
16	4
8	2
4	1

[Table/Fig-3]: MIC of imipenem in MBL producing isolates (n=29)

Polymyxin B	Colistin
25	28

[Table/Fig-4]: Susceptibility of MBL isolates to polymyxin B &colistin by disc diffusion method (n=29)

One isolate was found to be sensitive to imipenem on the basis of its MIC, but it was found to be positive for MBL by both the tests, thus indicating that MBL producers could show susceptibility to imipenem. The organisms can appear to be susceptible to carbapenems though they carry carbapenemases. Such organisms thus carry hidden MBL genes, whereby the microbiologist may remain unaware of their presence [22].

As per [Table/Fig-4], out of 29 MBL producing isolates, 25 were sensitive to both polymyxin B and colistin, 3 isolates were resistant to polymyxin B and sensitive to colistin and 1 isolate was resistant to both by the disc diffusion method. The sensitivity of the MBL producing strains to polymyxin B was found to be 86% and that of colistin was found to be 96.55% in our study; which correlated with the findings of A C Gales et al (2003) [11] and I M Heijden et al. (2007) [23] and it differed from the findings of G Agrawal et al. (2008) [24].

The development of simple screening tests which are designed to detect the β-lactamases will be a crucial step towards a large scale monitoring of these emerging resistant strains. Such institutional studies will help in the formulation of an antibiotic policy for a particular geographical area. A good infection control practice and a careful introspection during the prescription of beta-lactam drugs are necessary for the formulation of a good antimicrobial policy in a hospital. The combination of piperacillin and tazobactum and that of polymyxin B and colistin should be kept as reserve drugs and they should be used only in patients who have infections which are caused by MDR organisms, especially the strains which produce

MBLs.

The current data suggests that the MBLs are a heterogeneous group of enzymes which may prove it difficult in the designing agents that would be efficient against all MBLs. Combating the MBLs may prove to be a therapeutic challenge [25]. The mobile MBLs genes are capable of a horizontal spread among the nosocomial strains of *Enterobacteriaceae. P. aeruginosa* and other gram-negative nonfermenters may have considerably increased the attention which has been given to these enzymes, thus including them among the major threats for the 21st century in the field of microbial drug resistance [26].

As the phenotypic methods are easier to perform, they are able to discriminate among the various beta lactamases, which the automated systems fail to do. Hence, the phenotypic methods should be regularly performed where the molecular methods are not available. Strict infection control practices, the judicious use of antibiotics, an early detection of the MBL carriage, all will together help in extending the longevity of the carbapenems, which are the last resort antibiotics.

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FINANCIAL OR OTHER COMPETING INTERESTS:

None.

Date of Submission: **May 19, 2012**
Date of Peer Review: **Jul 17, 2012**
Date of Acceptance: **Aug 03, 2012**
Date of Publishing: **Sep 30, 2012**