Original article

Frequency and susceptibility pattern of metallo-beta-lactamase producers in a hospital in Pakistan

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Abstract

Introduction: 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.

Methodology: This descriptive study was conducted between January and August 2009 in the department of Microbiology, Army Medical College, National University of Sciences and Technology, Rawalpindi, to determine the frequency and susceptibility patterns of MBL-producers among carbapenem-resistant Gram-negative rods (GNRs) from clinical isolates of a tertiary care hospital. All clinical samples were processed according to standard microbiological methods. Isolated GNRs were subjected to susceptibility testing against various 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 the E-test MBL strip method.

Results: Out of 50 carbapenem resistant isolates, 39 (78%) of were confirmed to be MBL producers by the E-strip method. *Acinetobacter baumannii* were the most frequent MBL producers, followed by *Pseudomonas aeruginosa*. A total of 19 (37%) of the MBL producers were susceptible to cefoperazone-sulbactam.

Conclusion: The findings strongly suggest that there is a need to track the detection of MBL producers and that judicious use of carbapenems is necessary to prevent the further spread of these organisms.

Key words: carbapenem-resistant Gram-negative rods; metallo-beta-lactamase; antimicrobial resistance; extended-spectrum beta lactamase

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Introduction

Infection with the metallo-beta-lactamase (MBL) producing organisms is associated with higher rates of mortality, morbidity, and health care costs. MBL producing *Pseudomonas aeruginosa* was first reported in Japan in 1991 and since then has been found in various parts of the world including Asia, Europe, Australia, South America, and North America [1-8]. The introduction of carbapenems into clinical practice marked a great advance for the treatment of serious bacterial infections caused by beta-lactam-resistant bacteria [9]. Resistance to extended-spectrum beta-lactams has been frequently observed in non-fermenting bacilli such as *Pseudomonas aeruginosa* and *Acinetobacter* spp. Resistance is mediated by a lack of drug penetration for example due to porin mutations, efflux pumps or hydrolysis by β-lactamases [10]. Based on molecular studies, carbapenem hydrolyzing enzymes are classified into four groups: A, B, C and D. The MBLs, which belong to group B, are enzymes requiring divalent cations as cofactors for optimal enzyme activity, being inhibited by the action of a metal ion chelator [10]. The MBLs efficiently hydrolyze all β-lactams. Several phenotypic methods are available for the detection of MBL-producing bacteria. All these methods are based on the ability of metal chelators such as Ethylenediaminetetraacetic acid (EDTA) and thiol-based compounds to inhibit the activity of MBLs. These tests include the double disc synergy tests using various combinations such as EDTA with imipenem (IPM) or ceftazidime (CAZ) [10-12]; 2-mercaptopropionic acid with CAZ or IPM [15]; the Hodge test [12,13]; a combined disk test using EDTA with CAZ or IPM [14-16]; the MBL E-test (AB BioDisk) [17]; and a micro dilution method using EDTA and 1,10-phenanthroline with IPM [18].

Gram-negative bacilli associated with hospital infections are often difficult to eradicate because they are resistant to drugs. Therefore, detection of MBL-producing Gram-negative bacilli is crucial to control the spread of resistance and for the optimal treatment of patients, particularly the critically ill and hospitalized patients [19]. There is not much

information concerning MBL-producing isolates available in Pakistan. Therefore, this study was conducted to detect the presence of MBL in carbapenem-resistant isolates obtained from the clinical isolates from a tertiary care hospital, which has intensive care units with a heavy patient turnover and extensive antibiotic use.

Methodology

Duration and setting

This descriptive study was conducted between January and August 2009 in the Department of Microbiology, Army Medical College, National University of Sciences and Technology, Rawalpindi, after approval from the ethical committee of the institute.

Specimens

Routine specimens were used, including nasobronchial lavages, pus swabs, blood, sputum, catheter tips, and urine received from admitted patients in the Military Hospital, Rawalpindi. Most of the specimens were received from patients admitted in medical and surgical intensive care units who had serious underlying diseases and risk factors and who were already on antibiotics. All specimens were processed according to standard microbiological procedures. From a total of 2347 samples 1,430 consecutive non duplicate Gram-negative bacilli were recovered (61%) Isolated Gram-negative rods were subjected to antimicrobial sensitivity testing per the Clinical and Laboratory Standards Institute (CLSI) guidelines. [20]. All carbapenem-resistant isolates were subjected to detection of MBLs by the E-strip method as described by Yan *et al*. [14].

E-test

The E-test MBL strip (AB Biodisc, Sweden) contains predefined gradients of imipenem ranging from 1 to 256 µg/ml). Carbapenem-resistant Gram-negative rod colonies that were grown overnight were emulsified in normal saline to achieve a turbidity equivalent to 0.5 McFarland standards (1 McFarland if mucoid). A sterile swab was dipped into the inoculum suspension; excess fluid was removed. The entire Mueller Hinton agar surface was inoculated, and the strip was placed onto the inoculated agar surface. The plates were incubated at 37°C for 16 to 18 hours. When bacterial growth was clearly visible, the MIC values were read where the respective inhibition ellipses intersected the strip in accordance with the manufacturer's instructions (AB Biodisc, Sweden). *Pseudomonas aeruginosa* ATCC 27853 was used as a negative control for MBL and an MBL genotype-positive reference strain; *Stenotrophomonas maltophilia* ATCC 13636 (intrinsic MBL production) was used as a positive control.

Results

Among the 50 carbapenem resistant isolates, 39 (78%) were found to be MBL positive by the E-test strip method. Twenty-seven (84%) carbapenem-resistant *Acinetobacter species* were found to be MBL producers, whereas seven (78%) of carbapenem-resistant *Pseudomonas aeruginosa* were positive for MBL production and five (71%) carbapenem-resistant *Escherichia coli* were metallo-beta-lactamase producers. (Table 1). Most of the MBL producers were isolated from nasobronchial lavage samples followed in number by urine and pus samples. (Table 2). Nineteen (38%) of the MBL producers were sensitive to the cefoperazone-sulbactam combination and the aminoglycosides. Only seven (14%) MBL-producers were sensitive to the ampicillin-clavulanic acid combination. Thirteen percent of the MBL producers were sensitive to piperacillin-tazobactum combination and only 11% showed sensitivity to tetracycline group.

Table 1. Percentage of MBL producers among carbapenems resistant organisms

Carbapenem resistant Organism	Total	MBL + by Etest	Percentage of MBL production
Acinetobacter baumannii	32	27	84%
Pseudomonas aeruginosa	9	7	78%
Escherichia coli	7	5	71%
Providencia rettegeri	1	0	0%
Morganella morganii	1	0	0%

(n = 50)

Table 2. Number of MBL producers isolated from different clinical samples containing carbapenems resistant organisms.

Specimen	Total	Total MBL+ organisms isolated
NASOBRONCHIAL LAVAGE	22	22
PUS	10	6
URINE	6	3
CATHETER TIPS	4	3
BLOOD	3	2
SPUTUM	2	1
EAR SWAB	2	1
HIGH VAGINAL SWAB	1	1

Discussion

Japan reported the first plasmid-mediated MBL in *Pseudomonas aeruginosa* in 1991. This was followed by a report of transferable metallo enzyme in *Bacterioides fragilis* [1]. In addition to *Pseudomonas aeruginosa*, other Gram-negative bacteria including *Klebsiella pneumoniae, Escherichia coli, Enterobacter aerogenes, Enterobacter cloacae, Citrobacter freundii, Proteus vulgaris, Acinetobacter* spp. and *Alcaligenes xylosoxidans* have also been shown to produce MBL [16]. There are frequent reports of MBL production in *Pseudomonas aeruginosa* and *Acinetobacter* species from different parts of the world, particularly in military settings [21]. MBLs have been identified from clinical isolates worldwide with increasing frequency over the past few years, and strains producing these enzymes have been responsible for prolonged nosocomial outbreaks that were accompanied by serious infections [22].

A total of 56 (87.5 %) carbapenem resistant isolates were found to be MBL producers from hospitalized patients in Chennai, India, in 2005. Reports have also described the significant prevalence of MBLs in *Pseudomonas aeruginosa* and *Acinetobacter spp.* from India in a study conducted in 2008 [23]. Carbapenem-resistant Gram-negative bacteria that were isolated from nosocomial infections in patients in a university hospital in Turkey (15 of 52 *Pseudomonas aeruginosa* strains [29%], 5 of 24 *Acinetobacter baumannii* strains [21%], and 2 of 2 *Klebsiella pneumoniae* strains [100%] were found to be metallo enzyme producers using the E-test MBL technique. *In vitro* antibiotic susceptibility of the MBL-positive organisms was investigated by the E-test method. Of the ten drugs tested, isepamicin was the most active agent against the MBL-producing strains. Overall, the rank order of activity of the ten antibiotics, in terms of the percentages of susceptible strains was as follows: isepamicin: 73%; ciprofloxacin: 64%; amikacin: 59%; aztreonam: 18%; tobramycin: 18%; meropenem: 14%; cefoperazone-sulbactam: 5% [24].

The results are similar to those of a 2008 study conducted at Aga Khan University, Karachi, by Irfan *et al.*, in which 96.6% of the carbapenem resistant *Acinetobacter baumannii* were MBL producers and all the carbapenem-resistant *Pseudomonas spp.* were found to be MBL producers [25].

Emergence of MBL-mediated resistance in Pakistan is of serious concern. Carbapenems are effective therapeutic agents against highly resistant pathogens such as *Pseudomonas spp*. and *Acinetobacter spp*. Spread of this resistance among these pathogens and transfer to other Gram-negative bacteria would seriously restrict therapeutic options. This challenging situation is difficult to manage in a resource-limited country. Conversely, the situation continues to become more complicated by the indiscriminate use of antibiotics in the population.

The occurrence of an MBL-positive isolate in a hospital setting poses a therapeutic problem, as well as serious concern for infection control management. The accurate identification and reporting of MBL-producing bacteria will aid infection control practitioners in preventing the spread of these multidrug-resistant isolates.

Conclusion

The study results demonstrate the serious therapeutic and epidemiological threat of the spread of metallo-beta-lactamase producers. Early detection and infection control practices are the best defense against these organisms; therefore systematic surveillance to detect MBL producers is necessary. Judicious use of carbapenems is essential to prevent the spread of these organisms.

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References

1. Watanabe M, Iyobe S, Inoue M, Mitsuhashi S (1991) Transferable imipenem resistance in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 35: 147-51.
2. Lee K, Ha GY, Shin BM, Kim JJ, Kang JO, Jang SJ, Yong D, Chong Y (2004) Metallo-β-lactamase-producing Gram-negative bacilli in Korean Nationwide Surveillance of Antimicrobial Resistance group hospitals in 2003: continued prevalence of VIM-producing *Pseudomonas spp*. and increase of IMP producing *Acinetobacter spp*. Diagn Microbiol Infect Dis 50: 51-58.
3. Yan JJ, Hsueh PR, Ko WC, Luh KT, Tsai SH, Wu HM, Lin YT, Wu JJ (2001) Metallo-β-lactamases in clinical Pseudomonas isolates in Taiwan and identification of VIM-3, a novel variant of the VIM-2 enzyme. Antimicrob Agents Chemother 45: 2224-28.
4. Lagatolla C, Tonin EA, Monti-Bragadin C, Dolzani L, Gombac F, Bearzi C, Edalucci C (2004) Endemic carbapenem-resistant *Pseudomonas aeruginosa* with acquired Metallo-β- lactamase determinants in European hospital. Emerg Infect Dis 10: 535-38.
5. Libisch B, Gacs M, Csiszar K, Muzslay M, Rokusz L, Fuzi M (2004) Isolation of an integron-borne blaVIM-4 type Metallo-β-lactamase gene from a carbapenem-resistant *Pseudomonas aeruginosa* clinical isolate in Hungary. Antimicrob Agents Chemother 48: 3576-78.
6. Patzer J, Toleman MA, Deshpande LM, Kaminska W, Dzierzanowska D, Bennett PM, Jhons RN (2004) *Pseudomonas aeruginosa* strains harboring an unusual blaVIM-4 gene cassette isolated from hospitalized children in Poland (1998–2001). J. Antimicrob. Chemother 53: 451-56.
7. Poirel L, Naas T, Nicolas D, Collet L, Bellais S, Cavallo JD, Nordman P (2000) Characterization of VIM-2, a carbapenems hydrolyzing Metallo-β-lactamase and its plasmid- and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. Antimicrob Agents Chemother 44: 891-97.
8. Peleg AY, Franklin C, Bell J, and Spelman DW (2004) Emergence of IMP-4 Metallo-β-lactamase in a clinical isolate from Australia. J Antimicrob Chemother. 54: 699-700.
9. Kahan FM, Kropp H, Sundelof JG, Birnbaum J (1981) Thienamycin: development of imipenen-cilastatin. J Antimicrob Agents Chemother 12: 1-35.
10. Ambler RP (1980) The structure of beta-lactamases. Philos Trans R Soc London Bio Sci 289: 321-31.
11. Bush K, Jacoby GA, Medeiros AA (1995) A functional classification scheme for b-lactamases and its correlation with molecular structure. Antimicrob Agents Chemother 39: 1211-33.
12. Lee K, Chong Y, Shin HB, Kim YA, Yong D, Yum JH (2001) Modified Hodge and EDTA-disk synergy tests to screen Metallo-β-lactamase-producing strains of Pseudomonas and *Acinetobacter species*. Clin Microbiol Infect 7: 88-91.
13. Lee K, Lim YS, Yong D, Yum JH, Chong Y (2003) Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating Metallo-beta-lactamase producing isolates of *Pseudomonas spp*. and *Acinetobacter spp*. J Clin Microbiol 41: 4623-29.
14. Yan JJ, Wu JJ, Tsai SH, Chuang CL (2004) Comparison of the double-disk, combined disk, and Etest methods for detecting Metallo-β- lactamases in Gram-negative bacilli. Diagn Microbiol Infect Dis 49: 5-11.
15. Arakawa Y, Shibata N, Shibayama K, Kurokawa H, Yagi T, Fujiwara H, Goto M (2000) Convenient test for screening metallo-β-lactamase-producing Gram-negative bacteria by using thiol compounds. J Clin Microbiol 38: 40–43.
16. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y (2002) Imipenem-EDTA disk method for differentiation of Metallo-β-lactamase producing clinical isolates of *Pseudomonas spp*. and *Acinetobacter spp*. J Clin Microbiol 40: 3798-801.
17. Walsh TR, Bolmstrom A, Qwarnstrom A, Gales A (2002) Evaluation of a new E-test for detecting metallo-β-lactamases in routine clinical testing. J Clin Microbiol 40: 2755-59.
18. Migliavacca R, Docquier JD, Mugnaioli C, Amicosante G, Daturi R, Lee K (2002) Simple microdilution test for detection of metallo-β-lactamase production in *Pseudomonas aeruginosa*. J Clin Microbiol 40: 4388-90.
19. Richet HM, Mohammed J, McDonald LC, Jarvis WR (2001) Building communication networks: international network for the study and prevention of emerging antimicrobial resistance. Emerg Infect Dis 7: 319-22.
20. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing-19th informational supplement. (2009) Wayne, PA M100-S19. NCCLS.
21. Nordmann P and Poirel L (2002) Emerging carbapenemases in Gram negative aerobes. Clin Microbiol Infect 8: 32131.
22. Senda K, Arakawa Y, Nakashima K, Ito H, Ichiyama S, Shimokata K, Kato N, Ohta M (1996) Multifocal outbreaks of metallo-β- lactamase producing *Pseudomonas aeruginosa* resistant to broad-spectrum β-lactams, including carbapenems. Antimicrob Agents Chemother 40: 349–53.
23. Hemalatha V, Uma S, Vijay LK (2005) Detection of metallo betalactamase producing *Pseudomonas aeruginosa* in hospitalized patients. Indian J Med Res. 122:148-52.
24. Varsha G (2007) An update on newer beta lactamases. Indian J Med Res 26: 41727
25. Irfan S, Zafar A, Gauhar D, Hasan R (2006) Metallo beta lactamase producing clinical isolates of *Acinetobacter species* and *Pseudomonas aeruginosa* from intensive care unit of tertiary care hospital. Indian J Microbiol 26: 243-45.

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