



Original Research Article

In Vitro Activity of Polymyxin B for Metallobetalactamase Producing *Pseudomonas aeruginosa*: Drugs for the Bugs

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ABSTRACT

Pseudomonas aeruginosa is a leading cause of nosocomial infections, being responsible for 10% of all hospital acquired infections. The emergence of carbapenem resistant *P. aeruginosa*, in the form of metallo-beta lactamases (MBLs) is a cause of major concern for treating clinicians. Multidrug resistant MBL *P. aeruginosa* isolates, which are susceptible only to colistin or polymyxin B as a last treatment resort, have been described. The present study was undertaken to evaluate in vitro efficacy of polymyxin B against MBL *P. aeruginosa*. A total number of 120 *P. aeruginosa* isolates were obtained from different clinical specimens like pus, wound swabs, sputum, urine, blood, endotracheal tube secretions, various body fluids etc. Specimen processing and identification of isolates was done by standard microbiological methods. The antibiotic sensitivity test was performed by Kirby Bauer disc diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines using gentamicin (10 µg), ceftazidime (30µg), ciprofloxacin (5µg), aztreonam (30µg), imipenem (10µg), piperacillin (100µg), piperacillin-tazobactam (100µg/100µg), polymyxin-B (300 µg). Imipenem resistant strains were subjected to modified Hodge test for detection of carbapenemases. Modified Hodge test positive *P. aeruginosa* isolates were further tested for MBL production by combined disc test. The antimicrobial susceptibility pattern showed highest sensitivity for polymyxin B and lowest for piperacillin. Among the imipenem resistant isolates, 16.6% isolates were Modified Hodge test positive and 13.3% isolates were MBL positive by combined disc test. All the MBL positive isolates were sensitive to polymyxin B. Combined disc test should be routinely practiced for screening of MBL in imipenem resistant *P. aeruginosa* isolates. Polymyxin is an effective therapeutic option for MBL producing *P. aeruginosa*.

Keywords

Pseudomonas aeruginosa, multidrug-resistant, nosocomial, metallo-beta lactamases (MBLs), polymyxin B, Modified Hodge test, combined disc test

Introduction

Pseudomonas aeruginosa is a leading cause of nosocomial infections, being responsible for 10% of all hospital acquired infections

(Valerie Aloush *et al.*, 2006).The increasing incidence of multidrug-resistant (MDR) *P. aeruginosa* is a global health care problem

particularly among critically ill hospitalized patients. The increasing frequency of such multi-drug resistant *P. aeruginosa* strains is worrisome as effective therapeutic options are limited. Further, the emergence of carbapenem resistant strains of *P. aeruginosa*, in the form of metallo-beta lactamases (MBLs) has become a therapeutic challenge and a cause of concern for treating clinicians (Troillet N *et al.*, 1997; D'Agata *et al.*, 2006). High mortality rates and longer length of hospital stay are associated with MBL producing *P. aeruginosa* infections (Zavascki A *et al.*, 2006). Multidrug resistant, MBL producing *P. aeruginosa* isolates susceptible only to colistin or polymyxin B as last treatment option have been described (Mastoraki A *et al* 2008; Lee Y *et al.*,2007).

Early clinical reports suggested a high rate of toxicity, specifically nephrotoxicity and neurotoxicity associated with the polymyxins. Recent studies reveal that these adverse effects are not as frequent as reported earlier; therefore it should not discourage physicians from the use of these antibiotics. (Falagas ME *et al.*, 2006; Falagas ME *et al* 2005; Wolinsky E *et al.*, 1972). Therefore, the present study was undertaken to evaluate in vitro efficacy of polymyxin B against MBL producing *P. aeruginosa*.

Material and Methods

The study was conducted in the department of Microbiology, KIMS Amalapuram from January 2014 to June 2014. A total number of 120 *Pseudomonas aeruginosa* isolates were obtained from different clinical specimens like pus, wound swabs, sputum, urine, blood, endotracheal tube secretions, various body fluids etc. Specimen processing and identification of isolates was done by standard microbiological methods (Collee JG *et al.*, 2008; Winn WJ *et al.*,

2006). The antibiotic sensitivity test was performed by Kirby Bauer disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines using gentamicin (10 µg), ceftazidime (30µg), ciprofloxacin (5µg), aztreonam (30µg), imipenem (10µg), piperacillin (100µg), piperacillin-tazobactam (100µg/10µg), polymyxin-B (300µg) (Bauer AW *et al.*,1966; Clinical and Laboratory Standards Institute, 2013) *P. aeruginosa* ATCC 27853 strain was used as quality control. Imipenem resistant strains were subjected to modified Hodge test for detection of carbapenemases (Lee K *et al.*, 2001). Modified Hodge test positive *P. aeruginosa* isolates were further tested for MBL production by combined disc test (Yong D *et al.*,2002).

The Modified Hodge Test

(Lee K *et al.*, 2001)

In this test, a lawn culture of *E.coli* ATCC 25922 (1:10 dilution of 0.5 McFarland's standard broth) was done on a Mueller Hinton Agar (MHA) plate and allowed to dry for 2-5 minutes. A 10 µg meropenem disc was placed in the centre of the plate. 10 µl of 50mM zinc sulfate solution was added to meropenem disk. Further imipenem resistant *P. aeruginosa* isolate in disc diffusion method (test organism) was streaked from the edge of the disc to the periphery of the plate. After overnight incubation, the plates with the presence of a 'clover-leaf' shape indentation at the intersection of the test organism and the *E. coli* 25922, within the zone of inhibition of the meropenem susceptibility were interpreted as modified Hodge test positive.

The combined disc test

(Yong D *et al.*, 2002)

A lawn culture of the test organism (modified Hodge test positive) was done on

Mueller Hinton agar plates (opacity adjusted to 0.5 McFarland's standard). Two imipenem disks (10 μ g) were placed 15 mm apart on the inoculated plates. 10 μ l of 50mM zinc sulfate solution was added to each of the imipenem disks. Then, 5 μ l of 0.5M EDTA solution was added to one imipenem disk. After overnight incubation, an increase in zone size of 7mm around the Imipenem-EDTA disk as compared to imipenem disk alone was considered as a positive result for combined disc test indicating metallo- beta lactamase production by the test organism.

Results and Discussion

A total number of 120 *P. aeruginosa* isolates from various specimens were included in the present study. Out of these 120 isolates, 42 were isolated from pus, 24 from sputum, 22 from blood, 16 from urine, 10 from body fluids (ascitic fluid, pleural fluid) and 6 from ear swab.

The antimicrobial susceptibility pattern of *P. aeruginosa* isolated from various clinical samples showed highest sensitivity for polymyxin-B (100%). The percentage sensitivity to other antimicrobials is as shown in table 1.

Among imipenem resistant isolates, 20 (16.6%) isolates were Modified Hodge test positive and 16(13.3%) were MBL positive by combined disc test (Table 2). All the MBL positive isolates were sensitive to polymyxin B (Table 3).

P. aeruginosa is a leading cause of outbreak of nosocomial infections worldwide. *P. aeruginosa* is known for its intrinsic resistance to antibiotics as well as ability to acquire genes encoding resistance determinants leading to emergence of multidrug resistant strains (Bonomo RA *et al.*, 2006). In the present study, highest

resistance was noted for piperacillin (76.7%) followed by gentamicin (75%), ceftazidime (70%), ciprofloxacin (47.5%), piperacillin-tazobactam (45.9%), imipenem (35%) and aztreonam (30%). All the isolates were sensitive to polymyxin B. In a recent study, Kumar *et al* also reported high resistance to gentamicin (73%), ceftazidime (78%), and tobramycin (79%) as well as for aztreonam (87%) (Kumar R *et al.*, 2014). In the present study, sensitivity to imipenem was 65% which is comparable to study by Kumar *et al* which reported 68% sensitivity to imipenem. (Kumar R *et al.*, 2014). In contrast, various studies have reported less sensitivity to imipenem from 0% (Franco *et al.*, 2010), 18.6% (Picao RC *et al.*, 2008) to as high as 78% (JaviyaVA *et al.*, 2008), 85% (Hocquet D *et al.*, 2007). Carbapenems, mainly imipenem and meropenem, are useful agents for the treatment of infections due to multi drug resistant *P. aeruginosa* because these drugs are stable against extended-spectrum β -lactamases and AmpC- β -lactamases (Livermore DM *et al.*, 1995). The mechanism of resistance to carbapenems may be production of carbapenemases, deficiency of the outer-membrane protein OprD which forms a transmembrane channel accessible to carbapenems or overexpression of multidrug efflux pumps. (Burcher K H *et al.*, 1987; Livermore DM *et al.*, 2001; Quale J *et al.*, 2006).

The emergence of carbapenemases is a grave concern leading to pan drug resistant *P. aeruginosa* leaving no therapeutic options for serious infections. Carbapenemases are classified as the serine carbapenemases belonging to molecular Class A or D of Ambler and metallo beta lactamases (MBL) belonging to molecular class B of Ambler and Group 3 of enzymes proposed by Bush and Jacoby (Ambler RP *et al.*, 1980 ; Bush K *et al.*, 1995).

MBLs differ from other carbapenemases in having broad substrate profile, potential for horizontal transfer; require zinc ions for their activity and lack of inhibition by serine beta lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam. (Ambler RP *et al.*, 1980; Bush K *et al.*, 1995). In the last few years, metallo beta lactamases have been reported in several bacteria including *P. aeruginosa*, *Acinetobacter* species and members of the family Enterobacteriaceae (Docquier JD *et al.*, 2003). IMP-1 was the first MBL described in *P. aeruginosa* (Watanabe M *et al.*, 1991).

In the present study, detection of carbapenemase production by modified Hodge test revealed 16.6% positivity while

13.3% isolates were MBL producers as detected by combined disc test. Modified Hodge test positive but MBL negative may be due to type A or D carbapenemases. Imipenem resistance in MBL negative isolates is explained by other mechanism of carbapenem resistance as discussed earlier. Our findings are comparable with recent study by Kumar *et al* which reported 18% MBL positivity and. Khakhkhar *et al* reporting 11.11% MBL producing *P. aeruginosa* isolates (Kumar R *et al.*, 2014; Khakhkhar DVM *et al.*, 2012). In contrast, less incidence of MBL of 4.4% (Berges L *et al.*, 2007) to as high as 100% (Franklin C *et al.*, 2006) have also been reported by other studies.

Table.1 Antimicrobial susceptibility pattern of *P. aeruginosa*

Antibiotic	No. of resistant isolates (n=120)	% of resistant isolates
Piperacillin (100µg)	92	76.7
Gentamicin (10 µg)	90	75
Ceftazidime (30µg)	84	70
Ciprofloxacin (5µg)	57	47.5
Piperacillin-Tazobactam (100µg/10µg)	55	45.9
Imipenem (10µg)	42	35
Aztreonam (30µg)	36	30
Polymyxin-B (300 µg)	0	0

Table.2 Results of Modified Hodge test, Combined disc test in imipenem resistant isolates

Total no of <i>P. aeruginosa</i> Isolates	Imipenem resistant (n=120)	Modified Hodge test positive (n=120)	Combined Disc test positive (MBL positive) (n=120)
120	42 (35%)	20 (16.6%)	16 (13.3%)

Table.3 Sensitivity of MBL positive isolates to polymyxin B

No. of isolates	MBL positive	Sensitive to polymyxin B (n=16)
	16	16 (100%)

The spread of MBL genes among bacterial pathogens are matters of major concern with regard to the future of antimicrobial chemotherapy. Studies have shown a poor outcome in patients with serious infections due to MBL-producing organisms, which are treated with antibiotics to which the organism is resistant (Hirakata *et al.*, 2003; Poirel L *et al.*, 2000). In the present study, all the isolates including MBL producers were sensitive to polymyxin B. Data from in vitro studies suggest that polymyxin B or colistin represents the best treatment options (Livermore DM *et al.*, 2002). Various studies have also reported high sensitivity ranging from 97% to 100% for polymyxin B in MBL producers. (Deeba B *et al.*, 2011; Mai MZ *et al.* 2014; Tawfik AF *et al.*, 2012). This supports the evidence that polymyxin B has increasingly become a practical therapeutic option for MBL *P. aeruginosa* infections.

Current data regarding the treatment options in clinical practice for metallo beta lactamase producing organisms is limited. Identification of MBL in every microbiology laboratory is important to initiate appropriate treatment regimen. Although PCR is highly accurate and reliable choice for detecting MBL, it is beyond the limit of routine microbiology laboratories. Combined disc test is less costly and should be routinely practiced for screening of MBL in imipenem resistant isolates. Strict infection control practices, judicious use of antibiotics and early detection of MBL producers will help in extending the endurance of carbapenems as the last alternative antibiotic. Polymyxin is an effective therapeutic option for MBL producing *P. aeruginosa*. The revival of polymyxins stands as an example that old and largely overlooked antibiotics may prove to be useful in the treatment of critically ill patients.

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