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Original Research Article

Phenotypic Detection of Metallobeta-Lactamase (MBL) Producing Clinical Isolates of *Pseudomonas aeruginosa* in a Tertiary Care Hospital in North Karnataka India

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Abstract:

Introduction: When treating clinically challenging bacteria like *Pseudomonas aeruginosa* that are resistant to many drugs, carbapenems are frequently utilized with great success. However, there is a growing risk of developing resistance to carbapenems, primarily linked to the formation of metallo-β-lactamases (MBLs), which has been a clinical disaster. The prompt execution of infection control measures and the prevention of nosocomial transmission through proper treatment depend heavily on the early detection and identification of strains that produce MBL. Therefore this study was undertaken for screening MBL production in clinical isolates of *Pseudomonas aeruginosa*.

Materials and Methods: A total of 112 isolates of *Pseudomonas aeruginosa* isolated from different clinical samples were tested for Carbapenem resistance and MBL production. Imipenem disc (10 µg Himedia) was used for detection of Carbapenem resistance. All Imipenem resistant strains of *P. aeruginosa* by Kirby -Bauer disc diffusion test were screened for MBL production by phenotypic methods, (1). Imipenem+EDTA combined disc test (CDT) and (2). Imipenem and EDTA double disc synergy test (DDST).

Results: Out of 112 isolates of *P. aeruginosa*, 21 strains were Imipenem resistant, of which 18 isolates were detected as MBL producers by CDT and 14 isolates of *P aeruginosa* were detected as MBL producers by DDST. Out of 21 Imipenem resistant *P. aeruginosa*, 16 isolates were multidrug resistant and 5 strains were Non multidrug resistant. All Imipenem resistant *P. aeruginosa* were found to be 100% sensitive to Colistin and Polymyxin B.

Conclusion: Screening tests like CDT &DDST are a crucial step towards monitoring of these MBL producing strains of *P. aeruginosa* as they are multidrug resistant. All isolates of *P. aeruginosa* should be routinely screened for MBL production.

Keywords: CDT, DDST, Multidrug Resistant, *Pseudomonas aeruginosa*, Metallo-beta lactamases.

Introduction

Pseudomonas is the epitome of an opportunistic pathogen, often causing Hospital acquired infection. *P. aeruginosa* infection is prevalent among patients with burn wounds, cystic fibrosis, acute leukemia, organ transplants, and intravenous drug users. It causes spectrum of infections which include septicaemia, urinary tract infections, pneumonia, chronic lung infection, endocarditis, dermatitis and osteochondritis.[1]

Carbapenems are the antimicrobials of choice for severe Pseudomonas infections. However, resistance to this novel antimicrobial is increasing worldwide. Carbapenem resistance in *Pseudomonas aeruginosa* is most commonly due to production of metallo-beta-lactamases (MBLs). [2] MBL-producing gram-negative bacilli produce severe infections leading to high morbidity and mortality. The emergence of MBLs in gram-negative bacilli is becoming a therapeutic challenge as these enzymes render all penicillins, cephalosporins, and carbapenems ineffective.[3] MBL was reported for the first time in 1991 in Japan, and since then, nosocomial outbreaks due to MBL-producing gram-negative bacilli are being increasingly reported from different parts of the world.[4]

The benefits of early MBLs detection include timely implementation of strict infection control practices and treatment with alternative effective antimicrobials. [5] Various phenotypic methods are

used for MBL detection such as the combined disc method, double-disc synergy method, and Epsilometer test (E-test). MBL E-test is considered the phenotypic standard method for MBL detection, but the test is expensive.[6] Double-disc synergy and combined disc tests (CDTs) are economical and simple to perform.[6]

This study was undertaken to find the prevalence and antimicrobial sensitivity pattern of MBLs producing *Pseudomonas aeruginosa* in a tertiary care center.

Materials and Methods

After approval from the Institutional ethics committee, this prospective study was conducted from July 2021 to December 2021 in the Department of Microbiology at KIMS, Koppal.

A total of 112 Pseudomonas species isolated from various clinical materials like sputum, Endotracheal secretion, pus, urine, blood and body fluids of all age groups and both sexes received at microbiology section of central laboratory for routine culture and sensitivity were included in the study.

Isolates were identified as Pseudomonas based on standard microbiological techniques. The antibiotic susceptibility testing was performed by Kirby - Bauer disc diffusion method. Isolates of *Pseudomonas aeruginosa* resistant to Imipenem were tested for MBL production by CDT and DDST.

Antibiotic Susceptibility testing: Antibiotic susceptibility test for all *P. aeruginosa* isolates was performed by Kirby-Bauer disc diffusion method on Mueller-Hinton agar (MHA) plates as per clinical laboratory standards institute guidelines using following antibiotic discs from Hi-media imipenem disc10 µg ,Piperacillin /tazobactam(100µg/10 µg),Ceftazidime 30 µg, Amikacin 30 µg, Ciprofloxacin 5 µg, Gentamicin 10 µg, Polymyxin-B 300 units, Colistin 10 µg.

Phenotypic detection of MBL production: All isolates that were resistant to imipenem by disc diffusion method were subjected to MBL production test by following phenotypic methods.

1. Imipenem-EDTA combined disc test (CDT)
2. Impinem-EDTA double-disk synergy test (DDST)

Imipenem-EDTA combined disk method (CDT) was performed as described by Yong et al. Imipenem and imipenem + EDTA discs were placed approximately 30mm apart on lawn culture of test isolate on Mueller Hinton agar. After overnight incubation at 37^{O} C, an increase in zone of inhibition of ≥ 7mm around imipenem + EDTA combined disk in comparison to imipenem disk alone indicated production of Metallo Beta-Lactamase (MBL)

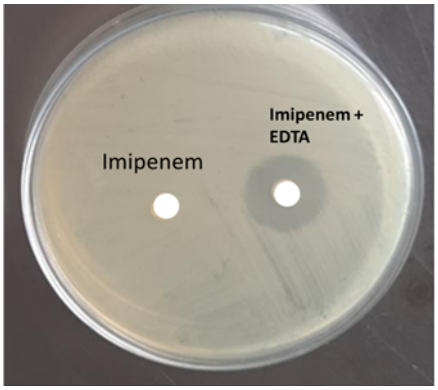

Figure 1: Combined disc test for the detection of MBL producer

Zone of inhibition surrounding the Imipenem + EDTA disc showing > 7mm enhanced zone when compared to Imipenem disc alone indicating MBL production.

CDT has less chance of subjective variation, as it measures the increase in inhibition zone above a cut off value

Imipenem-EDTA double disk synergy test (DDST) was performed as described by Lee et al.

Test isolates were inoculated on Muller- Hinton agar. An imipenem (10µg) disk is placed 20mm centre to centre from a EDTA (750µg) disk. Enhancement of the zone of inhibition in the area between imipenem and EDTA disk i.e synergy when compared with zone of inhibition on the side of imipenem disk was interpreted as a positive result for MBL production.

Results

Out of total 112 isolates of *Pseudomonas aeruginosa* screened for MBL production by imipenem disk diffusion test, 21 isolates were imipenem resistant and 91 isolates were sensitive to imipenem. All 21 PA strains showing resistance to imipenem 10 ug disk in the screening test were

further tested by imipenem-EDTA disk synergy test and combined disk test. Of the 21 strains of PA resistant to imipenem, 18 isolates were confirmed as MBL producers by CDT and only 14 isolates were confirmed by imipenem+ EDTA(DDST)synergy test. Diagram:1 Table 1 shows the age distribution of patients yielding *Pseudomonas aeruginosa* in their clinical specimens. Maximum were obtained from patients in the age group 51-60 years.

Table 1: Age distribution of patients infected with Pseudomonas

Age group	Total cases n=112	Percentage (%)
0-10	13	12
11-20	15	13
21-30	16	14
31-40	11	10
41-50	13	12
51-60	23	20
61-70	17	15
71-80	04	4
	112	100

Table 2 shows the gender wise distribution of patients yielding *Pseudomonas aeruginosa* in their clinical specimens. Maximum were obtained from male patients

Table 2: Distribution of cases by sex

Sex	Total Cases N=112	Percentage (%)
Male	79	70.5
Female	33	29.5
Total	112	100

Of the 112 isolates of PA, 55 were isolated from pus samples,28 were isolated from sputum ,2 were isolated from endotracheal secretion, 14 isolates from blood,10 from urine and 03 were isolated from stool samples as shown in Table 3

Table 3: Distribution of *Pseudomonas aeruginosa* in clinical samples

Sample type	Number of isolates n-112	Percentage (%)
Pus	55	49
Sputum	28	25
Endotracheal secretion	02	1.7
Blood	14	12.5
Urine	10	9
Stool	03	2.6

Susceptibility pattern of imipenem resistant PA strains is shown in Table 4. 100% sensitivity to Colistin and Polymixin B was observed.

Table 4: Susceptibility pattern of imipenem resistant *P.aeruginosa* strains to commonly use anti pseudomonal antibiotics.

Antibiotics	Sensitive	Intermediate	Resistant
Piperacillin /tazobactam(100µg/10 µg)	4	1	16
Ceftazidime 30 µg	0	3	18
Amikacin 30 µg	5	0	16
Ciprofloxacin 5 µg	1	4	16
Gentamicin 10 µg	0	0	21
Polymyxin-B 300 units	21	0	0
Colistin 10 µg	21	0	0

P aeruginosa with resistance to 3 or more antibiotic groups are considered multidrug resistant strains.
Table 5 compares the prevalence of multidrug resistance (MDR) among imipenem sensitive and imipenem resistant *P. aeruginosa*.

Table 5: Comparison of prevalence of multidrug resistance among imipenem sensitive and imipenem resistant *P aeruginosa*

Isolates of *P.aeruginosa*	MDR *Paeruginosa*	Non MDR *Paeruginosa*
Imipenem sensitive strains N = 91	15	76
Imipenem resistant strains N=21	16	5

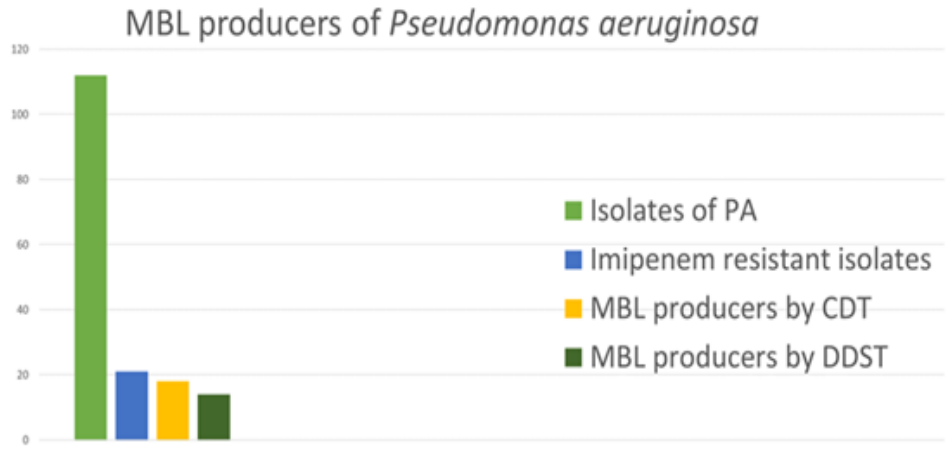

Figure 1: MBL producers of *P. aeruginosa*

Discussion

Carbapenems are typically employed as last resort antibiotics for treating infections caused by multidrug resistant Gram negative bacilli because they are stable and extended spectrum and Amp C-β lactamase producing Gram negative bacilli respond to it[7]. However, there have been rising reports of resistance to these life-saving antimicrobials in *Pseudomonas aeruginosa* [8,9].

Resistance to carbapenem is caused by decreased outer membrane permeability, increased efflux systems, changes in penicillin binding proteins, and the creation of carbapenem hydrolyzing enzymes known as carbapenemases. Resistance to carbapenem hydrolyzing enzymes such as metallo-beta-lactamases (MBL) may be chromosomally encoded or plasmid mediated, posing a risk of resistance propagation by gene transfer among gram negative bacteria [9]. The presence of an MBL-positive isolate in a hospital context presents a therapeutic challenge as well as a severe risk for infection control.

In our study, out of 112 *P.aeruginosa* isolates, 21 (18.75%) were imipenem resistant. Mehul et al [10] reported low Imipenem resistance i.e.5.30%. Bashir et al [11] reported 13.42% of imipenem resistance. Kumar R et al [12] reported 30% of imipenem resistance. The prevalence of MBL producing *Pseudomonas aeruginosa* was 16% and 12.5% by CDT and DDST respectively in our study. It was found in our study that 5 isolates were Carbapenem resistant non MBL producers. Isolates from patients aged 51-60 years produced the most MBL, followed by those aged 71-80 years. Shobha et al. found that isolates aged 40 to 75 years produced the most MBL [13]. In our investigation, MBL producing *Pseudomonas aeruginosa* isolates demonstrated 100% sensitivity to Colistin and Polymixin B and 16 imipenem resistant isolates out of 21 were multidrug resistant. Research suggests that MBL-producing bacteria are resistant to aminoglycosides and flouroquinolones, in addition to β-Lactams [14].

CDT & DDST are phenotypic methods for detection of MBL producing *Pseudomonas aeruginosa*, based on the ability of metal chelators such as EDTA to inhibit the activity of MBL [14] Sensitivity and specificity of these tests could not be calculated as PCR was not done which is the gold standard test with high sensitivity & specificity.

Conclusion

Early diagnosis and quick implementation of infection management measures are critical to avoid the spread of MBLs to other gram-negative rods. Because these MBL-producing strains of *Pseudomonas aeruginosa* are multidrug resistant, screening procedures such as CDT and DDST are critical for monitoring them. Additionally, it is critical to adhere to antibiotic limitation policies in order to avoid overuse of carbapenem and other broad spectrum antibiotics.

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