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Phenotypic Detection of Metallo-Beta Lactamases in Pseudomonas Aeruginosa in a Tertiary Care Hospital of Western Rajasthan

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Abstract

Introduction: *Pseudomonas aeruginosa*is an opportunistic gram negative bacilli, often causing Hospital acquired infection. *Pseudomonas* spp. infection is prevalent among patients with burn wounds, cystic fibrosis, acute leukemia and intravenous drug users and urinary tract infection etc.

Aim of the study: The present study was designed to determine the prevalence of Metallo -beta lactamase production in *Pseudomonas aeruginosa*and to evaluate the phenotypic test methods to detect the MBL production in imipenem-resistant clinical isolates of *Pseudomonas aeruginosa.*

Materials and Methods: The present study was conducted fromSeptember 2022 to December 2024 at the department of Microbiology ,Dr.S.N Medical college,Jodhpur.It was a hospital based prospective study.

Results: Totally 215,isolates of *Pseudomonas aeruginosa* were collected from various clinical specimens.Out of which 184 were impipenem resistant .Highest sensitivity and specificity was shown by E –test i.e 184(100%) followed by combined disc test 177 (96.17%),MHT 98(53.26%) and DDST 96(52.17%) respectively.

Keywords: Metallo-beta lactamases (MBL),Muller Hinton Agar (MHA),Combined disc test (CDT),Double disc synergy test (DDST) and E-test.

Introduction

*Pseudomonas aeruginosa*is an opportunistic gram negative bacilli, often causing Hospital acquired infection [1].*Pseudomonas*spp. infection is prevalent among patients with burn wounds, cystic fibrosis, acute leukemia and intravenous drug users and urinary tract infection etc. Its tendency to remain viable on both animate and inanimate objects around the patient including antiseptic solutions [2,3].

β-Lactams are the most widely used class of antibiotics. Since the discovery of benzylpenicillin in the 1929 by Sir Alexander Fleming , thousands of new penicillin derivatives and related β-lactam classes of cephalosporins, cephamycins, monobactams, and carbapenems have been discovered.Carbapenems are β-lactam antibiotics with the broadest spectrum of antibacterial activity, exhibiting bactericidal activity against many aerobic and anaerobic Gram-positive and Gram-negative bacteria, including non-fermentative pathogens [4-7]. Carbapenems were the only hope to for infections with these multi drug resistant (MDR)bacteria[8,9] ,However this last barrier was also broken by carbapenemases enzymes that started emerging worldwide and were able to destroy Carbapenems.

Metallo beta lactamase (MBL) has ability to hydrolyze a wide variety of beta lactam drugs, such as Penicillin, Cephalosporins and Carbapenems. MBL are inhibited by metal chelators, such as ethylene demine tetra acid (EDTA) and thiol-based compounds .There is an alarming increase of antibiotic resistance in bacteria [10,11]

Aim and Objective

The present study was designed to determine the prevalence of Metallo -beta lactamase production in *Pseudomonas aeruginosa*and to evaluate the phenotypic test methods to detect the MBL production in imipenem-resistant clinical isolates of *Pseudomonas aeruginosa.*

Material and Methods

The present study was conducted fromSeptember 2022 to December 2024at the department of Microbiology ,Dr.S.N Medical college,Jodhpur,Rajasthan.Present study was a hospital based prospective study.Institutional Ethical Clearance was taken .

Totally, 215 isolates of *P. aeruginosa* were isolated from various clinical samples. The isolates were identified by standard laboratory techniques.All these isolates were screened for imipenem resistant by Kirby-Bauer disc diffusion method as per Clinical and Laboratory Standard Institute guidelines.

All isolates that were resistant to imipenem(184/215) by disc diffusion method were subjected to MBL production test by following phenotypic methods.

1. **Imipenem-EDTA combined disc test (IMP EDTA CDT) :**In this method ,the test organism was lawn culture on Muller Hinton Agar (MHA) .Two disks were placed on MHA plate.One was 10 ugimipenem,another was imipenem+ EDTA (10/750 ug) combined disk.The increase in more than 7mm in the inhibition zone of the imipenem-EDTA disk over than imipenem disk alone, was considered as MBL positive.
2. **Double disk synergy test (DDST):** An imipenem (10 µg) disc was placed in the center of the plate a top a lawn culture of the test organism, and a sterile blank disc was placed 20 mm away from it. Then, the blank disc was covered with 10 µL of EDTA (750 µg). Enhancement of the zone of inhibition in the area between the imipenem and EDTA discs in comparison with the zone of inhibition on the far side of the drug was interpreted as a positive result for MBL production.
3. **Modified Hodge Test:***Escherichia coli* ATCC 25922 (an indicator organism sensitive to carbapenems) was cultured in peptone water to achieve 0.5 McFarland opacity standard and was lawn cultured onto a Mueller-Hinton agar plate using sterile cotton swab. After drying, 10 µgImipenem disc was placed at the centre of the plate on the lawn culture and an overnight growth of test strain was heavily streaked from the edge of the Imipenem disc outwards, to the periphery of the plate in four different directions. The plates were incubated at 37ºC overnight. The presence of a distorted zone (Clover-leaf shaped zone of inhibition) was considered as positive test.
4. **E-test Metallo -β-lactamases strips:**Single strips impregnated with a concentration gradient of imipenem (4–256 µg/mL) on one end and that of imipenem (1–64 µg/mL) fused with a stable concentration of EDTA on the other end were placed a top lawn cultures of the test organisms in culture plates. An 8-fold increase in the ellipse of imipenem and EDTA indicated the presence of MBL .

Result

Table 01 :Distribution of clinical samples and *Pseudomonas aeruginosa* isolates (N=215).

S.No	Sample type	Number of Isolates (%)
1	Pus	90 (41.86%)
2	Urine	72 (33.48%)
3	Burn wound	26 (12.09%)
4	Sputum	02(0.93%)
5	Blood	11(05.11%)
6	Endotracheal tube	05(02.32%)
7	Bronochoalveolarlavage	00 (00%)
8	Fluids and effusions	06(02.79%)
9	Vaginal swab	03 (01.39%)
	Total	215 (100%)

Table 02 :Distribution of imipenem –resistant and imipenem-sensitive isolates of *Pseudomonas aeruginosa*(N=215)

S.No		Bacteria	No of IMP-Resistant Isolates (%)	No of IMP-Sensitive Isolates (%)	Total
1		*Pseudomonas aeruginosa*	184 (85.58%)	31(14.41%)	215(54.70%)

Table 03 : Distribution of MBL and Non MBL producers according to Gender wise (N=215).

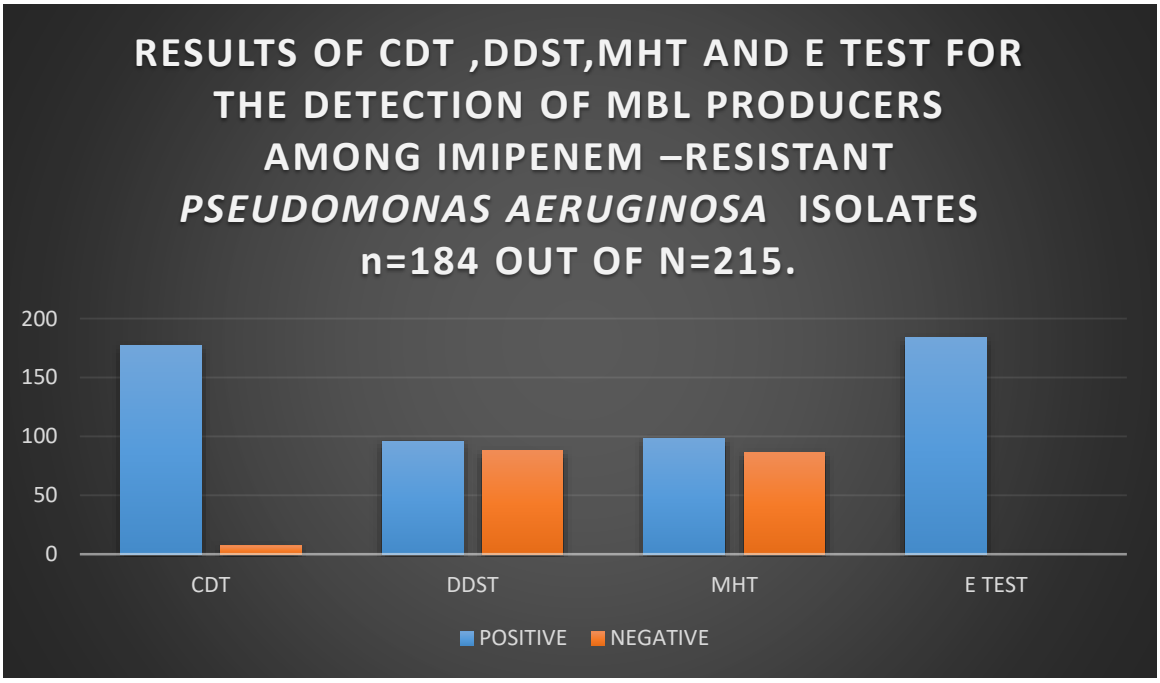
S.No	Gender	MBL producer (n=184)	MBL non producer (n=31)	Total isolates (N=215)
1	Male	98(53.26%)	25(80.64%)	123(57.20%)
2	Female	86(46.73%)	06(19.35%)	92(42.79%)
Total		184(85.58%)	31(14.41%)	215(100%)

Table 04 :Distribution of MBL and Non MBL producers according to Age wise (N=215)

S.NO	Age group	MBL producer(n=184)	MBLnon producer (n=31)	Total (N=215)
1	<10 year	04 (2.17%)	01(3.22%)	05 (2.32%)
2	11-20 year	23 (12.5%)	07(22.58%)	30(13.95%)
3	21-30 year	29 (15.76%)	03 (9.67%)	32(14.88%)
4	31-40 year	37 (20.10%)	10 (32.25%)	47(21.86%)
5	41-50 year	35 (19.02%)	03 (9.67%)	38(17.67%)
6	51-60 year	35 (19.02%)	04 (12.90%)	39(18.13%)
7	61-70 year	07 (3.80%)	01 (3.22%)	08(3.72%)
8	71-80 year	09 (4.89%)	01 (3.22%)	10(4.65%)
9	> 81 year	05 (2.71%)	01 (3.22%)	06(2.79%)
Total		184 (85.58%)	31 (3.22%)	215(100%)

Table 05 :Results of CDT ,DDST,MHT and E test for the detection of MBL producers among imipenem –resistant *Pseudomonas aeruginosa* isolates n=184 out of N=215.

S. No	Bacterial isolates	Resistant to Imipenem(n=184)	CDT		DDST		MHT		E-test	
			Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
1	*Acinetobacter baumanni*	184	177 (96.19%)	07(3.80 %)	96(52.17%)	88(47.82%)	98(53.26%)	86(46.73%)	184 (100 %)	00



Discussion

MBL-producing non fermenter gram-negative bacilli have now been reported in many geographic areas[12-14].In the present study, prevalence of MBL production in *Pseudomonas aeruginosa* was85.58% .The MBL producing *P. aeruginosa* was first reported from Japan in 1988[1] .Since then has been reported from various parts of the world. In India, MBL was first reported in 2002 by Navaneeth *et al*.[7], Since that the incidence of MBL production in *P. aeruginosa* has been reported from various parts of India.

In the present study total 215 *Pseudomonas aeruginosa* wereisolated.Imipenem resistant isolates was 184(85.58 %) and Imipenem sensitive was 31(14.41%). MBL E-test is the most sensitive method for the detection of MBL in *P. aeruginosa*[1,15]. The E-test, based on a combination of a β-lactam substrate and a β-lactam/MBL inhibitor, is specifically designed to detect various enzymes of Ambler' classification [16,17]

MBL E-test to be very sensitive for detection of MBLs. Since in the current study sensitivity of the E –test is 100%,which is similar to the study done by Shivaprasad A *et al*.[1] i.e 100%.After E test combined disc test is most sensitive phenotypic method i.e 96.15% is similar to the study done by Shivaprasad A *et al*. i.e 100%[1].

Conclusion

The present study has showed that E-test was the most sensitive phenotypic method followed by combined disc test. Strict antibiotic stewardship policy and infection control measures can decreases the positivity rate in the hospital settings as well as in community also . Early and easy detection techniques of MBLs are required in routine clinical labs

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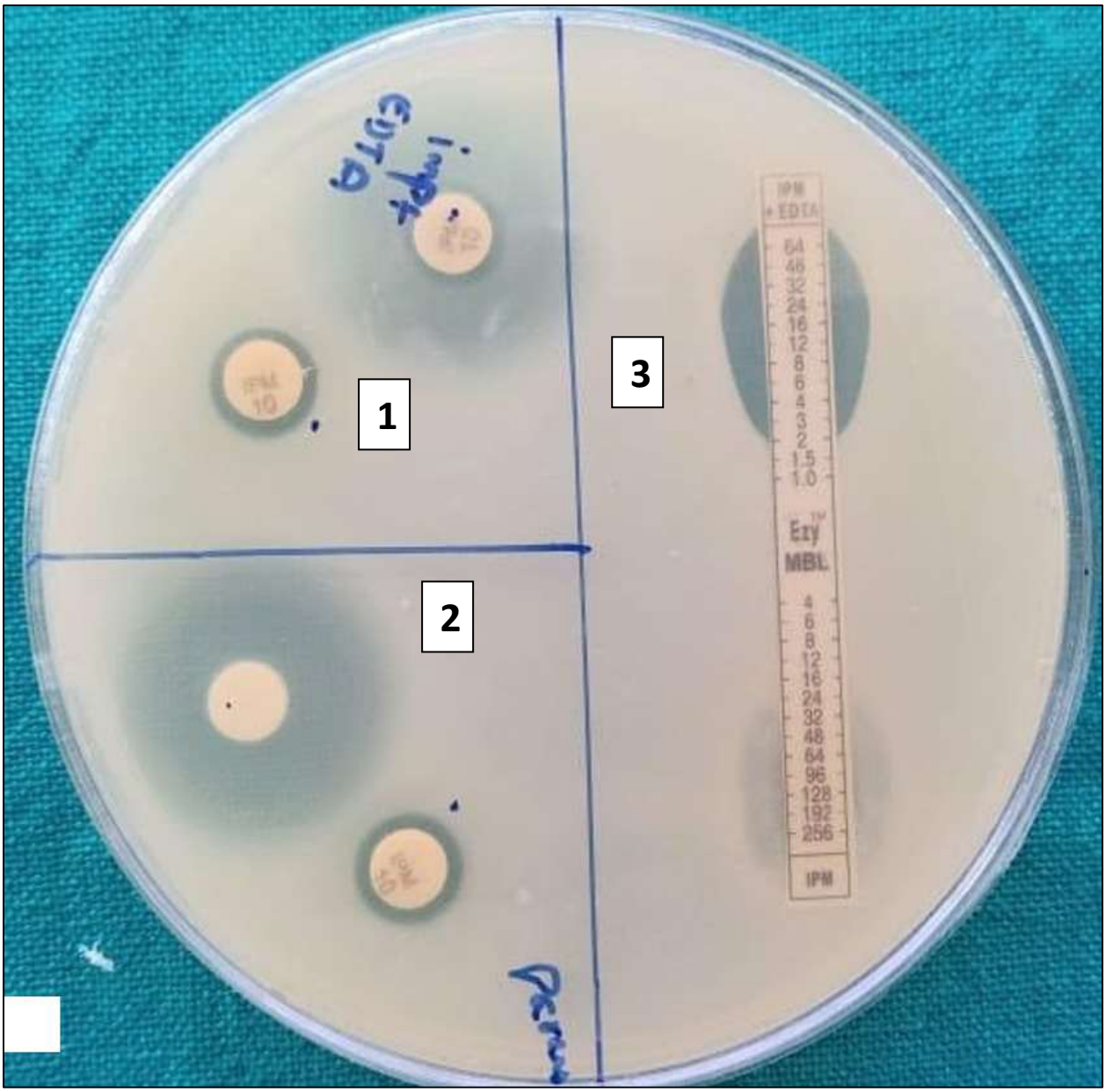

1. **Combined disc test**
2. **Double disc synergy test**
3. **E-test**