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ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *PSEUDOMONAS AERUGINOSA* AND THEIR PHENOTYPIC DETECTION OF EXTENDED SPECTRUM BETA LACTAMASES AND METALLO-BETA LACTAMASES IN ALL CLINICALLY SPECIMEN AT A TERTIARY CARE HOSPITAL, RAJASTHAN (INDIA).

Microbiology

Rachna — Department of Microbiology, National Institute of Medical Science and Research (NIMSR), Jaipur.

Bhawani Shankar Verma — Department of Microbiology, National Institute of Medical Science and Research (NIMSR), Jaipur.

Dr. Mohit Agrawal* — Associate Professor, Department of Microbiology, National Institute of Medical Science and Research (NIMSR), Jaipur. *Corresponding Author

ABSTRACT

Background: *Pseudomonas aeruginosa* continues to be a major cause of nosocomial infection. The emergence of resistance to β-lactam antibiotics is by the production of different classes of β-lactamases.

Objective: To investigate of Extended-Spectrum β-lactamase (ESBL), and Metallo-β-lactamase (MBL) enzymes in clinical isolates of *Pseudomonas aeruginosa* .

Material & methods: This prospective study was conducted in the Department of Microbiology National Institute of Medical science and research Jaipur, Rajasthan. Over a period from Nov 2019 to April 2020. A total of 141 clinical isolates of *Pseudomonas aeruginosa* were tested for the presence of ESBL and MBL enzymes. All the isolates of *Pseudomonas aeruginosa* which showed Resistance to Ceftazidime were evaluated for ESBL production by using the phenotypic confirmatory test by double-disc synergy test (PCDDT). To screen MBL producers in *Pseudomonas aeruginosa*, Imipenem and Meropenem resistant isolates were considered for phenotypic detection confirmatory test by EDTA Disk synergy test.

Results: Out of 141 isolates of *Pseudomonas aeruginosa*, ESBL production was seen by screening and confirmatory method 74(52.48%) and 61(43.26%) respectively. and MBL production was observed in 37(26.24%) and 32(22.69%) respectively. Out of 141 isolates, most of the samples were sputum 31.91%, Blood 21.28%, and urine 6.31%. *Pseudomonas aeruginosa*, isolates were 100% sensitive to Polymyxin B and Colistin for, ESBL & MBL followed by antipseudomonal drugs Tobramycin and Piperacillin-tazobactam were sensitively recorded as ESBL (73.77% & 77.05%) & MBL (78.12% and 71.88%) respectively.

Conclusions: A very high rate of MBL & ESBL production was observed, which suggested it to be an important contributing factor for Ceftazidime resistance among *pseudomonas aeruginosa* making an easy treat to infectious disease.

KEYWORDS

ESBL, MBL, PCDDT, EDTA & synergy

INTRODUCTION:

The genus *Pseudomonas* belongs to the family Pseudomonadaceae and consists of aerobic, Gram-negative bacilli, non-fermentative, non-sporing, oxidase-positive, and other biochemically identification [1]. *Pseudomonas aeruginosa* is generally not a member of the normal microbial flora in humans but colonization rates may exceed 50% during hospitalization [2].

The selection of the relevant antibiotic for the treatment of infection is complicated by its adaptability, high intrinsic antibiotic resistance, and the ability to develop resistance to multiple classes of antibacterial agents during treatment [3].

Resistance to β-lactam antibiotics is associated with the production of ESBL which can hydrolyze oxyimino β-lactams such as cefotaxime, ceftriaxone, ceftazidime, and monobactams, however, without any effect on cephamycins, carbapenems and related compounds [4,5].

Acquired resistance is mostly by the production of β-lactamase enzymes like extended-spectrum β-lactamases (ESBL), and Metallo β-lactamase (MBL) [6].

Infections due to *Pseudomonas aeruginosa* are infrequently encountered in healthy adults but in the last two decades, it has become the major cause of nosocomial infections in hospitalized patients [7]. It continues to be one of the lethal pathogens responsible for ventilator-associated pneumonia, infections in cystic fibrosis, wound infections, hospital-acquired catheter-associated urinary tract infections, and ulcerative keratitis [8,9].

Carbapenemase is β-lactamases, which include serine-β-lactamases and Metallo-β-lactamases (MBLs). The latter require metal ion zinc for their activity, which is inhibited by metal chelators like EDTA and thiol-based compounds but not by sulbactam, tazobactam, and clavulanic acid. MBL production is typically associated with resistance to aminoglycosides and fluoroquinolones, further compromising therapeutic options [10,11].

Further, the knowledge about the local antimicrobial susceptibility patterns will be helpful to start timely proper preliminary treatment.

MATERIALS AND METHODS:

This prospective study was conducted in the Department of Microbiology National Institute of Medical science and research Jaipur, Rajasthan. Over a period from Nov 2019 to April 2020.

A total of 141 isolates of *Pseudomonas aeruginosa* were obtained from all clinical samples. The cases which yielded growth of *Pseudomonas aeruginosa* from cultured on CLED agar, blood agar, and MacConkey agar were included in the study and they were further identified as per the standard microbiological procedures [12]. The isolates were further tested by employing the Kirby Bauer disc diffusion method to study their antimicrobial susceptibility pattern for Polymyxin-B (300units), Colistin (10µg), Imipenem (10µg), Meropenem (10µg), Norfloxacin (10µg), Gentamicin (10µg), Tobramycin (10µg), Piperacillin/tazobactam (100µg/10µg) and Ceftazidime (30µg). The results were recorded and interpreted as per the CLSI guidelines [13].

Extended Spectrum Beta-Lactamase (ESBL) Detection:

All the isolates of *Pseudomonas aeruginosa* which showed resistance to Ceftazidime were evaluated for ESBL production by using the phenotypic confirmatory test double-disc synergy test [13]. To put it briefly, a 0.5 McFarland's suspension of each isolate was spread on a Muller – Hinton agar (MHA) plate and Ceftazidime (30 µg) and Ceftazidime / Clavulanic acid (30 µg/ 10 µg) discs were placed aseptically on the agar plate. A distance of about 15mm was kept between the two discs (edge to edge) and the cultures were incubated at 37°C overnight. ≥ 5mm increase in the zone diameter for the observation of an antimicrobial agent which was tested in combination with clavulanic acid, versus its zone diameter when tested alone, confirmed the presence of ESBL production by the organism. The increase in the zone diameter was due to the inhibition of the β lactamase by clavulanic acid [12].

Phenotypic detection of MBLs: To screen MBL producer in *Pseudomonas aeruginosa* Imipenem and Meropenem resistant

isolates were considered for phenotypic detection confirmatory test by EDTA Disk synergy.

EDTA Disk synergy test: It's used for the detection of Metallo-β Lactamases in the Imipenem and Meropenem resistant clinical isolates. First of all, an EDTA solution with 0.5 M strength was prepared by dissolving 186.1 g of Disodium EDTA in one litter (1000 ml) of distilled water. The adjusted pH to 8.0 and the solution was sterilized by autoclaving. An overnight culture broth of the test isolate was adjusted to a turbidity of 0.5 McFarland standards and was spread on the surface of a Mueller Hinton Agar plate. A 10µg Imipenem disk (HI - MEDIA) was placed on the agar surface. A blank disk (6 mm diameter) was then kept on the inner surface of the lid of the Muller Hinton Agar plate and 10 µl of 0.5 M EDTA was poured onto it with the help of an auto pipette. This EDTA disk was then placed on the surface of the agar and was kept about 10 mm edge-to-edge apart from the Imipenem disk. After overnight incubation at 37°C, the presence of an expanded growth inhibition zone between the two disks was interpreted as positive for MBL production [14].

RESULTS:

The Department of Microbiology National Institute of Medical science and research Jaipur, Rajasthan. Over a period from Nov 2019 to April 2020.

A total of 141 isolates of *Pseudomonas aeruginosa* were obtained from all clinical. Out of 141 isolates of *Pseudomonas aeruginosa*, ESBL production was seen by screening and confirmatory method 74(52.48%) and 61(43.26%) respectively. and MBL production was observed in 37(26.24%) and 32(22.69%) respectively shown in Fig No.1.

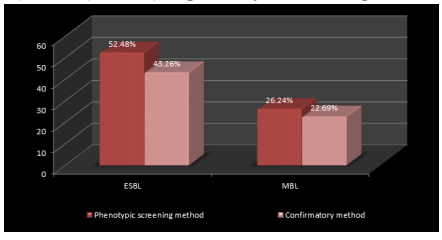

Fig no. 1 Distribution of ESBL and MBL isolates of *Pseudomonas aeruginosa with* phenotypic screening method and confirmatory method by PCDDT and EDTA disc synergy method respectively.

The total number of 141 isolates was *Pseudomonas aeruginosa*; those are the distribution of ESBL and MBL according to Sample-wise. Out of 141 isolates, most of the samples were sputum 31.91%, Blood 21.28%, and urine 16.31%. And they are out of 74 Ceftazidime resistance were ESBL by the Phenotypic Screening test sputum 13.48%, blood 14.89% & urine 9.22%. And out of 61 confirmatory tests with PCDDT are sputum 12.06%, blood 13.48% & urine 6.38%. Out of 37 Carbapenem resistance was MBL by the Phenotypic Screening test blood & Urine 7.09%, Sputum & ET 4.24%, and out of 32 confirmatory tests with PCDDT is blood 6.38%, Urine 5.67% and ET 4.24% as shown in Table No.1.

Table no. 1 Sample-wise distribution of ESBL and MBL isolates of *Pseudomonas aeruginosa*, by Phenotypic Screening test and confirmatory method by PCDDT and EDTA disc synergy method respectively.

Samples	Total	ESBL		MBL	
		Phenotypic Screening test	PCDDT	Phenotypic Screening test	EDTA disc synergy method
Urine	23 (16.31%)	13 (9.22%)	9 (6.38%)	10 (7.09%)	8 (5.67%)
Sputum	45 (31.91%)	19 (13.48%)	17 (12.06%)	6 (4.24%)	5 (3.55%)
Blood	30 (21.28%)	21 (14.89%)	19 (13.48%)	10 (7.09%)	9 (6.38%)
Fluids	5 (3.55%)	1 (0.71%)	1 (0.71%)	1 (0.71%)	1 (0.71%)
Pus	22 (15.60%)	10 (7.09%)	7 (4.96%)	4 (2.83%)	3 (2.14%)
ET	16 (11.35%)	10 (7.09%)	8 (5.67%)	6 (4.24%)	6 (4.24%)
Total	141	74 (52.48%)	61 (43.26%)	37 (26.24%)	32 (22.69%)

Pseudomonas aeruginosa, isolates were 100% sensitive to Polymyxin B and Colistin for, ESBL & MBL. Followed by antipseudomonal drugs Tobramycin and Piperacillin-tazobactam were sensitively recorded as ESBL (73.77% & 77.05%) & MBL (78.12% and 71.88%) respectively. Ceftazidime was 100% resistance for confirmed ESBL production and 100% resistance were Imipenem and Meropenem for confirmed MBL production is shown in Table no.2.

Table no. 2 Antimicrobial susceptibility of Pseudomonas *aeruginosa* ESBL (n=61) & MBL (n=32). *Only for urine sample.

Antibiotics	ESBL n=61 (43.26%)		MBL n=32 (22.69%)	
	Sensitive	Resistance	Sensitive	Resistance
Polymyxin-B	61 (100%)	0	32 (100%)	0
Colistin	61 (100%)	0	32 (100%)	0
Imipenem	43 (70.49%)	18 (29.51%)	0	32 (100%)
Meropenem	47 (77.05%)	14 (22.95%)	0	32 (100%)
Norfloxacin*	37 (60.65%)	24 (39.35%)	15 (46.87%)	17 (53.13%)
Gentamicin	18 (29.51%)	43 (70.49%)	9 (28.13%)	23 (71.87%)
Tobramycin	45 (73.77%)	16 (26.23%)	25 (78.12%)	7 (21.88%)
Piperacillin/ tazobactam	47 (77.05%)	14 (22.95%)	23 (71.88%)	9 (28.13%)
Ceftazidime	0	61 (100%)	0	32(100%)

DISCUSSION:

This study was aimed to study the prevalence rates of ESBL & MBL among various clinical isolates of *Pseudomonas aeruginosa*, Ceftazidime is a third-generation cephalosporin used frequently for the treatment of infections caused by *Pseudomonas aeruginosa*. Ceftazidime & Carbapenem resistance is mainly mediated by the production of beta-lactamases such as ESBL, MBL.

In the present study, the antibiogram of the 141 isolates of *Pseudomonas aeruginosa*, showed more resistance against Ceftazidime [52.48%], which was similar to the observations which were by Prashant DurWas PeshattiWar *et al* [15], who reported the Ceftazidime resistance 53.17%, Diwivedi *et al* [16], who reported the Ceftazidime resistance to be around 63% and Arya M *et al* [17]who reported a Ceftazidime resistance of 55.4% in isolates.

Our study showed that among the 141 *Pseudomonas aeruginosa*, isolates, 71 [43.26%] were ESBL producers, Goel *et al* [18]. And Rani *et al* [19] have reported ESBL production in *Pseudomonas aeruginosa*, as 42.3%and 37.2%, respectively.

In this study, 22.69% isolates of *Pseudomonas aeruginosa*, were positive for the production of MBL production. The results are similar to Upadhyay *et al* [20] who had reported MBL production in *Pseudomonas aeruginosa* as 20.8%. Amandeep Kaur *et al* [21] who had reported MBL production in *Pseudomonas aeruginosa* as 21.8%.

In the present study, Out of 141 isolates, most of the samples were ET 11.35%, Blood 21.28%, and urine 16.31%. The results are similar to Choudhary Vinita *et al* [22] who had reported ET 12.77%, Blood 17.77%, and urine 18.88%.

In the present study, *Pseudomonas aeruginosa* isolates were 100% sensitive to Polymyxin B and Colistin. The results are similar to Amandeep Kaur *et al* [21] who was observed with Polymyxin B (100%) and Colistin (100%) and Choudhary Vinita *et al* [22] who were observed with Polymyxin B (97.23%) and Colistin (94.45%) in this study as compared to another antipseudomonal antibiotic.

Ceftazidime was 100% resistance for confirmed ESBL production; the results are similar to Choudhary Vinita *et al* [22] who had reported 80% resistance. And 100% resistance was Imipenem and Meropenem for confirmed MBL production. The results are similar to Dr. Vipul M Khakhkhar *et al* [23], who had reported 100% resistance Imipenem and Meropenem in MBL.

Every effort needs to be made to carefully selected antibiotics, needed for a broad spectrum of empirical coverage of potential microorganisms with the need for preservation of antibiotic availability for when they are absolutely necessary to the right treatment.

CONCLUSION:

Pseudomonas aeruginosa is a major cause of nosocomial infections. In

the present study, we found an alarming number of multidrug resistances producing *Pseudomonas aeruginosa*. The production of ESBL and MBL would be most important in the reduction of mortality rate. The discover to be test is simple, easily performed, & economical state and can be done along with routine antimicrobial susceptibility testing. Most of the implementation of antibiotic constraint policies to avoid excessive and injudicious use of extended-spectrum antibiotics in every hospital.

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