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ESBL, MBL AND AmpC DETECTION IN MULTIDRUG RESISTANT PSEUDOMONAS AERUGINOSA (MDRPA) AND PANDRUG RESISTANT Pseudomonas aeruginosa (PDRPA) ISOLATED IN TERTIARY CARE HOSPITAL

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ABSTRACT

Pseudomonas aeruginosa is a leading cause of nosocomial infection. The rise of antibiotic emergence of resistance may vary with different antibiotic treatments. A variety of ß-lactamases which include ESBLs, AmpC ß-lactamases and metallo-ß-lactamases, have emerged as the most worrisome mechanism of resistance among the gram negative bacteria, which pose a therapeutic challenge to the health care settings. **Materials and Methods:** A total of 190 clinical isolates of *Pseudomonas aeruginosa* were studied. Detection of AmpC beta-lactamase was performed by three-dimensional method, whereas detection of ESBL was done by the combined disk diffusion method as per Clinical and Laboratory Standards Institute (CLSI) guidelines and MBL were detected by the combined disc diffusion test.

Result: A Total 25 strains of Multidrug resistance *Pseudomonas aeruginosa* (MDR PSA) and 07 of PAN drug resistant Pseudomonas aeruginosa (PDRPA) were studied in this study. In MDRPSA, 56% isolates were confirmed to be positive for AmpC beta-lactamase. Among them, 57% strains were extended spectrum beta-lactamase and both AmpC beta-lactamase and Extended Spectrum Beta-Lactamase co-producer were 24 % and None of enzyme producer were 18% respectively. While in PDRPA case, 86% isolates were confirmed to be positive for AmpC beta-lactamase. Among them, 86% strains were Extended Spectrum Beta-Lactamase and both AmpC beta-lactamase and Extended Spectrum Beta-Lactamase co-producer were 86 % and None of enzyme producer were 18% and MBL were 100%.

Conclusion: The study emphasizes the high prevalence of multidrug resistant and PAN drug resistant *Pseudomonas aeruginosa* producing beta-lactamase enzymes of diverse mechanisms. Thus, proper antibiotic policy and measures to restrict the indiscriminative use of cephalosporins and carbapenems should be taken to minimize the emergence of this multiple beta-lactamase producing pathogens.

KEYWORDS: *Pseudomonas aeruginosa*, PAN drug, metallo-**ß**-lactamases.

INTRODUCTION

An alarming rise in the rates of the antibiotic resistance has now become a serious and an increasingly common public health concern, with severe implications, especially in the intensive care units. A variety of ß-lactamases which include ESBLs, Amp-C ß-lactamases and Metallo-ß-lactamases, have emerged as the most worrisome mechanism of resistance among the gram negative bacteria, which pose a therapeutic challenge to the health care settings. [1] The β-lactamases inactivate β-lactam antibiotics by cleaving the structural β-lactam ring. Failure to detect these enzymes producing strains has contributed to their uncontrolled spread in Health Care setup and therapeutic failure. [2]

The newer beta-lactamases, including Extended-Spectrum Beta-lactamases (ESBL), AmpC beta-lactamases and Metallo-beta-lactamases (MBL), have emerged worldwide as a cause of antimicrobial resistance in gram-negative bacteria. The presence of ESBLs and AmpC beta-lactamases in a single isolate reduces the effectiveness of the beta-lactam-beta-lactamase inhibitor combinations, while MBLs and AmpC beta-lactamases confer resistance to carbapenems. Often, these enzymes are co-expressed in the same isolate. [3]

Pseudomonas aeruginosa is an important pathogen commonly implicated in nosocomial infections. The occurrence of multidrug-resistant (MDR) and pandrug resistant (PDR) *Pseudomonas aeruginosa* strains are increasing worldwide and limiting our therapeutic options. [4]

Pseudomonas aeruginosa strains showing resistance to three or more classes of antibiotics (e.g. Ciprofloxacin, Aminoglycosides and Carbapenem) are termed as Multidrug Resistance (MDR) strains. [5]

Pandrug resistance is defined as isolates intermediately-resistant or totally resistant to all antimicrobial agents available for clinical use according to routine disk diffusion susceptibility results. [6]

Infections caused by *Pseudomonas aeruginosa* are difficult to treat as the majority of isolates exhibit varying degrees of innate resistance. Acquired resistance is also reported by the production of plasmid mediated AmpC beta (β)-lactamase, extended spectrum β-lactamase and metallo β-lactamase (MBL) enzymes. [7] The multidrug resistant (MDR) and pandrug resistant (PAN) isolates that are present in the ICU and in the hospital environment pose not only therapeutic problems but also serious concerns for infection control management. [8]

MATERIALS & METHODS

There are lot of methods of detecting AmpC, ESBL & β lactamase in clinical samples. In this study. A total 190 isolates of Pseudomonas aeruginosa were processed. Out of which 25 *isolates were* MDRPA and 08 isolates were PDRPA. These drug resistant isolates were further tested for the presence of AmpC Beta-Lactamase, Extended Spectrum Beta-Lactamase (ESBL) and Metallo Beta-Lactamase (MBL) enzyme by following methods- **(MBL/ESBL/AmpC detection)**

Common initial steps

1. 4-5 colonies of the test strain were touched with a straight wire and transferred to 1ml of Normal saline to match turbidity to 0.5 McFarland standard.
2. Using this inoculum, excess broth was expressed by rotating the swab against the inner side of the suspension tube.
3. Lawn culture was made on cation balanced Muller Hinton Agar (MHA) plate with a sterile cotton swab.
4. Inoculum was allowed to dry for 15 minutes before putting the antibiotic disc or E test strips.

i. Detection of Extended spectrum Beta lactamase (ESBL)

Phenotypic confirmatory test for Extended spectrum Beta lactamase (ESBL)

1. Discs of Ceftazidime and Ceftazidime + Clavulanic acid were placed on the surface of MHA.
2. Overnight incubation was done at 37˚C.

Interpretation

An increase of ≥5mm in zone diameter of Ceftazidime + Clavulanic acid in comparison to the zone diameter of Ceftazidime alone confirmed the organisms to be an ESBL producers by disc diffusion method (Figure 1). [9]

Quality Control: *Pseudomonas aeruginosa* ATCC 27853

Figure 1: Extended spectrum Betal lactamase (ESBL).

ii. Detection of AmpC β-lactamase

Detection of AmpC β-lactamase by AmpC Disc Test

1. Using a sterile loop, the surface of 4-5 large or 5-10 small morphologically similar well isolated colonies were touched from an 18-24 hour non-inhibitory agar plate of ATCC *Escherichia coli* 25922 strain.
2. It was inoculated in 1ml of normal saline and the turbidity was adjusted to 0.5 McFarland standard.
3. MHA plate was seeded with this inoculums using sterile cotton swab.
4. Inoculum was allowed to dry for 5minutes and a cefoxitin disc of 30µg was placed in the centre of the plate.
5. A sterile plain disc was placed beside the cefoxitin disc (almost touching it) and moistened it with 20µl of sterile saline.
6. The plain disc was inoculated with several colonies of test organism (5-10 colonies) and incubated overnight at 36˚±1˚C.

Interpretation

A flattening or indentation of the cefoxitin inhibition zone in the vicinity of the disc was a positive test (Figure 2). [10]

Quality Control

Pseudomonas aeruginosa: ATCC 27853

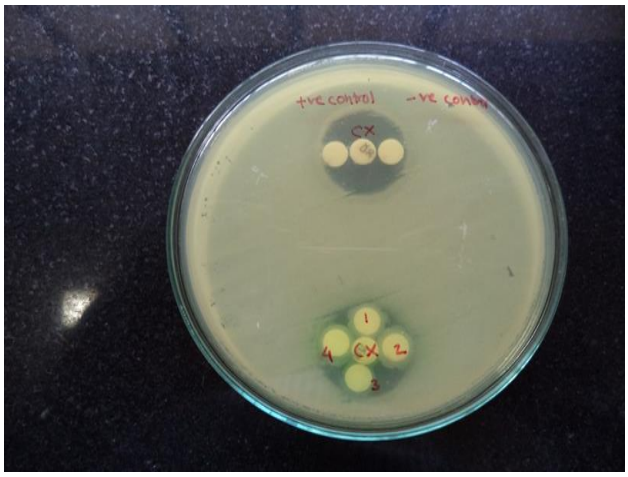


Figure 2: Detection of AmpC β-lactamase by AmpC Disc Test.

iii. Detection of Metallo-β-lactamases

1. Inoculum was allowed to dry for 5minutes and 2 imipenem discs one with 0.5M EDTA and the other plain are placed on the surface of the agar plate approximately 30 mm apart.
2. The plates were incubated overnight at 37˚C.

Interpreation

A ≥7mm increase in the zone diameter around imipenem disc with 0.5 EDTA alone indicated production of MBL (figure 3). [11]

Quality Control: *Pseudomonas aeruginosa* ATCC 27853

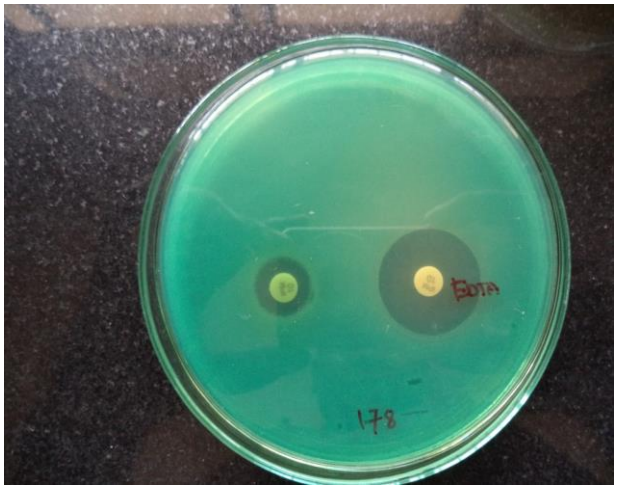


Figure 3: Metallo- betalactamase detection.

RESULT

A total 190 *Pseudomonas aeruginosa* were processed, out of which 25 (13%) were Multi drug resistant *Pseudomonas aeruginosa* (MDRPA), 07 (4%) were PDRPA and 158 (83%) were Non Drug resistant (Figure 4).

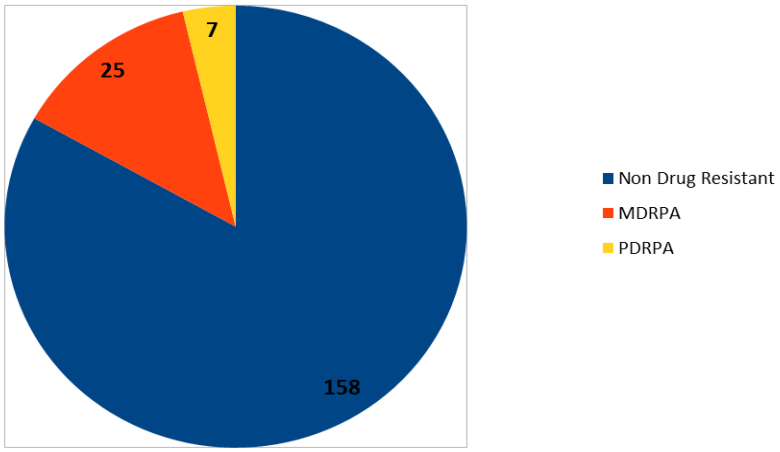


Figure 4: Distribution of Pseudomonas aeruginosa

Out of total 25 MDRPA, 16 were isolated from Male patients and 9 were Isolated from Female patients.

Table 1: Age and Sex distribution of MDRPA

S. No	Age groups	Sex	
		Male	Female
1.	0-10	02(12.5%)	--
2.	11-20	01(6.25%)	01(11.11%)
3.	21-30	07(43.75%)	02(22.22%)
4.	31-40	01(6.25%)	01(11.11%)
5.	41-50	--	02(22.22%)
6.	51-60	02(12.5%)	01(11.11%)
7.	61-70	02(12.5%)	02(22.22%)
8.	71-80	01(6.25%)	--
	Total	**16 (100%)**	**09 (100%)**

Out of total 07 PDRPA, 05 were isolated from Male patients and 02 were Isolated from Female patients.

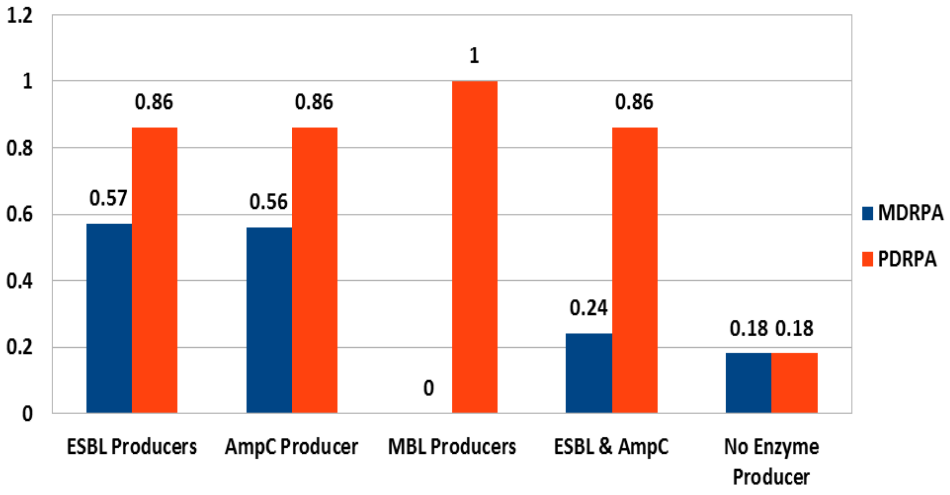
Table 2: Age and Sex distribution of PDRPA.

S. No	Age groups	Sex	
		Male	Female
1.	0-10	--	--
2.	11-20	--	--
3.	21-30	01(20%)	--

4.	31-40	01(20%)	--
5.	41-50	01(20%)	--
6.	51-60	01(20%)	01(50%)
7.	61-70	01(20%)	01(50%)
	Total	**05**	**02**

Table 3: Comparision of ESBL, AmpC & Metallo Beta lactamase producers in MDRPA & PDRPA.

S. No	Beta Lactamase Production		
1.		**MDRPA (n=25)**	**PDRPA (n=07)**
2.	ESBL Production	57%	86%
3.	AmpC Production	56%	86%
4.	MBL Production	0	100%
5.	Both ESBL & Ampc Production	24%	86%
6.	No enzyme production	18%	18%



DISCUSSION

Beta-lactamases have been grouped into four molecular classes, namely A, B, C, and D, based on the amino acids sequence homology according to Ambler classification. A, C, and D classes are called serine-beta-lactamases, and B class beta-lactamases are referred to as MBL. Newer beta-lactamases that hydrolyze cephamycins, oxyimino and zwitterionic cephalosporins, monobactams, or carbapenemsare of increasing concern because they restrict therapeutic options, cause treatment failures, and are increasing in occurrence. [12]

In this study, MDRPA rate was (13.15%) which correlates well with H.Goossens et al (2002) (12.2%), Gale et al (2001) and Ahmed Bakr et al (2013) reported less resistance (i.e.1.6% and 9.5%). [13,14,15] whereas DONG fang et al (2008) and Ahmed M S et al (2007) reported maximum MDR rate (i.e.24.1 % & 40%) in respective years. [16, 17] In this study, Male patients (64%) were predominant than female patients (36%).
Lodise et al. documented that among 351 MDRPA patients, majority were male (61%) and females (39%) [18]

In present study, 07/190 (3.68%) isolates showed resistance to all the anti pseudomonal antibiotics i.e. pandrug resistance. It correlates with Jaykumar et al (2007), from Coimbatore, India report 4% incidence of PDRPA.

C.Y. Wang et al (2006), Taiwan reported 19.56% pandrug resistance in National Taiwan University Hospital at Taipei, Taiwan, which is much higher than our study. [19]

With regards to ESBL producer (57%) in present study close to Silpi Bask et al (2008) and Varun Goel et al (2013) (40% and 42.3%) respectively. [20,21]

AmpC resistance (56%) in present study is close to with Silpi Bask et al (2008) and Varun Goel et al (2013) (42% and 48.72%) respectively. [20,21]

In this Study, 11.6% strains were AmpC producer and this result is supported by a study by M. Shahid et al. who reported that 20% strains were AmpC producer. [22]

Our study reports the ESBL, AmpC and MBL producers in Pseudomonas aeruginosa. Early detection of these beta-lactamase-producing isolates in a routine laboratory could help physician to provide better treatment plan.

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