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Biofilm Detection amongst Metallo Beta Lactamase (MBL) Producing Clinical Isolates of *Pseudomonas aeruginosa*

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A B S T R A C T

In recent decades the global emergence the development of multidrug-resistant and pan drug resistance in *Pseudomonas aeruginosa* has been observed. This opportunistic pathogen is responsible for causing various infections especially in the intensive care units, resulting for almost 10% of nosocomial infections. The resistance to Metallo beta-lactamase in *P. aeruginosa* strains has been related with bacterial biofilm formation. So, the aim of this study was to determine the rates of biofilm production and metallo beta-lactamase production (MBL) among the strains of *Pseudomonas aeruginosa***.** A total of 200 *P. aeruginosa* isolates were isolated from different clinical specimens were included in this study. Modified Kirby-Bauer disc diffusion technique was used for antimicrobial susceptibility testing. MBL production was detected by E-Test, while microtiter plate assay was used for detection of biofilm production. Out of 200 isolates, 21 isolates were MBL producing whereas, 32 were able to produce biofilm as shown microtiter plate assay. The highest tendency of biofilm production was shown by *Pseudomonas aeruginosa* isolated from urine followed endotracheal tube. Highest rates of susceptibility of *P.aeruginosa* were seen toward colistin. In our study, biofilm production and metallo beta-lactamase production were observed among *Pseudomonas aeruginosa*. However, no statistically significant association could be established between biofilm production and metallo beta-lactamase production.

Introduction

With the emergence of metallo- β-lactamase producing bacterial isolates, the clinical utility of drugs like carbapenem is under threat (1). MBL producing bacteria shows high-level resistance to all β-lactams except aztreonam (2). Antimicrobial resistance in case of biofilm associated infections presents a serious threat in clinical practice (3, 4). Biofilm producing isolates produces an extracellular matrix of polysaccharides which act as a protective barrier for the bacteria, preventing diffusion of antibiotics, immune cells and host proteins (5). Thus, it is important to screen biofilm producing bacteria for the treatment of infection and needs to be incorporated as a routine

laboratory procedure. Antibiotics used for the treatment of such infections should be directed against the biofilms rather than the planktonic forms (6). *Pseudomonas aeruginosa* are the common causes of life threatening infections mainly in hospitalized patients (7). In addition, biofilm and metallo-β-lactamase production among these bacteria may present as serious problem to the treatment of the infections caused by them. In this study, we determined the rates of biofilm production and MBL production among the strains of *Pseudomonas aeruginosa.*

Materials and Methods

This study was conducted in Department of Microbiology, M.L.B Medical College, Jhansi for a period of one year. A total of 200 isolates of Pseudomonas aeruginosa were included from various clinical samples. These isolates were studied for MBL screening test, phenotypic detection by E-Test was done to detect MBL, antibiogram and biofilm detection.

MBL detection

The E-test MBL Strip Himedia (EM078) were used for detection of MBL, it contains a double sided seven-dilution range of IP(Imipenem) (4 to 256 µg/ml) and Imipenem (1 to 64µg/ml) in combination with a fixed concentration of EDTA is considered as the most sensitive method for MBL detection). The E-test was done according to manufacturer's instructions. MIC ratio of IP/ IPI (Imipenem+EDTA) of >8 or >3 log dilutions indicates MBL production.

Biofilm detection

A quantitative test described by Christensen *et al.,* (1985) was performed. In brief, the test strains isolated from fresh agar plates were inoculated into 10 mL of TSB with 1% glucose. Inoculated broth media were incubated at 37°C for 24 hours. The cultures were then diluted 1:100 with fresh medium. Individual wells of sterile 96 well flat bottom microtiter plates (Sigma-Aldrich, USA) were filled with 200 µL of the diluted cultures. The positive control strains were treated in the same way and added to separate wells of microtiter plates, while negative controls contained only TSB. The plates were covered with a lid and incubated at 37°C for 24 hours. After incubation, the contents of each well were removed by gentle tapping. The wells were washed with 200 µL of PBS (pH 7.3) four times to remove free floating bacteria, dried in an inverted position and stained with 0.1% crystal violet for 15 minutes. Excess stain was removed with distilled water and the plates were kept for drying.

The optical density at 570 nm (OD 570) was determined using microplate reader. According to the absorbance values, the adherence capability of each isolate was classified into the following four categories: none (−), weak (+), moderate (++) and strong (+++) adherent cells. The cut-off absorbance value (ODc) was considered as three standard deviations above the mean OD of the negative control.

Results and Discussion

In this study total 200 isolates of Pseudomonas aeruginosa were included from various clinical samples. The isolates were collected for a period of one year. These isolates were studied for MBL phenotypic detection by E-test and biofilm detection.

Detection of MBL

Among 200 isolates overall percentage of MBL production was 33.8%. Of the 62 isolates that were positive in screening test, 21 isolates showed MBL activity that was confirmed by E-Test.

Detection of biofilm production

Biofilm was detected from all the clinically isolated Pseudomonas aeruginosa. Out of 200 isolates 15.5% (32) were found to be biofilm producers. Among them 15.5% of biofilm producers, the adherence was as follows (Figure 1).

1. Strongly adherent – 19
2. Weakly adherent- 13
3. Non adherent – 168

Table 1 showing higher percentage of Metallo-β-Lactamases in strong Biofilm forming isolates. Out of 32 Biofilm isolates 18 were found to MBL producing. Biofilm was predominantly isolated from urine sample (6 strongly positive for biofilm and 4 weakly positive) followed by endotracheal tube (4 strong positive and 3 weakly positive) and other isolates. Table 2 shows their no statistical association between biofilm and Metallo beta lactamase in our study.

Table.1 No of isolates forming biofilm & Metallo-β-Lactamases

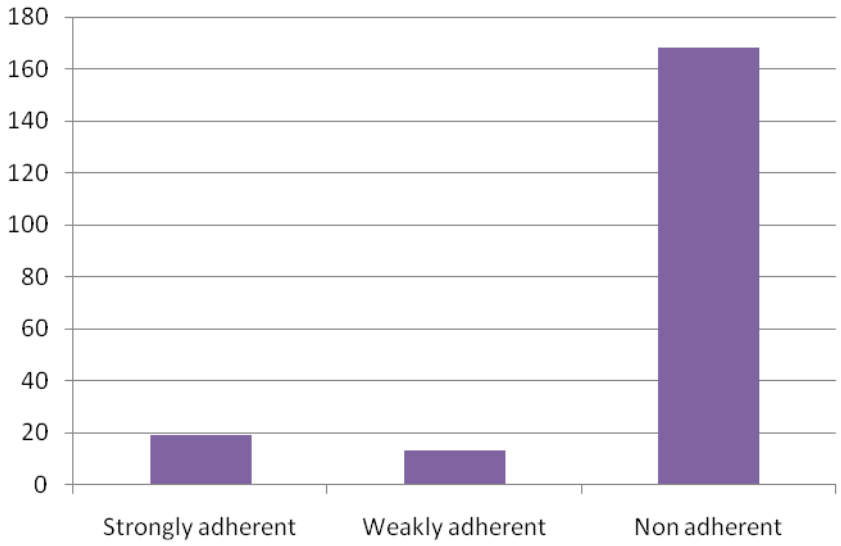
Biofilm formation	No of isolates	Metallo-β-Lactamases positive isolates
STRONG	19	15
WEAK	13	3
NEGATIVE	168	3
TOTAL ISOLATES	200	21

Table.2 Specimen wise distribution – Metallo β- Lactamases & Biofilm formation

S. no	No. of isolates	Total sample	Biofilm formation	Metallo-β-lactamases positive	p-valve*
1	**Pus**	75	4S 1W 70N	7 (9.33%)	>0.05
2	**Urine**	44	6S 4W 34N	8 (18.18%)	>0.05
3	**Sputum**	14	2S 0W 12N	1 (7.14%)	>0.05
4	**Endotracheal tube**	38	4S 3W 32N	2 (5.26%)	>0.05
5	**Blood**	9	1S 3W 5N	1 (11.1%)	>0.05
6	**Pleural fluid**	5	0S 0W 5N	-	
7	**BAL**	4	0S 1W 3N	-	
8	**Folley's catheter tip**	3	1S 0W 2N	1 (33.3%)	>0.05
9	**Vaginal swab**	2	0S 0W 2N	-	
10	**Nasal swab**	3	1S 1W 1N	-	
11	**Ascitic fluid**	1	0S 0W 1N	-	
12	**Bile**	1	0S 0W 1N	1 (100%)	>0.05
13	**Throat swab**	1	1S 0W 0N	-	
	TOTAL	**200**	**19 S 13W 168N**	**21 (10.5%)**	

Where, S: Biofilm production Strong, W: Biofilm production Weak, N: Biofilm production Negative
*p value: <= 0.05 is considered to be statistically significant.

Figure.1 No of isolates forming biofilm



Many bacterial species requires specific pH or temperature to grow and produce biofilm, *Pseudomonas* has been reported to produce EPS wherever conditions are suitable for bacterial colonization. In our study, Biofilm formation was found in 32(16%). Strong biofilm producer was shown by 19/200; weak biofilm producer in 13/200. Out of 32 biofilm producing isolates 18 isolates was found to be metallo beta lactamase producing by E-Test, in our study it shows high rate of biofilm detection. Similarly, Alicia Valéria Zaranza in their study documented biofilm production by the Congo Red Agar method in 52.0% and biofilm formation on polystyrene microplates, from 86.0% strains. Among them 22.1% were strongly adhered, 47.7% were moderate and 30.2% were weakly adhered. (8) Carlos J *et al.,* reported biofilm formation in *P. aeruginosa* in 83% of clinical strains & that biofilm formation was prevalent among isolates with a MDR phenotype (9). In our study we found higher antibiotic resistance in strong biofilm producers as compared to that of non-biofilm producing isolates.

In our sudy, 10/32 urinary *P.aeruginosa* isolates were biofilm producing whereas, study performed by Lucchetti *et al.,* showed similar finding that *P.aeruginosa* was the main isolated agent causing infections in the urinary tract, and according to epidemiologic data, 35.0% to 45.0% of all acquired nosocomial infections are urinary and 80.0% are related to catheter use (10).

Only 2 isolates from Sputum were strong biofilm producing and among them one of the strains was Metallo –β- lactamases positive. On the contrary, Drenkard in their study found that antibiotic-resistant *P. aeruginosa* have better capability to form biofilms among high frequency both in vitro and in the lungs of CF patients. They are also identified as a regulatory protein (PvrR) that controls the alteration between antibiotic-resistant and antibiotic-susceptible forming isolates (11).

Pseudomonas aeruginosa produces amature in vitro biofilm in almost 5–7 days. Development of these biofilm is initiated by freely moving bacteria that alternately attaches to a surface, At this stage, the bacteria are mostly susceptible to antibiotics The antibiotics MIC and MBC of biofilm-producing bacteria can be up to 100–1000-times higher as compared to these planktonic free living bacteria. Single treatment with

antibiotics such as lactams, can activate against *P. aeruginosa* and therefore not very efficient at eliminating biofilm infections increases horizontal gene transmission in biofilms. This condition explains how these biofilm-producing bacteria easily acquires multidrug resistant against lactam antibiotics, aminoglycosides and fluoroquinolones, which are detected by routine susceptibility testing in the clinical microbiology laboratory. (12)

In our study isolates those were MBL and Biofilm producing were mostly resistant to all the antibiotics except for colistin the similar findings were observed by Bagee *et al.*, they reported Colistin is only antimicrobial active against *P.aeruginosa* biofilms in vitro. Since the metabolically active surface layer of the biofilm is susceptible to ciprofloxacin in contrast to the inactive central part of the biofilm, he observed that combination therapy with ciprofloxacin and colistin was able to kill all cells in the biofilm *in vitro* (Potera *et al.*, 1999) Antibiotic resistance is increasing at an shocking rate, leading to increased morbidity, mortality and treatment costs.

References

1. Mahajan G, Sheemar S, Chopra S, Kaur J, Chowdhary D, Makhija SK. Carbapenem resistance and phenotypic detection of carbepenemases in clinical isolates of *Acinetobacter baumannii*. Indian J Med Sci. 2011; 65(1): 18–25.
2. Andrade SS, Picao RC, Campana EH, Nicoletti AG, Pignatari AC, Gales AC. Influence of disk preparation on detection of metallo-beta-lactamase-producing isolates by the combined disk assay. J Clin Microbiol. 2007; 45(6): 2058–60.
3. Hoiby N, Ciofu O, Johansen HK, Song ZJ, Moser C, Jensen PO, *et al.*, The clinical impact of bacterial biofilms. Int J Oral Sci. 2011;3(2):55–65.
4. Hall MR, McGillicuddy E, Kaplan LJ. Biofilm: basic principles, pathophysiology, and implications for clinicians. Surg Infect. 2014; 15(1): 1–7.
5. Kokare CR, Chakraborty S, Khopade AN, Mahadik KR. Biofilm: importance and applications. IJBT. 2009; 8: 159–68.
6. Kostakioti M, Hadjifrangiskou M, Hultgren SJ. Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the post antibiotic era. Cold Spring Harb Perspect Med. 2013; 3(4): a010306.
7. Navon-Venezia S, Ben-Ami R, Carmeli Y. Update on *Pseudomonas aeruginosa* and *Acinetobacter baumannii* infections in the healthcare setting. Curr Opin Infect Dis. 2005; 18(4): 306–13.
8. Alicia Valéria Zaranza, Francyelle Costa Morais, Monique Santos do Carmo, Adriana de Mendonça Marques, Cristina Andrade-Monteiro, Thiago Feitosa Ferro, Valério Monteiro-Neto, Patrícia de Maria Silva Figueiredo. Antimicrobial Susceptibility, Biofilm Production and Adhesion to HEp-2 Cells of Pseudomonas aeruginosa Strains Isolated from Clinical Samples. Journal of Biomaterials and Nanobiotechnology, 2013, 4, 98-106.
9. Carlos J Sanchez Jr, Katrin Mende, Miriam L Beckius, Kevin S Akers, Desiree R Romano, Joseph C Wenke and Clinton K Murray, BMC Infectious Diseases 2013, 13:47- 49
10. Lauretti L, M L Riccio, A. Mazzariol G. Cornaglia G. Amicosante, R. Fontana, G. M. Rossolini. Cloning and characterization of *bla*VIM, a new integron-borne metallo-β-lactamase gene from a *Pseudomonas aeruginosa* clinical isolate. Antimicrob. Agents chemother. 1999; 43:1584–90.
11. Drenkard E, Ausubel FM. Pseudomonas biofilm formation and antibiotic resistance are linked to phenotypic variation. Nature. 2002 Apr 18;

416(6882): 740-3.
12. Potera C. Forging a link between biofilms and disease. Science. 1999; 283: 1837–1838.
13. Bagge N, Schuster M, Hentzer M, *et al.*, Pseudomonas aeruginosa Biofilms Exposed to Imipenem Exhibit Changes in Global Gene Expression and ß-Lactamase and Alginate Production. *Antimicrob. Agents Chemother.* 2004; 48: 1175-1187.

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