JMSCR Vol||04||Issue||03||Page 9798-9801||March 2016

www.jmscr.igmpublication.org

Impact Factor 5.244
Index Copernicus Value: 5.88
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: http://dx.doi.org/10.18535/jmscr/v4i3.38

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

A Study on Antibiotic Sensitivity Pattern in Biofilm Positive and Negative Isolates of *Pseudomonas Aeruginosa* Isolated From Clinical Samples

Authors
Sowmya Nasimuddin[1], Jeevan Malaiyan[2], Mohanakrishnan Kandaswamy[3], Priyadharshini Parthasarathy[4], Sumathi Gnanadesikan[5], Savitha S[6], Kamal Raj M[7]
[1,2,3,4,5,6,7]Dept of Microbiology, Sri Muthukumaran Medical College Hospital & Research Institute, Chikkarayapuram, Chennai – 600069
Email: [1]*sowmyanasim@yahoo.co.in*, [2]*Jeevan1209@gmail.com*, [3]*Mohi2k2002@yahoo.co.in*, [4]*dharshinisarathi@gmail.com*, [5]*drgsumathi@gmail.com*, [6]*savithaknc@gmail.com*, [7]*Kamalraj2019@gmail.com*
Corresponding Author
Sowmya Nasimuddin
Dept of Microbiology, Sri Muthukumaran Medical College Hospital & Research Institute, Chikkarayapuram, Chennai – 600069

ABSTRACT
Pseudomonas aeruginosa, a potential hospital pathogen with the tendency to form biofilm and multidrug resistance. Our study is to observe the correlation between biofilm formation by Pseudomonas aeruginosa and an accelerated increase in frequency of multidrug resistance when compared to non-biofilm producers. A total of 100 isolates of Pseudomonas aeruginosa [Pus (59%), urine (21%), sputum (16%) and other samples (4%)] were taken for our study. Biofilm formations by tube method were found in 86 isolates (86%) with 85% (17/21) of the urinary isolates forming biofilm. Resistance to Carbapenam, Cephalosporins and other anti – Pseudomonal antibiotics were clearly observed to be more in biofilm producing isolates than non biofilm producers.
KEY WORDS: *Pseudomonas aeruginosa, Biofilm, Tube method.*

INTRODUCTION

Pseudomonas aeruginosa is gram negative, aerobic bacteria which can tolerate low oxygen conditions, survive with low levels of nutrients and grow at temperature ranging from 4 – 42^0 Celsius [1]. Biofilm are defined as community of microorganisms enclosed in an extracellular matrix and is responsible for the increased resistance to both antimicrobial agents and environmental stress. Colonisations of biofilm producing organisms upon medical devices have an enormous impact on healthcare, and are estimated to be associated with 65% of nosocomial infections [2]. *Pseudomonas aeruginosa* is one of the common and potential hospital pathogen with the tendency to form biofilm. Hence in this study, our prime objective is to establish a correlation between biofilm formation by *Pseudomonas aeruginosa* and an accelerated increase in frequency of multidrug resistance when compared to non-biofilm producers.

METHODOLOGY

SAMPLE COLLECTION & IDENTIFICATION [3]

Ethical committee approval and informed consent from the patient were taken prior to sample collection. A total of 100 isolates of *Pseudomonas aeruginosa* were obtained from clinical samples like pus, blood, urine, sputum and other respiratory samples. The samples were processed in blood agar, nutrient agar and Mac Conkey agar and were incubated at 37 ^{0}C for 24 - 48 hours. Identification of *Pseudomonas aeruginosa* were done by phenotypic methods such as production of catalase, oxidase, the presence of pigments (pyocyanin and pyoverdin), sodium citrate, growth at 42^0 C, nitrate reduction and arginine hydrolysis.

THE BIOFILM FORMATION

The qualitative assessment of biofilm formation was determined by tube method. TSB (Trypticase-soy broth) with 1% glucose (10mL) is inoculated with loop full of isolates from overnight culture plates and incubated for 24 hours at 37°C. The tubes were decanted and washed with Phosphate Buffer Solution (PBS - pH 7.3) and dried. Dried tubes were stained with crystal violet (0.1%). Excess stain was removed and tubes were washed with deionized water. Tubes were then dried in inverted position and observed for biofilm formation.

The adherence capabilities of the test strains were classified into two categories: non-adherent (0), or adherent (+). It was considered positive when a visible film lines the wall and bottom of the tube [4 - 7]. The data obtained were recorded. (Figure 1 & 2)

Figure 1- Positive for biofilm formation

Figure 2 – Negative for biofilm formation

ANTIBIOTIC SUCEPTIBILITY TESTING [8,9]

Antibiotic susceptibility test is performed using Kirby-Bauer disc-diffusion method according to Clinical Laboratory Standards Institute (CLSI). Five colonies of each strain are grown in peptone water at 37°C for 2-4 hours until reaching an optical density equivalent to 0.5 on the McFarland scale (NMF) A massive seeding is done from the bacterial suspension on Muller Hinton agar plates using a sterile swab. Discs with the antibiotics are immediately placed on the inoculated plates and incubated at 37°C for

24 hours . For susceptibility testing a total of 12 antibiotics are assessed - Piperacillin – Tazobactam (100/10 µg) , Piperacillin(100 µg), Tobramycin, Aztreonam(30 µg), Ceftazidime (30µg) , Polymyxin B(300 U) , Amikacin(30 µg) , Gentamicin(10 µg), Ciprofloxacin(5 µg), Imipenem(10 µg). Inhibition zones are determined and compared with the standard reference tables (CLSI).

RESULTS AND DISCUSSION

In our study, majority of *Pseudomonas aeruginosa* were isolated from pus (59%) followed by urine (21%), sputum (16%) and other samples (4%). Study by Lucchetti et al showed that *Pseudomonas aeruginosa* were isolated mainly from urinary tract (35 – 45%) [10]. In our study, biofilm formation were found in 86/100 isolates (86%). Carlos J et al reported biofilm formation in 83% of clinical strains of *Pseudomonas aeruginosa* [11]. It was of interest that, in our study 85% (17/21) of the isolates from

urine were able to form biofilm (Table 1). Prolonged catheterization and poor sanitary practices can be attributed to increased biofilm formation among urinary isolates.

In our study biofilm forming strains of *Pseudomonas aeruginosa* showed high resistance to Ceftazidime (91%) and Piperacillin (63%), high sensitivity to Imipenam (98%), Amikacin (94%), Polymyxin B (88%), Gentamicin (84%), Tobramycin (83%), moderate sensitivity to Ciprofloxacin (63%), Aztreonam (71%) and Piperacillin tazobactam (73%) (Table 2) when compared to non biofilm producers. Resistance to Carbapenam, Cephalosporins and other anti – Pseudomonal antibiotics were clearly observed to be more in biofilm producing isolates. Carlos J et al, reported maximum sensitivity to amikacin (75%); gentamycin(61%); tobramycin (77%) and resistance to ceftazidime (67%). Javiya VA etal and Neils etal also demonstrated maximum sensitivity to carbapenems and amikacin against pseudomonas species respectively [12, 13]

Table 1: Specimen wise distribution-Biofilm Formation

SAMPLE	NUMBER	BIOFILM FORMATION
1.Urine	21	17 Positive 4 Negative
2.Sputum	16	15 Positive 1 Negative
3.Pus	59	50 Positive 9 Negative
4.Miscellaneous	4	4 Positive 0 Negative
TOTAL	100	86 Positive 14 Negative

Table 2: Biofilm formation and antibiotic resistant pattern of isolates

ANTIBIOTICS TESTED	BIOFILM FORMATION			
	POSITIVE		NEGATIVE	
	SENSITIVE	RESISTANT	SENSITIVE	RESISTANT
Piperacillin tazobactam	63 (73%)	23 (27%)	12 (85%)	2 (15%)
Piperacillin	32 (37%)	54 (63%)	8 (66%)	6 (34%)
Imepenam	85 (98%)	1 (2%)	14 (100%)	-
Tobramycin	72 (83%)	14 (17%)	11 (78%)	3 (22%)
Amikacin	81 (94%)	5 (6%)	13 (93%)	1 (7%)
Gentamicin	72 (84%)	14 (16%)	12 (85%)	2 (15%)
Aztreonam	61 (71%)	25 (29%)	13 (93%)	1 (7%)
Polymyxin B	76 (88%)	10 (12%)	14 (100%)	-
Ceftazidime	7 (9%)	79 (91%)	5 (35%)	9 (65%)
Ciprofloxacin	55 (63%)	31 (37%)	10 (71%)	4 (29%)

CONCLUSION

Inappropriate use of antibiotics leads to increased resistance, which in turn increases morbidity, mortality and treatment costs. *Pseudomonas aeruginosa* is a common nosocomial pathogen, with a tendency to develop multidrug resistance (MDR) and causes life threatening infections in critically ill patients. Higher antibiotic resistances were seen in strong biofilm producers. Since the formation of mature biofilms takes 5-7 days, the bacteria in the initial stage of biofilm formation will still be susceptible to antibiotics. The routine practice of detecting and alerting the clinicians about biofilm forming *Pseudomonas aeruginosa* strains can reduce the morbidity in hospitalized patients by beginning early appropriate antibiotic prophylaxis or therapy.

REFERENCES

1. Marilyn PG, José VB, Santiago NC. Overview of Multidrug-Resistant *Pseudomonas aeruginosa* and Novel Therapeutic Approaches *Journal of Biomaterials and Nano biotechnology* 2012; 3: 519-27

2. Dardi CK, Dr.Wankhede SV A study of Biofilm formation & Metallo-β-Lactamases in *Pseudomonas aeruginosa* in a tertiary care rural hospital. International Journal of Scientific and Research Publications 2013;3 (10):2250-3153
3. Colle JG,Mles RB,Watt B 1996.Tests for identification of bacteria.In: Collee JG, Fraser AG, Marmon BP, Simmons A, Editors. Mackie and McCartney Practical Medical Microbiology,14 th ed. Churchill Livingstone, New York, Pp 131 – 49
4. Ryder C, Byrd M, Worzniak DJ Role of polysaccharides in *Pseudomonas aeruginosa* biofilm development Curr Opin Microbiol 2007;10:644-48.
5. Chaudhary M, Payasi A (2013) Rising Antimicrobial Resistance of Pseudomonas aeruginosa Isolated from Clinical Specimens in India. J Proteomics Bioinform 6:005-009. doi:10.4172/jpb.1000184
6. Sachinkumar W, Vivek I , Ghadge D.P, Bhore A.P Hospital based infections of Gram negative organisms: Study Report. Indian Journal of Basic & Applied Medical Research; June 2013: Issue-7, Vol.-2, P. 797-800.
7. Alicia VZ, Francyelle CM, Monique SC, Adriana de MM, Cristina AM, Thiago FF, Valério MN, Patrícia de MSF. 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.
8. CLSI (Clinical and Laboratory Standards Institute). (2007). Performance standards for antimicrobial disk susceptibility tests, 9th edn. Approved Standard M2 , A9.Wayne,PA: document M100 - S17.
9. Koneman EW, Allen SD, Janda WM, Schreckenbergr PC, Winn WC. Antimicrobial susceptibility testing, chapter 15. In: color Atlas and Textbook of Diagnostic Microbiology, 5th edition (Lippicott, Philadelphia) 1997 ;785.
10. G. Lucchetti, A. J. Silva, S. M. Y. Ueda, M. C. D. Perez and L. M. J. Mimica, "Infecções Do Trato Urinário: Análise da Freqüência e Do Perfil de Sensibilidade Dos Agentes Causadores de Infecções Do Trato Urinário Em Pacientes Com Cateterização Vesical Crônica," Journal Brasileiro de Patologia e Medicina Laboratorial , Vol. 41, No. 6, 2005, pp. 383-389.
11. Carlos J Sanchez Jr, Katrin Mende, Miriam L Beckius, Kevin S Akers, Desiree R Romano,Joseph C Wenke and Clinton K Murray, Biofilm formation by clinical isolates and the implications in chronic infections BMC Infectious Diseases 2013, 13:47
12. Javiya VA, Ghatak SB, Patel KR, Patel JA. Antibiotic susceptibility patterns of Pseudomonas aeruginosa at a tertiary care hospital in Gujarat, India. Indian J Pharmacol 2008;40:230-4
13. Niels Hoiby, Thomas Bjarnsholt, Michael Givskov, Soren Molin, Oana Ciofu. Antibiotic resistance of bacterial biofilms. International Journal of Antimicrobial Agents 35 (2010) 322–32.