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Detection of biofilm among clinical isolates of *Pseudomonas aeruginosa* by tissue culture plate (TCP) method

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ABSTRACT

Pseudomonas aeruginosa are the notorious pathogen frequently isolated from different hospital settings. It's increasingly associated with morbidity and mortality in debilitated individuals. They tend to exhibit wide array of virulent factors to establish the infection and to evade from other host defense mechanisms. One of the main factor is formation of biofilm. A total of 20 clinical isolates of *P. aeruginosa* were collected from different clinical specimens and subjected to antibiotic sensitivity testing and biofilm formation by TCP method. All the isolates were totally resistant to all antibiotics used. 25% of isolates were strong and 35% moderate biofilm producers of our isolates. We conclude that there has been a positive association between drug resistance and biofilm formation of *P. aeruginosa*.

KEY WORDS: *Pseudomonas aeruginosa*, biofilm, tissue culture plate (TCP) method.

1. INTRODUCTION

Pseudomonas aeruginosa has been established nosocomical bacterial pathogen causes wide spectrum of clinical conditions. This pathogen associated with increased percentage of morbidity and motility particularly in clinical settings. This pathogen has become notorious due to increased percentage of antibiotic resistance and tendency to form biofilms. *P. aeruginosa* exhibits an array of virulent determinants which contribute to its complicity, pathogenicity in different infection what they produces. These virulent factors include pili, flagella, lipo polysaccharides, elastase, alakaline protease, pyocyanine, pyoverdin, haemolysin etc. Bioflim significantly increases the ability of pathogen to evade from both host defenses and antibiotics. They have been implicated in its pathogenesis.

They implicated with variety of stubborn infections include chronic middle ear infections, bone infections, heart valve infections related to implanted medical device, prosthesis and lung infection in people with cystic fibrosis.

2. MATERIALS AND METHODS

Bacterial isolates: A total of 20 non repetitive clinical isolates of *Pseudomonas aeruginosa* were collected from Saveetha medical college, Thandalam. They were processed for a battery of standard bio chemical tests and confirmed. Isolates were preserved in semi-solid trypticase soy broth stock and stored at 4^0C until for that use.

Antibiotic susceptibility testing: Antibiotic susceptibility testing was determined for this isolates to routinely used antibiotics such as to piperacillin-tazobactam, Cefotaxime, ceftazidime, tetracycline, cotrimoxazole, aztreonam, gentamicin and imipenem by Kirby Bauer disc diffusion method as per CLSI guidelines.

Detection of biofilm by TCP method: Overnight grown cultures of *P. aeruginosa* from agar plates were inoculated in 0.5% of glucose and incubated at 37˚C overnight. Individual wells of sterile polystyrene 96 well flat bottom tissue culture plates were filled with 200µl of culture suspension from afore mentioned broth. Uninoculated broth served as negative control. The plates were incubated at 37˚C for overnight. After incubation, content of each well was gently discarded by tapping the plates downwards. The wells were washed three times with 200 µl of PBS (pH 7.2) in order to remove planktonic bacteria. Biofilms are formed by adherent sessile isolates in plates were fixed with 2% sodium acetate and stained with 0.1% W/V crystal violet. Excess stain was removed by washing the wells with distilled water and plates were kept for drying at an inverted position. Optical density of stained adherent bacteria was determined with an ELISA reader (Bio Rad) at wavelength of 570nm. These OD values were taken as index of bacteria adhering the surface and formed biofilm. Experiments were carried in triplicate and their mean was taken for the analysis. The below mentioned interpretation charge was applied to categorize the ability of *P. aeruginosa* to form biofilm.

Mean OD values	Adherence	Biofilm Formation
<0.120	Non	Non / weak
0.120-0.240	Moderately	Moderate
>0.240	Strong	High

3. RESULTS

Sample wise distribution of clinical isolates of *P. aeruginosa*: Of the 20 isolates of clinical isolates of *P.aeruginosa*, 9/20 (45%) isolates were from sputum, 5/20 (25%) from blood, 3/20 (15%) from urine, 3/20 (15%) from pus.

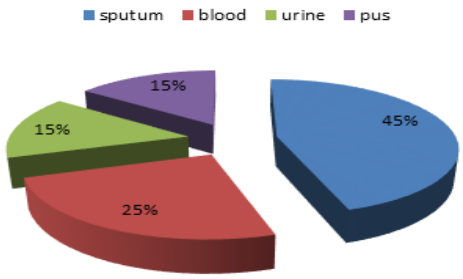

Figure.1. Sample wise distribution of clinical isolates of *P.aeruginosa*

Result of antibiotic susceptibility testing: In our isolates, we have observed an increased percentage of isolates were shown to be resistant to most of the routinely used antibiotics. Only 2/20 (10%) isolates showed sensitivity to imipenem. Other than that, for all other antibiotics such as piperacillin-tazobactam, cefotaxime, ceftazidime, tetracycline, cotrimoxazole, aztrionum, gentamicin isolates showed complete reistance 20/20 (100%). The detailed resistant pattern of *P. aeruginosa* isolates were showed in table.1.

Table.1. Results of antibiotic susceptibility pattern of *P. aeruginosa*

Antibiotics	Sensitivity (20) (%)	Intermediate (20) (%)	Resistant (20) (%)
piperacillin-tazobactam	0 (0)	0 (0)	20 (100)
cefotaxime	0 (0)	0 (0)	20 (100)
ceftazidime	0 (0)	0 (0)	20 (100)
tetracycline	0 (0)	0 (0)	20 (100)
cotrimoxazole	0 (0)	0 (0)	20 (100)
aztreonam	0 (0)	0 (0)	20 (100)
gentamicin	0 (0)	0 (0)	20 (100)
Imipenem	2 (10)	1 (5)	17 (85)

Results of biofilm production by *P. aeruginosa*: All isolates were subjected for biofilm assay by tissue culture plate method. Of 20 *P. aeruginosa* isolates 7/20 (35%) of isolates were found to be moderate biofilm producers. 5/20 (25%) were found to be strong biofilm formers.

Table.2. Results of biofilm formation of *P. aeruginosa*

Biofilm category	*P. aeruginosa* isolates (20%)
Non / Weak	**8 (40)**
Moderate	**7 (35)**
Strong	**5 (25)**

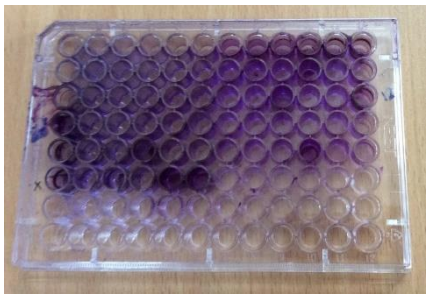

Figure.2. Representative picture for biofilm production by *P. aeruginosa* isolates using TCP method

DISCUSSION

The main problem associated with infections produced by biofilm formers is multitude of resistance to different antibiotics. Study conducted by Rao (2008), employed both tube and tissue culture plate (TCP) methods, showed 62% as strong producers of biofilm, 25.4% weakly adherent by tube method were additionally picked up by TCP method confirming its sensitiveness. However, as adherence alone may not complete the cycle of process of biofilm production and there might be several other mechanisms which could involve in adherence.

Study performed by Rodreguez-Ban (2008), documented 63% of biofilm positivity in their isolates, which was incomparable with our results, as our study showed 35% and 25% of moderate and strong biofilm producers respectively. Biofilm forming isolates were less frequently resistant to carbapenem and ciprofloxacin in his study. Whereas, our study showed positive association between production of biofilm and multiple antibiotic resistance, especially in some antibiotics.

Compared to non-biofilm producers our study also detected significantly higher resistance to piperacillin-tazobactam, Cefotaxime, ceftazidime, tetracycline, cotrimoxazole, aztreonam, gentamicin and imipenem among biofilm formers.

4. CONCLUSION

Overall, our *P. aeruginosa* isolates were shown to be resistant to most of the routinely used antibiotics. This demonstrated a higher propensity among the clinical isolates of *P. aeruginosa* to form biofilm and there were a significant correlation between biofilm and multiple antibiotic resistance.

REFERENCES

Christensen GD, Simpson WA, Bisno AL, Beachey EH, Adherence of slime producing strains of *Staphylococcus epidermidis* to smooth surface, Infect Immun., 37, 1982, 318-26.

Christensen GD, Simpson WA, Younger JJ, Baddour Barrett, Adherence of coagulase-negative *Staphylococci* to medical devices, J Clin Microbiol., 22, 1985, 996-1006.

Costerton JW, Stewart TS, Greenverg EP, Bacterial biofilms: A common cause of persistent infection, Science, 284, 1999, 1318-22.

Hoiby N, Kroghjohansen H, Moser C, Song Z, Ciofu O, *P. aeruginosa* and the *invitro* and *invivo* biofilm mode of growth, Microbes Infect., 3, 2001, 23-35.

Lewis K, Riddle of biofilm resistance, Antimicrob Agents Chemother., 45, 2001, 999-1007.

Mathur T, Singhal S, Khan S, Upadhyay DJ, Fatma T, Rattan A, Detection of biofilm formation among the clinical isolates of *Staphylococcus*: an evaluation of three different screening methods, J Med Microbiol., 24, 2006, 25-29.

Mikkelsen H, Duck Z, Lilley KS, Interrelationship between colonies biofilm, and planktonic cells of *P. aeruginosa*, Agenetics Microbiol Mol Biol Rev., 64, 2006, 847-67.

Rao RS, Karthika RU, Singh SP, Shashikala P, Kanungo R, Jayachandran S, Prashanth K, Correlation between biofilm production and multiple drug resistance imipenem resistant clinical isolates of Acinetobacter baumannii, Indian J Med Microbiol., 26, 2008, 333-337.

Rodrguez-Ban OJ, Marthi S, Soto S, Fernandes Cuenca F, Cisneros JM, Pachon J, Biofilm information in Acinetobacter baumannii: Assoicated features and clinical implications, Clinical Microbiol Infect., 14, 2008, 276-8.

Winn W, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P, Koneman's color atlas and textbook of diagnostic microbiology, 6th ed. Philadelphia, Lippincott Williams and Wikins, 2006, 101-102.