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Study of Biofilm Production in Pseudomonas, Klebsiella, Coagulase negative Staphylococci and *Escherichia coli*

Devesh S	Final Year Post-graduate Resident
Vinod R	Associate Professor
J. M. Easow	Head of Department Microbiology

KEYWORDS :

INTRODUCTION:

Infection poses a serious health problem with respect to drug resistance and high recurrence rate. Pseudomonas is an opportunistic pathogen posing a major threat to long term hospitalized patients. In case of urinary tract infection *Escherichia coli* is the predominant organism[1]. These organisms form intracellular community, live in close proximity and create various form of shelter in order to protect themselves from external threats like action of antimicrobials. The ability to attach to solid surface and the subsequent formation of an organized bacterial biofilm community are also important steps in the establishment of chronic bacterial infections and persistence in the host tissue [2]. This study was done to find out the prevalence of biofilm production among the commonly implicated biofilm producing organisms such as *Pseudomonas aeruginosa, E. coli,* Klebsiella, Coagulase negative Staphylococci (CoNS).

MATERIALS AND METHODS:

A total of 126 clinical isolates comprising of Pseudomonas*, E. coli,* Klebsiella, and CoNS isolated from blood culture, indwelling devices, urine, ear swab and exudates submitted to the microbiology laboratory in Sri Venkateshwaraa Medical College and Hospital for routine culture and sensitivity from October 2013 to April 2014 were included in the study. Isolates were identified to the species and genus level by standard protocols. Tissue culture plate method (microtitre plate) described by Christensen et al for detection of biofilm formation was used and its optical density measured by an ELISA reader at 570nm and the same compared by the Tube method (TM)[3]. Results were interpreted after 24 hrs and 48 hrs of incubation.

DETECTION OF BIOFILM PRODUCTION
TISSUE CULTURE PLATE METHOD (TCP)

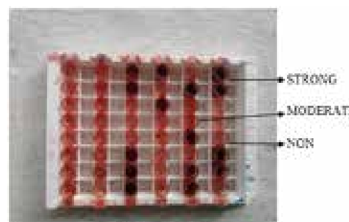
The TCP assay as described by Christensen et al is the most widely used and considered as standard test for detection of biofilm formation[3]. In the present study, we screened all isolates for their ability to form biofilm by TCP method as described by Christensen et al with a modification in duration of incubation which was extended by another 24 hours (48 hours). Individual sterile, polystyrene wells of the microtitre plates with flat bottom were used. Each well was filled with 0.2 ml of BHI broth and colonies of test organism were inoculated into each labelled well. Only broth served as control to check sterility and non specific binding of media.

The tissue culture plates were incubated for 24 hours and 48 hours at 37ºc. After incubation contents of each well was removed by gently aspirating with micropipette from the plate were fixed with 2 % sodium acetate and stained with safranine. Excess stain was rinsed off by gentle washing with deionised water and plates were kept for drying. Adherent cells usually formed biofilm on all side of the wells and were uniformly stained with safranine. Optical density (OD) of stained adherent bacteria was determined with a micro ELISA auto reader at wavelength 570 nm[3, 4]. These OD values were considered as an index of bacteria adhering to surface and forming biofilm.

TUBE METHOD (TM)

Tube method was done as previously described by Christensen et al. BHI (10 ml) was inoculated with loopful of microorganism and kept for incubation 24 hours and 48 hours at 37ºC. The tubes were decanted and was washed with PBS (pH 7.2) and dried. Dried tubes were stained with safranine. Excess stain was removed and tubes were washed with deionised water. Tubes were than dried in inverted position and observed for biofilm formation[3].

Biofilm formation was considered positive when a visible film lined the wall and bottom of the tube. Ring formation at the liquid interface was not indicative of biofilm formation. Tubes were examined and the amount of biofilm formation was scored as 0- absent, 1-weak, 2-moderate, and 3- strong[4].



STRONG
MODERATE
NON

RESULT:

A total of 126 isolates were tested for biofilm formation. Out of which 43(34.2%) were Pseudomonas, 25(19.8%) *E. coli*, 25 (19.8%) Klebsiella and *33 (26.2%)* CoNS. Biofilm formation was observed in both TCP and TM. Biofilm production was more pronounced in TCP and the same was observed better after 48 hours of incubation. We observed that 30(22.2%) out of 126 had produced biofilm. 15(34.9%) Pseudomonas, 4(16%) *E. coli,* 3(9.1%) CoNS, 8(32%) Klebsiella produced biofilm in both the methods and they were better appreciated with TCP method after 48 hrs of incubation.

LIST OF ISOLATES FROM VARIOUS DEPARTMENTS

Dept.	TOTAL NO OF ISOLATES	PSEUDOMONAS	KLEBSIELLA	E.COLI	CoNS
SURGERY	40 (31.7%)	16 (40%)	5 (12.5%)	6 (15%)	13 (32.5%)
ORTHO	13 (10.3%)	8 (61.5%)	3 (23.1%)	0 (0%)	2 (15.4%)
MEDICINE	6 (4.7%)	0 (0%)	1 (16.7%)	4 (66.6%)	1 (16.7%)
OBS & GYNE	18(14.3%)	4 (22.2%)	4 (22.3%)	7 (38.9%)	3 (16.7%)
PAED	4(3.2%)	0 (0%)	1 (25%)	2 (50%)	1 (25%)

SPC WARD	4(3.2%)	2 (50%)	0	1 (25%)	1(25%)
ICU, IMCU	7(5.6%)	2 (28.6%)	2 (28.4%)	0	3 (42.9%)
SICU	6(4.7%)	2 (33.3%)	3 (50%)	1 (16.7%)	0
UROLOGY	7(5.6%)	0	3 (42.9%)	3 (42.9%)	1 (14.3%)
ENT	6(4.7%)	4 (66.7%)	0	0	2 (33.3%)
EMERGENCY WARD, CASUALTY	15(11.9%)	5 (33.3%)	3 (20%)	1 (6.7%)	6 (40%)
TOTAL	126	43(34.2%)	25(19.8%)	25(19.8%)	33(26.2)

LIST OF ISOLATES FROM VARIOUS SPECIMENS

SPECIMENS	TOTAL n (%)	PSEUDOMONAS n (%)	KLEBSIELLA n (%)	*E.COLI* n (%)	CoNS n (%)
PUS	21(16.7%)	10(47.6%)	3(14.3%)	2(9.5%)	6(28.5%)
URINE	54(42.8%)	6(11.1%)	18(33.3%)	22(40.7%)	8(14.8%)
WOUND SWAB	33(26.2%)	16(48.8%)	1(3%)	1(3%)	15(45.5%)
CENTRAL LINE	3(2.4%)	1(33.3%)			2(66.7%)
DRAIN TIP	2(1.6%)	2(100%)			
EAR SWAB	7(5.5%)	5(71.4%)			2(28.6%)
TREACHEAL TUBE	1(0.8%)	1(100%)			
VAGINAL SWAB	1(0.8%)	1(100%)			
PLACENTA SWAB	1(0.8%)	1(100%)			
FOLEY'S CATH	1(0.8%)		1(100%)		
BLOOD	1(0.8%)		1(100%)		
SPUTUM	1(0.8%)		1(100%)		
TOTAL	126	43(34.2%)	25(19.8%)	25(19.8%)	33(26.2)

LIST OF ISOLATES PRODUCED BIOFILM BY TCP AFTER 48 HOURS OF INCUBATION

ISOLATES	TOTAL n (%)	POSITIVE n (%)	NEGATIVE n (%)
PSEUDOMONAS AERUGINOSA	43(34.2%)	15 (34.9%)	28(65.1%)
ESCHERICHIA COLI	25 (19.8%)	4 (16%)	21(84%)
KLEBSIELLA	25 (19.8%)	8 (32%)	17 (68%)
COAGULASE NEGATIVE STAPHYLOCOCCI	33(26.2%)	3 (9.1%)	30 (90.9%)

BIOFILM PRODUCTION BY BOTH TCP AND TM METHODS

ISOLATES	POSITIVE BY TISSUE CULTURE PLATE (TCP)	POSITIVE BY TUBE METHOD (TM)
PSEUDOMONAS AERUGINOSA	15 (34.9%)	4 (9.3%)
ESCHERICHIA COLI	4 (16%)	1 (4%)
KLEBSIELLA	8 (32%)	2 (8%)
COAGULASE NEGATIVE STAPHYLOCOCCI	3 (9.1%)	0 (0%)
TOTAL	30 (23.8%)	7 (5.6%)

DISCUSION:

Biofilms are the preferred method of growth by most organisms in the environment and in patients, as they originate due to selective pressures when their survival is at risk in the environment they grow. Formation of biofilms affects transition from acute infection to chronic[5]. Common molecules for all microbes, which are responsible for sending out an alarm signal, are referred as (p)ppGpp (peptide 1018)[5]. Biofilms increase the opportunity for gene transfer between/among bacteria. This is important since bacteria resistant to antimicrobials or chemical biocides can transfer the genes for resistance to neighboring susceptible bacteria. Gene transfer can convert a previous avirulent commensal organism into a highly virulent pathogen[5].

Slime production has been reported in strains of *Klebsiella, Pseudomonas, E. coli,* and CoNS which are associated with the infection of biomedical devices [2, 3, 6, 7, 8, 9, 10]. In our study, 126 clinical isolates were tested for biofilm formation. We observed that 30(22.22%) isolates in the study had produced biofilms. We had used two *in vitro* screening methods for the detection of biofilm production. Tissue culture plate assay with BHI medium seemed to be the better method for discrimination between biofilm producer and non producer. An extended incubation for 24 hours could discriminate between mild and strong biofilm producers.

The tube method correlates well with the tissue culture plate method for strong biofilm producing strains, but it was difficult to discriminate between weak producer and non producer, Biofilms are not recovered or recognized in the laboratory routinely neither is there any standard protocols for identification of biofilms. Currently we are unaware of the role played by these biofilms in our routine susceptibility testing which in turn affects the success of antimicrobial. The TCP method can be used as a reliable and reproducible method for the detection of biofilm production. Extending the incubation by 48 hours (total incubation time) could lead to a better discrimination between non biofilm producing and biofilm producing organisms. There is a need for better understanding of biofilm formation, methods for their detection *in vivo* and *in vitro* and methods to identify the genes responsible.

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