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Comparative Study Of Phenotypic Methods For The Detection Of Biiofilm Formation Among Urinary Isolates

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Abstract

Urinary tract infection (UTI) is one of the most common human infections in both catheterized and non catheterized patients. Uropathogenic organisms may produce intracellular bacterial communities called biofilm within the bladder, largely consisting of polysaccharides which prevent the access of antibiotics, antibodies, and white blood cells. The biofilms have major medical significance as they decrease the susceptibility to the antimicrobial agents. Furthermore, the proximity of cells within a biofilm can facilitate a plasmid exchange and hence enhance the spread of antimicrobial resistance. To establishment, standardization and validation of various in vitro phenotypic detection and methods for screening and confirmation of biofilm formation by urinary isolates. We evaluated 500 urinary isolates by four methods (that can be used in routine clinical laboratories) for their ability to form biofilms. Out of the 500 isolates, the TCP method detected biofilm in 137 isolates (45.6%), TM detected biofilm in 118 isolates (39.3%), CRA and MCRA detected biofilm in 33 isolates (11%). The TCP was found to be most sensitive followed by the TM, CRA and the MCRA method. TCP method is the ideal method for detection of bacterial biofilm formation by uropathogens. MCRA method is superior to CRA and not to TCP or TM for detection of the staphylococcal biofilm formation.

Key words: TM, Uropathogens, Staphylococcal, UTI, MCRA,TCP, CRA.

INTRODUCTION

Biofilm is a structured community of bacterial cells enclosed in self-produced polymeric matrix adherent to an inert or living surface [1-3]. As implant devices are increasingly used in medical practice, staphylococcal infections are now considered one of the major nosocomial infections [4]. Biofilm associated infections are characteristically refractory to different stresses including host immune defense and antibiotics, leading to persistent infections [5,6]. There is also a practice of categorizing UTI into first infection and recurrent infection. Recurrent infections are again subdivided into unresolved bacteriuria, bacterial persistence, and reinfection. Pain, fever, and discomfort are the common manifestations due to the urinary tract infections and it can be easily treated. Treatment becomes tough when the infection spread to the kidneys.

Biofilm is an aggregate of micro-organism in which the cells are irreversibly attached to substratum or to each other. They are embedded in a matrix of extra cellular polymeric substances (EPS) in which they have produced and exhibit an altered phonotype with respect to growth rate and gene transcription[7]Availability of key nutrients, chemotaxis towards surface, motility of bacteria, surface adhesins and presence of surface bacteria are some factors which influences biofilm formation [8,9]. The bacteria enclosed within the biofilm are extremely resistant to treatment. This may be because the drug concentration obtained may be insufficient in certain areas of the film[10]. The bacteria located at the base of the biofilm are metabolically inactive and are thus resistant to certain antibiotics and possess active antibiotic degradation mechanism that contribute to avoid the accumulation of an effective drug concentration.

MATERIALS AND METHODS

Sample Collection

A total of 2000 clinical isolates from urine samples will be subjected to different biofilm detection methods. The study will be conducted at the Department of Microbiology, L.N. MEDICAL COLLEGE, Bhopal from June 2019 to March 2020.

Tissue culture plate method:

Isolates from fresh agar plates were inoculated in brain heart infusion (BHI) broth with 2% sucrose and incubated for 18–24 h at 37°C in a stationary condition. The broth with visible turbidity was diluted to 1 in 100 with fresh medium. Individual wells of flat bottom polystyrene plates were filled with 0.2 ml of the diluted cultures, and only broth served as a control to check sterility and nonspecific binding of the medium. These plates were incubated for 24 h at 37°C. After incubation, the content of the well was gently removed and then were washed 4 times with 0.2 ml of phosphate buffer saline (PBS pH 7.2) to remove free- floating "planktonic" bacteria. Biofilms formed by adherent "sessile" organisms in plate were fixed with sodium acetate (2%) for half an hour and stained with crystal violet (0.1% w/v) for half an hour. Excess stain was rinsed off by thorough washing with deionized water and plates were kept for drying. Adherent bacterial cells usually formed a biofilm on all side wells and were uniformly stained with crystal violet. Optical densities (OD) of stained adherent bacteria were determined with a micro Enzyme- Linked Immuno sorbent Assay auto reader at wavelength of 570 nm (OD 570 nm) and were graded These OD values were considered as an index of bacteria adhering to the surface and forming biofilms. The experiment was performed in triplicate.[11]

Tube method:

BHI broth with 2% sucrose (10 ml) was inoculated with loop full of microorganism from overnight culture plates and incubated for 24 h at 37°C[12]. The tubes were then decanted and washed with PBS (pH 7.3) and dried. Dried tubes were then stained with crystal violet (0.1%) for half an hour. Excess stain was removed, tubes were then dried and observed for biofilm formation. Biofilm formation was considered positive when a visible film lined the wall and bottom of the tube. Tubes were examined, and the amount of biofilm formation was scored as absent, moderate or strong. The experiment was performed in triplicate.

Congo Red Agar method

This requires the use of a specially prepared solid medium BHI broth supplemented with 5% sucrose and Congo red. The CRA plate was inoculated with the microorganism from an overnight culture plate and incubated at 37°C for 24–48 h. Positive result was indicated by black colonies with a dry crystalline consistency. The experiment was performed in triplicate.[13]

Modified Congo Red Agar method:

In the MCRA the CRA is modified in the form of changing the concentration of Congo red dye and sucrose, omission of glucose, replacement of BHI Agar by an alternative agar, that is, Blood Base Agar. The MCRA plate was inoculated with organisms from a fresh plate with overnight growth, and then it was incubated for 48 h at 37°C and subsequently 2–4 days at room temperature. Positive result was indicated by black colonies with a dry crystalline consistency. The experiment was performed in triplicate.[14]

RESULTS AND DISCUSSION

Biofilm was detected by TCP method, CRA method and Tube method. shows the biofilm detection by different phenotypic methods and the sensitivity, specificity, PPV and NPV of congo red agar

method and tube method as compared to tissue culture plate method. The difference between the biofilm detection rates by CRA method and Tube method were statistically not significant

Spectrum of organisms isolated:
From the study total 2000 samples were collected such Staphylococcus epidermidis ATCC 35984 isolates by 300 (10%), Staphylococcus aureus ATCC 35556 isolates by 300 (10%) Pseudomonas aeruginosa ATCC 27853 isolates by 500 (30%) Escherichia coli ATCC 35218 isolates by 500 (40%) and Staphylococcus epidermidis ATCC 12228 isolates by 400 (10%).

Table 1: Spectrum of organisms isolated

Organism	Isolates	Percent
Staphylococcus epidermidis ATCC 35984	300	10%
Staphylococcus aureus ATCC 35556,	300	10%
Pseudomonas aeruginosa ATCC 27853,	500	30%
Escherichia coli ATCC 35218	500	40%
Staphylococcus epidermidis ATCC 12228 (non-slime producer)	400	10%

Organism wise distribution of biofilm production
From the total isolates biofilm producers were distributed as 50,50,50,300,50 to Staphylococcus epidermidis ATCC 35984, Staphylococcus aureus ATCC 35556, Pseudomonas aeruginosa ATCC 27853, Escherichia coli ATCC 35218 and Staphylococcus epidermidis ATCC 12228 (non-slime producer) respectively.

Table 2: Organism wise distribution of biofilm production

Organism	Total Isolates	Biofilm producers	Percent
Staphylococcus epidermidis ATCC 35984	300	50	10%
Staphylococcus aureus ATCC 35556,	300	50	10%
Pseudomonas aeruginosa ATCC 27853,	500	50	30%
Escherichia coli ATCC 35218	500	300	40%
Staphylococcus epidermidis ATCC 12228 (non-slime producer)	400	50	10%

Detection of biofilm production by different phenotypic methods
For detection of biofilm production here Tube method (150) and Tissue culture plate method (150) , Congo red agar (100), Modified Congo Red Agar method(100)

Table 3: Detection of biofilm production by different phenotypic methods

	Bio film producers	Percent
Congo red agar	100	10%
Tube method	150	40%
Tissue culture plate method	150	40%
Modified Congo Red Agar method	100	10%

Out of the 500 isolates TCP method detected biofilm production in (54.3%) cases. The rate of biofilm detection by TM (60.3%) was not significantly different from that by TCP (P = 0.14). However, in CRA and MCRA the rate of detection was 11% each which is significantly lower than that in TCP (P = 0.0001). Except for staphylococcus aureus we could not find any difference in the rate of biofilm detection between CRA and MCRA. However, MCRA detected biofilm production in 38.4% of staphylococcus isolates as compared to 23% by CRA. TCP method detected more strong (11%) and moderate (34.7%) biofilm producers when compared to other methods. TM was 81% sensitive, 95.1% specific, with positive and negative predictive value, and accuracy of 93.3%, 85.6% and 88.7% respectively as compared to CRA and MCRA methods

Table 4: Grading of biofilm formation in various isolates by the four different methods

Number of isolates	Bio film formation	TCP n(%)	TM n(%)	CRA n(%)	Modified CRA n(%)
n=500	High	40(11.0)	42(10.7)	18(1.3)	16(2.0)
	Moderate	150(34.7)	138(29.0)	22(9.7)	24(9.0)
	Weak/none	310(54.3)	320(60.3)	460(89.0)	460(89.0)

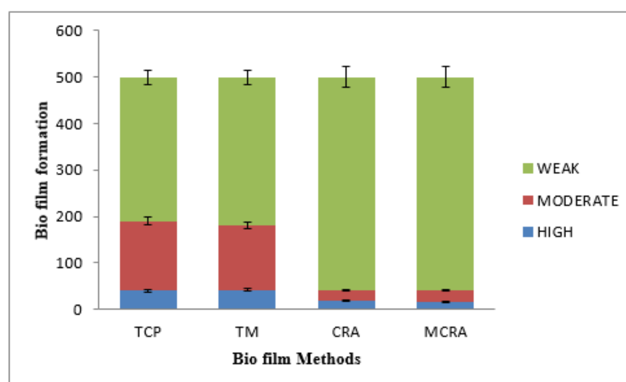

Figure 1: Grading of biofilm formation in various isolates by the four different methods

Table 5: Statistical evaluation of TM, CRA, modified CRA methods for detection of biofilm formation

Screening methods	Sensitivity	Specificity	PPV	NPV	Accuracy
TCP	11.0	34.7	54.3	55.6	42.1
TM	81	95.1	93.3	85.6	88.7
CRA	16.8	93.9	67.9	57.3	58.6
Modified CRA	17.5	94.5	72.7	57.7	59.3

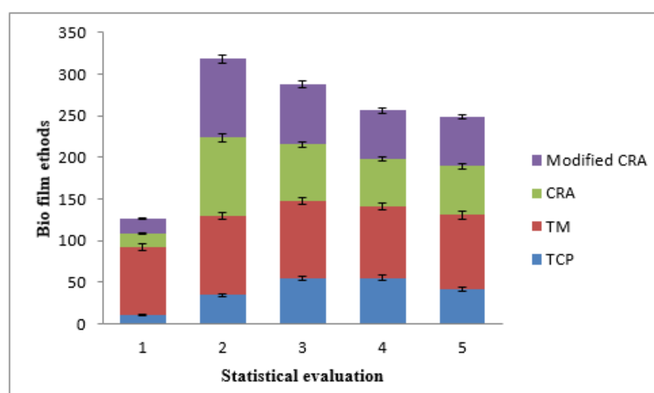

Figure 2: Statistical evaluation of TM, CRA, modified CRA methods for detection of biofilm formation

CONCULSION

To conclude biofilms are major cause of recurrent and recalcitrant UTI leading to increase morbidity in the patient increased duration of hospital and increase in economic burden and sources. The role of biofilms is converted of uncomplicated UTI due partial clearance of the infection needs. A suitable method which is cost effective easy to do and requiring less technical expertise is the need of the hour. The TCP was found to be most sensitive followed by the TM, CRA and the MCRA method. TCP method is the ideal method for detection of bacterial biofilm formation by uropathogens. MCRA method is superior to CRA and not to TCP or TM for detection.

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