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RESEARCH ARTICLE

EVALUATION OF TWO NEWER METHODS OF BIOFILM FORMATION IN BACTERIA OF MEDICAL IMPORTANCE

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ABSTRACT

Introduction: Biofilms formation plays an important role in bacterial and fungal pathogenesis. Biofilms have been considered a virulence factor contributing to bacterial infections. Therefore, a reliable method for their diagnosis is necessary. **Materials and Methods:** In this study, biofilm formation of 86 isolates of bacteria and yeasts were detected by Test tube method, polystyrene petri dish method and glass slide method and the results were compared. **Results:** Slide method and Petri dish method were found better than the test tube method for studying biofilm formation with better sensitivity but poorer specificity. **Conclusion**: Slide method and Petri dish method can be safely used to find out pattern of biofilm formation by bacterial isolates and yeasts.

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INTRODUCTION

The growth form of microorganisms that is associated with a surface is called a biofilm. The human microbiota plays a role in human metabolism and in understanding the pathogenesis and the optimized therapy for many diseases (Chakravarthi and Haleagrahara, 2011). Bacterial biofilms are notoriously known for their high resistance to antibiotics, disinfectant, chemicals,and components of the innate and adaptive inflammatory defense system of the body (Høiby *et al.,* 2011). Fungi being eukaryotic cells and more complex than bacteria cause infections that are often difficult to diagnose and treat, and carry unacceptably high mortality rates (Perlroth *et al.,* 2007). Antibiotic tolerance in biofilms is10-to1,000-fold higher than in corresponding planktonic bacteria (Hill *et al.,* 2005). Biofilm-reduced susceptibility to antibiotics arises from the combination of several mechanisms, including slow antibiotic penetration in the biofilm matrix, slow bacterial growth in an altered microenvironment (nutrient gradients and oxygen restriction), resort of quorum sensing mechanisms by bacteria, and existence of a population of persister microorganisms (Stewart, 2002; Stewart *et al.,* 2001). Candida bloodstream infections (CBSIs) are the fourth most common infections among hospitalized patients, accounting for 30% to 81% of hospital- acquired Blood stream infections (Wisplinghoff *et al.,* 2004).

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They are considered high-morbidity infections (Bassetti *et al.,* 2007; Leroy *et al.,* 2009) with significant hospital costs (Morgan *et al.,* 2005; Zaoutis *et al.,* 2005), largely due to increased hospital length of stay and costs for antifungal therapy (Pfaller and Diekema, 2007). Use of broad spectrum antibiotics, neutropenia, parenteral nutrition, indwelling catheter are risk factors contributing to increased disease burden (Dixon *et al.,* 1996). In addition, the cells of a true biofilm produce their own extracellular matrix material and manifest phenotypes that are distinct from the phenotypes of cells growing in suspension (called planktonic cells). However, in their natural ecosystems, most microbes exist as attached communities of cells within an organized biofilm and rarely as planktonic organisms (Costerton *et al.,* 1999). Thus, a biofilm is defined as a surface associated and highly structured community of microorganisms that are enclosed within a protective extracellular matrix. Microbial biofilms cannot only form in nature but also inside a host, and in recent years there has been an increased appreciation of the role that microbial biofilms play an important in human medicine: it is now estimated that about 65% of all human infections have a biofilm etiology (Costerton *et al.,* 1999). Formation of biofilms, therefore should preferably be assessed in vitro before or during therapy for optimum cure.

Objective

To isolate and identify the microbes, and perform biofilm testing by test tube, petri dish and glass slide method.

MATERIALS AND METHODS

This was a laboratory-based observational study, which was carried out in the Department of Microbiology, All India Institute of Medical Sciences, Patna

Time of study: from April 2017 to September 2017

86 different clinical isolates of bacteria and yeasts were retrieved from samples like urine, blood, and pus in the laboratory of the department and subjected to biofilm detection methods. The bacterial isolates for testing biofilm were 20 isolates each of *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia,* 10 isolates of *Staphylococcus aureus, Candida albicans*, and 6 samples of *Acinetobacter baumannii,* from different samples. Isolates were identified by standard microbiological procedures, staining and biochemical tests. Candida albicans were identified by conventional methods like germ tube test, microscopic morphology by Dalmau technique [on Rice extract agar], growth at 44℃, sugar fermentation and sugar assimilation tests. Biofilm detection were tested by Test tube method (TM), petridish method and slide method.

Methods

Slide method vs Petri dish method vs Test Tube Method: Peptone water with 1% (weight/volume) glucose was prepared and autoclaved at 110 ℃ at 10 Ibs/in2 pressure. In 3ml of this media each in 3 glass test tubes, 0.5 Mc Farland turbidity (standard turbidity) of suspension of each isolates was made. One tube was incubated at 37℃ overnight as such and contents of the other two was dispensed in polystyrene disposable, sterile, 90 mm petri dish (Tarsons Inc.). In one of these two petridishes, one sterile glass slide was placed on the bottom of the petri dish .Then the petri dishes were incubated at 37 ℃ overnight. Next day, Liquid contents of both test tube and the petri dishes were drained off and test tube and petri dish with and without the sterile glass slide kept inside were washed thrice with sterile 0.9% normal saline. After that 3 ml of 0.5% aqueous Safranine was poured in both test tube and the petridishes and kept for 1 minute. Following this, Safranine was drained from all of them. Again they were washed thrice with 0.9% normal saline. After that the tube and the petri dishes were kept for drying. Test tube was observed by naked eye for biofilm formation and the petri dishes with and without the glass slide were observed by naked eye and also microscopically at 10X and 40X microscope objective.

Table 1. Results of Slide method Vs Petri dish method Vs Test tube method

Serial no.	Isolates	Slide method	Petri dish method	Test tube method
1-20	*Pseudomonas aeruginosa*	bs	bs	b
		Uniform, few bacilli	Uniform, few bacilli	b
		ul	ul	bns
		bs	bs	b
		bs	Uniform , few bacilli	b
		ul	ul	bns
		ul	ul	bns
		ul	ul	bns
		bs	bs	bns
		Uniform, few bacilli	Uniform , few bacilli	b
		bs	bs	b
		Few bacilli seen	Uniform layer	bns
		Uniform, few bacilli	Uniform , few bacilli	b
		ul	ul	bns
		bs	bs	bns
		ul	ul	bns
		bs	bs	b
		ul	ul	bns
		Uniform, few bacilli	Uniform, few bacilli	bns
		bs	bs	b
1-20	*Escherichia coli*	ul	ul	bns
		Few bacilli	Uniform , few bacilli	bns
		ul	ul	bns
		bs	bs	b
		ul	ul	bns
		bs	bs	b
		Few bs	Uniform, few bacilli	bns
		Few bacilli	Uniform, few bacilli	b
		ul	ul	bns
		Few bacilli	Uniform, few bacilli	b
		bs	bs	b
		bs	bs	b
		ul	ul	bns
		ul	ul	bns
		ul	ul	bns
		bs	bs	b
		ul	ul	bns
		bs	Uniform, few bacilli	b
		ul	ul	bns
1-20	*Klebsiella pneumonia*	bs	bs	b
		bs	bs	b
		ul	ul	bns
		ul	ul	bns
		bs	bs	b
		Few bacilli	Uniform , few bacilli seen	bns
		ul	ul	bns
		bs	bs	b

……. Continue

1-20		Very few bacilli seen	ul	bns
		ul	ul	bns
		bs	bs	b
		bs	bs	b
		Few bs	Few bacilli	bns
		Few bs	Uniform , few bs	b
		ul	ul	bns
		bs	bs	b
		Few bacilli	Uniform, few bs	b
		ul	ul	bns
		bs	bs	bns
		ul	ul	bns
1-10	*Staphylococcus aureus*	Few cocci	Very few cocci	bns
		Cocci in clusters	Cocci in clusters	b
		ul	ul	bns
		ul	ul	bns
		Few cocci	Uniform, few cocci	b
		ul	ul	bns
		ul	ul	bns
		Few cocci	Uniform, few cocci	b
		ul	ul	bns
		ul	ul	bns
1-10	*Candida albicans*	Uniform , few in clusters	Uniform , few in clusters	b
		Uniform , few in clusters	Uniform , few in clusters	b
		by	by	bns
		by	Budding, uniform layer	b
		ul	ul	bns
		by	by	bns
		by	Budding, uniform layer	b
		ul	ul	bns
		ul	ul	bns
		ul	ul	bns
1-6	*Acinetobacter baumannii*	bs	bs	b
		ul	ul	bns
		bs	bs	b
		ul	ul	bns
		bs	bs	bns
		ul	ul	bns

b– Biofilm seen ; bns - Biofilm not seen ; ul- Uniform layer ; bs- bacilli seen ; by- budding yeast

The petridish method and slide method of detecting biofilm were better than the Test tube method: Among petridish method and slide method, the results were almost comparable.

DISCUSSION

There are different methods of studying biofilms in vitro, of which microtiter plate or tissue culture method is a good method (Pierce *et al.,* 2008). Also such expensive techniques are not commonly available for use in routine and peripheral clinical microbiology laboratories. The present study, therefore, evaluated three simple and cost effective alternatives methods for the identification of micro-organisms. Test tube method can be a good method for this purpose, but it has high degree of subjective variability in reading and cannot detect moderate to weak biofilm producers (Mathur *et al.,* 2006). Glass slides and Polystyrene petri dishes are cheap and easily and widely available, strong biofilm producers. If these methods are successful, it can even be done in bedside, and this will be helpful since treatment can then be modified accordingly. We can even grade degree of biofilm formation in this method (PDM or petri dish method), much like test tube method. These newer tests are simple and cost effective that will aid routine identification. So these methods can be a simple, yet better option for detecting assessing biofilm formation. Polystyrene petri dish method with and without glass slide is equally good for biofilm detection as compared to test tube method. Also we were able to grade biofilm formation microscopically as 1+, 2+ etc. Thus gradation of biofilm formation can be done. Also, we can study the effect of methylene blue on biofilms to see metabolic activity of the biofilm cells.

Conflict of interest: none

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