

ISOLATION AND IDENTIFICATION OF BIOFILM PRODUCERS FROM THE IMPLANT INFECTED INDIVIDUALS

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Abstract

Mostly the UTI infections are caused by short term and long term catheterization. To treat those infections, the biofilm forming organisms were identified and treated with specific antibiotics. So the urinary catheter was isolated from the UTI infected patient. The urinary catheter was identified for gram negative rods using gram staining. Further Congo red agar test was performed to isolate the organism. To identify the biofilm formation, trypticase soy broth and microtiter method was done. Biofilm formation was observed. Various biochemical tests were performed to identify the biofilm forming organism. Finally as a confirmatory test, MacConkey agar and motility were performed and the organism was found to be *Pseudomonas*. In future, antibiotic coated catheters can be used against *Pseudomonas aerogens* to reduce the UTI associated infections. The present study is to isolate and identify the UTI causing organisms.

Key words: UTI, *Pseudomonas aerogens*, biofilm.

INTRODUCTION

In seventeenth century, Anton van Leeuwenhoek first observed “animalcules” swarming on living and dead matter. He discovered these “animalcules” in the tartar on his own teeth and even after meticulous cleansing. The remaining opaque deposits isolated between his teeth were still “as thick as if it were batter”. These deposits contained a mat of various forms of “animalcules” that we now know were the bacteria of dental plaque. It is reasonable to suggest that this early study of dental plaque was the first documented evidence of the existence of microbial biofilms. Now such biofilms are defined as microbial communities adhered to a substratum and encased within an extracellular polymeric substance (EPS) produced by the microbial cells themselves. Biofilms may form on a wide variety of surfaces, including natural aquatic systems living tissues, indwelling medical devices and industrial/potable water system piping. These biofilms can be benign or pathogenic, releasing harmful products and toxins, which become encased within the biofilm matrix. Biofilms may exist as beneficial epithelial communities in rivers and streams, wastewater treatment plant trickling beds or in the alimentary canal of mammals. The surface of implants that contact the body might be made of a biomedical material such as titanium, silicone or apatite. In a biofilm the adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS). Biofilm EPS, which is also referred to as slime.

To identify the biofilm forming organisms, various tests have been carried out. Nowadays, urinary tract infections are becoming a threat due to the usage of catheters. And so we decided to identify and isolate the biofilm forming organisms from the urinary catheter. We collected a urinary catheter from a female patient because UTI infections are more prominent in female. It was collected from Bethel Hospital, Coimbatore. Normally many biofilm forming organisms can be found in a used urinary catheter such as *Pseudomonas*, *E.coli*, *Klebsiella* etc. To isolate a particular organism we made the catheter into pieces and put it into the nutrient broth. Various other methods were followed such as Congo red method, Trypticase soy broth method, Microtiter method. The organism growth was found and biofilm formation was seen in all the three methods. To identify the organism, biochemical tests were done. The results indicated that the organism was *Pseudomonas*. To confirm the above result, confirmatory tests were done. MacConkey agar test, Nitrate reduction test and motility tests were performed. In MacConkey test, non lactose fermenting colonies were obtained. In nitrate test, the

nitrate reduction was found and in hanging drop method, the motility was seen clearly. Thus it was identified that the biofilm forming organism is *Pseudomonas*.

MATERIALS AND METHODS

Nutrient broth preparation

Nutrient broth was prepared in 25 ml of distilled water. It was kept for sterilization for about 15 lbs/sq.inch. Urinary catheter was made into small pieces and it was put inside the nutrient broth. Then it was kept for incubation for 37°c at 24 hrs.

Gram staining method

A clean grease free glass slide was taken and the isolated culture was smeared. It was covered with few drops of the crystal violet. And after 1 minute exposure the slide was washed with water. Then smear was treated with few drops of Gram's iodine and allowed for 1 minute. Then slide was again washed with water and then decolorized with ethyl alcohol for 30 seconds. After decolorization the smear was washed and it was finally treated with safranin for 1 minute. The slide was washed with water and excess water was removed using a blotting paper, the slide was air dried and heat fixed before observing under oil immersion microscope.

Congo red agar medium (50 ml)

Congo red agar was prepared. It was kept for sterilization for about 15lbs/sq inch along with the petriplates. Congo red agar was poured in petriplates and allowed to solidify. After that each petriplate was inoculated with various organisms by quadrant streak. Then it was kept for incubation.

Trypticase soy agar broth

Tube method procedure

Trypticase soy broth was prepared and poured in test tubes. It was kept for sterilization for about 15lbs/sq inch. After 24 hrs it was inoculated with 3 organisms. It was kept for incubation. Then the tubes were taken, they were decanted. Crystal violet was added to the tubes. After 5 mins they were drained. Then distilled water was poured into the tubes and drained. And so biofilm formation was observed.

Microtiter method

Microtiter plate was taken and it was inoculated with 6 organisms. It was kept for incubation. After 72 hrs microtiter plates were decanted. Crystal violet was added. After 10 mins it was washed. Then it was washed with distilled water.

Biochemical tests

To identify the organism IMViC test, carbohydrate fermentation test, starch hydrolysis, catalase test, casein hydrolysis and oxidase test were performed.

Confirmatory tests

To confirm the organism MacConkey test were performed.

RESULT AND DISCUSSION

Gram's staining

From the sample that was obtained from the nutrient broth, Pink coloured rods were obtained when observed through microscope. It is shown in figure 1.1.

Congo red method

White colonies were formed on Congo red plate. Non lactose fermenting colony growth was formed. It is shown in figure 1.2.

Trypticase soy broth

Visible stained biofilm were found to adhere the sides and bottom of the tube in sample 1. This surface may be inert, non living or living tissue biofilm. It is shown in figure 1.3.

Microtiter method

Biofilm formation by microorganisms was visible through crystal violet stain. The biofilm formation was more in sample 1. It was shown in figure 1.4.

Biochemical test

From biochemical test it was concluded that the biofilm was formed by *pseudomonas*. The results of various biochemical tests are shown in the table 1.1

Confirmatory test

MacConkey agar test

This test was done to confirm that the biofilm forming organism is *pseudomonas*. White coloured colonies were formed. Thus it was a non lactose fermenting organism. It is shown in figure 1.5.

TABLE.1.1- Biochemical Test Results

TEST	RESULT
1.Indole	- ve
2.Mr	+ve
3.Vp	-ve
4.Citrate	+ve
5.Gelatin	+ve
6.Catalase	+ve
7.Oxidase	+ve
8.Carbohydrate a. glucose b. dextrose c. lactose	-ve
9.caesin	-ve
10.starch	+ve

FIGURE 1.1: GRAM STAINING

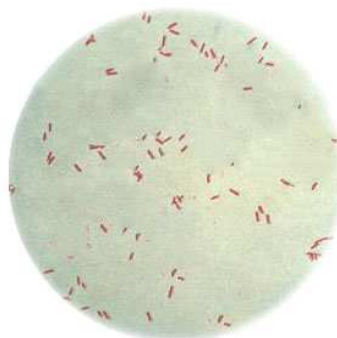


FIGURE 1. 2: ORGANISM GROWTH IN CONGO RED



FIGURE 1.3-BIOFILM FORMATION IN SAMPLE 1

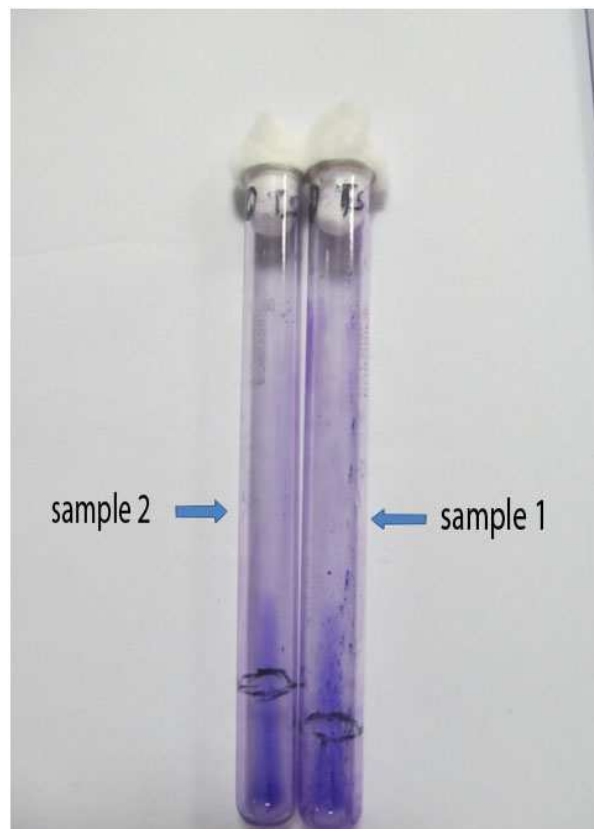


FIGURE 1.4-BIOFILM FORMATION IN SAMPLE 1

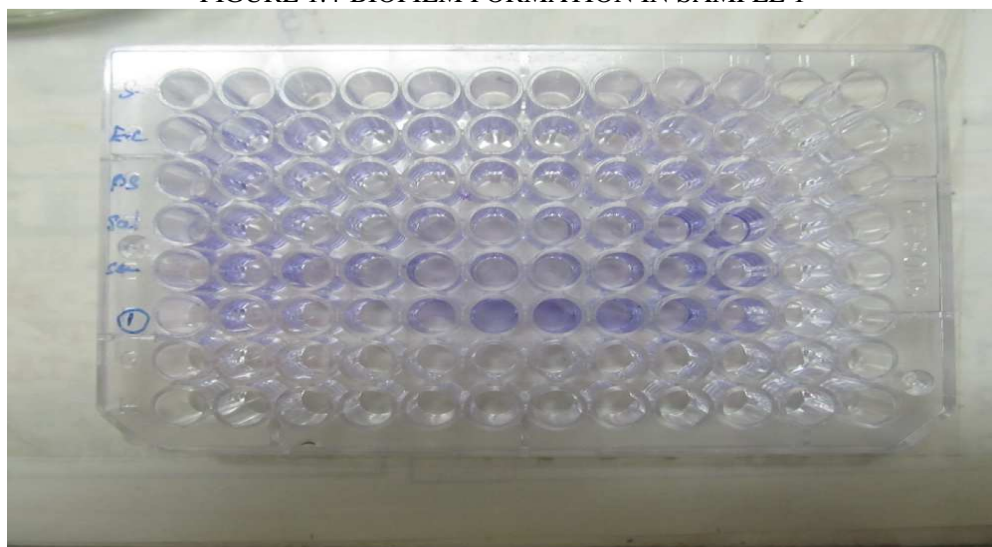
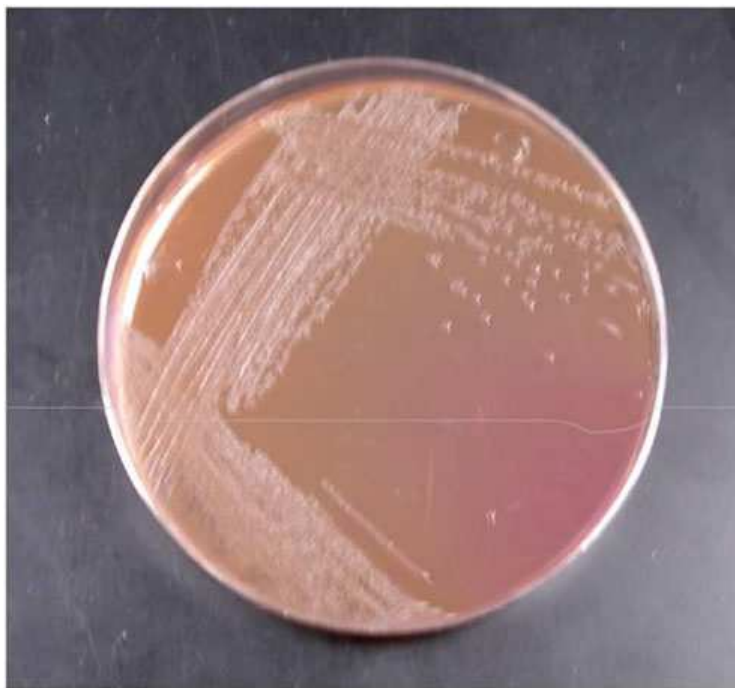


FIGURE 1.5- MACCONKEY AGAR



DISCUSSION

Quantification of the biofilm depends on the number of organisms recovered by contact with the agar surface. Resistant to antimicrobial agents than plank tonic cells because of the diminished rates of mass transport of antimicrobial molecules to the biofilm associated cells or because biofilm cells differ physiologically from plank tonic cells. Microbial biofilms may pose a public health problem for persons requiring indwelling medical devices. The microorganisms in biofilms are difficult or impossible to treat with antimicrobial agents; detachment from the device may result in infection. Although medical devices may differ widely in design and use characteristics, specific factors determine susceptibility of a device to microbial contamination and biofilm formation.

CONCLUSION

Thus from various tests it was confirmed that the biofilm forming organism is *Pseudomonas*. To better understand and control biofilms on indwelling medical devices, research must progress in several key areas. More reliable techniques for collecting and measuring biofilms should be developed.

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