

In Vitro Detection of Biofilm Formation by Uropathogenic Escherichia Coli in A Tertiary Care Hospital

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Abstract

Introduction: Biofilm are surface associated bacterial communities surrounded by a matrix of exo-polymers and are responsible for development of clinical infections. Urinary tract infections are considered to be one of the most common bacterial infections. Uropathogenic strains of *Escherichia coli* account for 70-95% of the UTIs. The bacteria enclosed within the biofilm are extremely resistant to treatment. **Objective:** To study Biofilm formation of Uropathogenic *Escherichia coli* by Tube method and Tissue Culture Plate method. **Material and Methods:** The study was carried out at Department of Microbiology, MGM Medical College, Kamothe, Navi Mumbai from October 2015 – September 2016. Total 200 urine samples were processed. Out of 200 samples, 100 isolates of Uropathogenic *Escherichia coli* were included in this study. They were identified by standard microbiological procedures. These isolates were subjected to biofilm production by Tube method and Tissue culture plate method. **Results:** Out of 102 Uropathogenic *Escherichia coli* isolates 40.19% were biofilm producers by Tube Method and 47.05% by Tissue culture Plate Method. **Conclusion:** Tube Method correlated well with Tissue Culture Plate method for strong biofilm detection in Uropathogenic *Escherichia coli*. **Keywords:** Biofilm, uropathogenic *Escherichia coli*, antimicrobial resistance, Tube Method, Tissue Culture Plate Method.

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INTRODUCTION

Escherichia coli are one of the most prevalent pathogens among gram-negative bacteria, capable of causing complicated and uncomplicated UTI's. Uropathogenic *E.coli* are the primary cause of community acquired urinary tract infections (70% -95%) and nosocomial UTI (50%) [1].

Uropathogenic *E.coli* forms intracellular bacterial communities with many biofilm like properties within the bladder epithelium [2].

The transition from planktonic growth to biofilm occurs in response to environmental changes and involves multiple regulatory networks, which translate signals to concerted gene expression changes thereby mediating the spatial and temporal reorganization of the bacterial cell [3]. Bacteria attach to surface aggregate in a hydrated polymeric matrix of their own synthesis to form biofilms [4].

Biofilms have role in up to 60% of human infections and they are difficult to eradicate with antimicrobial treatment. They largely consist of

polysaccharides, which prevents the access of antibacterial agents and antibodies. Planktonic cells are highly susceptible to antibiotics than the sessile bacterial cells in the biofilms which can withstand the host immune responses. Biofilm forming bacteria are more resistant to antimicrobial agents leading to the limited effectiveness of current antibiotic therapies. Hence, detection of biofilm production by uropathogens is important and it can help in initiating appropriate intervention in cases of symptomatic UTI.

Inclusion Criteria

- Urine samples with pure cultures showing significant bacteriuria (10^5 CFU/ml).

Exclusion Criteria

- Mixed cultures and asymptomatic bacteriuria.

MATERIAL AND METHOD

102 urine samples fulfilling inclusion criteria were selected from urine samples in duration from October 2015 – September 2016.

Biofilm detection was done by the following methods.

Tube method(TM)

A qualitative assessment of biofilm formation was determined as described by Christensen *et al.*, [5] TSBglu (10ml) was inoculated with loopful of microorganism from overnight culture plates and incubated for 24hrs at 37°C. The tubes were decanted and washed with PBS (PH 7.3) and dried. Dried tubes were stained with crystal violet (0.1%). Excess stain was removed and tubes are washed with deionized water. Tubes were then dried and kept in inverted position and observed for biofilm formation.

Biofilm formation was considered positive when a visible film lined the wall and bottom of the tube. Ring formation at the air- liquid interface is not indicative of biofilm formation. Experiments were performed in triplicate [6].

Tissue Culture Plate Method

Isolates of uropathogenic *E.coli* were inoculated in brain heart infusion (BHI) broth with 2% sucrose and incubated for 18-24 hrs at 37 °C in a stationary condition. The broth with visible turbidity was diluted to 1 in 100 with fresh medium.0.2ml of diluted cultures were added to the flat bottom wells of sterilized polystyrene plate and incubated for 24hrs at 37°C.Plain broth served as a control to check sterility and nonspecific binding of medium. Following incubation, the contents of the plate were gently aspirated. The plates were washed with 0.2 ml of sterile phosphate -buffered saline four times at pH 7.2. Biofilm

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 removed by washing the plate under distilled water and then plates were dried. 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 Immunosorbent Assay auto reader at wavelength of 570nm. The experiment was performed in triplicates [7].

RESULTS

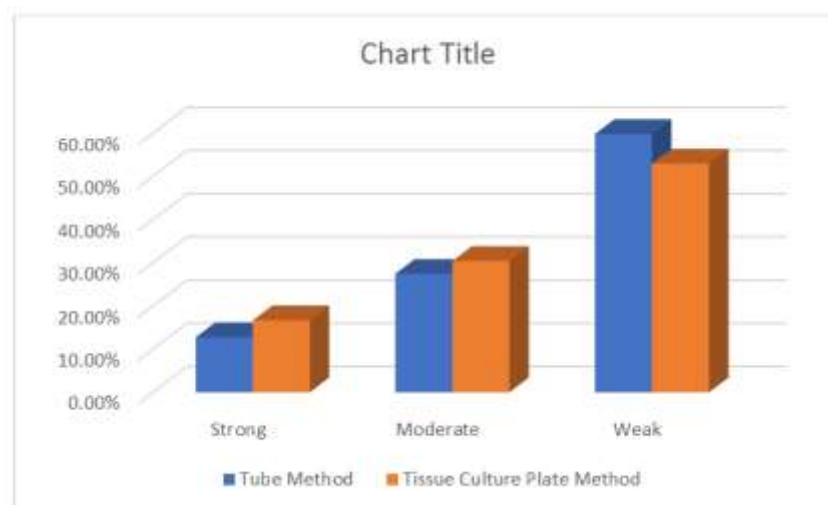
Total 200 urine samples were processed .Out of 200 samples,102 samples with *E.coli* isolates were tested for biofilm formation.

Among 102 uropathogenic *E.coli* strains 41(40.19%) were positive for biofilm production by Tube Method and 48(47.05%) by Tissue culture Plate Method.

Under optimized conditions, biofilm positive phenotype strains in Tube method were classified as strong positive 13(12.74%), moderate positive 28(27.45%) and weakly positive 61(59.80%). While in Tissue Culture Plate method, biofilm positive phenotype strains were also classified as strong positive17(16.66%), Moderate positive 31(30.39%) and weakly positive 54(52.94%).

Table-1: Biofilm Formation in Uropathogenic *E.coli* by Tube Method and Tissue Culture Plate Method

Sr, No.	Methods	Tube Method	Tissue Culture Plate Method
1.	Strong biofilm producers	13(12.74%)	17(16.66%)
2.	Moderate biofilm producers	28(27.45%)	31(30.39%)
3.	Weak biofilm producers	61(59.80%)	54(52.94%)



Graph-1: Biofilm formation by Tube Method and Tissue Culture Plate Method

Statistical Analysis

Data collected, compiled, tabulated and analysis was carried out for comparison between two methods by using Chi-Square Test and significance level was set at 95% and $p < 0.05$ was considered significant.

DISCUSSION

Escherichia coli is the most frequent microorganism involved in urinary tract infection (UTI). Acute UTI caused by uropathogenic *E. coli* (UPEC) can lead to recurrent infection, which can be defined as either re-infection or relapse [8].

Bacterial biofilm are often associated with long-term persistence of organism in various environments. Biofilm formation protects bacteria from hydrodynamic flow in the urinary tract against phagocytosis, host defence mechanisms, as well as antibiotics. Bacteria in biofilm display dramatically increased resistance to antibiotics [9]. Easier methods for diagnosing and quantifying biofilm associated infection and ideal device surface would surely help in the fight against biofilm formation [10].

In our study, we studied 102 strains of uropathogenic *Escherichia coli* out of which were 40.19% were biofilm producers by Tube Method and 47.05% by Tissue culture Plate Method.

Baqai *et al.*, reported biofilm production in 75% of the isolates as detected by Tube method while with Congo red agar method only 10% isolates [11].

Murugan *et al.*, found 81 out of 96 (84.3%) isolates of *E. coli* formed biofilm as detected by tube method while only 33 out of 96 (34.3%) produced biofilm by congo red agar method [12].

Our findings are similar with study done by Mathur *et al.*, who detected biofilm production 47.3, 41.4% and 5.2% in 152 isolates by TCP, TM and CRA method [13].

Biological and technical factors were responsible for differences in the result as tube method was performed in the glass tubes and Tissue Culture Plate method was in polystyrene microtitre plate.

Technical factors influencing biofilm production depend on the type of medium, atmosphere of incubation and the nature of the solid surface [14].

CONCLUSION

Urinary tract infections are common infections encountered in the clinical practice. UTI caused by biofilm producing *E. coli*, may promote the colonization and increased the incidence rate of UTI. Thus different methods should be employed to avoid biofilm formation on various surfaces. In present study, Tube

Method correlated well with TCP method for strong biofilm detection. It was difficult to discriminate moderate and weak biofilm production by Tube Method. Tube Method required subjective observer's assessment as compared with Tissue Culture Plate Method which was based on accurate objective assessment.

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