



Comparative evaluation of methods for detection of Biofilm formation in urinary catheter tips and their antibiogram

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Abstract

Background: A biofilm is an accumulation of microorganisms embedded in an exopolysaccharide matrix of microbial host origin called polysaccharide intracellular adhesion (PIA). They pose a significant threat to indwelling devices, causing slow persistent infections, thereby interfering with antimicrobial therapy.

Objectives: This study was conducted to isolate and compare three conventional methods for the detection of Biofilm formation and their antimicrobial susceptibility pattern.

Materials and Methods: A total of 120 urinary catheter tips were collected from medical and dialysis units from May 2019 to November 2019. Biofilm production was detected by Congred agar, Tube method, Tissue culture methods. Antibiotic susceptibility test of Biofilm producing organisms was performed according to CLSI guidelines.

Results: Out of 120 samples 52 (43.33%), showed culture positivity, out of which bacteria are 37(71.2%) which includes *E.coli* 15(28%), *klebsiella* 10(19%), *pseudomonas* 6(11.5%), *proteus* 4(7%), *Enterococci* 2 (3.8%), and fungal culture revealed *Candida* 15 (28.8%). Out of 52 culture positives, 23(44.23%) produced Biofilm. The rate of detection by CRA, TM TCP methods was 23%, 36%, 44%, respectively. Higher antibiotic resistance was observed in Biofilm producing bacteria.

Conclusion: The TCP method of biofilm detection is quantitative and more reliable than TM and CRA methods; it can be recommended as a general screening method for the detection of Biofilm producing bacteria in laboratories.

Keywords: Biofilm of urinary catheter tips, Nosocomial infections, TCP, TM, CRA methods.

Introduction

Urinary tract infection (UTI) is the most significant human infections in catheterized patients. Uropathogenic organisms produce intracellular bacterial communities called Biofilm within the bladder aligned with polysaccharides, which prevent the entry of antibiotics, antibodies, and white blood cells¹.

Biofilms are defined as microbially derived sessile communities characterized by the cells that are

irreversibly attached to a substratum or each other.

A biofilm is an aggregation of microorganisms embedded in an exopolysaccharide matrix of microbial and host origin called Polysaccharide Intracellular Adhesion (PIA)^{2,3,4}. Biofilm forming microorganisms are capable of adhering irreversibly to each other or catheter surface^{2,5}. They show an altered phenotype concerning growth rate and gene transcription^{2,6}. Within a biofilm production, bacteria communicate by

producing chemotactic particles or pheromones, a quorum-sensing phenomenon⁷. Microorganisms growing in a biofilm are intrinsically high resistant to antimicrobial agents than planktonic cells. High dose antimicrobial concentrations are required to inactivate organisms growing in Biofilm, as antibiotic resistance can increase 1,000 fold^{2,8}. This is because of the failure of antibiotics to penetrate the polysaccharide matrix.

Some cells in Biofilm may experience nutrition deprivation; therefore, they exist in a slow-growing or starved state, displaying reduced susceptibility to antimicrobial agents⁵. The proximity of cells within Biofilm can facilitate the exchange of plasmids responsible for drug resistance and enhance the spread of antimicrobial resistance⁹. Biofilm impedes penetration of antibiotics and various disinfectants, emphasizing that their characterization is an essential aspect of infection control^{4,10}. Usually, the urinary tract is protected from microbial colonization by sterile flushing urine, the sloughing of uroepithelial cells, and the glycosaminoglycan layer¹¹. If an indwelling catheter is present, then Biofilm producing bacteria attach to the surface of the catheter⁹. Gram-negative bacilli, Gram-positive cocci, and *Candida* are capable of forming biofilms.

The main aim and objectives of this study are, To isolate and identify bacteria and fungi from urinary catheter tips. To determine their antimicrobial susceptibility pattern. To detect their capability to produce Biofilm by three different methods, i.e., Tube Method (TM), Congo Red Agar (CRA) method, Tissue culture plate (TCP) method.

Materials and Methods

A total of 120 urinary catheter tips were collected under strict aseptic conditions into sterile universal containers from patients admitted into medicine wards and were processed.

Organisms are initially isolated on routine culture media like Blood agar, Mac-Conkey agar and were identified by standard microbiological

procedures, i.e., cultural characteristics, Grams stain, catalase test, oxidase test, motility, and other biochemical tests. Antimicrobial susceptibility testing is done by the modified Kirby-Bauer disc diffusion method on Muller Hinton agar. Thus the organisms identified were subjected to three different tests to detect their ability to produce biofilms.

Biofilm detection methods

Tube Method(TM)

This is a qualitative method for assessment of biofilm formation was described by Christensen et al.¹².

Five milliliters of trypticase soy broth (TSB) in 1% glucose is mixed with a loopful of the test organism from overnight culture plates. Incubate the Inoculated broths at 37°C for 48 hours. The contents were gently emptied and washed with phosphate buffer saline (pH 7.3) and dried at room temperature. Stain the tubes with 0.1% safranin. Gently rotate each Tube to ensure uniform staining, and after 1 minute, its contents were decanted. They were dried in an inverted position and look for the formation of the Biofilm formation. When a clear film lined the wall and bottom of the Tube, then biofilm formation was considered positive. The appearance of a ring at the liquid interface does not indicate the formation of Biofilm. Tubes must be examined, and the results were scored visually as (Figure 2)

0 – Absent, 1 – Weak biofilm producer, 2 – Moderate biofilm producer, and 3 – Strong biofilm producer.

Congo Red Agar (CRA) Method

Based on the property of cultural morphology of biofilm-forming bacteria on Congo-red agar medium is the method, it was developed by Freeman et al.¹³. The CRA media contains BHI broth 37 gm/L; Sucrose 50 gm/L; Agar 10 gm/L; Congo red 0.8 gm/L

The congo red stain's concentrated aqueous solution is prepared and autoclaved separately without adding other media constituents. When the temperature gets to 55°C, it is then mixed to

the autoclaved BHI agar with sucrose. Plates are inoculated with the test organism and incubated overnight at 37°C. Black dry crystalline colonies indicated strong biofilm production (Figure 1). Red colonies with darkening at the center are Weak biofilm producers, and non-biofilm producers usually remained pink to red.

Tissue Culture Plate (TCP) Method

This test was described by Christensen et al.; it is a quantitative test. Overnight growth of bacteria in trypticase soy broth (TSB) with 1% glucose was diluted 1:100 with a fresh medium. Individual wells of 96 well, sterile, polystyrene microtitre plate was filled with 200 µl of diluted cultures.

Biofilm producer *Pseudomonas aeruginosa* ATCC 27853 strain is used as a positive control, and sterile TSB is used as a negative control. Incubate the plates at 37°C for 24 hrs. The contents of each well were demounted by light clicking after incubation. The wells are washed with phosphate buffer saline (pH 7.3) three times to remove 'planktonic' bacteria. Biofilm formed by bacteria adhesive to the wells is fixed with 2% sodium acetate and was stained with 0.1% safranin. Excess stain was removed by thorough washing with deionized water, and plates were air-dried (Figure 3)

The optical density of the stained adhesive Biofilm was measured at 570nm using an ELISA reader. The interpretation of the biofilm production was made depending on the criteria of Stephanovic et al.

OD value Biofilm production :

≤ 2x OD_{non}/weak biofilm producer

2x OD_c to ≤ 4x OD_c moderate biofilm producer

≥ 4x OD_c strong biofilm producer

(Optical density cut-off value (OD_c) = OD of negative control + 3SD of negative control)

Results and Discussion

In the present study, out of 120 samples processed, 52 (43.3%) were culture positive for both bacterial and fungal isolates, and 68 were culture sterile (56%)(Diagram 1).

Out of 52 isolates obtained, bacterial isolates 37(71.2%), Fungal isolates 15(28.8%). Bacterial isolates include *Escherichia coli* 15(29%), *Klebsiella* 10 (19%), *Pseudomonas* 6 (12%), *Proteus* 4(7%), *Enterococcus* 2(3.8%), Fungal isolate is *Candida* 15 (29%).

52 isolates, 23(44%) isolates showed positive biofilm production. Among them, 23(44%) isolates showed biofilm production by the Tissue culture plate method, 19 (36%) by the Tube method, and 12(23%) by Congo red agar method. This finding suggests that the TCP method detects more biofilm producers than the Tube method and CRA method (Diagram 2).

The 23 isolates that showed positive biofilm production in the present study were *Escherichia coli* (10), *Klebsiella* spp. (7), *Pseudomonas* sp.(3) *Enterococci* (1) and *Candida* (2) (Diagram 3).

On Muller Hinton agar, the Antimicrobial susceptibility testing is done by the Modified Kirby Bauer disc diffusion method by using the antibiotics Nalidixic acid 30 µg, Nitrofurantoin 300 µg, Norfloxacin 10 µg, Amikacin 30 µg, Ceftazidime 30 µg, and Amoxycylav 30 µg/disc. The zones of inhibition are measured and compared with that of the standard zone size interpretation chart (Table 1), susceptibility was decided.

According to CLSI M44-A2 guidelines, Antifungal susceptibility testing for *Candida* was done on Mueller Hinton agar, added with 2% glucose and methylene blue. Antifungals include Amphotericin-B - 20µg, Fluconazole -10 µg, Itraconazole - 10µg, Clotrimazole - 10µg, Ketoconazole -10µg and Nystatin - 100units/disc (Table 2).

In the present study, the Tissue Culture Plate (TCP) method detected more number of isolates (42%) capable of producing Biofilm when compared to the Congo red agar method and the Tube method. Identical findings were observed by Hassan A et al. (2011), Anuradha De et al. (2012). In the present study, 1% glucose was supplemented to trypticase soy broth in the TCP method and tube method, and 10% sucrose was

added to agar in the CRA method. Thus, the addition of sugar facilitates biofilm formation. Similar findings were reported by Mathur T et al., 2006 and Bose S et al. (2009). In the present study, isolates showing biofilm production were *Escherichia coli*, *Klebsiella* spp. *Enterococcus faecalis* and *Pseudomonas* spp. These findings are compatible with that of Stickler et al. (1996), who isolated *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp. along with *Proteus* and *Enterococcus faecalis* from the tips of urinary catheters.

In conclusion, Biofilm producing bacteria are responsible for many recalcitrant infections and are notoriously difficult to eradicate¹³. The information on the clinical isolate's capacity to

result in Biofilm would help a clinician assess its virulence and devise an appropriate treatment plan for the patient¹⁴.

Detection of biofilm formation can help prevent potentially fatal and persistent infections¹⁴. The tissue Culture Plate method can be advised as a general screening method for the detection of Biofilm producing bacteria in laboratories².

Wise usage of indwelling urinary catheters in patients and their timely replacement, preferably every two to three days, help prevent the formation of biofilms. For all post-operative cases, the catheter must be removed as soon as possible, preferably within 24 hours, to prevent biofilm formation.

Table 1 Antimicrobial susceptibility testing for Gram-Negative bacterial isolates producing Biofilm and nonbiofilm producers

Antibiotics	Non Biofilm producers (29)		Biofilm producers (23)	
	Susceptible	Resistant	Susceptible	Resistant
Nitrofurantoin	83.3%	16.7%	65.2%	37.8%
Nalidixic acid	72.9%	27.1%	52.1%	47.9%
Norfloxacin	85.3%	14.7%	68.4%	31.6%
Amikacin	88.2%	11.8%	67.1%	32.9%
Imipenem	86.4%	13.6%	70.4%	29.6%

Table 2: Antifungal susceptibility testing for fungal isolates producing biofilms

Antifungals	Susceptible	Resistant
Amphotericin B	50%	50%
Fluconazole	-	100%
Cotrimazole	50%	50%
Ketoconazole	50%	50%
Nystatin	50%	50%

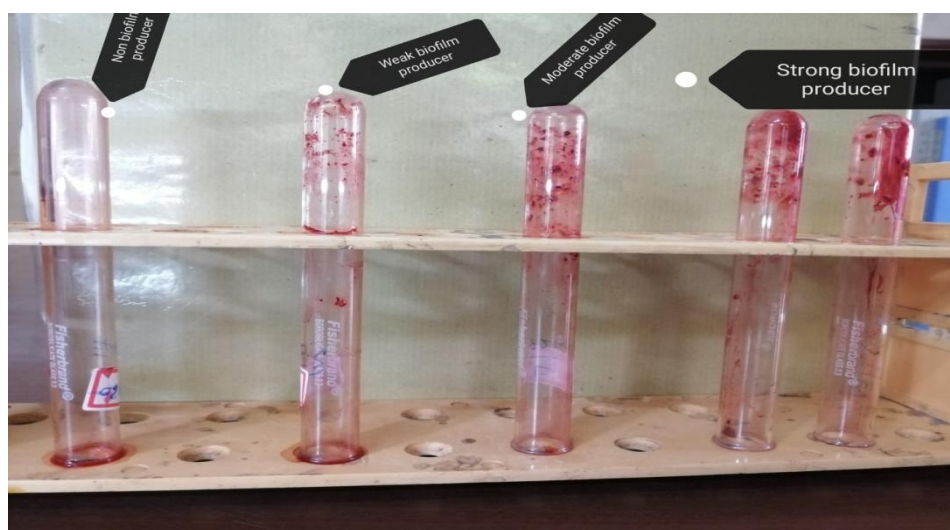


Figure 1 Tube method



Figure 2 Congo red agar

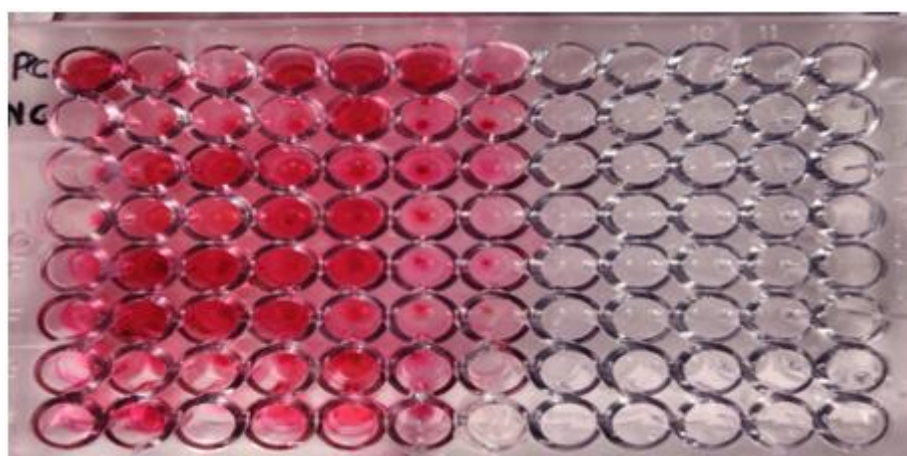


Figure 3

Diagram 1: Diagram showing the percentage of culture positivity

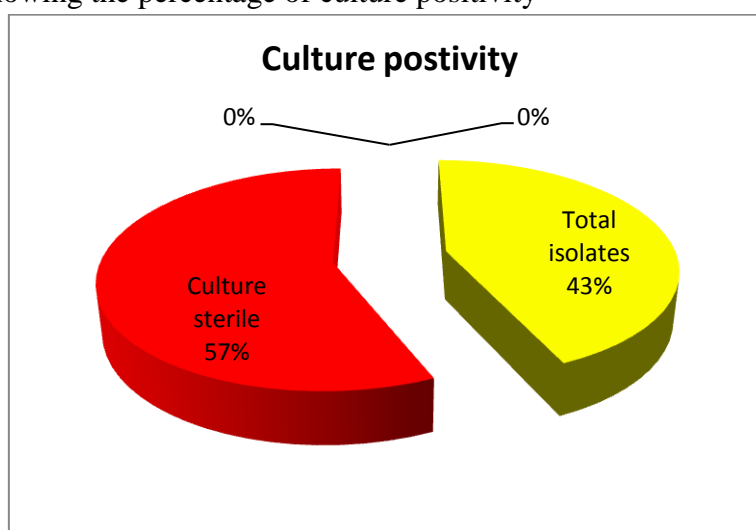
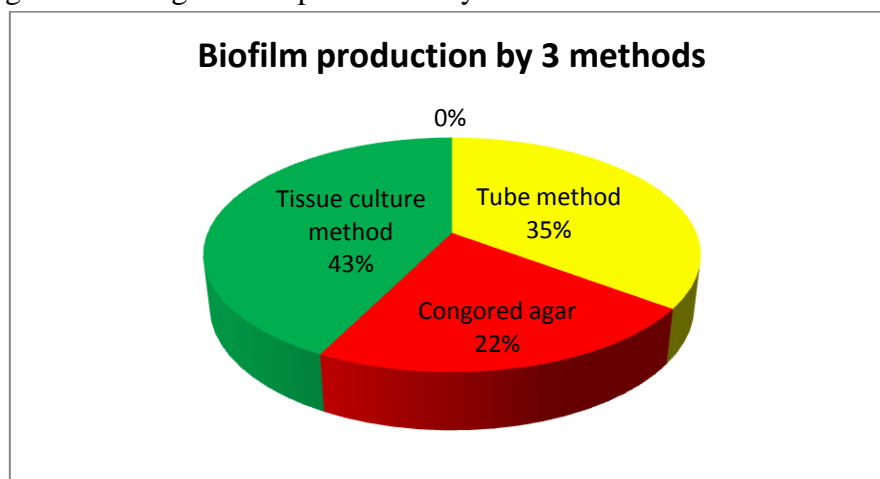
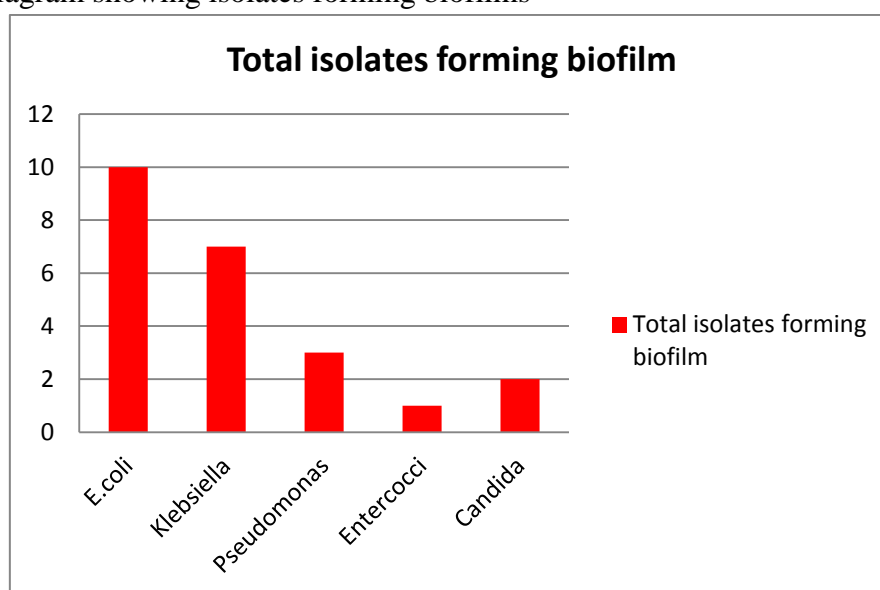


Diagram 2: Pie diagram showing biofilm production by three different methods**Diagram 3:** Bar diagram showing isolates forming biofilms

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