Original Research Article

Isolation of Uropathogensin Catheterized Patients and Their Biofilm Formation Capacity and Comparison of Biofilm Formation by Two Different Methods in a Tertiary Care Hospital of North India

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Abstract

Introduction: Urinary tract infections **(**UTIs) account for 30% of all hospital acquired infections (HAI). Of these 30% infections, 80% of them are estimated to be catheter-associated. According to the CDC, Catheter associated urinary tract infections (CAUTIs) are defined as an UTI developing in a patient after 48 hours of implantation of an indwelling urinary catheter. In most of the cases, the underlying cause of CAUTI is formation of a pathogenic biofilm on the surface of the indwelling urinary catheter.Most important medical significance of biofilm is decrease in susceptibility of antimicrobial agents. **Materials and Methods:** The present study was a cross sectional study**,** conducted over a period of one year.A total of 468 subjects (patients) were included in this study.After taking informed written consent from each patient, Urine samples of catheterized patients were received and sentfor bacterial culture and sensitivity in the Microbiology section of Central laboratory (an ISO 15189:2012 certified, NABL accredited Laboratory) of SMIH, Patel Nagar, Dehradun. Samples were collected taking all aseptic precautions, out of which 100 samples were processed and reported as per the standard methods. **Results:** Taking Tissue culture plate (TCP) as gold standard, sensitivity of Tube method (TM) method was found to be 76.4%; specificity 71.5%.64.1% of *Escherichia coli* isolates were positive by both tube method and tissue culture plate method; However, 75% of *Klebsiella pneumonia* isolates were positive by tissue culture plate method. *Pseudomonas aeruginosa* showed a positivity of 88.8% by tissue culture plate method. **Conclusion:** Based on this study, we conclude that Catheter associated urinary tract infections are the most common nosocomial infections. Most aspects of the diagnosis, treatment, and prevention of CAUTI are influenced by the tenacity of biofilm-associated uropathogens. Based on our study results, we can conclude that TCP is a quantitative and reliable method to detect biofilm forming micro-organisms. When compared to TM, the TCP can be recommended as a general screening method for detection of biofilm producing bacteria in laboratories.

Keywords: Biofilm, CAUTI, Tube Method, Tissue Culture Method, UTI.

Introduction

In the human body, bacteria reside as biofilms on skin, oropharynx, nose; intestine and indwelling medical devices[1]. Biofilms are associated with many medical conditions including indwelling medical devices, dental plaque, upper respiratory tract infections, peritonitis, and urogenital infections[2]. Bacterial biofilms are responsible for the failure of many medical devices and are associated with many infectious and non-infectious complications[3].

Gram-positive and Gram-negative bacteria both have the capability to form biofilms. Most commonly involved bacteria involved in biofilm formation include Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus viridans, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis and Pseudomonas aeruginosa[4].

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Urinary tract infections (UTIs) are one of the most important causes of morbidity among hospital acquired infections affecting persons of all ages, including young women, children, and the elders[5].

The risk of developing urinary tract infections increases significantly with the use of indwelling devices such as catheters and urethral stents[6].

Surgical-site infections (SSIs) were identified to be the most common hospital acquired infections (HAI) (23.94%), followed by hospital-acquired pneumonia (HAP) (18.31%), urinary tract infection (UTI) (16.9%), catheter-related bloodstream infection (BSI) (16.9%), ventilator-associated pneumonia (VAP) (9.85%), septicemia (8.45%) and others (5.65%)[7].

UTIs account for 30% of all hospital acquired infections (HAI). Of these 30% infections, 80% of them are estimated to be catheter-associated. According to the CDC, Catheter associated urinary tract infections (CAUTIs) are defined as UTI developing in a patient after 48 hours of implantation of an indwelling urinary catheter[8].

Indwelling urinary catheters are standard medical devices utilized to relieve urinary retention and urinary incontinence. Due to their frequent and unnecessary use many patients are placed at risk of complications like Catheter-Associated UTI (CAUTI) which has got considerable economic impact. In most of the cases, the underlying cause of CAUTI is formation of a pathogenic biofilm on the surface of the indwelling urinary catheter[9].

Most important medical significance of biofilm is decrease in susceptibility of antimicrobial agents. Microorganisms those are apparently fully sensitive to antibiotics may become fully resistant in the biofilm mode[10]. Furthermore, the proximity of cells within a biofilm can facilitate a plasmid exchange and hence enhance the spread of antimicrobial resistance[11].

Thus, with the above background, present study had been undertaken to isolate and detect the biofilm forming capacity of uropathogens in catheterized patients with presumed urinary tract infections and two methods were compared for their sensitivity and specificity.

Materials and methods

After taking clearance from Institutional Ethical Committee, A one year cross – sectional study from 1st October 2015 to 30th September 2016 was carried out in the Department of Microbiology & Immunology, at Shri Guru Ram Rai Institute of Medical and Health Sciences (SGRRIM&HS), & Shri Mahant Indiresh Hospital, Dehradun.

Inclusion Criteria

Catheterized Patients with catheterization of greater than 48 hours duration and with clinical suspicion of Urinary tract infections.

Exclusion Criteria

Non catheterized patients with UTIsandPolymicrobial growth.

Selection of Subjects

Over a period of one year from 1st October 2015 to 30th September 2016, a total of 468 urine samples from catheterized patients were collected. Out of 468, 100 were culture positive and were further processed. Cases from all age group and of all sexes admitted in SGRRIM&HS were selected for study.

Study Tools

An informed written consent was taken from all the participants of this study beforehand. A structured patient pro-forma was prepared. Patients were evaluated according to predetermined protocol and history was taken regarding various socio-demographic factors as name of the patient, age, sex, occupation, residence. Relevant medical history was taken for the study purpose.

Study Protocol

Urine samples of catheterized patients from various wards & ICUs received for bacterial culture and sensitivity in the Microbiology section of Central laboratory (an ISO 15189:2012 certified, NABL accredited Laboratory) of SMIH, Patel Nagar, Dehradun. All samples were collected taking all aseptic precautions, followed by their processing and reporting as per the standard methods.

Sample Collection

Under aseptic conditions, catheter specimens of urine were obtained by clamping off above the port to allow collection of freshly voided urine.The wall of the tubing was vigorously cleaned with 70% ethanol.Urine was aspirated via sterile syringe. And hence the integrity of closed drainage system was maintained.Urine which had been standing in the catheter drainage bag was not used.

Sample Transport

As urine is an excellent medium for growth of bacteria, urine was immediately transported to laboratory in sterile Uricol (HiMedia) for processing.

Standard Strains

No.	Strain	Control	Source
1.	**Staphylococcus epidermidisATCC 12228 (non-biofilm producer)**	Negative control	HiMedia
2.	**Pseudomonas aeroginosa ATCC 27853 (Moderate biofilm producer)**	Positive control	HiMedia

Identification of Organisms

All the suspected colonies were subjected to gram staining for initial identification of organism according to their gram reaction and morphology. The isolates were identified as lactose fermenter and non-lactose fermenter on the basis of colony morphology on CLED media.

Biofilm Study

A number of tests are present to detect in-vitro biofilm formation. We have used the following methods:

1. Tube Method (TM)
2. Tissue culture plate (TCP) method

1. Tube Method (TM)

Organism isolated from overnight culture of nutrient agar were inoculated on Trypticase soy broth (TSB) and incubated at 37 ºC for 24 hours. After decanting, the cultures tubes were washed with phosphate buffer saline (pH 7.2). Then the tubes were dried and stained with 0.1% crystal violet. The visible stained film seen with lining the wall and bottom of the tube was taken as positive. Ring formation at the liquid-air interface was considered negative for slime production.

2. Tissue Culture Plate (TCP) Method

Isolates from fresh nutrient agar plates were inoculated in Trypticase soy broth (TSB) with 1% glucose and incubated for 24 hours at 37ºC in stationary condition. Then each test was diluted 1 in 100 with fresh medium. Individual wellsofsterile, polystyrene, 96 well flat bottom issue culture plates wells were filled with 0.2 mlaliquots of the diluted culture sandonly brothserved as control to check sterility and non-specific binding of media. After incubation content of each well was gently removed by tapping the plates. The wells were washed with 0.2ml of phosphate buffer saline (PBS pH 7.2).All wells were fixed with sodium acetate (2%) for 15minutes. Each well was stained with crystal violet (0.1%) for 15 minutes. Excess stain was rinsed off by deionized water and plates were kept for drying.

Opticaldensity (OD) of stainedad herent bacteria was determined with a ELISA auto reader at wave length of 630 nm (OD630nm).

Average OD Value	O.D value	Biofilm Production
≤ ODc/ ODc<-≤ 2× ODc	< 0.12	Non/Weak
2× ODc< -4× ODc	0.12-0.24	Moderate
>4× ODc	>0.24	Strong

Optical density cut off value (ODc) = average OD of negative control + 3× SD of negative control.

Data Management and Statistical Analysis

Data was entered and analyzed on Microsoft Excel and interpreted by descriptive methods in terms of frequency distribution in percentages, proportions, rates ratios etc. Nonparametric tests i.e., chi square was applied to ascertain significance of association.

Results

In our study, maximum numbersof cases werein the age-group of 71-80 years (18%), followed by 41-50 & 61-70 years (16%) each, and

least numbers of cases in age-group of 1-10 years & 91-100 years (1%).

47% cases were Male, and 53% cases were Female with a Male: Female ratio of 0.8:1.

In our study, only 11% of catheterized patients had symptoms of UTI whereas 89% of cases were asymptomatic.

Table 1 shows that isolation of Escherichia coli was maximum (53%) followed by Klebsiella Pneumoniae (16%). Minimum (1%) isolation was seen for Enterococcus casseliflavus.

Table-2 shows that the positivity for biofilm production was more for the groups with duration of catheterization less than 4 days, followed by 4-7 days. None of the case in this study was catheterized for more than 7 days.

Table 3showed that taking TCP as gold standard, sensitivity of TM method was 76.4% and specificity 71.5%.

Table 4 showed that 64.1% of Escherichia coli isolates were positive by tube method and tissue culture plate method, however 75% of Klebsiella pneumonia isolates were positive by tissue culture plate method. Pseudomonas aeruginosa showed a positivity of 88.8% by tissue culture plate method.

Table 1: Spectrum of organisms isolated from catheterized patients (n=100)

Organism isolated	Number of cases	Percentage of Cases
Escherichia coli	53	53%
Klebsiella pneumoniae	16	16%
Pseudomonas aeroginosa	9	9%
Enterococcus faecium	9	9%
Enterococcus faecalis	3	3%
Enterococcus casseliflavus	1	1%
Proteus mirabilis	3	3%
Enterobacter aerogenes	2	2%
Enterobacter cloacae	2	2%
Acinetobacter baumannii	2	2%
Total	**100**	**100%**

Table 2: Duration of Catheterization and Positivity percentage(n=100)

Duration of catheterization	Number of cases	Percentageof cases	Biofilm positive	Positivity (%)
<4 days	64	64%	46	63.8%
4-7 days	36	36%	26	36.1%
>7 days	0	0	0	0
Total	**100**	**100%**	**72**	**100%**

Table 3: Comparison of biofilm detection using Tissue culture plate (TCP) and Tube method (TM) (n=100)

Method	Biofilm production	Sensitivity	Specificity	PPV	NPV	Accuracy
Tissue Culture Plate method (Taken as gold standard for comparison)						
Positive	72(72%)					
Negative	28(28%)					
Tube Method		76.4%	71.5%	87.3%	54%	75%
Positive	63 (63%)					
Negative	37 (37%)					

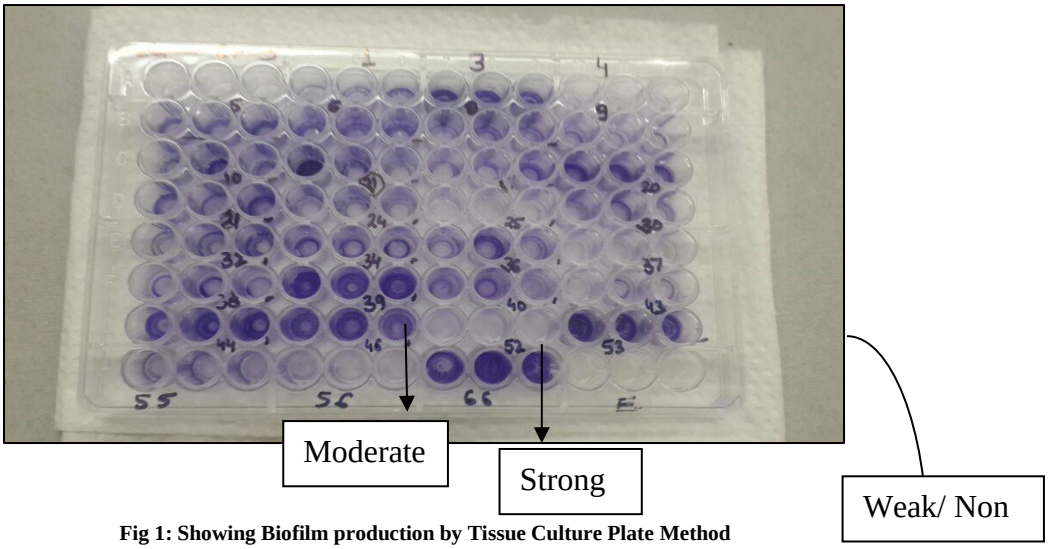

Fig 1: Showing Biofilm production by Tissue Culture Plate Method

Fig 2: Shows Biofilm Production by Tube Method

Table 4: Organism wise evaluation of different methods for biofilm detection (n=100)

Organisms (n)	Positive by Tube method (n%)	Positive by Tissue Culture Plate method (n%)
Escherichia coli (53)	34 (64.1)	34 (64.1)
Klebsiella pneumoniae (16)	8 (50)	12 (75)
Enterococcus faecium (9)	5 (55.5)	7 (77.7)
Pseudomonas aeruginosa (9)	6 (66.6)	8 (88.8)
Proteus mirabilis (3)	2 (66.6)	3 (100)
Enterococcus faecalis (3)	3 (100)	1 (33.3)
Enterococcus casseliflavus (1)	1 (100)	1 (100)
Enterobacteraerogenes (2)	2 (100)	2 (100)
Enterobacter cloacae (2)	1 (50)	2 (100)
Acinetobacter baumannii (2)	1 (50)	2 (100)
Total (100)	**63 (63)**	**72 (72)**

Discussion

The current study was carried out at SGRRIM & HS, Patel Nagar Dehradun. Out of total of 468 urine samples from catheterized patients, 100 were culture positive and were further processed.

In this study it was shown that maximum numbers of cases were in the age-group of 71-80 years (18%), followed by 41-50 years and 61-70 years (16%) each and least number of cases was in the age-group of 1-10 years & 91-100 years (1%) each. This is in concurrence with a study by NivedithaS. et al, wherein, UTIs in catheterized patients were found to be more common in elderly patients (aged > 60 years)[12]. Taiwo S.S. and Aderounmu A.O.A. have also reported the age group of 61-70 years as the most vulnerable group[13]. Our findings also agree with another study by Oni *etal*, wherein 61-70 years was the most affected[14].

Advanced age predisposes to catheter associated UTI. This is because the elderly are more prone to acquired structural abnormalities and neurogenic bladder secondary to stroke or autonomic neuropathy of diabetes than the young people[14]. Thus presence of co-morbidities in the elderly increases the risks of catheter associated UTIs.

This study also showed that 47% cases were Male and 53% cases were Females with a M:F ratio of 0.8:1.In a study by Abdullah N M A et al, the frequency of UTI was also greater in women as compared to men, as 66% of the patients were females and 34% were males principally owing to anatomic and physical factors[15]. Similar results were shown by kashef et al[16]. Our study is also in concurrence with a study by Syed M.A, Devanand P et al[17]. This may be explained by the fact that females are more prone to develop UTIs, probably due to their anatomical &physiological changes like short urethra, its proximity to anus, dilatation of the urethra and stasis of urine during pregnancy[18].

Our work shows that only 11% of catheterized patients had symptoms of UTI (fever, lower abdominal pain, and dysuria) whereas 89% of cases were asymptomatic. Majumder M.I et al. in their study also reported that 92% patients were asymptomatic, 06% had lower abdominal pain, 03% had fever with chills and rigor, and 02% had loin pain[19].

In our study, Considering TCP as gold standard,data from TM was compared. Parameters like sensitivity ,specificity, negative predictive value (NPV) andpositive predictive value (PPV) and accuracy were calculated.True biofilm producers were positive by both TCP and TM. Sensitivity of TM method was found to be 76.4%; specificity 71.5%; PPV 87.3%;NPV 54% and accuracy 75%. Results with slight variation have been reported by many authors[20-24].

The current study showed that out of the 13-gram positive isolates, 69.2% showed biofilm production, whereas out of 87-gram negative organisms, 72.4 % were biofilm producers. A study by Zubair et al and Swarna et al reported 80% and 91% of biofilm detection in gram negative bacilli respectively[25]. Devaraj C. in his study has also reported biofilm production in 76% of 25-gram positive organisms isolated and 93% of 45-gram negative isolates[26].

According to the present study,64.1% of *Escherichia coli* isolates were biofilm positive by both tube method and tissue culture plate method. However, 75% of *Klebsiella pneumonia* isolates were positive by tissue culture plate method and 50% by tube method. *Pseudomonas aeruginosa* showed a positivity of 88.8% by tissue culture plate method. By the TCP method *Enterococcus faecium* showed positivity of 77.7%. Except for *Enterococcus faecalis*, wherein 100% isolates were positive by Tube method, for rest of the isolates TCP method showed a higher positivity than tube method. In a study by Devaraj C., by the TCP method, positivity percentages of 95%, 100%, 80% and 71.5% were seen for Escherichia coli, Pseudomonas aeruginosa,Klebsiella pneumoniaand Enterococcus Spp respectively[24].

Conclusion

Based on this study, we conclude that Catheter associated urinary tract infections are one of the most common nosocomial infections. Most aspects of the diagnosis, treatment, and prevention of CAUTI are influenced by the tenacity of biofilm-associated uropathogens. The complications caused by biofilms can undermine the patient's quality of life and threaten their health. The high incidence of CAUTI and the consequent complications warrants the development and application of effective control strategies.

Based on our study results, we can also conclude that TCP is a quantitative and reliable method to detect biofilm forming micro-organisms. When compared to TM, the TCP can be recommended as a general screening method for detection of biofilm producing bacteria in laboratories.

To establish a standard guideline for the indwelling urinary catheter management in our tertiary hospital, similar types of large-scale prospective studies are required. Also, there is a need to establish standard guidelines on the care of catheters for all the units in the hospital, with a view to prevent the nosocomial infections which are associated with the devices in the patients.

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