

Efficacy of disinfectants on biofilm production in catheter related infections

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Abstract

Background: Biofilms are complex microbial communities that are frequently connected with the colonisation of clinically relevant medical devices such as intravascular and urinary catheters. Approximately 82 percent of nosocomial septicemias are caused by biofilm-producing bacteria colonising these catheters. Disinfectants with broad range multiple target activity are widely used in hospitals for skin antisepsis in order to successfully prevent nosocomial infections. The physician can combat catheter-related infections earlier if the right disinfectant strength and contact duration are used. **Objectives:** To isolate and identify various biofilm producing organisms among catheter-related infections and to evaluate the effect of disinfectants i.e., 0.5% chlorhexidine, 70% alcohol and povidone-iodine against such organisms. **Methodology :** Over a 12-month period, 110 catheter samples taken from 100 in-patients were investigated at the Department of Microbiology, Govt Medical College, Nalgonda. The samples were handled in accordance with industry standards. The microtitre plate method was used to detect biofilm formation, and the effect of disinfectants was studied by incubating biofilms with 0.5 percent chlorhexidine, 70% alcohol, and povidone-iodine for 1, 5, and 10 minutes. **Results :** A total of 50 organisms were isolated. Predominant organisms isolated from intravascular catheters were *Candida* spp. (78.4%) and Coagulase negative staphylococci (18.42 %); from urinary catheters were Coagulase negative staphylococci(30%) and *P. aeruginosa* (10 %). 96.2% of isolates were biofilm producers. Incubation with 0.5% chlorhexidine, 70% alcohol led to a reduction of biofilm optical density after a contact time of 1 min and for povidone-iodine, biofilm optical density reduction was observed after 5 min. Chlorhexidine exhibited significant biofilm reduction in 96.4% clinical isolates, followed by povidone-iodine 92.8% and 70% alcohol (91.6%). **Conclusion:** The disinfectants reduced the biofilm at the different time intervals investigated, but none of them completely eradicated the biofilm. In catheter-related infections, chlorhexidine was more efficient than povidone-iodine and 70% alcohol at reducing biofilm. This study underlines the need for skin antisepsis prior to catheter insertion and strict hand hygiene procedures before the formation of biofilms.

Keywords: catheter-related infection, biofilm, MTP assay, disinfectants.

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Introduction

Biofilms are complex mono- or polymicrobial assemblages that are anchored to abiotic or biotic surfaces[1]. The presence of indwelling catheters introduces an artificial substratum into the body, resulting in biofilm growth[2]. Biofilms increase pathogen pathogenicity and account for a considerable proportion of all human microbial illnesses. Nosocomial infections are the fourth most prominent cause of death. 1, 3 Approximately 60-70 percent of nosocomial infections are related to some form of the implanted medical device[3]. According to a CDC research from 2007, health-care linked illnesses account for an estimated 1.7 million infections. Thirty-two percent are urinary tract infections (UTIs), 22 percent are surgical site infections, 15 percent are pneumonia, and 14 percent are bloodstream infections. According to CDC and NIH data, biofilm infections are predicted to be between 65 and 80 percent.

Biofilm production has been seen in organisms such as Coagulase-negative staphylococci, *S. aureus*, *Enterococcus* spp., *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis*, *Enterobacter* spp., *Candida*, *Acinetobacter* spp., *Citrobacter freundii*, *S. marcescens*, *Streptococci*, and others[4]. Biofilms pose a range of clinical problems to disorders involving uncultivable species, prolonged inflammation, delayed wound healing, and recalcitrance to host immune defence mechanisms. Antimicrobial resistance developed quickly, as did the spread of infectious emboli[3,5]. Regardless of the sophistication of

the implant, all medical devices are prone to microbial colonisation and infection. Currently, research efforts are focused on eradicating or minimising medical device colonisation[5]. The molecular biologist Paul Stoodley explains why biofilms are so tough to produce. The requirement to acquire such demanding laboratory techniques has discouraged many scientists from attempting to deal with biofilms. Although research on biofilms has increased in the previous 20-30 years, the majority of biofilm research to far has concentrated on exterior biofilms. Antibiotics and disinfectants are broad-spectrum biocidal compounds that inactivate micro-organisms on living tissue and inanimate surfaces[6]. Techniques used for skin preparation prior to catheter insertion appear to influence the risk for infection. The CDC guidelines recommend disinfecting skin before catheter insertion and during dressing changes using tincture of iodine and iodophores, 70% alcohol, or preferably, a 2% chlorhexidine based preparation[4].

Objectives

1. To isolate the organisms causing catheter-related infection.
2. To study the biofilm-forming ability of these organisms.
3. To test the efficacy of disinfectants like 0.5% chlorhexidine, 70% isopropylalcohol and povidone-iodine on biofilm formation in catheter-related infections.

The present study was conducted on in-patients from Ayaan Institute of Medical Sciences, Hyderabad.

Materials

Clinical samples were collected from the catheterized patients of both sexes. Specimens included catheter samples (intravascular and Foley's urinary catheters), blood and urine samples from 110 patients. An informed consent was taken from all the catheterized patients under study. Detailed clinical history such as fever with or without chills, burning micturition, frequency of micturition, lower

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abdominal pain, swelling, pain at catheter site and duration of catheterization were recorded in the proforma.

Inclusion Criteria

All the in-patients who have been catheterized for more than 48 hours showing clinical signs of sepsis.

Signs of BSI: Fever, hypothermia, chills, rigors, tachycardia, hypotension, tachypnea.

Signs of UTI : Fever, dysuria, frequency, urgency, suprapubic tenderness.

Exclusion Criteria

- All the catheterized patients without any signs of sepsis.
- All the catheterized patients < 48 hours.

Methods

1. a. Collection of Intravenous Catheters

At the time of catheter removal the site was examined for the presence of swelling, erythema, local rise in temperature and tenderness. The site was cleaned with an alcohol pledget and the catheter was withdrawn with sterile forceps, the externalized portion being directed upward and away from the skin surface. After removal, the site was examined and milked to express any exudate.

For short catheters (< 6 cm), the entire length of the cannula was cut 1 cm below the surface/catheter junction aseptically. For long catheters, two 5cm segments were collected: the tip and the intracutaneous segment. The catheter segments were transported to the laboratory in sterile, dry containers[7].

b. Collection of Blood sample

The venepuncture site was disinfected and with standard aseptic precautions, 5ml of blood was drawn. The sampling needle was safely detached and discarded; then a fresh needle was fitted and the drawn blood was inoculated into the blood culture bottle[8].

c. Gram Staining

Catheter segments were air dried and clotted blood if present was removed with sterile wire. Sterile forceps was used to handle the segment. Opaque catheters were cut in half longitudinally. The staining procedure was done in a series of different sterile petri dishes, each containing Crystal violet, Lugol's iodine solution and dilute carbol fuchsin. It was then air dried and examined under oil immersion at 1000x after being taped firmly on a glass slide[9].

d. Culture of Catheter sample[10]

Catheters were cultured by using the semiquantitative method described by Maki et al. Flamed forceps were used to transfer the entire catheter segment onto the surface of a 5% sheep blood agar plate and the catheter was rolled back and forth four times across the agar surface. Plates were incubated at 37° C for 48 hours, inspected for microbial growth and colonies were enumerated.

Growth >15 colonies on agar plate indicates infection, 1-14 colonies on agar plate indicates contamination. Samples which grew > 15 colonies on plate were considered for the study. All the colony types

were identified by standard microbiological methods.

Catheter segment was inoculated into 5ml trypticase soy broth (TSB) and incubated overnight at 37° C. Subculture was done from the broth onto Blood agar and Mac Conkey agar, incubated for 24 hours and colonies were enumerated and identified.

2. a. Collection of Urine from Catheterized Patients[11]

Urinary catheterization will allow collection of bladder urine with less urethral contamination. Specimen collection from such patients was done with strict aseptic techniques. A pair of gloves was worn while handling urinary catheter. The catheter tubing was clamped off above the port to allow collection of freshly voided urine. The catheter port or the wall of the tubing was then cleaned vigorously with 70% ethanol and urine aspirated with a sterile needle and syringe, the integrity of closed system was maintained to prevent introduction of organisms into the bladder.

Removal of Foley's Catheter

Using another syringe (without the needle), the water or saline injected initially during catheter insertion was drained out. Care was taken to see to it that the entire fluid was removed. Initially one or two gentle tugs were given on the catheter and it was slowly withdrawn. With the help of sterile scissor, a 5 cm portion of the catheter tip was cut off and placed in a sterile test tube and plugged. It was then taken to the laboratory and processed.

Urine culture

A 5% sheep blood agar and a Mac Conkey agar were used for plating. Before inoculation, urine was mixed thoroughly and the top of the container was then removed. The calibrated loop was inserted vertically into the urine in the container. The loop is touched to the centre of the plate. Without flaming or re-entering urine, the loop is drawn across the entire plate, crossing the first inoculum streak numerous times to produce isolated colonies.

A colony count of > 10³ CFU/ml was taken as indicative of a positive culture as all urine samples collected were catheterized urine samples.

b. Processing of Urine Catheters

The catheters were placed in 10ml of 0.15M phosphate buffer saline with 0.1% Tween-80 and sonicated for 30 minutes at room temperature to detach adherent microorganisms. The microbial suspension was vortexed vigorously for 15 seconds to break up clumps. Tenfold serial dilutions of each suspension were plated on 5% blood agar, incubated at 30° C for 18 hours and the mean number of colony forming units was determined.

3. Identification of the organisms by Biochemical reactions[86]

All isolates so obtained were identified by biochemical reactions as per standard protocol.

4. Antibiotic susceptibility testing

Antibiotic susceptibility testing was done on Mueller Hinton agar using Kirby-Bauer disc diffusion method.

Antibiotics used

| Gram positive isolates | Gram negative isolates |
|------------------------------|--|
| i) Penicillin(10 IU) | i) Ampicillin (10 µg) |
| ii) Gentamicin(10 µg) | ii) Ciprofloxacin(5 µg) |
| ii) Ciprofloxacin(5 µg) | iii) Gentamicin(10 µg) |
| Cotrimoxazole(1.25/23.75 µg) | iv) Cotrimoxazole(1.25/23.75 µg) |
| v) Cefoxitin(30 µg) |) Amoxycillin/clavulanic acid(20/10µg) |
| i) Erythromycin(15 µg) | vi) Cefoxitin(30 µg) |
| vii) Clindamycin(2 µg) | vii) Cefotaxime(30 µg) |
| viii) Vancomycin(30 µg) | viii) Ceftazidime(30 µg) |
| ix) Linezolid (30 µg) | ix) Imipenem (10 µg) |

Demonstration of biofilm formation by Microtitre plate Assay[12]

This is also called as the Tissue Culture Plate Assay. Isolates from fresh agar plates were inoculated in 5ml trypticase soy broth with 1% glucose and incubated at 37°C for 18 hours and then diluted 1 in 100 in fresh medium. Individual wells of sterile, polystyrene, 96 well flat bottom tissue culture plates were filled with 0.2 ml aliquots of the diluted cultures and only broth served as control to check sterility.

The tissue culture plates were incubated for 18 hours at 37° C. After incubation, content of each well was gently removed by tapping the plates. The wells were washed four-times with 0.2ml of phosphate buffer saline (PBS pH 7.2) to remove free-floating „planktonic“ organisms. Biofilms formed by adherent sessile organisms in plate were fixed with 2% sodium acetate and stained with 0.1% crystal violet. Excess stain was rinsed off by thorough washing with

deionized water and plates were kept for drying. Adherent cells usually formed on all side wells and were uniformly stained with crystal violet. Optical density (OD) of stained adherent bacteria was determined with a spectrophotometer at a wavelength of 570nm. These OD values were considered as an index of bacteria adhering to surface and forming biofilms. OD value < 0.12 were weak/no biofilm producers, 0.12 – 0.24 were moderate and > 0.24 were strong biofilm producers.

Monitoring the Effect of Disinfectants on Biofilms[13]

The disinfectants used in the present study were: 5% povidone-iodine, 0.5% chlorhexidine and 70% isopropyl alcohol. To test the anti-

biofilm effect of the disinfectants, the biofilms were incubated with 100µl of the solutions for 1, 5 and 10 minutes at 35°C in ambient air. For calculation of the decrease of the biofilm OD, a ratio of the biofilm OD of the isolate incubated with disinfectant solution to the biofilm OD of the same isolate without disinfectant solution was calculated.

Results

The present study was carried out in the Department of Microbiology, Govt Medical College, Nalgonda from April 2019 to March 2020 to look for the effect of disinfectants on biofilm production in catheter-related infections.

Table 1: Age and sex-wise distribution

| Gender | Neonates No. (%) | Adults No. (%) | Total No. (%) |
|--------|------------------|----------------|---------------|
| Male | 32(32%) | 30 (30%) | 62 (62%) |
| Female | 30(30%) | 8 (8%) | 38 (38%) |
| Total | 62 (62%) | 38 (38%) | 100 |

Out of 100 cases, maximum number of samples were from neonates 62 (62%). 62(62%) were males and 38 (38%) were females. The male: female ratio was 4:3

Table – 2: Distribution of samples

| Type of catheter | |
|-------------------------|--|
| Intravascular catheter | |
| Urinary catheter | |
| Central venous catheter | |
| Total | |

A total of 110 catheter samples obtained from 100 patients were studied. Out of the 100 catheters, 79 (79%) were peripheral intravascular catheters (IVC), 20(20%) were Foley's urinary catheters and 1(1%) was a central venous catheter (CVC).

Table 3: Comparison between catheter duration and colonization

| Catheter duration | Total No. of Cases | Catheter Colonization No. (%) |
|-------------------|--------------------|-------------------------------|
| 3 days | 12 | 3 (25%) |
| > 3 days | 88 | 42 (47.72%) |

Maximum number of catheter colonization was observed in 42 (47.72%) with catheter duration of more than 3 days.

Table 4: Relationship between direct catheter staining with Gram stain and semi-quantitative culture

| Types of Catheter | Gram stain Positive No. (%) | Catheter culture positive |
|-------------------|-----------------------------|---------------------------|
| Peripheral IVC | 19 (48.71%) | 39 |

Gram stain was applied to the vascular catheters and not to the urinary catheters. Among 39 culture positive catheter samples, Gram stain was positive in 48.71%. Gram stain did not show any organisms in culture negative cases.

Table 5: Distribution of culture positive samples

| Samples | Culture positive No. (%) | Culture negative No. (%) |
|--------------------------------|--------------------------|--------------------------|
| Intravascular catheters (n=79) | 35 (44.30) | 44 (55.5) |
| Blood (n=79) | 16(20) | 63 (79.75) |
| Urinary catheter (n= 20) | 5 (25) | 15 (75) |
| Urine (n=20) | 2(10) | 18(90) |

35 (44.30%) and 16(20%) of intravascular catheters (IVC) and blood samples yielded growth respectively. Culture was positive in 8 (16.6%) urinary catheters and 2(25.0%) urine samples.

In 3 patients, only blood culture yielded growth but not in catheter culture, indicating the primary site of infection other than vascular catheter.

Table 6: Isolation from the Catheter-tips and corresponding samples

| Catheter type | No. of Positive cultures from | |
|------------------|-------------------------------|---------------------------|
| | Catheter tip | Both tip & Sample*No. (%) |
| Peripheral IVC | 40 | 12 (30) |
| Urinary Catheter | 8 | 2 (25) |
| Total | 48 | 15(31) |

Among 40 patients with positive IVC tip cultures, 12 (30%) organisms were grown from both the catheter culture and blood culture. 2(25%) had same growth on urinary catheter as well as urine, indicating catheter related infection.

Table 7: Distribution of organisms associated with Catheter colonization

| Isolates | IVC (38) No. (%) | Urinary Catheter (10) No. (%) | Total (51) No. (%) |
|----------------------------------|------------------|-------------------------------|--------------------|
| Coagulase negative staphylococci | 7 (18.42) | 3 (30) | 10 (19.60) |
| <i>E. coli</i> | 1 (2.6) | 2 (20) | 1 (1.96) |
| <i>Klebsiella</i> spp. | 2 (5.2) | 1 (10) | 2 (3.92) |
| <i>Enterobacter</i> spp. | - | 1 (10) | 1 (1.8) |
| <i>P. aeruginosa</i> | - | 1 (10) | 2 (3.7) |
| <i>Acinetobacter</i> spp. | - | 1 (10) | 2 (3.7) |
| NF-GNB | - | 1 (10) | 2 (3.92) |
| Candida | 30 (78.94) | - | 31 (60.78) |

A total of 51 isolates were obtained. Of these, 40 isolates were obtained from IVC and 8 isolates were from urinary catheter.

The commonest organisms colonizing IVC were *Candida* spp. 30 (78.94%), CONS 8(19.5%) and *Klebsiella* spp. 2(3.92%), NF-GNB 2 (3.92%). catheter samples were commonly colonized with CONS 3(25%), *P. aeruginosa* and *Acinetobacter* spp. 2(3.7%).

Table 8: Biofilm formation among the clinical isolates

| Organisms | Total | Producers No. (%) |
|------------------------|-----------|-------------------|
| CONS | 10 | 10 (100) |
| Gram negative bacteria | 12 | 8 (66.8) |
| <i>Candida</i> | 30 | 28 (96.5) |
| Total | 50 | 48 (96) |

Out of 50 clinical isolates, 48 (96%) were found to produce biofilm. Based on Optical density values, bacterial adherence were grouped as weak (< 0.12), moderate (0.12 – 0.24) and strong (> 0.24) biofilm producers. In the present study, 48(96%) were strong biofilm producers.

Table 9: Antibiotic susceptibility pattern of biofilm producing organisms

| Organisms | | Antibiotics | | | | | | | | | | | | |
|---------------------------------------|-----|-------------|-----|-------|------|------|------|------|------|-----|----|------|------|-----|
| | | A | CN | G | E | CD | CF | CO | VA | LZ | AC | CE | CA | I |
| Coagulase negative staphylococci (11) | No. | 0 | 1 | 4 | 7 | 9 | 3 | 5 | 10 | 11 | - | - | - | - |
| | % | 0 | 8.1 | 33.2 | 58.3 | 83.3 | 25.0 | 41.6 | 91.5 | 100 | - | - | - | - |
| <i>E. coli</i> (1) | No. | 0 | 0 | 1 | - | - | 1 | 0 | - | - | 0 | 1 | 0 | 1 |
| | % | 0 | 0 | 100 | - | - | 100 | 0 | - | - | 0 | 100 | 0 | 100 |
| <i>Klebsiella</i> spp. (3) | No. | 0 | 0 | 1 | - | - | 1 | 1 | - | - | 0 | 2 | 2 | 3 |
| | % | 0 | 0 | 33.31 | - | - | 33.3 | 33.3 | - | - | 0 | 66.7 | 66.7 | 100 |
| <i>Enterobacter</i> spp. (1) | No. | 0 | 0 | 1 | - | - | 1 | 1 | - | - | 0 | 0 | 0 | 1 |
| | % | 0 | 0 | 100 | - | - | 100 | 100 | - | - | 0 | 0 | 0 | 100 |
| <i>P. aeruginosa</i> (2) | No. | 0 | 0 | 1 | - | - | 1 | 2 | - | - | 0 | 0 | 1 | 2 |
| | % | 0 | 0 | 50.0 | - | - | 50.0 | 100 | - | - | 0 | 0 | 50.0 | 100 |
| <i>Acinetobacter</i> spp. (2) | No. | 0 | 0 | 0 | - | - | 1 | 1 | - | - | 0 | 0 | 0 | 2 |
| | % | 0 | 0 | 0 | - | - | 100 | 100 | - | - | 0 | 0 | 0 | 100 |
| NF-GNB (2) | No. | 0 | 0 | 1 | - | - | 2 | 1 | - | - | 0 | 1 | 1 | 2 |
| | % | 0 | 0 | 50.0 | - | - | 100 | 50.0 | - | - | 0 | 50.0 | 50.0 | 100 |

Coagulase negative staphylococci were 100% resistant to Ampicillin. 91.6% were sensitive to vancomycin and 100% sensitive to linezolid. Gram negative bacteria were 100% resistant to Ampicillin and Amoxycillin – clavulanic acid. 63.6% were sensitive to ciprofloxacin and 100% sensitive to imipenem respectively.

Table 10: Number of clinical isolates showing biofilm reduction by different disinfectants

| Organisms | Contact time (min) | Disinfectants | | | P value* |
|-----------------------------|--------------------|---------------------|----------------------------|-------------------------|----------|
| | | 70% alcohol No. (%) | 0.5% chlorhexidine No. (%) | Povidone-iodine No. (%) | |
| Staphylococci=11 | 1 min | 10 (90.7) | 11 (100) | 6 (53.4) | 0.0213 |
| | 5 min | 7 (62.6) | 11 (100) | 8 (72.7) | 0.1 (NS) |
| | 10 min | 4 (35.3) | 9 (81.7) | 5 (45.3) | 0.1 (NS) |
| Gram Negative Bacteria n=11 | 1 min | 5 (45.2) | 10 (90.7) | 3 (27.2) | 0.0013 |
| | 5 min | 3 (27.2) | 6 (54.5) | 3 (27.2) | 0.1(NS) |
| | 10 min | 0 | 6 (54.5) | 1 (9.0) | 0.02 |
| <i>Candida</i> n=29 | 1 min | 17(58.6) | 20 (68.9) | 13 (44.6) | 0.2 (NS) |
| | 5 min | 8 (27.5) | 18 (62) | 15 (51.5) | 0.031 |
| | 10 min | 4 (13.5) | 17 (58.4) | 9 (31) | 0.0021 |

- Chlorhexidine was highly effective against CONS 11(100%) after 1 & 5 min followed by gram negative bacteria 10(90.9%) after 1 min of incubation.
- 70% Alcohol was more effective against CONS 10(90.9%) after 1 min compared to gram negative bacteria and *Candida*.
- Povidone iodine reduced biofilm in 8(72.7%) CONS after 5 min of contact time. 50% or less gram negative bacteria and *Candida* isolates showed biofilm reduction after 5 min of contact time.

Discussion

The presence of biofilms on intravascular catheters and their role in catheter-related infections is well accepted. Many biofilm infections develop slowly, producing very few symptoms initially, but in the long run, they may produce immune complex sequelae and may act as reservoir of infection. Early detection of biofilm associated infections and implementation of preventive measures are needed to reduce the complications. Antiseptics and disinfectants are biocidal agents that inactivate microorganisms on living and inanimate surfaces. Antiseptic measures is superior to the antibiotic use for biofilm elimination and in view of the increasing microbial resistance to antibiotics, it is of particular significance. In the present study, an attempt has been made to evaluate the efficacy of disinfectants against various clinical isolates producing biofilm in catheter-related infections. Out of 53 isolates, 96.2% were biofilm producers. Chlorhexidine effectively reduced biofilm formation among 100% CONS and 90.9% gram negative bacteria at 1 min. 70% alcohol reduced biofilm in 90.9% CONS at 1 min and povidone-iodine

reduced biofilm in 72.7% gram negative bacteria at 5 min contact time. In the present study, out of 83 peripheral intravascular catheters (IVC) studied, 30% catheters were positive on semi-quantitative culture (SQC). This finding correlates with studies of Subba Rao et al[14] (52.5%) The rate of colonization of urinary catheters in the present study was 31.8%. However, Akash et al in their study have shown 69.6% urinary catheter colonization.

Comparison between catheter duration and colonization

In the present study, 12 catheters were placed for 3 days, 25% were infected. Out of the 88 catheters placed for more than 3 days, 47.7% were infected. The catheter-related infection increased with duration of catheterization, similar to the study of Harsha et al[90], who showed 12.5% catheters infected that were placed 3 days and 34.2% catheters infected which were placed > 3 days. Subba Rao et al[14] has reported 51.2% and 60.5% colonization from catheter duration of 48-96 hours and >96 hours respectively.

Comparison of catheter culture and Gram stain

In the present study, the Gram stain was positive in 51.2% of the

culture positive cases which is comparable with the study of Francois et al[9] who showed sensitivity of 44%. However Cooper et al has reported 100% sensitivity. In the present study, maximum colonization was with *Candida* spp. 30 (78.4 %). It is more, compared to the studies of Akash et al[15](3.7%), Patricia et al (3.7%), Shaimaa et al[17] (5%) and Parameswaran et al[16] (11.4) respectively. In the present study, *Candida* spp. was the predominant isolate from IVC. This may be due to the patient population wherein majority of our patients were from NICU. In our hospital set up, we encounter a higher rate of candidemia in neonates. Coagulase negative staphylococci accounted for 18.42 % colonization which is similar to the studies conducted by Akash et al[15](18.8%) but more compared to the study of Shaimaa et al[17] (10%). *Klebsiella* spp. was isolated in 5.2 % which is similar to the study conducted by Patricia et al[91] (3.7%) but less compared to the study of Akash et al[15] (20.7%). *E. coli* accounted for 2.6% of isolates, similar to the studies of Subba Rao et al[14] (3%), but less compared to the studies conducted by Akash et al[15] (7.5%) and Shaimaa et al[17] (5%). In the present study, most common urinary catheter colonization was seen with Coagulase negative staphylococci (18%), which is more compared to the study of Abdallah et al[18] (11.7%). *P. aeruginosa* accounted for 16.6% of isolates, more than the studies of Akash et al[89] (6.8%) and Abdallah et al[18]. (6.7%). *E. coli* accounted for 20 % of isolates which is less compared to the studies conducted by Akash et al[15] (34.4%), Sangita et al[19] (30%) and Abdallah et al[18] (31.7%). This may be due to the fewer samples in the present study.

Catheter-related infection

In the present study, 30 % samples had same organisms grown from both the IVC culture and simultaneous blood culture indicating catheter-related infection. This study correlates with the studies of Akash et al[15] (43.1%) and Harsha et al[20] (28.5%). 25% of the patients in the present study with urinary catheter had the same growth on catheter tip as urine, similar to the study of Carlos et al[21] (20.3%), but less compared to the study of Akash et al[15] (56.5%).

Biofilm formation among the clinical isolates

In the present study, out of 50 isolates, 96% were found to be biofilm producers, which is in accordance with the studies of Sangita et al[19] (88.8%) and Singhai et al[22] (81.5%).

Antibiotic susceptibility pattern of biofilm producing organisms

In the present study, Coagulase-negative staphylococci were 91.6% resistant to methicillin and 100% resistant to Ampicillin. 91.7% of CONS were sensitive to vancomycin and 100% sensitive to linezolid. This is similar to studies of Harsha et al[20], and Singhai et al[22].

In the present study, all gram-negative bacteria were 100% resistant to Ampicillin and Amoxy-clavulanic acid, similar to Harsha et al[20]. All were susceptible to imipenem, correlating with the investigations of Singhai et al[22], and Srinivasa et al[23].

Effect of disinfectants on biofilm-producing isolates

In the present study, the following disinfectants were selected due to common application in our hospital environment and to help clinicians in choosing the better biocide in regular antiseptics. 0.5% chlorhexidine was the most effective disinfectant found to reduce the biofilm optical density against all clinical isolates at 1 min, which is following the studies of Takeo et al[24], and Theraud et al[6]. Hence, chlorhexidine can be used on biofilms, on implants, on the implant surrounding tissue or the skin surface. 0.5% Chlorhexidine and 70% alcohol were more effective at 1 min, and povidone-iodine was effective at 5 min in reducing biofilm formation. Increasing their contact times had no significant effect on biofilm. The disinfectants were found to reduce the biofilm formation, but none of them completely removed the biofilm. Among the three disinfectants, 0.5% chlorhexidine significantly reduced biofilm formation compared to 70% isopropyl alcohol and 5% povidone-iodine with biofilm reduction more than 90% clinical isolates at 1 min. The study conducted by Maki et al[25], showed similar results.

Conclusion

Catheter-associated infections caused by microbial colonisation and biofilm formation have received increased attention. The use of catheters has resulted in a rise in the number of nosocomial infections.

As a result, detecting biofilm formation in catheter-related infections is critical because it leads to persistent infections with significant antibiotic resistance that are difficult to eliminate. Appropriate skin antiseptics to combat catheter-related infections caused by biofilm-producing microorganisms in the hospital setting, efficient disinfectants should be used before catheter insertion and during subsequent dressing changes, as well as stringent hand hygiene measures and the removal of unneeded catheters. In this investigation, 96.5 percent of the isolates colonising catheters generated biofilm, and 0.5 percent chlorhexidine efficiently decreased biofilm compared to 70% alcohol and 5% povidone-iodine at 1 minute. As a result, for catheter insertion where a short contact duration (1 minute) for skin disinfection is achievable, the following ranking for the tested disinfectants in terms of effective biofilm reduction may be established: 0.5 percent chlorhexidine > 5% povidone-iodine > 70% isopropyl alcohol

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