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The Impact of Different Disinfectants on Microbial Biofilm Formation in Catheter Related Infections

[1]H. Kavitha, [2]Pooja Mansabdar, [3]B. Amrutha Kumari and [4]H. Kavitha

[1,4]Department of Microbiology, KLE JGMMMC, KLE Academy of Higher Education and Research, Hubli, Karnataka, India

[2]Department of Microbiology, Gandhi Medical College, Secunderabad, Telangana, India

[3]Department of Microbiology, Mysore Medical College and Research Institute, Mysore, Karnataka, India

ABSTRACT

The high prevalence of nosocomial infections is related to the use of medical insertion devices such as central venous catheters (CVCs). Most of the microorganisms causing nosocomial infections are biofilm producers, this characteristic allows them to adhere to abiotic surfaces and cause initial catheter infections. Disinfectants with broad range multiple target activity are widely used in hospitals for skin antisepsis in order to successfully prevent nosocomial infections. The clinicians can combat catheter-related infections earlier if appropriate disinfectant with right strength and contact duration are used. This study was conducted to evaluate the effect of disinfectants i.e., 0.5% chlorhexidine, 70%alcohol and povidone-iodine against various biofilm producing organisms among catheter-related infections. A total of 106 catheter samples obtained from 105 patients were processed by standard microbiological methods. The micro titre 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. Of the 106 catheters, 53 isolates were obtained. Out of 53 clinical isolates. 96.2% were found to produce biofilm. Chlorhexidine was highly effective against CONS (100%) after 1 and 5 min followed by gram negative bacteria (90.9%) after 1 min of incubation. 70% Alcohol was more effective against CONS (90.9%) after 1 min compared to gram negative bacteria and Candida. Povidone iodine reduced biofilm in 72.7% CONS after 5 min of contact time but around 50% or less gram negative and Candida isolates showed reduction.

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Key Words

Disinfectants, biofilm formation, catheter-related infections, nosocomial infections, central venous catheters

Corresponding Author

H. Kavitha,
Department of Microbiology, KLE JGMMMC, KLE Academy of Higher Education and Research, Hubli, Karnataka, India

Author Designation

[1,2,4]Assistant Professor
[3]Professor and Head

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INTRODUCTION

Colonization is an important first step in infections and biofilms may be an important mechanism of attachment to host tissue and indwelling medical devices (IMDs). Initial colonization involves bacterial adhesions such as surfaces proteins. (e.g.: teichoic acid, lipoteichoic acid) and pili[1,2]. Biofilms allow members of the microbial community to withstand the shear forces of blood and urine flow, keeping the microorganisms in a nutrient-rich environment[3]. Approximately 60-70 percent of nosocomial infections are related to some form of the implanted medical device[4]. 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 arepredicted 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[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[6]. Antibiotics and disinfectants are broad-spectrum biocidal compounds that inactivate micro-organisms on living tissue and inanimate surfaces[7]. Techniques used for skin preparation prior to catheter insertion appear to influence the risk for infection. The CDC guidelines recommend disinfecting skin before catheter insertionand during dressing changes using tincture of iodine and iodophores, 70% alcohol, orpreferably,a 2% chlorhexidine based preparation[5].

MATERIALS AND METHODS

The present study was carried out in the Department of Microbiology, to test the efficacy of disinfectants like 0.5% chlorhexidine, 70% isopropyl alcohol and povidone-iodine on biofilm formation in catheter -related infections. Samples analyzed were intravascular catheters. Foley's catheters, blood and urine samples from 105 patients. An informed consent was taken from all the catheterized patients under study. Detailed clinical history such as fever with or without chills, burning maturation, frequency of maceration, Lower abdominal pain, swelling, pain at catheter site, duration of catheterization, duration of hospitalization 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 7 BSI: Fever, Hypothermia, Chills, Rigors, Tachycardia, Hypotension, Tachypnea.

Signs of UTI: Fever, Dysuria, frequency, urgency, suprapublic tenderness.

Exclusion Criteria:

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

Collection of Intravenous Catheters: All 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 pledged and the catheter was withdrawn with sterile forceps, the externalized portion being directed upward and away from the stain surface. After removal, the site was examined and milked to express any exudate. For short catheters (<6cm). The entire length of the Cannala was cut 1cm below the surface/catheter junction aseptically. For long catheter, two 5cm segments were collected: the tip and the intracutaneous segment. The catheter segments were transported to the laboratory in sterile. dry containers[8].

Collection of Blood Sample: The venepucture site was cleaned/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 inoculated with the drawn blood into the blood culture bottle[9].

Culture of Catheter Sample: The catheter segment was aseptically cut into 3 fragments: one for culture, another for gram's stain and third one was inoculated into TSB. Catheters were cultured by using the semiquantitative method described by Maki *et al.* Flamed forceps were used to transfer the entire catheter segment into the surface of a 5% sheep blood plate and the catheter was rolled back and forth four times across the agar surface. Plates were incubated at 37o C for 48 hours and 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 calories on plate were considered for the study. All the colony types were identified by standard microbiological methods[10].

Gram's 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 100 after being taped firmly on a glass slide[11].

Collection of Urine from Catheterized Patients: Urinary catheterization will allow collection of bladder urine with less urethral contamination. Specimen collection from such patients was done with strict aseptic techniques. A part of gloves were worn while handling urinary catheter. The catheter tubing was clamped off above the port to allow collection of freshly voided urine. The catheters port or the wall of the tubing was then cleaned vigorously with 70% ethanol and urine aspirated via a sterile needle and syringe, the integrity of closed system was maintained to prevent introduction of organisms into the bladder[12].

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 5cm 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 in oculum 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.

Processing of Urine Catheters: The catheters were placed in 10ml of 0.15m phosphate buffered 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 30oc for 18 hours and the mean number of colony forming units were determined.

Identification by Biochemical Reactions: All isolates so obtained were identified by standard biochemical reactions.

Demonstration of Biofilm Formation by Microtitre Plate Assay: 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 0.2ml aliguots 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 fived with sodium acetate 2% and stained with crystal violet 0.1% Excess stain was rinsed off by thorough washing with deionized water and plates were kept for drying. Adherent cells usually formed on all side walls and were uniformly stained with crystal violet. Optical density (OD) of stained adherent bacterial were determined with a spectrophotometer at a wavelength of 570nm. These OD values were considered as an index of bacterial adhering to surface and forming biofilms. O.D. value <0.12 were weak/no biofilm producers, 0.12-0.24 were moderate and >0.24 were strong biofilm producers[13].

Monitoring the Effect of Disinfectants on Biofilms: The disinfectants used in the present study were: providone-iodine (5%), 0.5% chlorhexidine and 70% isopropyl alcohol. To test the anti-biofilm effect of the disinfectants, the biofilms were incubated with 100ul of the solutions for 1, 5 and 10 minutes at 35oc 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 (control) was calculated[14].

RESULTS AND DISCUSSIONS

A total of 106 catheter samples obtained from 105 patients were studied. Of the 106 catheters. 83 were peripheral intravascular catheters (IVC), 22 were Foley's catheters and 1 was a central catheter, central arterial catheters were not obtained. Out of 105 cases, maximum number of samples were from neonates. 65 were males and 40 were females. The male: female ratio was 1:6:1. Positive tip culture was found in 46.98% peripheral intravascular catheters (IVC) and 31.8% urinary catheters. A total of 53 isolates were obtained. Of these, 41 isolates were obtained from IVC and 12 isolates were from Foley's catheter. 4 isolates were obtained from blood culture only, indicating the primary site of infection other than vascular catheter (not included for the study). The commonest organism

Table 1: ODr of the Biofilms of Staphylococci (mean± SD)

Disinfectants	Incubation time (Min)			
	1	5	10	P*
70% Alcohol	0.555±0.285	0.751±0.349	1.016±0.369	<0.0001
0.5% Chlorhexidine	0.393±0.187	0.467±0.236	0.604±0.307	<0.0001
Povidone- iodine	0.941±0.390	0.774±0.321	0.979±0.395	<0.0001
P**				
AL Vs CH	0.2	0.045	0.023	
AL Vs PI	0.017	0.82	0.6	
CH Vs PI	0.001	0.039	0.028	

ODr= OD of the treated biofilm/ OD of the untreated biofilm

* Friedmann test

**Mann-whiteney test

Table 2: ODr of the Biofilms of Gram Negative Bacteria (Mean±SD)

Disinfectants	Incubation time (Min)			
	1	5	10	P*
70% Alcohol	1.105±0.361	1.270±0.431	1.483±0.519	<0.0001
0.5% Chlorhexidine	0.561±0.371	0.805±0.371	1.016±0.510	<0.0001
Povidone- iodine	1.320±0.413	1.082±0.365	1.461±0.399	<0.0001
P**				
AL Vs CH	0.008	0.023	0.07	
AL Vs PI	0.15	0.56	0.56	
CH Vs PI	<0.001	0.12	0.04	

ODr= OD of the treated biofilm/ OD of the untreated biofilm

* Friedmann test

**Mann-whiteney test

Table 3: ODrof the Biofilms of Candida (Mean±SD)

Disinfectants	Incubation time (Min)			
	1	5	10	P value*
70% Alcohol	1.239±0.981	1.484±1.173	1.798±1.450	<0.0001
0.5% Chlorhexidine	0.763±0.700	0.909±0.860	1.097±1.070	<0.0001
Povidone- iodine	1.231±0.981	1.038±0.823	1.472±1.372	<0.0001
P value**				
AL Vs CH	0.002	0.001	0.001	
AL Vs PI	0.8	0.017	0.06	
CH Vs PI	0.004	0.2	0.036	

ODr= OD of the treated biofilm/ OD of the untreated biofilm

* Friedmann test

**Mann-whiteney test

Table 4: ODr of the Biofilms of Different Isolates (Mean± SD)

		Incubation Time (min)			
Isolates	Disinfectants	1	5	10	P-value*
Staphylococci	70% alcohol	0.555±0.285	0.751±0.349	1.016±0.369	<0.0001
	0.5% chlorhexidine	.393±0.187	0.467±0.236	0.604±0.307	<0.0001
	Povidone- iodine	.941±0.390	0.774±0.321	0.979±0.395	<0.0001
Gram Negative Bacteria	70% alcohol	1.105±0.361	1.270±0.431	1.483±0.519	<0.0001
	0.5% chlorhexidine	0.561±0.371	0.805±0.371	1.016±0.510	<0.0001
	Povidone- iodine	1.320±0.413	1.082±0.365	1.461±0.399	<0.0001
Candida	70% alcohol	1.239±0.981	1.484±1.173	1.798±1.450	<0.0001
	0.5% chlorhexidine	0.763±0.700	0.909±0.860	1.097±1.070	<0.0001
	Povidone- iodine	1.231±0.981	1.038±0.823	1.472±1.372	<0.0001

ODr= OD of the treated biofilm/ OD of the untreated biofilm

*Friedmann test

Table 5: Comparison Between Disinfectants at Different Contact Time

		Contact Time (min) P value *		
Isolates	Disinfectants	1	5	10
Staphylococci	70% alc vs 0.5% CH	0.9 (NS)	0.26 (NS)	0.009 (NS)
	70% alc vs PI	0.01	0.7 (NS)	0.6 (NS)
	0.5% CH vs. PI	<.001	0.014	0.01
Gram Negative Bacteria	70% alc vs 0.5% CH	0.005	0.001	0.3 (NS)
	70% alc vs PI	0.1 (NS)	0.6 (NS)	0.4 (NS)
	0.5% CH vs. PI	<0.001	0.026	0.006
Candida	70% alc vs 0.5% CH	0.002	0.001	0.001
	70% alc vs PI	0.8 (NS)	0.017	0.06 (NS)
	0.5% CH vs. PI	0.004	0.2 (NS)	0.036

*Mann-Whitney test

Table 6: Effect of Disinfectants on Different Biofilm Producers

		Disinfectants			
Organisms	**Contact time(min)**	**70% alcohol**	**0.5% chlorhexidine**	**Povidone-iodine**	**P value***
Staphylococci n=11	1	10 (90.9)	11 (100)	6 (54.4)	0.02
	5	7 (63.6)	11 (100)	8 (72.7)	0.1 (NS)
	10	4 (36.3)	9 (81.8)	5 (45.4)	0.1 (NS)
Gram Negative Bacteria n=11	1	5 (45.4)	10 (90.9)	3 (27.2)	0.001
	5	3 (27.2)	6 (54.5)	3 (27.2)	0.1
	10	0	6 (54.5)	1 (9)	0.02
Candida n=29	1	17(58.6)	20 (68.9)	13 (44.8)	0.2 (NS)
	5	8 (27.5)	18 (62)	15 (51.7)	0.03
	10	4 (13.7)	17 (58.6)	9 (31)	0.002

*Chi square test

colonizing IVC were Candida spp. (73.1%), CONS (19.5%) and Klebsiella spp. (4.8%). Urinary catheter samples were commonly colonized with CONS (25%), P. aeruginosa and Acinetobacter spp. (16.6%). Of the 39 patients with positive IVC tip cultures, 35.8% had similar organisms grown from both the tip culture and simultaneous blood culture indicating catheter related infection. Out of 7 positive urinary catheter cultures, 28.5% had same growth on catheter tip as urine. Maximum number of catheter colonization (48.8%) was observed with catheter duration of more than 3 days. Out of 53 clinical isolates. 51 (96.2%) were found to produce biofilm.

Effect of Disinfectants on Different Microbial Biofilms: Alcohol and chlorhexidine were more effective at 1 min and no further effect was observed at longer contact time. The effect of povidone iodine after contact time of 5 min showed better results than contact time of 1 min and 10 min. Significant difference in efficacy was observed between alcohol and chlorhexidine after 5 and 10 min as well as between chlorhexidine and povidone iodine at all contact time ($p<0.05$). Difference in the effect of alcohol and povidone iodine was observe dafter 1 min. For gram negative isolates, chlorhexidine and alcohol showed biofilm reduction after 1 min contact time but no effect on increasing their contact times. The effect of povidone iodine was high at 5 min when compared after 1 and 10 min. Significant difference in efficacy was observed between alcohol and chlorhexidine after 1 and 5 min as well as between chlorhexidine and povidone iodine after 1 and 10 min ($p<0.05$). No difference in the effect of alcohol and povidone iodine was observed.

After contact time of 1min, chlorhexidine exhibited significant effect than after 5 and 10min. Povidone iodine reduced biofilm OD better after 5min while alcohol showed less reducing effect. Significant difference in efficacy was seen among the three disinfectants. Compared to alcohol, chlorhexidine showed significant reduction at all contact times and after 1 and 10 min when compared to povidone iodine. While difference in effect between alcohol and povidone iodine was after 5 min ($p<0.05$).

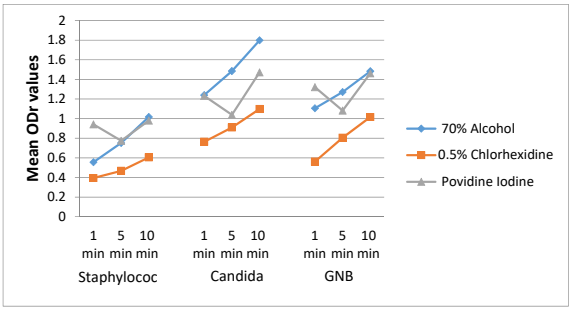

Fig. 1: Contact Time in Minutes in Different Bacteria

Biofilm ODr of the clinical isolates were significantly different.

- Alcohol showed significant reduction in biofilm OD after 1min of incubation., no further reduction was seen at the later time points.
- Chlorhexidine significantly reduced biofilm OD after 1min when compared after 5 and 10min.
- Povidone iodine reduced biofilm OD after 5min. Less effect was seen after 1 and 10min.
- For staphylococcal biofilms effect of alcohol vs.povidone iodine showed significant reduction at 1 min and chlorhexidine vs.povidone iodine was more effective at 1.5 and 10 min ($p<0.05$).
- For biofilm produced by gram negative bacilli, alcohol vs. chlorhexidine showed significant effect at 1 and 5min: chlorhexidine vs. PI exhibited significant effect at 1, 5 and 10min.
- For Candida biofilms, alcohol vs.chlorhexidine showed significant effect at 1, 5 and 10min, alcohol Vs exhibited effect at 1min and chlorhexidine vs. povidone iodine at 1 and 10min.
- Chlorhexidine was highly effective against CONS (100%) after 1 and 5min followed by gram negative bacteria (90.9%) after 1min of incubation.

- 70% Alcohol was more effective against CONS (90.9%) after 1 min compared to gram negative bacteria and Candida.
- Povidone iodine reduced biofilm in 72.7% CONS after 5 min of contact time but around 50% or less gram negative and Candida isolates showed reduction.

In the present study, 0.5% chlorhexidine was the most effective disinfectant found to reduce the biofilm OD against all clinical isolates at 1 min, in accordance with the studies of Takeo[15]. and Theraud[7]. 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. Increasing of 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 against more than 90% clinical isolates at 1min. The study conducted by Maki[16] showed similar results. To increase the efficacy of antiseptic and disinfectant agents on biofilms. Takeo[15] recommended increasing the concentration and/or contact time. All these difficulties emphasize the importance of regular disinfection, before biofilm starts.

CONCLUSION

Catheter associated infections due to microbial colonization and biofilm formation has gained more attention. The incidence of nosocomial infections as a result of catheter use has increased. Therefore, it is necessary to detect the biofilm production in catheter-related infections as they lead to persistent infections, show high antimicrobial resistance and are difficult to eradicate. Hence, appropriate skin antisepsis before catheter insertion and during subsequent dressing changes, strict hand hygiene measures and removing unnecessary catheters are important to combat catheter related infections due to biofilm producing microbes in the hospital set-up. In this study, most of the isolates colonizing catheters produced biofilm (96.2%) and chlorhexidine efficiently reduced biofilm when compared to alcohol and povidone iodine at 1 min. Hence, for catheter insertion where a short contact time for skin disinfection is feasible, the following ranking for the investigated disinfectants regarding their effective biofilm reduction may be set : 0.5% chlorhexidine >70% isopropyl alcohol ^{3}5% povidone iodine.

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