Original Research Article

Antibiotic sensitivity pattern of staphylococcal biofilms formed on hemodialysis catheters

Malleshappa Pavan[1*], J.V. Sathish[2]

[1]Associate Professor, Division of Nephrology, Department of Medicine, Adichunchanagiri Institute of Medical Sciences, B. G. Nagara, Karnataka, India
[2]Professor, Department of Microbiology, Chamarajanagar Institute of Medical Sciences, Yadapura, Chamarajanagar, Karnataka, India

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Abstract

Introduction: Initiation of hemodialysis is commonly performed using non-tunneled dialysis catheters. Biofilms formed by staphylococcus aureus within these catheters are the major cause for catheter related blood stream infections. **Objective and Aim:** Our study aimed at analyzing the effect of ofloxacin, oxacillin and vancomycin on the biofilms formed in non-tunneled hemodialysis catheters. **Materials and methods:** A total of 50 adult patients with end-stage renal disease receiving hemodialysis through non-tunneled catheters, whose catheters were removed for catheter- related blood stream infection were enrolled into this study.Results: Catheter cultures were positive in 32 patients. Staphylococcus aureus (S.aureus) biofilm was found in 25 patients.All 25 strains of S.aureus were susceptible to vancomycin. Ofloxacin and vancomycin significantly decreased the production of biofilm whereas oxacillin did not affect the production of biofilm.**Conclusion:** Our study shows that staphylococcus aureus is the most common organism responsible for biofilm formation in the non-tunneled HD catheters.Ofloxacin and vancomycin significantly reduce biofilm production by S.aureus, indicating that these antibiotics may have role in treatment of infections associated with biofilm.

Keywords: Biofilm; Hemodialysis; Ofloxacin; Oxacillin; Staphylococcus aureus; Vancomycin.

Introduction

Staphylococcal species are associated with hemodialysis catheter related infections that are often difficult to treat[1]. Staphylococcal virulence is caused by a complex process that involves cell-to-cell communication through the release and response to chemical signals in a process called quorum sensing[2]. Staphylococcal biofilm depends on the production of a polysaccharide intercellular adhesin (PIA) encoded by the *ica* operon comprising *icaA, icaB, icaC* and *icaD* genes [3]. Biofilm associated staphylococcal infections are difficult to treat and may require antibiotic concentration 100-1000 times higher than that needed to treat planktonic bacteria[3].The antibiotics that are active against the biofilm-associated bacteria and reduce biofilm formation will be useful in treatment of catheter-associated infections. Studies on the effect of antibiotics on biofilm formation have shown conflicting results[4].Our study aimed at analyzing the effect of different antibiotics on staphylococcal biofilms formed on hemodialysis catheters.

Materials and methods

This study was conducted at Adichunchanagiri Institute of Medical Sciences between May 2014 and April 2015. We studied the effect of antibiotics on biofilm production by Staphylococcus aureus and Staphylococcus epidermidis. Non-tunneled HD catheters were collected from both outpatients and inpatients who were clinically proven to have catheter related blood stream infection[5].

Specimen collection: The tip of the catheter is rolled across the surface of a blood agar plate and the resulting colonies are counted after overnight incubation. A statistical association of >15 CFU with catheter-associated sepsis was established[6].All of the procedures were in accordance with the Helsinki Declaration of 1975.

Idenficiation of Staphylocci

Staphylococcus species isolated from HD catheters were identified using standard procedure[7].The phenotypic characteristics tested were colony morphology, gram staining, catalase test, coagulase test and mannitol fermentation and Novobiocin sensitivity test.Gram-positive cocci of about 1µm diameter arranged irregular clusters, catalase positive, coagulase positive, fermenting mannitol were considered S.aureus. Gram-positive cocci of about 1µm diameter arranged irregular clusters,catalase positive, coagulase negative, mannitol non-fermenter and novobiocin sensitive were considered S.epidermidis (Staphylococcus epidermidis).

Coagulase

The coagulase test used to differentiate coagulase producing Staphylococcus from other Staphylococcus species. EDTA plasma was used.

Slide Coagulase Test

The slide coagulase test was used for the detection of bound coagulase or clumping factor, a drop of saline was placed on each end of a slide, colonies of staphylococcus were emulsified in each of the drops to make two thick suspensions. A loopful of rabbit plasma was added to one of the suspensions mixed and slide was rotated. Formation of visible clumps within 10 sec was considered positive. No plasma was added to second suspension. This was used to see the autoagglutination of the organism. Negative (No clumping within 10 sec) were confirmed by tube coagulase test[9].

Tube Coagulase Test

The tube coagulase test was used to detect free coagulase as a confirming test. Rabbit plasma (1 in 5 dilution) was dispensed in a volume of 0.5 ml in each of 3 sterile test tubes. To the first tube (test)

**Correspondence*
Dr. Malleshappa Pavan
Associate Professor,Division of Nephrology, Department of Medicine,Adichunchanagiri Institute of Medical Sciences, B. G. nagara, Karnataka, India.
E-mail: dr_pavanm@yahoo.co.in

0.5ml of overnight broth culture of the test organism was added, to the second tube (positive control), 0.5ml of broth culture of S.aureus ATCC 25923 was added and to third tube (plasma control) 0.5ml of sterile broth was added. The tubes were incubated at 37°C for 6 h. The tubes were examined for clot at hourly intervals. Formation of clot was considered positive. The samples, which were negative after 6h, were incubated at room temperature overnight and observed[8].

Antibiotic Susceptibility Testing

The antibiotic susceptibility pattern of S.aureus was determined by modified Kirby-Bauer disk diffusion method using the following antibiotics[9].Ofloxacin, Oxacillin and Vancomycin antibiotic disks were tested. The zone of inhibition around each disk was measured in mm and interpreted as per CLSI criteria[10]. S.aureus ATCC 25922 was used as control.

Determination of MIC and Subinhibitory Concentration of antibiotics

The MIC of ofloxacin, oxacillin and vancomycin to staphylococci was determined using broth dilution method[11].

Media preparation

Mueller Hinton broth was dispensed in a volume of 1ml in each of 7 test tubes and sterilized in autoclave at 121°C for 15 min at 15 lb/in^2 pressure. The stock antibiotics solution (1ml) was added to the first tube and than serially diluted in 2-folds to achieve required final concentration.

Inoculation Procedure

Four or five colonies were picked from overnight growth on blood agar and inoculated into 5ml of nutrient broth. The tubes were incubated at 37°C for 2 to 6h and the turbidity was matched with McFarland 0.5 standard (Bacterial concentration $1.5x10^8$ cfu/ml).The tubes were inoculated with 0.01 ml of standardized suspension, resulting in the final inoculum of approximately 10^6 cfu/ml. Growth control was used with each test. And an aliquot of the inoculum was plated to check for purity and inoculum density. S.aureus ATCC 25923 was used as control.

Incubation: The tubes were incubated at 37°C for 18-24 h.

Interpretation of Results

Before reading and interpreting the results, growth control and results with quality control strains were checked. The lowest concentration of antibiotic that inhibited visible growth was recorded as MIC.According to CLSI guidelines S.aureus stains which had MIC of vancomycin ≤4µg/ml were considered susceptible, 8-15µg/ml as intermediate and ≥ 32µg/ml as resistant. S.aureus strains which had MIC of oxacillin ≤ 2µg/ml were considered susceptible, ≥4µg/ml as resistant and S.aureus strains which had MIC of ofloxacin≤ 2µg/ml were considered susceptible, 4 µg/ml as intermediate and ≥8µg/ml as resistant. The next higher dilution to MIC (MIC/2) was considered as subinhibitory concentration of ofloxacin, oxacillin and vancomycin respectively and the subinhibitory concentration (MIC/2) of antibiotics used are as per Table 1.

Effect of antibiotics on biofilm production

Three antibiotics namely ofloxacin, oxacillin and vancomycin were used in the present study to determine their effect on biofilm production by staphylococci using standard procedure.[12]Bacteria were grown in trypticase soy broth at 37°C for 18 h and diluted 1 in 100 in fresh medium supplemented with subinhibitory concentration (MIC/2) of antibiotics and transferred in volume of 0.2ml to 96- well tissue culture plates. The plates were incubated at 37°C for 24 h. Following incubation, the cultures were poured out. The plates were washed three times with phosphate buffered saline (PBS) and remaining bacteria were fixed with Sodium acetate solution (2% w/v) and stained with crystal violet (0.1w/v). Excess stain was rinsed off by thorough washing with deionized water and plates were kept for drying. The optical density of the adherent biofilm was determined at 570 nm (OD_{570}) in an enzyme linked immunosorbent assay reader.Values of > 0.120 were regarded as biofilm positive.

Statistics

Statistical analysis of the results was done using Wilcoxon Signed Rank, Kruskul-Wallis test, and Chi-Square test and p values <0.05 were considered significant.

Results

A total of 50 adult patients with end-stage renal disease receiving hemodialysis (HD) through non-tunneled HD catheter whose catheters were removed for catheter-related bacteremia are studied. Catheter-related bacteremia was defined as the presence of bacteremia in an HD patient with a non-tunneled catheter and in whom no other obvious source of infection was evident. Catheters were removed by a Nephrologist under strict aseptic precautions, after taking informed consent from the patients. Patient's demographic data and clinical information were shown in table 1. Catheter cultures were positive in only 32 patients (Table 3). Staphylococcal biofilm was found in 25 patients (Table 4).All the 25 biofilms were positive for Staphylococcus aureus.Antibiotic susceptibility pattern was studied by disk diffusion method for 25 biofilm positive isolates. Among 25 isolates of S.aureus, four had ofloxacin MIC of 4µg/ml, 6 isolates showed of MIC of 2µg/ml, while 4 showed MIC of 1µg/ml, 7 isolates had MIC of 0.5µg/ml and 4 isolates had MIC of 0.25µg/ml. One isolate showed a oxacillin MIC of 4µg/ml, 9 isolates showed MIC of 2µg/ml, 6 isolates showed MIC of 1µg/ml, 9 isolates had MIC of 0.5µg/ml and none had MIC of 0.25 µg/ml.All 25 strains of S.aureus were susceptible to vancomycin. None of the strains had MIC of vancomycin ≥8µg/ml. The MIC of vancomycin was 4, 2, 1, 0.5µg/ml for 4, 7, 3, and 11 strains respectively (Figure1).The effect of the subinhibitory concentration (MIC/2) of antibiotics on biofilm production by 25 strains of S.aureus is shown in Table5.ofloxacin and vancomycin significantly decreased the production of biofilm. Although there was some strain-to-strain variation, oxacillin did not affect the production of biofilm.

Discussion

We determined the MIC of ofloxacin, oxacillin and vancomycin for all 25 biofilm forming S.aureus isolates by broth dilution method. Our results showed that all 25 strains of S.aureus were susceptibile to vancomycin, 4 strain showed intermediate susceptibility to ofloxacin and one strain was resistant to oxacillin. Our result regarding vancomycin is in agreement with previous reports[13].The biofilm formation is a complex multistep process that can arbitrarily be subdivided into the stages of attraction, adhesion and aggregation. These stages of adherence are mediated by a variety of nonspecific factors, such as hydrophobicity and electrostatic charge, and specific factors, such as bacterial adhesions[14].Because of the importance of bacterial adherence and biofilm formation, a number of investigators have studied the effect of antibiotics on this process[15,16]. A variety of antibiotics have been demonstrated to affect the biofilm formation of gram-negative bacilli and fungi[17,18]. Of particular interest, studies regarding the effect of antibiotics on biofilm formation by staphylococci are less numerous and the conclusions less clear-cut[19,20].Subinhibitory concentration of antibiotics can influence the adherence of a variety of microorganism[21]. The morphologic and ultrastructural alterations of staphylococci in the presence of subinhibitory concentration of antibiotics have been reported.[22]In the present study, ofloxacin, oxacillin and vancomycin were chosen for a number of reasons. The active fluoroquinolone ofloxacin was chosen because of the interesting activity of fluoroquinolones on biofilm elaboration and adherence by gram-negative bacilli[23].Also, there are limited data suggesting that fluoroquinolones decrease biofilm production by staphylococci[24]. Glycopeptide antibiotic such as vancomycin is frequently used to treat patients with staphylococcal infections and also as a prophylactic agent during the implantation of prosthetic devices.In the present study, biofilm formation by S.aureus was significantly decreased by ofloxacin and vancomycin. Although there was some strain-to-strain variation, subinhibitory concentration of oxacillin did not affect the biofilm formation. With regard to vancomycin our observations are in agreement with observations

reported by previous studies[25,26].A previous study on effect of ofloxacin on biofilm production by staphylococcus could not show inhibitory effect of the antibiotic on biofilm formation[27]. Our results of oxacillin is also in disagreement with the previous study,[21]wherein oxacillin and other beta –lactam antibiotics have been shown to enhance of adherence and biofilm formation by staphylococci. Ofloxacin is a nucleic acid inhibitor, which inhibits the DNA synthesis by inhibiting DNA gyrase (topoisomerase). Vancomycin is a glycopeptide that inhibits cell wall synthesis by blocking transpeptidation by binding directly to D-alanyl-D-alanine portion of the pentapeptide which blocks the transpeptide,whereas oxacillin binds to transpeptidase itself.Our study has several limitations. Firstly, only 50 patients were studied. This is a small number considering high prevalence of catheter related bacteremia among the HD patients. Secondly, only limited number of antibiotics were studied. Thirdly, only patients with catheter related bacteremia were studied.

Conclusion

In conclusion,present study show that biofilms can be formed by S.aureus and can pose problems in the treatment of infections associated with biofilm formed on non-tunneled HD catheters. Ofloxacin and vancomycin reduce biofilm production by S.aureus, indicating that these antibiotics may have role in treatment of infections associated with biofilm.

Table1: Subinhibitory Concentration (MIC/2) of Antibiotics

Species	Strain Number	Antibiotics		
		Subinhibitory concentration (µg/ml) (MIC/2)		
		ofloxacin	Oxacillin	Vancomycin
S.aureus	1	0.25	0.25	2
	2	0.25	0.25	1
	3	0.25	0.25	1
	4	0.25	1	0.25
	5	1	1	2
	6	0.25	0.25	0.5
	7	0.25	0.25	0.25
	8	0.5	0.5	0.25
	9	1	1	0.25
	10	0.25	0.25	1
	11	1	1	0.25
	12	0.5	0.5	0.25
	13	0.5	0.5	0.25
	14	0.5	0.5	2
	15	1	1	1
	16	0.5	0.5	0.5
	17	1	1	1
	18	0.5	0.5	0.25
	19	2	2	0.25
	20	0.25	0.25	1
	21	0.25	0.25	0.5
	22	1	1	1
	23	0.25	0.25	2
	24	1	1	0.25
	25	1	1	0.25

Table 2: Patient Characteristics

	Patients with Catheter Related Blood Stream Infections
Number	50
Age (years)	54 +/- 15
Sex -M/F	38/12
BMI (Kg/m^2)	22.4 +/- 2.1
History of DM	41 (50)
Duration of catheter (days)	25+/- 12
Hemoglobin (g/dL)	9.7+/-6.4

Table 3: Biofilms formed on Non-tunneled Hemodialysis Catheters

Sex group	Biofilm Positive		Biofilm Negative	
	Number of isolates	Percentage (%)	Number of isolates	Percentage (%)
Male	20	62.5	15	83.33
Female	12	37.5	3	16.66
Total	32	100	18	100

Table 4: Organisms Isolated from the Biofilm

Organisms Isolated	Number (32)	Percentage (%)
S.aureus	25	78.1
Gram Negative	4	12.5
Polymicrobial	3	9.3

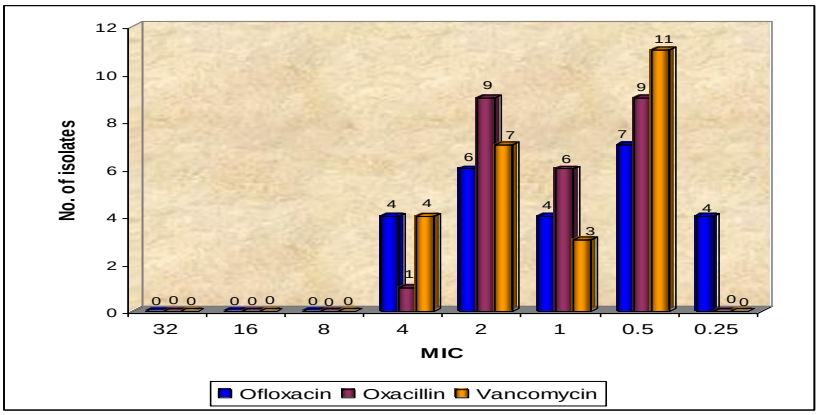

Fig 1: MIC of antibiotics to S.aureus (n=25)

Table 5:Effect of subinhibitory concentration (MIC/2) of antibiotics on biofilm production by *S.aureus*

Condition	OD_{570} value (Mean ± SD)	p value	Statistical significance
Medium with no antibiotic	0.14 ± 0.03		
Subinhibitory concentration(MIC/2) of ofloxacin	0.09 ± 0.02	0.001	Highly significant
Subinhibitory concentration (MIC/2) of oxacillin	0.12 ± 0.05	0.172	Not significant
Subinhibitory concentration(MIC/2) of vancomycin	0.09 ± 0.02	0.001	Highly significant

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