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Original Article

Microbiology Section

The Bacterial Biofilms in Dialysis Water Systems and the Effect of the Sub Inhibitory Concentrations of Chlorine on Them

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ABSTRACT

Introduction: The presence of bacteria in the form of biofilms poses a problem in the fluid pathways of haemodialysis plants and procedures which are aimed to detach and neutralize biofilms are necessary to improve the patient safety and the quality of the healthcare.

The present study was therefore aimed at isolating the organisms which colonized dialysis water systems as biofilms, as well as to study the effect of the sub inhibitory concentrations of chlorine on the biofilms which were produced by these isolates.

Methods: Swabs were used to collect the biofilms which were produced on the internal surface of the dialysis tubing from the dialysis units. This study was conducted at the Department of Microbiology, Kasturba Medical College (KMC), Mangalore, India. The cultures were performed on MacConkey's agar and blood agar. The organisms which were isolated were identified and antibiotic sensitivity tests were performed. The biofilm production was done by the microtitre plate method of O'Toole and Kolter. The biofilm production was also studied in the presence of sub inhibitory concentrations of chlorine.

Results: *Acinetobacter spp* and *Pseudomonas aeruginosa* were the two predominant organisms which colonized the dialysis water systems as biofilms. The sub inhibitory concentrations of chlorine did not bring about any decrease in the biofilm production by the isolates. On the contrary, there was an increase in the biofilm production.

Conclusion: Our study highlighted the importance of using appropriate methods to improve the quality of the water in dialysis units. This in turn, may help in reducing the biofilm formation in the water systems of dialysis units and thus, contribute to the prevention of hospital acquired infections in the patients who need haemodialysis.

Key Words: Biofilms, Dialysis water, Chlorine, *Pseudomonas aeruginosa, Acinetobacter spp*.

INTRODUCTION

The development of a biofilm is a very effective method which helps bacteria to survive in hostile conditions and to resist biocides and antimicrobial substances. Bacteria attach to surfaces and they aggregate in a biopolymer matrix to form biofilms [1]. Studies on biofilms have shown their presence in many prosthetic devices which are used in nephrology as well as in the fluid pathways of haemodialysis plants and monitors. The biofilm formations in dialysis systems may be relevant, because they continuously release bacterial compounds and are resistant to disinfection [2]. Once it is present, this community of bacteria increases the resistance to biocides due to slime production and, as a result, the chemical products for dialysis monitor disinfection and descaling procedures do not result in an effective treatment [3]. The bacterial fragments which are generated by biofilms are able to cross the dialysis membrane and stimulate an inflammatory response in the patient. Such an inflammation has been implicated in the mortality and the morbidity which are areassociated with dialysis [4]. An ultrapure dialysate is a goal in modern haemodialysis, and ultrafiltration is used to obtain sterile and apyrogen fluids. A microbial colonization of the ultrafilters may occur if, due to inadequate disinfection protocols, the membrane is exposed to a persistent bacterial contamination, and biofilms are allowed to form and to grow. As more and more data link the final dialysate microbial contamination to the clinical effects of the bioincompatibility from the chronic inflammation in dialysis patients, attention has to be focused on the possibilities for the avoidance of biofilms [3].

A germ- and endotoxin-free dialysate does not exclude the risks and hazards of bacteria and an endotoxin discharge from the biofilms, which may have developed on the fluid pathway tubing, may act as a reservoir for a continuous contamination. Efforts in the optimization of the cleaning and disinfection procedures which are used for haemodialysis systems should aim at detaching and neutralizing biofilms whenever necessary [5].

The composition of the dialysis fluid is crucial in the normalization of the electrolyte composition of plasma water, homeostasis and the acid-base balance, and it should be individualized to the patient's requirements in the same way as the blood and dialysate flow rates are individualized, to ensure an optimal comfort and minimal complications which are associated with the procedure [6].

Monitoring the infections and the antibiotic resistance patterns in dialysis populations is an important component of the efforts which are made to improve the patient safety and the quality of health care [7]. There have been very few reports from India with regards to the biofilms in dialysis units and the methods which are used for the prevention of such biofilms.

This study was aimed at detecting and isolating the organisms which colonized dialysis water system as biofilms and at studying their biofilm production as well as the effects of the sub inhibitory concentrations of chlorine on the biofilm production.

MATERIALS AND METHODS

Swabs were used to collect the material from the internal surfaces of the dialysis tubing from the dialysis units at Government Wenlock Hospital and KMC Hospital, Ambedkar Circle. A total of 100 swabs were collected (with a 95% confidence level and 80% power, the sample size came up to 96).The samples were processed at the Department of Microbiology, Kasturba Medical College, Mangalore within 1 hour. An institutional ethics committee clearance was obtained for the study. A gram smear examination of the smears which were prepared from the swabs was performed. The swabs were then inoculated onto MacConkey's agar and blood agar. The heterotrophic bacterial counts which employed the pour plate method, were performed. The plates were incubated at 37^{O}C for 18 hours [8].

The bacterial growth was identified by checking for the standard biochemical reactions [9]. Antibiotic sensitivity tests were performed by the Kirby Bauer disk diffusion method by using antibiotic disks from Hi Media, India [10].

The biofilm production was done by the microtitre plate method of O'Toole and Kolter and it was quantified spectrophotometrically by using an ELISA reader [11]. The organisms were grown on Trypticase soya agar for 48 hours. The colonies of the organisms were emulsified by using phosphate buffered saline (PBS; pH 7.4). The turbidity was compared with the Mc Farlands 0.5 standard (10^8 org/ml). Each microwell was inoculated with 200µl of the suspension. The microtitre plates were incubated for 24 hours at 37^{O}C, after which the contents were aspirated. Bouin's fixative was added and they were kept for 10min at room temperature, after which the contents were aspirated and the plates were stained with crystal violet for 1 minute. The OD_{490} values were recorded spectrophotometrically.

Effects of the sub inhibitory concentrations of chlorine: To study the effects of the sub inhibitory concentrations of chlorine on the biofilm production, the microorganisms were grown in nutrient broth for 24 hours. The sub inhibitory concentration of chlorine was determined by the MIC method [10]. The biofilm production was studied in the presence of the sub inhibitory concentrations of chlorine. All the results were tabulated and a statistical analysis was performed.

STATISTICAL ANALYSIS

This was done by the Wilcoxon signed rank sum test. P values of <0.05 were considered to be significant. A statistical package, SPSS, vers.16.0 was used to do the analysis.

RESULTS

Of the 10 organisms which were isolated, 6 isolates were identified as *Acinetobacter spp* and 4 were identified as *Pseudomonas aeruginosa*. All the isolates were sensitive to amikacin, ceftazidine, ciprofloxacin, cotrimoxazole, gentamicin, imipenem and piperacillin. However, 33.3% of the *Acinetobacter spp* and 50% of the *Pseudomonas aeruginosa* isolates showed resistance to gentamicin.

The biofilm production of the isolates before and after the exposure to chlorine has been shown in [Table/Fig-1].

The mean and standard deviation of the OD_{490} values of the isolates before and after the exposure to chlorine has been shown in [Table/Fig-2].

DISCUSSION

The organisms which were isolated were *Acinetobacter spp* and *Pseudomonas aeruginosa*. These two organisms are predominant opportunistic pathogens which may cause infections in dialysis patients. So, it is important to consider their presence as biofilms in dialysis units and also to prevent their existence in the form of biofilms. In the United States, forty-three outbreaks of waterborne nosocomial infections had been reported between January 1, 1966 and December 31, 2001, and an estimated 1400 deaths occur each year in the United States as a result of waterborne nosocomial pneumonias which are caused by *Pseudomonas aeruginosa* alone [12]. Because of the seriousness of these nosocomial waterborne infections and the availability and the proven effectiveness of sterile water, it has been recommended that hospitalized patients who are at a high risk for infection must avoid exposure to hospital water and use sterile water instead [12].

The sub inhibitory concentrations of chlorine did not bring about any decrease in biofilm production of various isolates. A particular study showed a reduction in the adherence in 500ppm sodium hypochlorite [13]. However, in our study, we did not find any decrease and this may be due to the increased adherence of bacteria as a protective mechanism, to evade the action of chlorine. On the contrary, there was an increase in the biofilm production as per the data in [Table/Fig-2].

Sl. No.	Isolated organism	Biofilm production (before exposure to chlorine) (OD_{490})	Biofilm production (after exposure to chlorine) (OD_{490})
1	*Acinetobacter spp*	0.160	0.320
2	*Pseudomonas aeruginosa*	0.096	0.492
3	*Acinetobacter spp*	0.127	0.345
4	*Pseudomonas aeruginosa*	0.101	0.397
5	*Acinetobacter spp*	0.126	0.382
6	*Acinetobacter spp*	0.161	0.217
7	*Acinetobacter spp*	0.361	0.264
8	*Pseudomonas aeruginosa*	0.160	0.540
9	*Acinetobacter spp*	0.138	0.723
10	*Pseudomonas aeruginosa*	0.124	0.475

[Table/Fig-1]: Biofilm production of the isolates before and after exposure to chlorine

	N	Mean	Std. Deviation	Paired differences Mean ± SD	P
Before exposure to chlorine	10	0.155	0.076	0.260 ± 0.191	0.009 HS
After exposure to chlorine	10	0.415	0.148		

[Table/Fig-2]: Mean and standard deviation OD_{490} values and the comparison between the isolates before and after exposure to chlorine
SD- Std. deviation, HS- Highly significant

One of the previous studies has reported that the bacteria showed an increase in size in the presence of the sub inhibitory concentrations of chlorine [14]. Our study indicated that in the presence of adverse conditions, the bacteria may change their morphologies and shift towards the production of more biofilms as a protective mechanism, to overcome the adverse conditions. It is likely that the appropriate concentrations of the inhibitory agent may kill the

bacteria, while the sub inhibitory concentrations may facilitate a biofilm production.

A study which was conducted on the quality of the dialysis water showed that the risk of a chemical contamination was due mainly to the primary pollution of the municipal water, whereas the most important microbiological problem was the control of bacterial growth in the water treatment and distribution system [15]. The dialysis water treatment implies various levels of pre-treatment, a final purification module (which, in many cases, is Reverse Osmosis {RO}) and a hydraulic circuit for the distribution of the purified water. The RO-based treatment systems produce water of an optimal chemical and microbial quality, and so, the dialysis units need to concentrate on maintaining this quality level in the long term, by means of effective maintenance and disinfection strategies. The most widely accepted standards for water purity are those which are recommended by the Association for the Advancement of Medical Instrumentation and the European Pharmacopea, which respectively allow bacterial growths of <200 and <100 c.f.u./ml, and endotoxin concentrations of <2 and <0.25 IU/ml. However, a number of multicentre studies have reported that 7-35% of the water samples have bacterial growth of >200 cfu /ml, and that up to 44% have endotoxin levels of >5 IU/ml. This study indicated that the microbial quality of the dialysis fluids is still a too often neglected problem, particularly, as there is an evidence of a possible relationship between the dialysis fluid contamination and the long-term morbidity [15]. It has been found that The Japanese Society for Dialysis Therapy (JSDT) standard was the most stringent in the world and that its compliance rate was excellent. It guarantees an ultra-pure dialysis fluid and this was obtained by installing Endotoxin Retention Filters (ETRFs) in the dialysis machines. The bacteriological water qualities of the dialysis fluid are extremely high in most of the Japanese dialysis facilities and these might have a close relationship with the high dialysis patient survival rates in Japan [16]. Further research on the role of the ETRFs in the prevention of biofilms, is warranted.

Hence, our study highlights the importance of using appropriate methods to improve the quality of the water in dialysis units. This in turn, may help in reducing the biofilm formation in the water system of the dialysis units and thus, contribute to the prevention of hospital acquired infections.

CONCLUSION

The *Acinetobacter spp* and *Pseudomonas aeruginosa* were the two predominant organisms which colonized the dialysis water systems as biofilms. Most of the isolates were sensitive to all the antibiotics except gentamicin.

The sub inhibitory concentrations of chlorine did not bring about any decrease in the biofilm production of various isolates. On the contrary, there was an increase in the biofilm production. This may be due to the increased adherence of bacteria, as a protective mechanism, to evade the action of chlorine. Our study highlighted the importance of using appropriate methods to improve the quality of the water in dialysis units. This in turn, may help in reducing the biofilm formation in the water system of dialysis units and thus, contribute to the prevention of hospital acquired infections in patients who need haemodialysis.

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