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RESEARCH ARTICLE

Effect of chlorhexidine on oral airway biofilm formation of *Staphylococcus epidermidis*

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ABSTRACT

Objective: Biofilm formation of microorganisms on the surface of airways may lead to supraglottic colonization that may cause lower respiratuar tract infections. Studies searching the efficiency of local disinfectants on biofilm formation are limited. The aim of this study was to investigate the effects of chlorhexidine coated airways on biofilm formation of *Staphylococcus epidermidis*.

Methods: Culture and electron microscopy methods were used for biofilm assessment. Airways were divided into two groups to investigate the effects of chlorhexidine on number of bacteria attached to the airway and biofilm formation. Group 1(control): naive material, *S. epidermidis*, Group 2: chlorhexidine coated material, *S. epidermidis*. No process was applied in Group 1. Chlorhexidine gluconate (0.2%) was sprayed on the surface of naive material for four seconds and then left to dry in air, in Group to. Number of bacteria attached to the airway were counted by microbiological methods and biofilm formation was shown by Scanning Electron Microscope (SEM). Mann-Whitney u test was performed for statistical analyses.

Results: In Group 2, bacteria numbers were 1x102-8x102 cfu/ml, whereas they were 3x103-1x104 cfu/ml in Group 1. Chlorhexidine decreased number of microorganisms attached to the airways with statistical significance (p=0.04). The results of the electron microscopic evaluation were in accordance with the bacteriological findings.

Conclusion: This study has shown that chlorhexidine coating can successfully reduce the number of adhered bacteria and biofilm formation on airways. *J Microbiol Infect Dis 2015;5(4): 162-166*

Key words: Chlorhexidine; oral airway; biofilm; *Staphylococcus epidermidis*

Klorhekzidinin Staphylococcus epidermidis'in airway üzerinde biofilm oluşturmasına etkisi

ÖZET

Amaç: Airway yüzeyinde biofilm oluşması supraglottik kolonizasyona, bu da alt solunum yolu enfeksiyonlarına neden olabilir. Lokal dezenfektanların biofilm oluşumuna etkisini araştıran pek fazla çalışma bulunmamaktadır. Bu çalışmanın amacı airwayleri klorhekzidinle kaplamanın *Staphylococcus epidermidis*'in biofilm oluşturmasına etkisinin araştırılmasıdır.

Yöntemler: Biofilm deneyi için kültür ve elektron mikroskobi yöntemleri kullanıldı. Airwayler, klorhekzidinin biofilm oluşumu ve bakteri sayısına etkisini araştırmak üzere, iki gruba ayrıldı. Grup 1 (Kontrol Grubu): Katkısız airway, *Staphylococcus epidermidis*, Grup 2: Klorhekzidinle kaplanmış airway, *Staphylococcus epidermidis*. Grup 1'e bir işlem yapılmazken, Grup 2'ye 4 saniye boyunca % 0,2 konsantrasyonda klorhekzidin sprey sıkıldı ve kurutuldu. Materyale tutunan bakteri sayısı mikrobiyolojik yöntemle incelendi, biofilm oluşumu elektron mikroskobik inceleme ile gösterildi. İstatistik analiz için Mann-Whitney U testi kullanıldı.

Bulgular: Grup 2'de bakteri sayısı 1x102-8x102 cfu/ml iken Grup 1'de 3x103-1x104 cfu/ml idi. Klorhekzidinin airwaye tutunan bakteri sayısını istatistiksel olarak anlamlı oranda azalttığı görüldü (p=0,04). Elektron microskobik incelemede de sonuç uyumluydu.

Sonuç: Bu çalışmada, klorhekzidinin airway üzerinde biofilm oluşumu ve tutunan bakteri sayısını azaltmada etkili olduğu gösterildi.

Anahtar kelimeler: Klorhekzidin, airway, biofilm, *Staphylococcus epidermidis*

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INTRODUCTION

Oral airway devices may be the source of nosocomial infections, while being used for airway control during anesthesia and in Intensive Care Units (ICUs). Infectious microorganisms may form a biofilm layer by adhering to the surface of the foreign objects. Biofilm is a microorganism group adhering on the layer of the embedded extra-cellular polysaccharide matrix.[1-3] Biofilm formations developing on airways may lead to supraglottic tracheal colonization and may increase the risk of ventilator associated pneumonia (VAP).[1]

Biofilm formation is an essential factor that creates significant difficulty for the treatment of some infections like cystic fibrosis in lungs, oral cavities and medical implants. Staphylococcus aureus and *Staphylococcus epidermidis* are bacteria commonly encountered in medical implant infections.[4] It is possible to reduce microbial attachment, avoid colonization and decrease equipment related infection intervals by coating the polymer structure inside medical equipment such as antimicrobial coated intravascular catheters. A chlorhexidine and sulfadiazine combination forms synergistic antimicrobial effect against especially pseudomonas, proteus and staphylococci. Another imperative aspect is that there is no evidence for bacteria becoming more resistant through this application.[5]

The most recurrent nosocomial infection encountered in intensive care units is pneumonia. This is known to be related with long-term mechanical ventilation and endotracheal intubations. The oropharynx is an early colonization site for bacterial pathogens. There is a strong relationship between oropharyngeal colonization and nosocomial pneumonia. Endotracheal tubes (ETT) and airways are the devices that supply environments for this colonization to initiate. Bacteria loaded biofilm in the airway apparatus which make ventilator parts become colonized rapidly, are the source of infection especially in VAP. Biofilm formation and being resident within the biofilm, precipitate bacteria with resistance to antibiotics and host defense.[5,6]

Studies about the effects of local disinfectant use on bacterial biofilm are limited. Antiseptics have been used for a long time during clinical applications. However, exposure to antiseptic agents may have negative effects on human health.[7]

Chlorhexidine chlorophenyl is a biguanide structured cationic biocide. It is often used in hospitals as a topical antiseptic and for periodontal illnesses. Chlorhexidine is the most effective antimicrobial agent with a wide antibacterial activity spectrum and low toxicity. It only contacts with the lipid layer superficially and changes viscosity. Chlorhexidine adheres to the skin and mucosal membranes. The positive charged chlorhexidine molecules interact with the negative charged extracellular matrix of the cell surface. Cytoplasmic content loss, membrane damage and enzyme inhibition occur. Chlorhexidine decreases virulence by adhering bacterial components such as lipopolysaccaride and proteases, thereby inhibits biofilm formation. It shows bactericide effects at early stages and bacteriostatic effects in the long run.[2,3,8-10]

It has been identified that chlorhexidine with a 0.12 concentration regulates oral hygiene and reduces oral colonization of intubated patients receiving mechanical ventilation in ICUs. This concentration of chlorhexidine reduced VAP incidence in elective cardiac surgery patients although it is ineffective for trauma patients.[8,-12] Since high dosages result in extreme damage, 0.2% concentration is advised mostly.[12]

The aim of this study was to investigate the effect of airway coating with chlorhexidine spray on biofilm formation caused by *Staphylococcus epidermidis*.

METHODS

This study was conducted at the Kırıkkale University Faculty of Medicine, Department of Clinical Microbiology and Infection together with the Kırıkkale University Electron Microscopy Center. The study was approved by the Ethics Committee of the Kırıkkale University.

In order to investigate the effects of chlorhexidine on bacteria counts and biofilm formation, airways were divided into two groups. These study groups; Group 1 (Control Group): 10 naive materials, *S. epidermidis* and Group 2: 10 chlorhexidine coated materials, *S. epidermidis*.

Coating method: No application was done on airways in Group 1. In Group 2, chlorhexidine gluconate 0.2% was sprayed on the surface of the naive material for four seconds in a sterile environment. The airways were left to dry then.

In-vitro microbiological method

For the formation of biofilm on airways, a previously validated method was used.[13] A slime positive *S. epidermidis* (clinic isolate) suspension at 0.5 McFarland turbidity was prepared in a sterile cup (About

80 ml suspension was prepared for each material in order to coat the whole surface of the airway). Airways in the groups (chlorhexidine not sprayed and sprayed) were incubated for 6 hours, at 36.5°C. After the removal of airways from the suspensions, they were flushed in sterile distilled water and then vortexed for 1 minute in 20 cc. normal saline (NS). One hundred mcl was taken from the NS and was cultured in sheep blood agar and incubated for 24 hours at 37°C. Clinical and Laboratory Standards Institute (CLSI) standards were used for microbiological methods.[14]

Analyzed parameters

Bacteria counts isolated from airway surfaces were quantified with a standard microbiological method. After the procedures, one additional specimen from each group was coated with gold palladium alloy using a sputter-coating technique (Hummer VII; Anatech Ltd, Alexandria, Va) and examined by scanning electron microscopy (SEM) (JSM-5600 Scanning Microscope; JEOL Ltd, Tokyo, Japan) between x6000 and x8000 magnification to observe the topographic patterns. The assessor was blind to the design of the study.

Statistical analysis was performed with the SPSS 15.0 statistical software. Mann-Whitney U test was used for the data analyses, and statistical significance was considered at $p<0.05$.

RESULTS

When the culture results of the biofilm formation of the groups were evaluated it was identified that chlorhexidine coating reduced biofilm forming bacteria counts with statistical significance ($p=0.04$). Median bacteria count for Group 1 was 7.1 x 103 and 2.8 x 102 for Group 2 (Table 1).

Table 1. Bacteria counts in the groups

Group 1	Group 2
$5x10^3$ cfu/ml	$2x10^2$ cfu/ml
$8x10^3$ cfu/ml	$8x10^2$ cfu/ml
$3x10^3$ cfu/ml	$1x10^2$ cfu/ml
$1x10^4$ cfu/ml	$1x10^2$ cfu/ml
$7x10^3$ cfu/ml	$2x10^2$ cfu/ml
$8x10^3$ cfu/ml	$4x10^2$ cfu/ml
$6x10^3$ cfu/ml	$2x10^2$ cfu/ml
$9x10^3$ cfu/ml	$3x10^2$ cfu/ml
$8x10^3$ cfu/ml	$4x10^2$ cfu/ml
$7x10^3$ cfu/ml	$1x10^2$ cfu/ml

Cfu=Colony-forming unit, Group 1; naive material, *S. epidermidis*, Group 2; chlorhexidine coated material, *S. epidermidis*

Electron microscopic investigation that evaluated the bacteriological study expressed similar results. Figure 1 shows the electron microscopic images of naive airways. Naïve material, biofilm formation in chlorhexidine non-coated (Group 1) and coated (Group 2) airway images are shown in Figures 1, 2 and 3, respectively. Chlorhexidine coated airways reduced biofilm formation significantly.

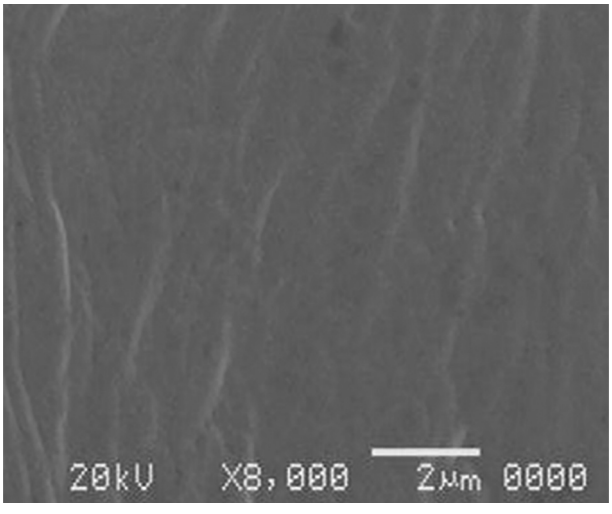

Figure 1. Shows the electron microscopic images of naive airways

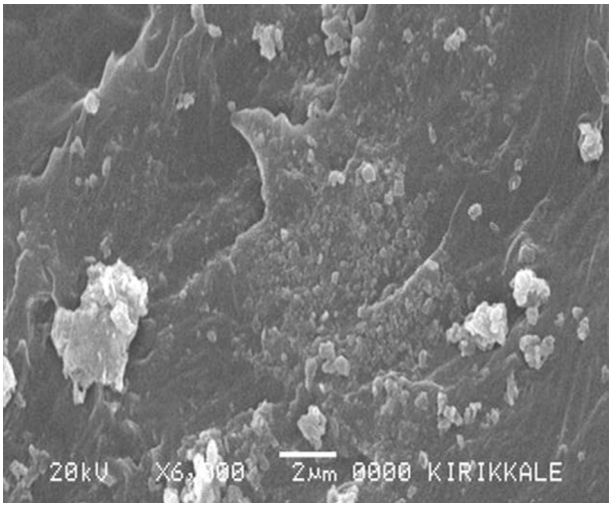

Figure 2. Biofilm formation in chlorhexidine non-coated material (Group 1)

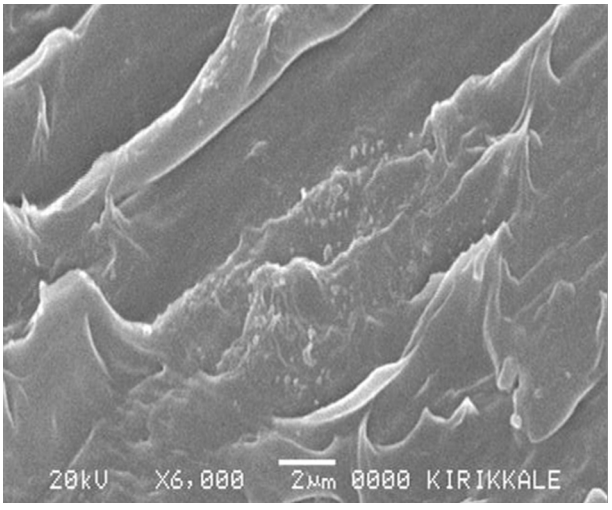

Figure 3. Biofilm formation in chlorhexidine coated material (Group 2)

DISCUSSION

VAP, one of the most common nosocomial infections in ICU, effects length of hospital stay and may cause mortality. One of the most essential reasons of VAP is oropharyngeal colonization with endogenous flora or with exogenic pathogens acquired from the ICU. Nosocomial infections related to non-invasive mechanical ventilation are lower, when compared with invasive mechanical ventilation. In ICU patients, oral flora slides to the domination of gram positive pathogens like S. aureus and Gram negative bacilli. Oropharyngeal colonization of patients undergoing mechanical ventilation is followed by tracheobronchial colonization. A significant reason for endemic VAP is nosocomial organisms and especially the aspiration of oropharyngeal secretions colonized with these microorganisms. Reducing oral micro-organisms contributes to the prevention of VAP. It has been shown that oral care with the chlorhexidine antiseptic solution reduces oral microbial colonization and VAP.[1]

ETT and airways are reservoirs for the biofilm forming micro-organisms through adhering to foreign particles. Biofilms show high resistance to antibiotics and host defenses. They establish areas for colonization with antibiotic resistant nosocomial pathogens. There are in vitro studies that shows significant reductions in number of microorganisms in antiseptic (chlorhexidine and silver or chlorhexidine and Gentian Violet) soaked ETT's.[1,15-16]

Chlorhexidine chlorinated phenolic disinfectant, changes permeability of bacterial cell membrane and results in the death of damaged bacteria. It inhibits bacterial adhesion. There are also several studies which show the insufficient effects of chlorhexidine against oral biofilm formation.[17,18]

Biofilm formation on airways is known as colonization. Any foreign particles colonized on this area become a cause of infection. Therefore, biofilm formations need to be prevented. Changing the surface electricity, altering the surface geography and coating the surface with antibiotics, antiseptics and other biofilm preventers are the avoiding strategies of the biofilm formation.[4,19-21] While some of these methods are currently in use at clinical environments, experimental studies are still continuing for the others.[22,23]

This experimental study demonstrated the reducing effect of chlorhexidine on bacterial adherence and biofilm formation on the airways. However, further experimental or clinical studies related to chlorhexidine coated materials are needed.

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