RESEARCH ARTICLE

Effect of chlorhexidine on oral airway biofilm formation of Staphylococcus epidermidis

Ünase Büyükkoçak¹, Canan Ağalar², Turgut Deniz³, Sabahat Çeken⁴, Fatih Ağalar⁵

¹ Kırıkkale University Faculty of Medicine, Department of Anesthesiology and Reanimation, Kırıkkale, Turkey
² Fatih Sultan Mehmet Research and Training Hospital, Dept. of Infectious Diseases and Clinical Microbiology, İstanbul, Turkey
³ Kırıkkale University Faculty of Medicine, Department of Emergency Medicine, Kırıkkale, Turkey
⁴ Dr Abdurrahman Yurtaslan Oncology Research and Training Hospital, Department of Infectious Diseases and Clinical Microbiology, Ankara, Turkey
⁵ Kırıkkale University Faculty of Medicine, Department of General Surgery, Kırıkkale, Turkey

ABSTRACT

Objective: Biofilm formation of microorganisms on the surface of airways may lead to supraglottic colonization that may cause lower respiratory 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 2. 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


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

Correspondence: Sabahat Çeken, Dr Abdurrahman Yurtaslan Oncology Research and Training Hospital, Department of Infectious Diseases and Clinical Microbiology, Ankara, Turkey  Email: sabahatceken@yahoo.com

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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.\textsuperscript{1,3} Biofilm formations developing on airways may lead to supraglottic tracheal colonization and may increase the risk of ventilator associated pneumonia (VAP).\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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 lipopolysaccharide and proteases, thereby inhibits biofilm formation. It shows bactericide effects at early stages and bacteriostatic effects in the long run.\textsuperscript{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.\textsuperscript{8,12} Since high dosages result in extreme damage, 0.2% concentration is advised mostly.\textsuperscript{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 Kirikkale University Faculty of Medicine, Department of Clinical Microbiology and Infection together with the Kirikkale University Electron Microscopy Center. The study was approved by the Ethics Committee of the Kirikkale 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 glucosinate 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.\textsuperscript{13} A slime positive S. epidermidis (clinical 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 ml 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.

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).

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<thead>
<tr>
<th>Group 1</th>
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<tr>
<td>5x10º cfu/ml</td>
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<td>8x10³ cfu/ml</td>
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<td>3x10³ cfu/ml</td>
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<td>1x10⁴ cfu/ml</td>
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<td>7x10¹ cfu/ml</td>
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<td>8x10³ cfu/ml</td>
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<td>7x10² cfu/ml</td>
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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.

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

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Figure 1. Shows the electron microscopic images of naive airways

Figure 2. Biofilm formation in chlorhexidine non-coated material (Group 1)
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.\textsuperscript{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.\textsuperscript{4,19-21} While some of these methods are currently in use at clinical environments, experimental studies are still continuing for the others.\textsuperscript{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.

REFERENCES

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