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Antimicrobial effects of chlorhexidine, matrica drop mouthwash (chamomile extract), and normal saline on hospitalized patients with endotracheal tubes

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Abstract

Background: The functions and use of mouthwashes are variable depending on their type. Oral care in patients with endotracheal tubes is important to prevent side effects such as pneumonia. The aim of this study was to determine the antimicrobial effects of chlorhexidine, drop of Matrica mouthwash (chamomile extract), and normal saline on hospitalized patients with endotracheal tube in an intensive care unit (ICU).

Materials and Methods: In this clinical trial, 39 patients admitted to the ICU were selected by convenience sampling, were matched based on age and sex, and randomly assigned to three groups (chlorhexidine, Matrica, saline). Mouth washing was performed every 8 to 48 hours. The samples were taken at time zero (before the intervention) and 48 hours after the intervention for bacterial culture. Antibacterial activity of each mouthwash on microorganisms was measured based on the growth of *Staphylococcus aureus*, *Pneumococcal*, *Enterococcus*, *Pseudomonas*, and *Escherichia coli*. The obtained data were then analyzed using Chi-square and Fisher's exact tests with the Statistical Package for the Social Sciences Package version 18.

Results: Chlorhexidine mouthwash was more effective in preventing colonization of bacteria in the mouth (point probability = 0.06) in comparison with chamomile and saline mouthwashes. Nevertheless, none of the tested mouthwashes were able to remove pathogens, including *Staphylococcus aureus*, *Pseudomonas*, *Klebsiella*, and *Acinetobacter*.

Conclusions: 0.2% chlorhexidine mouthwash has a significant effect on the bacterial colonization rate in comparison with Matrica and normal saline mouthwashes in ICU hospitalized patients with endotracheal tube.

Key words: Chlorhexidine mouthwash, ICU, Matrica, normal saline, pathogenic microorganisms

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Introduction

Provision of oral health care is one of the main tasks of health care providers and a fundamental aspect of nursing care in intensive care units (ICU).[1] This creates a sense of comfort and health and prevents dangerous complications such as ventilator-associated pneumonia (VAP).[2] Mouth is among the most critical organs in maintaining patients' health in ICUs because the artificial airway and nasogastric tube pass through this organ. However, these interventions result in opened mouth

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and impaired airway defense.[3] The earlier mentioned treatments make patients susceptible to developing plaque, oral health problems, and VAP due to decreased fluid intake, decreased salivation, lack of spontaneous motion in the tongue and jaw, difficulty in swallowing, and drug side effects.[4] According to the Center for Disease Control and Prevention (CDC), procedures that lead to hospital-acquired pneumonia include aspiration of pre-existing organisms in the oropharynx, inhalation of bacteria containing particles, infection spread through blood to other places, and transfer of bacteria from the gastrointestinal tract. Organisms' aspiration from the oropharynx is considered as the most pivotal procedure; reduction of hospital-acquired pneumonia is also caused by decrease of bacteria in the oropharynx.[5] Decontamination of mouth and throat using antifungal and antibacterial drugs is a common method to prevent aspiration of pre-existing bacteria in the mouth and throat. Commercial mouthwashes consist of a chemical formula including bis-biguanide (chlorhexidine and alexidine), antibiotics, enzymes, salts of heavy metals, halogens, sarcosines, and plant alkaloids that are consumed in the form of mouthwashes, gels, ointments, and toothpaste. In summary, using the abovementioned products increases the local concentration of drugs up to 100-fold greater than the systemic medication. Mouthwashes are oral irrigator solutions that are used for particular oral health conditions.[6] Chlorhexidine is a chemical agent that is widely used to reduce dental plaque. It is an antiseptic and disinfectant bis-biguanide that is effective against a wide range of bacteria as well as some fungi and viruses. Because of its lack of microbial resistance and carcinogenic effect, it can be utilized as a proper mouthwash solution.[7] Chlorhexidine prevents sticking of bacteria to the teeth and oral mucosa and causes damage to bacteria by increasing the permeability of bacterial cell walls and changing osmotic balance.[8] It has an inhibiting effect on both Gram-negative and positive bacteria and yeasts.[9] In addition, it has anti-microbial effects due to its gradual release in 12 h.[10] Although chlorhexidine is recommended as the most effective anti-plaque,[10] it is not yet recommended by the CDC due to lack of evidence in terms of its effectiveness and side effects (tooth discoloration, irritation of the mucous, and mucosal lesions).[8] A study by DeRiso *et al*.[11] regarding the effect of chlorhexidine mouthwash on ventilator-associated pneumonia in post-cardiac surgery patients, revealed that this procedure reduced hospital infections, especially respiratory infections, and decreased the use of antibiotics. Genuit *et al*.[12] also showed that use of chlorhexidine mouthwash leads to decreased removal time of endotracheal tube and reduces ventilator-associated pneumonia. Reduced incidence rate of pneumonia due to the use of chlorhexidine mouthwash was also observed in a study by Houston *et al*.[13] However, because of the side effects of using the said mouthwash, medical science has been recently more inclined to plant extracts because of their appropriate therapeutic effects and low side effects.

Chamomile is suggested as a standard medicinal plant because of its numerous treatment benefits. It has anti-bacterial, anti-viral and anti-fungal effects, as well as contains compounds that are effective against *Staphylococcus aureus* and *Candida*. In a study by Pourabbas *et al*.,[14] the authors aimed at determining the effect of Matrica mouthwash on plaque of 25 patients with gingivitis, the average reduction of plaque and gums due to mouthwash provided by chamomile was significantly more than that of the mouthwash used in the control group.

According to a research regarding the use of this mouthwash, Kamillosan maintains the natural condition of the mouth and reduces the severity of mucositis and accelerates re-epithelialization of tissue in the mouth. No patients were allergic or complained about its taste after chamomile consumption.[15] Therefore, this study aimed to investigate the anti-bacterial effects of the three abovementioned mouthwashes on mouth bacterial colonies.

Materials and Methods

A double-blind, randomized clinical trial with a control group was implemented. The study participants were newly admitted patients to the ICU of the fifth Azar Hospital in Gorgan (Northern Iran) who had an endotracheal tube during the study and were also expected to remain connected to the ventilator for more than 48 h. Inclusion criteria included hospitalization in the ICU, age of 15 years or more, having an endotracheal or nasogastric tube, mechanical ventilation for 48 h, lack of aspiration, lack of antibiotic treatment before hospitalization, lack of sensitivity to the mouthwashes, asthma, allergic inflammation of the nose and skin, lack of radiation exposure, and immunosuppressive drugs such as corticosteroids, and no consumption of any antimicrobial mouthwash in 2 months prior to hospitalization. In total, 39 eligible participants were included in the study. Re-intubation, lung infection, reaction to any mouthwashes, death, or transfer of the patient to another unit were the excluding criteria. The Iranian Registry of Clinical Trial code for the study is IRCT201204287821N1.

Sample size

According to the methods reported by Taraghi *et al*.[16] 13 samples were assigned to each group. First, samples were selected by convenient sampling from hospitalized patients in the ICU, and then they were randomly distributed between the two groups. Written consent form was obtained from the patients. Each hospitalized eligible participant randomly selected a sheet from a box containing 13 sheets mentioning chlorhexidine mouthwash, 13 sheets mentioning Matrica mouthwash, and 13 sheets mentioning saline. The patients were then divided into three groups after matching age and gender. Data were collected using a bipartite checklist including demographic characteristics (age and gender) and clinical history (disease diagnosis, consumed drugs, and comorbid diseases). Laboratory tools were sterile swabs, Falcon tubes, blood agar culture medium, MSA (Mannitol Salt Agar), EMB (Eosin Methylene Blue Agar), MHA (Mueller Hinton Agar), Bile esculin, in vitro diagnostics, TSI (Triple Sugar Iron Agar), OF (Oxidation Fermentation), MRVP (Methyl Red Voges Proskauer) SIM (Sulfide Indole Motility), CS (Citrate Simmons), urea, diagnostic discs, sheep blood, and disposable plates.

Study design

The researcher was attending the ICU daily and the selected eligible hospitalized. Then, demographic and consent forms were filled by the participants. Before intervention, researcher washed hands for 30 s with soap and water.

In the first experimental group, 0.2% chlorhexidine mouthwash and in the second group Matrica mouthwash were used, whereas the control group received normal saline. At the time of admission, four swabs were taken from the upper posterior oropharynx and at the bedside; one swab was cultured on the blood agar medium and the remaining three swabs were transferred to a tube containing 0.5–1 ml transport medium, and finally, mouthwash was consumed. In all the study groups, the entire mouth surface, teeth, tongue, palate, and inside the cheeks were washed three times per day for 6 min with 10 ml of mouthwash, following which oropharynx sterile suctioning was carried out. Mouthwash was given every 8 h for 48 h, and then the patients were checked again. Other three swabs were transferred to the Laboratory of Microbiology located in the Faculty of Medicine for a maximum time of 1 h. In the laboratory, samples were cultured in EMB, MSA, Bile Esculin (BB), and BA and were put on a candlestick whereas others were put in an incubator at 37°C for 24 h. According to the microbial growth of *Pneumococcus*, *S. aureus*, *Enterococcus*, *Pseudomonas*, *Escherichia coli*, after 24 h, plates were evaluated for the identification of bacteria and its type by colony morphology and initial screening. To prevent bias, all testing processes were carried out by a lab specialist who was unaware of the intervention. Finally, all the findings were coded (double-blinded study was implemented). It implies that the laboratory and statistical analysis experts were unaware of the sample assignment in the groups. For data analysis, Chi-square and Fisher tests were carried out using the Statistical Package for the Social Sciences (SPSS Inc. version 18) and *P* value and test power of 0.05 and 90%, respectively, were considered as statistically significant.

Ethical considerations

The ethical and scientific contents of this study have been approved by research ethics committee of Golestan University of Medical Sciences. This study registered at Iranian Registry of Clinical Trials with Registry code: IRCT201204287821N1.

Results

Among the 39 enrolled patients, 21 were males (53.84%) and 18 were females (46.15%). Subjects' age was in the range of 20 to 68 years with the mean and standard deviation of 43.64 ± 15.01. With regards to matching at the first stage, there was no significant difference between age ($P = 0.595$), gender ($P = 0.758$), diagnosis ($P = 0.407$), and level of consciousness ($P = 0.066$) with received antibiotics, antacids, and sedation in the three groups [Table 1].

Bacterial culture of the obtained samples before mouthwash consumption indicated that bacterial growth was only observed in 11 (28.2%) patients whereas 2 (5.1%), 4 (10.3%), 3 (7.7%), and 2 (5.1%) had *S. aureus*, *Pseudomonas*, *Klebsiella*, and *Acinetobacter*, respectively

Table 1: Demographic information and clinical characteristics of the studied ICU patients in all three test groups (Chlorhexidine, Matrica, Normal Saline)

	Variable	Chlorhexidine	Matrica	Saline normal	*P* value
Age	Mean±SD	42.92 (15.33)	46.15 (15.23)	41.84 (15.35)	0.595
Gender	Male	7 (17.94%)	7 (17.94%)	7 (17.94%)	0.001
	Female	6 (15.38%)	6 (15.38%)	6 (15.38%)	
Antibiotic	Yes	13 (33.33%)	13 (33.33%)	13 (33.33%)	
	No	0 (0)	0 (0)	0 (0)	
Antacid	Yes	13 (33.33%)	10 (25.64%)	13 (33.33%)	0.094
	No	0 (0)	3 (7.69%)	0 (0%)	
Sedation	Yes	12 (30.76%)	11 (28.20%)	12 (30.79%)	0.001
	No	1 (2.56%)	2 (5.12%)	1 (2.56%)	

[Table 2]. Results of the bacterial cultures, 48 h after the usage of mouthwash, revealed that 18 patients (46.2%) in the second culture had no growth of any bacteria compared to the first 28 patients. The most grown pathogen was *Klebsiella* (20.5%) followed by *Pseudomonas* (17.9%). Findings also revealed that patients infected by *S. aureus*, *Pseudomonas*, *Klebsiella*, and *Acinetobacter* before mouthwash consumption, after 48 h had no change in their bacterial condition and mouthwash was not able to eliminate these pathogens. Mouth bacterial condition after mouthwash consumption indicated that 11 patients before the application of chlorhexidine mouthwash had no growth of some bacteria, and 48 h after usage, only one positive sample was found. For Matrica mouthwash, 8 patients before mouthwash usage and 5 patients 48 h after the consumption were positive. Finally, in normal saline, 9 patients before consumption and 6 patients after application were positively infected [Table 3].

Table 2: The frequency distribution of ICU patients with intubation, based on mouth bacterial condition at the time of admission and 48 hours after intervention

Mouth bacterial condition before mouthwash usage	Number	Percent	Mouth bacterial condition 48 hours after mouthwash usage	Percent
Lack of growth	28	71.8	18	46.3
Staphylococcus aureus	2	5.1	2	5.1
Enterococcus	-	-	2	5.1
Pseudomonas	4	10.3	7	17.9
Klebsiella	3	7.7	8	20.5
Acinetobacter	2	5.1	2	5.1
Total	39	100	39	100

Discussion

The results of this study revealed that 0.2% chlorhexidine mouthwash is more effective in preventing bacterial colony growth compared to Matrica mouthwash and normal saline. The primary colonization rate in this study was 28.2%, in the chlorhexidine, Matrica, and normal saline group, it was 5.12, 12.82, and 10.25%, respectively. After 48 h, colonization rates were 53.7, 7.69% for the first group, 20.51% for the second group, and 25.64% for the third group. The findings of Ozcaka *et al*.[17] also represented the effectiveness of 0.2% chlorhexidine on on reducing the incidence of ventilator-associated pneumonia. Scannapieco *et al*.[18] also demonstrated that chlorhexidine reduces the number of *S. aureus* bacteria in ventilated patients, whereas it was unable to reduce other pathogens.

Gram-negative bacteria were the most common isolated microorganisms from the samples and the majority of grown pathogens were *Klebsiella* and *P. aeruginosa*. These results are relevant with that of Ghazvini *et al*. in which *K. pneumonia* was the most frequently isolated bacteria from the throat, followed by *Acinetobacter baumannii*, *P. aeruginosa*, and different strains of Enterobacteriaceae.[19] In Panchabhai[20] study, the most common isolated pathogens were *P. aeruginosa*, *A. baumannii*, *K. pneumonia*, and *S. aureus*.

In our study, on the first day, sampling was carried out at two different times and after 48 h, and then changes in bacterial colonization were assessed in the three test groups. The findings revealed that colonization rate increased on the second day, whereas results of Berry *et al*.[21] indicated that colonization increased and there was no difference

Table 3: The frequency distribution of mechanically ventilated ICU patients, based on isolated type of microorganisms from secretions of samples before and 48 hours after intervention

Type of solution	Chlorhexidine			Matrica			Saline normal		
Type of microorganism isolated from medium	Before	After	Point probability	Before	After	Point probability	Before	After	Point probability
Lack of growth	11	10	0.06	8	5	0.001	9	3	0.01
	84.6%	76.9%		61.5%	38.5%		9.2%	23.1%	
Staphylococcus aureus	1	1		1	1		0	0	
	7.7%	7.7%		7.7%	%7.7%		0	0	
Enterococcus	0	0		0	0		0	2	
	0	0		0%	0%		0%	15.4%	
Pseudomonas	1	1		1	3		2	3	
	7.7%	7.7%		7.7%	23.1%		15.4%	23.1%	
Klebsiella	0	1		2	3		1	4	
	0	7.7%		15.4%	23.1%		7.7%	30.8%	
Acinetobacter	0	0		1	1		1	1	
	0	0		7.7%	7.7%		7.7%	7.7%	

between the three groups on the fourth day compared to the first day of colonization. In this study, 23.1% of the normal saline group and 76.9% in the chlorhexidine group had no bacterial growth and *K. pneumonia* was the most common pathogen. Seyedalshohadaee *et al*.[22] reported that 72.3% of patients in the saline group had no pathogen growth whereas in the chlorhexidine group, no microorganism growth was found in 81.5% of the patients. The most common pathogen in both groups was *A. baumannii*. The gradual release of chlorhexidine leads to relatively long-term effects of antimicrobial substances in the mouth. Chlorhexidine stays active for 6 h in the tissues. According to the Dental Association of America, the standard procedure of chlorhexidine usage is 10 ml of 0.12% chlorhexidine mouthwash for 1 min twice daily in healthy individuals. In several clinical trials, concentration of 0.12 and 0.2% for 1–4 times daily usage as a mouthwash or gel has been implemented because there is no agreement on its concentration and frequency of usage in the ICU. No significant difference was observed in the elimination of pathogens and the incidence of pneumonia related to ventilator in the said studies; in contrast, 2% chlorhexidine has a significant impact on reducing the incidence of ventilator-associated pneumonia. These findings suggest the usage of higher concentrations of chlorhexidine in ICUs.[23] In a study by Munro,[24] 0.12% chlorhexidine swab was not effective on bacteria colonization, however, in a study by Forier,[25] 0.2% chlorhexidine gel resulted in a decrease in positive cultures on days 5, 7, and 10, with no effect on colonization after 10 days. In this study, 0.2% chlorhexidine was used three times daily for every 8 h, which was more effective for bacteria colonization compared to the other two tested mouthwashes. However, in the study by Ranjbar *et al*.[26] and Faridian[22] where 0.2 and 0.12% chlorhexidine mouthwash were used, results revealed no statistically significant difference between chlorhexidine and saline group in terms of type and amount of isolated bacteria from patients with ventilator-associated pneumonia.

The oropharynx of patients in our study receiving mechanical ventilation was quickly colonized by Gram-negative microorganisms. Although it is believed that the maximum growth of microorganisms is within 96 h after intubation, 28.2% of our participants were colonized by these microorganisms during the first 12 h of admission. Because the admitted patients to the ICU usually have unstable conditions in the first 24 to 48 h, most nursing cares are focused on stabilizing the patient's hemodynamic status; thus, oral care is not a high priority.[27] The study by Grap *et al*.[28] indicated that 0.12% chlorhexidine in the forms of swab or spray may prevent the development of ventilator-associated pneumonia immediately after intubation and reduce microorganisms' growth in the oral cavity.

In our study, while there was no significant relationship between the diagnosis and microorganisms' colonization, 48 h after mouthwash usage, Cendrero[29] revealed that trauma increases the risk of *S. aureus* colonization. There is no consensus regarding the effects of age on ventilator-associated pneumonia, however, some studies have hypothesized that age over 70 years can be a risk factor for nosocomial pneumonia.[26] In this study, the age of over 65 years was introduced as a risk factor of ventilator-associated pneumonia; however, age is an independent risk factor of colonization with Gram-negative bacilli.[26] The mean age of patients in this study was 43.64 ± 15.01, and there was no significant relationship between age and colonization of bacteria. There is no agreement on the impact of gender on the incidence of ventilator-associated pneumonia.[26] Some studies mentioned the male and others female gender as a risk factor for VAP. The results of this study, showed no significant difference between the two sexes in terms of growth of microorganisms. It might be the result of inadequate samples in our research and it is also recommended to implement more studies with various designs. Effects of 0.2% chlorhexidine on mouth bacteria with Matrica mouthwash have been previously studied in healthy individuals, but no identical study has yet been conducted on ICU patients. Therefore, our results are only compared to a few *in vitro* studies that have been implemented on healthy individuals. The results of the study by Atai[30] revealed that herbal mouthwash has significantly less antibacterial effects than chlorhexidine mouthwash; meanwhile, the antibacterial effects of Matrica mouthwash was substantially more efficient compared to other mouthwashes. In our study, the antibacterial effect of 0.2% chlorhexidine mouthwash was higher than normal saline and Matrica mouthwash.

Conclusion

This study shows that use of 0.2% chlorhexidine three times daily has more ability to prevent the growth of bacterial colonies in mouth compared to saline and Matrica mouthwashes. However, further research with larger sample sizes are recommended in order to determine the effects of Matrica mouthwash.

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Conflicts of interest

There are no conflicts of interest.

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