

## Evaluation of the Antibacterial Effect of Persica ® Mouthwash in Mechanically Ventilated Icu Patients: A Double Blind Randomized Clinical Trial

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**Abstract:** Oropharyngeal colonization of bacteria is one of the most important risk factors for ventilator associated pneumonia. One of the ways to prevent accumulation of bacteria in the throat is to use mouthwash. Among the oral rinse, chlorhexidine has been introduced as the gold standard but has a variety of side effects. The aim of this study is to determine and compare the antibacterial effects of persica® mouthwash 10% (*miswak extract*) and chlorhexidine gluconate 0.2% in mechanically ventilated patients in intensive care unit (ICU). In this double blind randomized clinical trial, 60 patients who were admitted in a surgical ICU and met the inclusion criteria were enrolled and were randomly divided in two equal intervention and one control groups. In the first intervention group, chlorhexidine gluconate mouthwash 0.2% was used, in the second one, the researchers used persica® herbal mouthwash 10% and finally in the control group, normal saline was used. In order to culture *Staphylococcus aureus* and *Streptococcus pneumoniae*, immediately before and after 6 minutes of mouth washing, saliva samples were taken without any stimulation. Data were analyzed using Chi-square and ANOVA tests in SPSS 17 software. Decrease of bacterial counts was significant in all three groups after intervention ( $p < 0.001$ ). Unlike normal saline, chlorhexidine and persica® mouthwashes had significant antibacterial effects on *Staphylococcus aureus* (respectively  $p < 0.001$  and  $P = 0.008$ ) and *Streptococcus pneumoniae* ( $p < 0.001$  and  $p < 0.001$ ). The findings of this study indicated that herbal persica® mouthwash can be considered as an alternative for chlorhexidine in ICU patients due to high resistance of the bacteria to synthetic mouthwashes and side effects of these drugs.

**Key words:** Mouthwash • Chlorhexidine • Persica® • Normal Saline • Antibacterial Effect • VAP

### INTRODUCTION

More than 500 species of bacteria are found in the oral cavity, among them approximately 22 dominant species are recognized. In healthy adults, *Streptococcus viridians* is the dominant aerobic microorganism in the oral flora but in intensive care unit (ICU) patients it changes into *Streptococcus pneumoniae* and *Staphylococcus aureus* that cause ventilator associated pneumonia (VAP) [1, 2]. Colonization of these bacteria in the oropharynx and its subsequent micro aspiration into the lower respiratory tract are two important factors which

cause VAP [3, 4]. VAP occurs at least 48 hours after starting mechanical ventilation with tracheal tube and is the most common nosocomial infection in ICU patients [5] with an incidence rate of 9-40% [6, 7]. It increases hospitalization time [8] and costs [9] and leads to 15-50% of mortality [10].

Mouthwashes decrease the risk of VAP via reducing the number of microorganisms, their transmission and colonization in the patient's lung. Among the mouthwashes, chlorhexidine is considered as the gold standard. Nevertheless, it has several adverse effects such as mucosal irritation, dryness and injuries, allergies

and even the occurrence of anaphylactic shock, acute respiratory distress syndrome (ARDS), cytotoxic effects and if ingested it causes negative systemic effects [11-13]. Therefore, there has always been a tendency to use mouthwashes that have antibacterial effects like chlorhexidine and at the same time have less unwanted effects.

Various studies have been done in order to find antibacterial materials with plant origin [14] and one of the agents considered as an alternative to chlorhexidine is a plant called *Salvadora persica* or miswak. *Persica*® herbal mouthwash contains three medicinal plants, *Salvadora persica*®, Yarrow and Mint. Plants used in *Persica*® formulation have been used as food for centuries. Since its benefits have been supported by several studies [15-17], the consumption of *Persica* is allowed and unlike chemical components does not have any adverse effects [18, 19]. Furthermore, the World Health Organization (WHO) recommends and encourages the use of chewing *Persica* sticks (miswak) as an effective oral hygiene procedure [20, 21]. To choose a mouthwash for preventing infection, in addition to the type of mouthwash and its antibacterial effects, the sensitivity of pathogenic microorganisms to it must be considered as an important issue.

It should be noted that most of the studies conducted on the antibacterial effects of *Persica*® are *in vitro* and on the pathogens that are important in dentistry. However, the type of oral microorganisms in ICU patients is different from dental patients and body and oral immunity of these patients also differs from conscious patients. Furthermore, adverse effects and resistance to synthetic mouthwashes has been reported as a problem in these patients [22, 23]. Thus, the aim of this study was to evaluate the antibacterial effects of *Persica*® herbal mouthwash on *Staphylococcus aureus* and *Streptococcus pneumonia*, the most common pathogens causing early VAP (48 to 96 hours after intubation) [24] in ICU patients.

## MATERIALS AND METHODS

This study was a double blind randomized clinical trial (RCT) that was done to determine and compare the immediate antibacterial effects of *Persica*® mouthwash 10% and chlorhexidine gluconate 0.2%. Among the patients admitted to surgical ICU of Imam Khomeini Educational Hospital, Sari, Iran, 60 cases were enrolled based on the inclusion criteria and then were randomly allocated into three equal groups, chlorhexidine, *Persica*®

and normal saline. Approval to conduct the study on human subjects was obtained from the Research Council and Ethics Committee of Mazandaran University of Medical Sciences. All patients were enrolled in the study only after their families were completely informed of the aims and methods of the study and gave an informed written consent.

Inclusion criteria were: age of 15-65 years, the third or fourth day of the first admission to ICU, a period of three to four days of intubation and mechanical ventilation, a nasal or oral gastric tube used for 3 to 4 days and GCS (Glasgow Coma Scale) of less than 8.

Patients who had one of the following conditions were not included in the study: re-intubation, previous antibiotic therapy over two weeks before hospitalization, pulmonary aspiration, history of pulmonary and systemic infections, autoimmune diseases and malignancy, previous radiation therapy and use of immunosuppressive drugs such as corticosteroids, history of sensitivity to the used mouthwash, asthma and drug allergy, use of any antibacterial mouthwash over the two weeks before hospitalization, oral mucosal lesions and advanced periodontal disease and negative cultures of *Staphylococcus aureus* and *Streptococcus pneumonia* before use of mouthwash.

Moreover, if any sensitivity to the mouthwashes or any side effects were detected during the study, the intervention would be stopped and the patients would be excluded from the study.

In the first intervention group, chlorhexidine gluconate mouthwash 0.2% (the product of Shahre Daru Pharmaceutical Company, Tehran, Iran) with license production number of 019-SH-72 was used, in the second intervention group, the researchers used herbal *Persica*® mouthwash 10% (manufactured by Poursina Pharmaceutical Company with the registration number of: 1228013232, Iran) and finally in the control group normal saline was used.

In all groups the whole surfaces of mouth, gums, tongue, throat and teeth were precisely swabbed with the mouthwashes in 6 minutes. Mouthwash volume in the three groups was 10 cc and at the end of rinsing, solution was removed from the mouth of patients by sterile catheter. Immediately before and 6 minutes after mouth rinsing, saliva samples were taken without any stimulation for culturing *Staphylococcus aureus* and *Streptococcus pneumonia* [25, 26].

The time devoted to the three groups was in the morning shift and the patients had supine position during the mouthwash. Moreover, sterilization tips such as

complete and frequent hand washing and use of sterile gloves during mouth washing were done. Samples were taken directly from the tonsil areas and the posterior-upper part of oropharynx using sterile swabs and then they were immediately placed on the blood agar medium. Another swab was inoculated onto TSB (Tryptic Soy Broth) liquid medium for counting bacterial colonies. The plate and the TSB culture were transferred to the Microbiology laboratory within two hours [25, 27].

The blood agar medium, made by Merck Company, Germany, was used for culturing *Staphylococcus aureus* and *Streptococcus pneumoniae*. TSB fluid media made in Quelab Company, Canada (163505) with pour plate technique was used for counting the total colony number of bacteria which was represented by CFU (colony forming unit). For counting bacterial colonies, liquid TSB medium was incubated for about 24 hours at 37°C and then the total number of bacteria was estimated by using the standard curve. Method of pour plate in successive dilutions was used to confirm the test results [27]. Logarithm of the numbers was used due to the large numbers and easy calculations of the statistical tests.

Evaluation of antibacterial properties was based on two things, the presence or absence of *Streptococcus pneumoniae* and *Staphylococcus aureus* in samples after the mouth washing and the significant difference in total colony counts between the two samples before and after oral rinse ( $P < 0.005$ ).

## RESULTS

Only patients with positive samples of *Staphylococcus aureus* and *Streptococcus pneumoniae* were included and during the study no patients were excluded. The results of a Chi-Square test showed that there was no significant difference in demographic and clinical characteristics of the three groups. In order to find any differences between the groups with respect to their age, a t-test was conducted and the results showed no significant differences between the three groups (Table 1).

The results of the study showed that each of these three mouthwashes (chlorhexidine, persica®, saline) significantly decreased the number of bacterial colonies after intervention (Table 2).

Table 1: Demographic and clinical characteristics of patients in the three groups (chlorhexidine, persica®, saline)

Groups					
	Variables	Chlorhexidine	Persica	Saline	P value*
Age (y)	( Mean $\pm$ SD)	49.6 $\pm$ 1.31	52.35 $\pm$ 1.51	52.7 $\pm$ 1.24	P=0.932*
Sex	Male	11(55%)	11(55%)	12(60%)	P=0.934
	Female	9(45%)	9(45%)	8(40%)	
Total Parenteral Nutrition	Yes	8(40%)	11(55%)	10(50%)	P=0.324
	No	12(60%)	9(45%)	10(50%)	
Hx of Diabetes	Yes	13(65%)	10(50%)	8(40%)	P=0.189
	No	7(35%)	10(50%)	12(60%)	
Duration of Diabetes	<3y	7(35%)	8(40%)	3(15%)	P=0.227
	3y and more	6(30%)	2(10%)	5(25%)	
Duration of Hospitalization	<6D	12(60%)	9(45%)	11(55%)	P=0.626
	6D and more	8(40%)	11(55%)	9(45%)	
Antibiotics	Yes	19(95%)	18(90%)	19(95%)	P=0.765
	No	1(5%)	2(10%)	1(5%)	
Gastric PH suppressor	Yes	18(90%)	19(95%)	18(90%)	P=0.804
	No	2(10%)	1(5%)	2(10%)	
CNS Depressants	Yes	19(95%)	19(95%)	18(90%)	P=0.765
	No	2(10%)	2(10%)	2(10%)	

\*P value measured with Chi-Square Test (age with independent t-test)

Table 2: Comparison of the colony counts in the three studied groups (chlorhexidine, persica®, saline), before and after intervention

Colony count(CFU Log)				
Groups	Before intervention (Mean $\pm$ SD)	After intervention (Mean $\pm$ SD)		P value*
Chlorhexidine	5.9926 $\pm$ 0.0180	4.7039 $\pm$ 0.1403		p<0.001
Persica	5.9883 $\pm$ 0.0246	5.1826 $\pm$ 0.1101		p<0.001
Saline	5.9919 $\pm$ 0.0188	5.3457 $\pm$ 0.3132		p<0.001

\*P value measured with paired t - test

Table 3: Comparison of the antibacterial effects of chlorhexidine, persica® and normal saline after intervention

Groups	Agents	N(%) of patient with Positive Culture After intervention	P value*
Chlorhexidine	S. aureus	4(20%)	p<0.001
	Strep. pneumoniae	2(10%)	p<0.001
Persica	S. aureus	8(40%)	P=0.008
	Strep. pneumoniae	5(25%)	p<0.001
Normal Saline	S. aureus	19(95%)	P=1
	Strep. pneumoniae	17(85%)	p=0.25

\*P value measured with Chi-Square Test

The ANOVA test did not show any significant differences between three groups in the colony counts (log CFU) before intervention. However, the results of ANOVA showed significant decrease in the number of oral bacterial colonies after intervention between three groups ( $p<0.001$   $F=741.66$   $df=2$ ). Also Scheffe test showed a significant difference regarding the number of bacterial colonies between each two groups (chlorhexidine and persica®, chlorhexidine and normal saline, persica® and normal saline) after intervention ( $p<0.001$ ). It means that the effect of chlorhexidine mouthwash on colony counts reduction was significantly more than normal saline and persica® ( $p<0.001$ ) and persica® was more effective than normal saline ( $p<0.001$ ).

In this study, statistical analysis using Chi-square test showed that the effect of mouthwashes on *Streptococcus pneumonia* ( $X^2=23.17$   $p<0.001$ ) and *Staphylococcus aureus* ( $X^2=17.143$   $p<0.001$ ) after intervention was significantly different between three groups (Table 3).

Fisher test showed significant differences between the two intervention groups (chlorhexidine and persica®) in the presence of *Staphylococcus aureus* after intervention ( $X^2=6.66$   $P=0.02$ ). But no significant difference was observed between the two groups in the presence of *Streptococcus pneumonia* after intervention ( $X^2=1.558$   $P=0.407$ ).

## DISCUSSION

The purpose of this study was to determine and compare the immediate antibacterial effects of persica® mouthwash 10%, chlorhexidine gluconate 0.2% and normal saline in mechanically ventilated ICU patients. The results showed that all three mouthwashes reduced the colony count numbers after the intervention. Both chlorhexidine gluconate 0.2% and persica® 10% were effective on *Staphylococcus aureus* and *Streptococcus*

*pneumonia* and no significant difference was observed between the effect of these two mouthwashes on *Streptococcus pneumonia*.

Potential pathogens causing early onset VAP (i.e. *Staphylococcus aureus* and *Streptococcus pneumonia*) are found in the oral cavity in ICU patients [22]. Indeed, the accumulation of these pathogens in the oropharynx is one of the strongest independent predictors for accumulation of bacteria in the trachea and bronchi [28, 29]

Veksler *et al.* [30] showed the effect of chlorhexidine mouthwash in reducing the number of oral bacterial colonies. In Veksler's study, chlorhexidine 0.12% was employed, while in the current study chlorhexidine 0.2% was used, but similar results were obtained. It can be concluded that the lower concentration of chlorhexidine has a good effect on the number of oral bacterial colonies in patients on mechanical ventilation, as well. In this study, it was observed that all three mouthwashes could reduce the number of bacterial colonies; however, chlorhexidine 0.2% was more effective than persica® and normal saline. Similarly, in Veksler's study, normal saline mouthwash reduced the number of bacterial colonies after intervention.

Several studies have demonstrated the effect of chlorhexidine mouthwash on Gram-positive and negative agents [31-33]. In Scannapieco's study, oral pathogens considered as a source of bacterial growth in dental plaque were evaluated, whereas in the present study, oropharyngeal pathogens causing VAP in mechanically ventilated patients were studied. It is controversial whether dental plaque or oropharyngeal colonization is the main source of pathogens which causing VAP in mechanically ventilated patients [24, 34].

Similar to our study, Salehi *et al.* [27] found that although persica® reduced the number of oral bacterial colonies, the effect of chlorhexidine on the oral bacterial colony count was more than persica®. The difference between the two studies is that whereas he

worked on healthy people, our patients were under mechanical ventilation and had different and more resistant flora.

In the present study, persica® 10% showed good antibacterial effects on *Staphylococcus aureus* and *Streptococcus pneumonia* which may be due to the long time (6 minutes) usage of persica® mouthwash.

In an *in vitro* study it has been shown that metanolic persica® extract is effective on Gram-positive organisms but it has no effect on Gram-negative ones [35]. However, other studies showed antibacterial effects of persica® on the Gram-positive and negative agents [26, 36- 37]. The present study showed persica® 10% had effect on Gram-positive *Streptococcus pneumonia* and *Staphylococcus aureus* which are weaker than the Gram-negative ones.

Several clinical studies comparing the effect of chlorhexidine and persica® has been done on dental caries and periodontal pathogens [16, 26, 27]. These studies indicated that chlorhexidine was more effective than persica®, but a study similar to ours has not yet been conducted on ICU patients. Therefore, the results of the present study have only been compared with studies conducted in laboratory and on healthy people and our findings have been in line with the results of these studies.

In the present study no side effect was observed with the use of persica® mouthwash and chlorhexidine. According to the results of this study, using persica® 10% mouthwash for six minutes is effective on *Staphylococcus aureus* and *Streptococcus pneumonia* of oropharyngeal area which are common agents causing early VAP. So perhaps after further studies persica® 10% can be used in special cases, such as contraindications or side effects of chlorhexidine to prevent early VAP.

At the end it is recommended that other researchers design studies with the aim of comparing persica® and chlorhexidine to investigate if persica® can prevent the occurrence of VAP.

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