Comparison of the antibacterial effects of matrica & Persica™ and chlorhexidine gluconate mouthwashes in mechanically ventilated ICU patients: a double blind randomized clinical trial

Hadi Darvishi Khezri, Mohammad Ali Haidari Gorji, Ali Morad and Heidari Gorji

Comparación del efecto antibacteriano de aseos bucales con matrica & Persica® y gluconato de clorhexidina en pacientes de UCI bajo ventilación mecánica: ensayo clínico doble ciego y aleatorio

Background & Aim: Accumulation of bacteria in the pharynx is one of the risk factors of pneumonia due to ventilation. One of the methods of prevention from accumulation of bacteria in the pharynx is the use of oral solutions. Chlorhexidine is considered as the gold standard, but it has various side effects. Present study was aimed to determine and compare anti-bacterial effects of the chlorhexidine gluconate 0.2%, herbal mouthwash of matrica (chamomile extracts) 10%, Persica™ 10% and normal saline in intensive care unit patients. Methods: In this double blind randomized clinical trial, 80 patients who were admitted in ICU divided into four groups of 20 patients each one. Researchers applied Persica™ to group one, chlorhexidine gluconate mouthwash 0.2% to group two and third group received matrica, finally in the control group, normal saline were used. In order to culturing of Staphylococcus aureus and Streptococcus pneumoniae, salivary samples were obtained without any stimulation after six minimums oral rinsing. The data were processed in SPSS software and analyzed by appropriate statistical tests. Findings: Decreased rate of bacterial colonies after intervention in the whole four groups was significant (p < 0.001). The mouthwash of chlorhexidine (p = 0.001), Persica™ (p = 0.008) and matrica (p = 0.01) had a significant antibacterial effect on S. aureus and S. pneumoniae (p < 0.001). Conclusion: Herbal oral mouthwash of Persica™ and matrica has the effect on S. pneumoniae and S. aureus of oropharynx area in mechanical ventilation patients. However, there is a need for further research to be considered as an alternative to chlorhexidine for prevention of VAP in ICU patients.

Key words: Oral mouthwash, chlorhexidine, Persica™, matrica, normal saline, antibacterial.

Palabras clave: Aseo bucal, clorhexidina, persica, matrica, solución salina fisiológica, efecto antibacteriano.

Introduction

More than 500 types of bacteria have been discovered in oral cavity, out of them, 22 dominant types have been identified. These normal flora turns are known as gram positive such as Streptococcus pneumoniae and Staphylococcus aureus and negative organism that causes ventilator associated pneumonia (VAP)1,2. Colonization of these bacteria in the oropharynx and subsequently microaspiration into the lower respiratory tract, are two important factors, which leads to VAP1,2. It’s prevalence rate is 9-40%3-7, and leads to prolongs the period of hospitalization and consequently, increase in the cost8 and causes to 15-50% mortality9.

Accumulation of bacteria in the throat is one of the most important risk factors for VAP, and the relation between VAP and oral micro flora is thoroughly known10. Decrease in concentration of oral microorganisms is an effective factor in prevention from VAP11. Policies for prevention of VAP via oral hygiene including suction, removal of dental plaque and microorganisms through mechanical intervention (to brush) as well as chemical intervention (the use of mouthwashes). Interventions to prevent VAP begin at the time of intubation and should be nonstop until extubation12. Mouthwashes decrease the risk of VAP via reducing the number of microorganisms, their transmission and colonization in the patient’s lung. Among them mouthwashes, chlorhexidine is considered as the gold standard. Nevertheless, it has several adverse effect such as creation of dental pigments, 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 effects13,14. 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. Salvadora persica or miswak has attracted attention as an alternative for chlorhexidine.
mouthwashes. Persica™ herbal mouthwash contains three medicinal plants, Salvadora persica, Yarrow and Mint, and the plants are in the formulation oral drop of Persica™ and do not have the side effect of chemical substances, and it is an advantage for it. World Health Organization (WHO) has also recommended miswak plant as an effective tool for oral health.

Matrica is another mouthwash with commercial name of Camicell™. This product contains herbal extract of chamomile flower (scientific name: Matricaria chamomilla) from the Compositae Family. Antifungal effects of Chamomile particularly against Candida albicans and herpes virus, and so its anti-bacterial and anti-inflammatory effects have provided a unique position regarding the application of this mouthwash. It should be noted that the research on antimicrobial effects of Persica™ and matrica oral solution has mainly been concerned with in vitro as well as pathogens which are the focus of studies in dentistry. Considering the fact the types of microorganisms, the resistance of body, oral immunity of patients hospitalized in ICU is different from those of outpatients and also the side effects of resistance against industrial disinfectant is considerable. Various clinical studies on comparison between chlorhexidine and Persica™ on periodontal pathogens and tooth decay have been conducted. It has been pointed out that chlorhexidine has more effect than Persica™ and matrica, but as our knowledge it has not been conducted on patients hospitalized in ICU in Iran. Therefore, the researcher are unable to make comparison between antibacterial effects of these four mouthwash on S. pneumoniae and S. aureus, and the fact that there has not been much research on antibacterial effects of Persica™ and matrica oral solution on factors causing VAP, the present study is to determine the antibacterial effects of herbal oral solution of Persica™ and matrica on S. aureus and S. pneumoniae which are among the most common causes of early onset VAP, in patients under mechanical ventilation of ICU.

**Materials and Methods**

This randomized double blind clinical trial study was conducted on patients admitted to ICU wards of Imam Khomeini Educational and therapeutic Center in Sari/ Iran during 2011. 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.

To determine and comparison of the antibacterial effects of chlorhexidine and matrica mouthwashes in ICU patients, they were selected on the basis of inclusion criteria by purposeful method during second six months of 2011, and they were allocated into four groups: chlorhexidine, Persica™, matrica, and normal saline (Table 1 and Figure 1).

**Inclusion criteria were**

- Age between 15-65 years.
- Third or fourth day of ICU stay, tracheal tube and mechanical ventilation for 3 to 4 days, have nasogastric or orogastric tube for 3 to 4 days (Regarding change from oral micro flora into pathogens causing VAP, occurs 48-72 hours after intubation and hospitalization in ICU).
- Glasgow Coma Scale less than 8.

**Exclusion criteria were**

- Absence of consent.
- Re-intubation.
- Re-admission to the ICU.

---

**Table 1. Groups interventions**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mouth wash</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Chlorhexidine gluconate mouthwash 0.2%</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>(The product of ShahreDaru Pharmaceutical Company, Tehran, Iran with license production number of 019-SH-72)</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Herbal Persica™ mouthwash 10%</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>(Manufactured by Poursina Pharmaceutical Company with the registration number of: 1228013232, Iran)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Herbal mouthwash of matrica</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>(Made by Baryj Pharmaceutical Company) (Registration No: 1608) with a concentration of 1 to 10 (10 drops of matrica mouthwash in 9 cc water)</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Normal saline</td>
<td>20</td>
</tr>
</tbody>
</table>

**Figure 1. Sampling.**
- Autoimmune and malignant diseases.
- History of radiation therapy and immunosuppressive diseases and drugs such as corticosteroids.
- History of allergy to mouthwash, asthma, allergic rhinitis and dermatitis.
- History of antibiotic treatment over two weeks before hospitalization.
- Use of any antimicrobial mouthwash over two weeks before hospitalization.
- Oral mucositis and advanced periodontal disease
- Coagulopathy or anticoagulant drugs and pulmonary and systemic infections.

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

In all groups the whole surfaces of mouth, gums, tongue, throat and teeth were precisely swabbed with the mouthwashes during 6 minutes. Mouthwash volume in the four groups was 10 cc and at the end of rinsing, solution was removed from the mouth of patients by sterile catheter. Immediately before and after mouth rinsing, saliva samples were taken without any stimulation for culturing S. aureus and S. pneumoniae. The time devoted to the 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 (EMB/The product of Merk Company of Germany). 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. For culturing S. aureus and S. pneumoniae, we utilized coagulase test and Novobiocin and Bacitracin antibiotic disks in order to diagnosing S. aureus, and optochin test for diagnosing S. pneumoniae, and 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 colony forming unit (CFU). 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.

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 S. pneumoniae and S. 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). T test and ANOVA statistical test were used to make comparison between the number of colonies in each between chi-square and McNemar Test were used to investigate S. aureus and S. pneumoniae.

Results

Patients in four groups in terms of sex, age, receiving TPN (total parenteral nutrition), history and duration of diabetes, were no significant difference (Table 2). The results of the study indicated that the whole four mouthwash of chlorohexidine, Persica™, matrica and normal saline significantly in the decrease of bacteria after intervention (Table 3).

ANOVA test did not show significant difference in the number of colonies of bacteria before intervention between the four groups and the number of colonies of bacteria before intervention was similar in the four groups of chlorohexidine, Persica™, matrica, and normal saline (p = 0.372 f = 1.057). The results of the research indicated that the decrease in the number of the colonies of oral bacteria.

After intervention between the four groups by variance analysis test was significant (p < 0.001 $f = 1/243$). Scheffe statistical test indicated that among the mouthwash, chlorohexidine had significant difference with other mouthwashes for decrease in the number of the colonies of bacteria (p < 0.001). Persica™ mouthwash caused significant decrease in the number of colonies of bacteria as compared to matrica and normal saline (p < 0.001). Matrica mouthwash also showed significant difference in decreasing for S. pneumoniae and S. aureus (Figures 2 a and b).

All the initial samples of this study were positive for S. aureus and S. pneumoniae.

Statistical analysis by Chi-Square test indicated that the effect the mouthwash on S. aureus ($\chi^2 = 26/15$ p < 0.001) and S. pneumoniae ($\chi^2 = 23/7$ p < 0.001) in the whole four groups after intervention is significant. In comparison with other oral solutions, chlorohexidine had significant difference with matrica in the decrease of S.pneumoniae by Fisher Test ($\chi^2 = 6/14$ p = 0.03) but in comparison with Persica™, the difference was not significant ($\chi^2 = 1/558$ p = 0.407).

Fisher test showed significant difference between the two groups of chlorohexidine and Persica™ ($\chi^2 = 23/17$ p < 0.001), and chlorohexidine with matrica ($\chi^2 = 8.28$ p < 0.001) for the presence of S. aureus after intervention but no significant difference was showed between the Persica® and Matrica® groups in the presence of S. aureus after intervention ($\chi^2 = 0.1$, p = 1).
### Table 2. Demographic and clinical characteristics of patients in the four groups (chlorhexidine, Persica™, matrica, saline)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Chlorhexidine</th>
<th>Persica™</th>
<th>Matrica</th>
<th>Saline</th>
<th>Groups</th>
<th>Matrica</th>
<th>Saline</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>(Years) (Mean ± SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>11 (55%)</td>
<td>11 (55%)</td>
<td>12 (60%)</td>
<td>12 (60%)</td>
<td></td>
<td></td>
<td></td>
<td>0.41*</td>
</tr>
<tr>
<td>Female</td>
<td>9 (45%)</td>
<td>9 (45%)</td>
<td>8 (40%)</td>
<td>8 (40%)</td>
<td></td>
<td></td>
<td></td>
<td>0.97</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TPN (Total parenteral nutrition)</strong></td>
<td>Yes</td>
<td>8 (40%)</td>
<td>11 (55%)</td>
<td>9 (45%)</td>
<td></td>
<td></td>
<td></td>
<td>0.75</td>
</tr>
<tr>
<td>No</td>
<td>12 (60%)</td>
<td>9 (45%)</td>
<td>9 (45%)</td>
<td>10 (50%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>History of diabetes mellitus</strong></td>
<td>Yes</td>
<td>13 (65%)</td>
<td>10 (50%)</td>
<td>10 (50%)</td>
<td>8 (40%)</td>
<td></td>
<td></td>
<td>0.46</td>
</tr>
<tr>
<td>No</td>
<td>7 (35%)</td>
<td>10 (50%)</td>
<td>10 (50%)</td>
<td>12 (60%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Duration of diabetes</strong></td>
<td>&lt; 3 y</td>
<td>7 (35%)</td>
<td>8 (40%)</td>
<td>6 (30%)</td>
<td></td>
<td></td>
<td></td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>3 y and more</td>
<td>6 (30%)</td>
<td>2 (10%)</td>
<td>4 (20%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Duration of hospitalization</strong></td>
<td>&lt; 6 D</td>
<td>12 (60%)</td>
<td>9 (45%)</td>
<td>9 (45%)</td>
<td>11 (55%)</td>
<td></td>
<td></td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>6 D and more</td>
<td>8 (40%)</td>
<td>11 (55%)</td>
<td>11 (55%)</td>
<td>9 (45%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Antibiotics</strong></td>
<td>Yes</td>
<td>19 (95%)</td>
<td>18 (90%)</td>
<td>19 (95%)</td>
<td>19 (95%)</td>
<td></td>
<td></td>
<td>p = 0.88</td>
</tr>
<tr>
<td>No</td>
<td>1 (5%)</td>
<td>2 (10%)</td>
<td>1 (5%)</td>
<td>1 (5%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gastric pH suppressor</strong></td>
<td>Yes</td>
<td>18 (90%)</td>
<td>19 (95%)</td>
<td>19 (95%)</td>
<td>18 (90%)</td>
<td></td>
<td></td>
<td>p = 0.88</td>
</tr>
<tr>
<td>No</td>
<td>2 (10%)</td>
<td>1 (5%)</td>
<td>1 (5%)</td>
<td>2 (10%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CNS depressants</strong></td>
<td>Yes</td>
<td>19 (95%)</td>
<td>19 (95%)</td>
<td>19 (95%)</td>
<td>18 (90%)</td>
<td></td>
<td></td>
<td>p = 0.86</td>
</tr>
<tr>
<td>No</td>
<td>1 (5%)</td>
<td>1 (5%)</td>
<td>1 (5%)</td>
<td>2 (10%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significant level were obtained in * with independent t test and in others with chi-square test.

### Table 3. Comparison of the colony counts in the four studied groups (chlorhexidine, Persica™, matrica and normal saline), before and after intervention

<table>
<thead>
<tr>
<th>Groups</th>
<th>Colony count (CFU Log) Before intervention (Mean ± SD)</th>
<th>After intervention (Mean ± SD)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorhexidine</td>
<td>5.9926 ± 0.0180</td>
<td>4.7039 ± 0.1403</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Persica™</td>
<td>5.9883 ± 0.0246</td>
<td>5.1826 ± 0.1101</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Matrica</td>
<td>5.9523 ± 0.1649</td>
<td>5.2792 ± 0.2492</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Saline</td>
<td>5.9919 ± 0.0188</td>
<td>5.3457 ± 0.3132</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

p value measured with t-test.

### Discussion and Conclusion

The purpose of this study was to determine and compare antibacterial effects of the chlorhexidine gluconate 0.2%, herbal mouthwash of matrica (chamomile extract) 10%, Persica™ 10% and normal saline in ICU patients. The results of the present research indicated that chlorhexidine gluconate 0.2% Persica™ and matrica 10% had effect on reduction of oropharyngeal S. aureus and S. pneumoniae of patients under mechanical ventilation. Among oral solutions, chlorhexidine 0.2% was more effective than other mouthwashes, and Persica™ 10% had more effect in comparison with matrica 10%, although whole four mouthwashes decreased the number of colonies of bacteria’s after intervention.

The results showed the significant effect of the whole four mouthwashes on decrease in the number of colonies. The effect of
chlorhexidine 0.2% was higher than Persica™, matrica and normal saline. Vekslar AF et al pointed out to the effect of chlorhexidine oral solution on decreasing the colonies of oral bacteria. This is in harmony with our findings, although, they have used chlorhexidine 12%, while in the present study chlorhexidine was 0.2%. In explanation the chlorhexidine with any concentration has considerable effect on the number of colonies of oral bacteria of mechanical ventilation patients, and also we applied mouth wash for longer time (6 minutes).

In a meta-analysis, Pineda et al. demonstrated that the use of oral decontamination with CHX neither caused significant reduction in the incidence of VAP, nor altered the mortality rate23.

Koeman et al24 and Ozçaka et al25 suggested that topical oral decontamination with CHX reduced the incidence of VAP. In a systematic review and meta-analysis, Labeau et al. showed that the use of CHX resulted in a significant risk reduction of VAP relative risk (RR): 0.67; 95% confidence interval (CI): 0.50-0.88; (p = 0.004)26. Another systematic review and meta-analysis by Zamora reported similar findings27. It seems that moderating oropharyngeal colonization by CHX, at least theoretically, reduced the probability of VAP.

Many clinical studies demonstrated the affect of mouth washes on positive and negative agents26-29. In Scannapieco’s study, the effect of chlorhexidine 0.2% on S. aureus and S. pneumoniae clearly has been indicated28. Similar to our study, Salehi et al. founded that although Persica™ reduced the number of oral bacterial colonies, the effect of chlorhexidine on the oral bacterial colony count was more than Persica™41. Although in Salehi’s study, the effect of matrica was stronger than Persica™, there is a point that, whereas them sample included normal patients while ours were ICU patients and also we prescribed Persica™ 10% for 6 minutes which is more longer time.

In another study conducted by Ashghari (2006) it has been shown that metanolic Persica™ extract is effective on Gram-positive organisms but it has no effect on Gram-negative ones30. However, other studies showed antibacterial effects of Persica™ on both Gram-positive and negative agents also39,40. The present study also points out to the useful effect of Persica™ 10% on Gram negative organisms (data not presented).

Mozaffari et al., in a study on the antibacterial effects of Persica™ and chlorhexidine mouth wash showed that Persica™ has less antibacterial effect on Streptococcus mutans, Streptococcus sanguis and Lactobacillus casei and according to the researcher it is because of short contact of the mouth wash with microorganism31.

Atai et al in an in-vitro study comparing the antifungal and antibacterial effects of mouthwashes Persica™, matrica, and Iral wax™ with chlorhexidine demonstrated that among these herbal mouthwashes, matrica had more antibacterial effects against S. sanguis, Streptococcus salivarius, Streptococcus sobrinus, Actinomyces viscosus, and Candida albicans, but this difference was not statistically significant32. This study, unlike to Atai’s study, the antibacterial effect of Persica™ was higher than matrica. It might be due to two times using and concentration of Persica™ in this study. Lee et al. in a laboratory study showed that Chamomile extract had anti-bacterial effects on non-oral strains but they did not clarify the amount and intensity of this effect32. There was no side effect was observed in using Persica™ and matrica solutions.

Considering the obtained results, Persica™ and matrica10% were effective on S. aureus and S. pneumoniae of oropharynx which is one the common factors of pneumonia resulting from premature ventilation. Therefore, it might be possible to use Persica™ 10%, matrica 10% to prevent early onset VAP under especial circumstances such as prevention from using chlorhexidine or because of its side effects, but steel there is a need for further researches.

Acknowledgments. We would like to thank the voice chancellor of Mazandaran Medical Science University, Iran, for his scientific helps and advices.

References

Healthcare Associated Infections


