Comparative efficacy of honey 12.5% and chlorhexidine 0.2% mouthwashes on the oropharyngeal bacterial colonization in mechanically-ventilated patients: a randomized controlled trial

Halimeh Amiri, Farhad Ramezani-Badr, Naseh Sigari, Karim Nasseri

Background:
Oropharyngeal colonization and the associated aspiration are more common in patients hospitalized in Intensive Care Unit (ICU) than in other wards. Several

Methods:
This study was a randomized, single blind, phase III controlled clinical trial. Sixty patients newly admitted to internal and trauma Intensive Care Units of the two educational hospitals of Sanandaj city affiliated with Kurdistan University of Medical Sciences were selected by convenience sampling and allocated to two groups of 30 patients using random blocks design. In each group, the mouthwash was applied twice a day for four consecutive days. Swab samples were taken from the mouth and throat of all patients three times a day (pre- intervention, two days, and four days after the intervention) and then the samples were transferred onto the blood agar and eosin methylene blue (EMB) culture plates and investigated for bacterial growth and colonization after 24-48 h.

Results:
The findings showed that oropharyngeal colonization was not significantly different between the two groups, pre-intervention, two days, and four days after the intervention (P > 0.05). Rinsing with honey mouthwash 12.5% led to the inhibition of Staphylococcus aureus and Pseudomonas aeruginosa on the fourth day of the intervention in all samples.

Conclusion:
None of the studied solutions contributed to the reduction of oropharyngeal bacterial colonization. It seems that the growth inhibition of Staphylococcus aureus and Pseudomonas aeruginosa by the honey 12.5% mouthwash in mechanically-ventilated patients need further investigation.

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Keywords: Mouthwashes; Honey; Chlorhexidine; Ventilators, mechanical; Oropharyngeal colonization; Intensive Care Unit; Randomized controlled trial

INTRODUCTION
Oropharyngeal colonization and the associated aspiration are more common in patients hospitalized in Intensive Care Unit (ICU) than in other wards. Several
studies have shown that approximately 30%-60% of mechanically-ventilated patients admitted to the ICU are diagnosed with oropharyngeal colonization by pathogenic organisms such as gram-negative bacilli. In the first 48 h of hospitalization in ICU, the normal oropharyngeal flora composition which is often Streptococcus viridans is replaced by Streptococcus pneumonia and Staphylococcus aureus (S. aureus) as well as gram-negative organisms such as Pseudomonas aeruginosa most of which are fatal. Oropharyngeal colonization by these bacteria and subsequent micro aspiration in the lower respiratory system can cause ventilator-associated pneumonia (VAP). Available evidence suggests that oropharyngeal colonization is the most important pathogenic mechanism and one of the most serious risk factors for VAP.

O’Reilly declared that keeping good oral hygiene is necessary to reduce the risk of nosocomial infection, improve patient comfort and discharge outcomes in critically-ill patients. Washing mouth regularly with a good solution is essential for oral hygiene. Various types of mouthwashes such as chlorhexidine, sodium chloride 0.9%, hydrogen peroxide, sodium bicarbonate and povidone-iodine are used for oral hygiene. Despite their impact on microorganisms and dental plaques, it seems that each of these mouthwashes causes some complications. Malhotra et al showed that chlorhexidine has a significant impact on gram-positive bacteria. However, Haffajee et al suggested that chlorhexidine is less effective against gram-negative bacteria. Chlorhexidine has side effects such as temporary teeth discoloration, vilt taste destruction, stomatitis, gingivitis and xerostomia. Other solutions such as hydrogen peroxide and sodium bicarbonate can change the mouth pH and consequently the normal flora and if not properly diluted, cause irritation and damage to the mucosal membrane of the mouth as well. Results of various studies indicated that regarding the uncertainty of sodium chloride 0.9% impact on oropharyngeal colonization, and its contribution to xerostomia, it is not recommended as a mouthwash. Also, there is scant evidence indicating that VAP can be reduced by povidone iodine rinse.

Despite the importance of oral care by nurses in mechanically-ventilated patients hospitalized in ICU, there is insufficient evidence about appropriate standard mouthwashes and their instructions with higher efficacy and fewer side effects. Honey has been reported to have broad spectrum antibacterial effects without significant side effects. Most of the studies conducted on the effects of honey as an antibacterial have been in vitro, or on patients undergoing radiotherapy with oral mucositis and also as an inhibitor against the formation of dental plaque. However, to the best of our knowledge there is no reliable information on the impact of honey mouthwash on oropharyngeal colonization in intubated mechanically-ventilated patients in ICU. So, the aim of current study was to compare the effect of chlorhexidine solution 0.2% as a routinely used mouthwash and honey mouthwash 12.5% on the oropharyngeal colonization rate of mechanically-ventilated patients.

MATERIALS AND METHODS

This study was a single blind randomized phase III clinical trial (Blind Microbiologist) conducted on mechanically-ventilated patients hospitalized in internal and trauma ICUs of Tohid and Be‘sat educational hospitals affiliated to Kurdistan University of Medical Sciences in Iran. After obtaining the Ethics Committee approval of Zanjan University of Medical Sciences and registering at Iranian Registry of Clinical Trial (2016012412257N4), and also after getting informed written consent, 60 ventilated patients were included. The sample size was calculated according to the confidence interval of 95%, test power of 80%, and sample loss of 10%. Using convenient sampling and considering the inclusion and exclusion criteria, patients were selected from January to September 2015. Then each patient was placed into one of the two groups of chlorhexidine mouthwash (n = 30) or honey mouthwash (n = 30), using random assignment blocks. Inclusion criteria were as follows: age between 18 to 65 years; new patients admitted to the ICU with an endotracheal tube intubation and mechanical ventilation of less than 12 h; and requiring at least 4 d of mechanical ventilation. Also, exclusion criteria were as follows: re-adsmission to the ICU and re-intubation; transferred patients to ICU from other ICUs; patients with pneumonia or other systemic infections; a confirmed diagnosis of lung cancer; evident signs of airway aspiration; thrombocytopenia (platelet count < 40 × 10⁹ and/or INR > 2) or other coagulopathies; a confirmed diagnosis of immunosuppressive disease; high fasting blood sugar (FBS); known allergy to chlorhexidine, honey and other complications of mouthwash solutions used in this study; leukopenia; HIV; pregnancy; organ transplants; oral mucositis; advanced periodontal disease; long term steroid therapy; oromaxillofacial surgery; extensive burns; contraindication for semi fowlers’ position.

On the first day of study, in order to determine the baseline oropharyngeal microbial population, cotton swab samples were collected from the surface of upper and lower teeth, tongue, gums and throat (upper tonsils) of all participants. The collected samples in Cary Blair transport medium were transferred immediately to the microbiology laboratory. Sampling was repeated after the intervention in the same way on the 2nd and 4th days. In each group, the mouthwash was administered by three trained nurses in the same manner every 12 h (8 am-8 pm) for 4 consecutive days. For the mouthwashing, patients were placed on the semi-sitting position to prevent the aspiration. Then, using a cotton applicator, the surface of the mouth, buccal mu-
cosa, gums, tongue, throat, teeth and palate were rinsed by sterile technique (including hand wash and use of sterile gloves and cotton applicators) for 5 min using 10 mL mouthwash. Five min after the rinse, excess solution of the throat and mouth were suctioned by sterile technique. Every 3 h after performing the mouthwash and before the next intervention, patients were examined in terms of allergic reactions to mouthwash (redness and swelling). If allergy symptoms were diagnosed, the patient was excluded (Figure 1). Collected samples were cultured on Blood Agar (SBA; Merck, Darmstadt, Germany) and Eosin Methylene Blue Agar (EMB, Merck, Darmstadt, Germany) media for the isolation of Staphylococcus aureus and gram-negative bacilli, respectively. Cultured petri dishes were incubated at 37 °Celsius for 24-48 h. Bacterial pathogens such as Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter species (Acinetobacter spp) and gut bacteria including Escherichia coli (E. Coli), Proteus mirabilis, Klebsiella species (Klebsiella spp.), Enterobacter species, and Serratiam arcescens were investigated on cultured petri dishes. Bacterial growth was reported as semi-quantitative colony-forming unit (CFU) in ranges of 0 to +4; +1 = 10^1 CFU, +2 = 10^2-10^3 CFU, +3 = 10^4-10^5 CFU, +4 > 10^5 CFU, and growth ranges of +3 and +4 were considered as oropharyngeal colonization. The following morphological and biochemical tests were used to identify and confirm the isolates: Gram stain, Catalase, Oxidase, Coagulase and Urease, Indole production, VogesProskauer reaction, growth on Simmons citrate agar, Sugar fermentation on triple sugar iron agar and Motility test.

Honey used in this study was native honey from Zagros Mountains of Kurdistan province in the West of Iran, with a dark brownish yellow color prepared from the herbs Thyme and Astragalus. After confirmation of honey by the laboratory of the department of Agriculture in Kurdistan province affiliated to Iran’s Jihad Agriculture ministry in terms of standard honey factors (Table 1), the honey mouthwashes 12.5% were prepared on a daily basis using the following formula. Notably, all production stages were completely sterile.

Chlorhexidine solution 0.2% used in this study (Hexo-dine brand) was manufactured by World Health Company, Iran with license number of 44/13186 approved by the Ministry of Health and Medical Education of Iran.

60 patients randomly assigned using the block method

Control group (n = 30)
Receiving chlorhexidine mouthwash 0.2% every 12 h

Intervention group (n = 30)
Receiving honey mouthwash 12.5% every 12 h

Obtaining swab samples of oropharyngeal secretions right at the beginning, first two days and second two days after the intervention

Analysis

Assessed for eligibility (n = 70)
Excluded (n = 10)
- Not meeting inclusion criteria (n = 4)
- Declined to participate (n = 6)

Analyzed (n = 30)
Analyzed (n = 30)

Figure 1 Study overview of the patients entered into the trial
Iran. Data were analyzed using the SPSS version 23 (IBM Corp., Armonk, NY, USA). Results are expressed as mean ± standard deviation. Pearson $\chi^2$ test and Independent-samples $t$-test were used to compare the two groups in terms of colonization and some demographic and clinical factors, and the Related Samples Qochran’s $Q$ Test was applied to compare the colonization in each group on the 1st, 2nd and 4th days, respectively. The $P$ value < 0.05 was considered significant for data analysis.

## RESULTS

All 60 patients enrolled in the study participated in the whole process until the end. The comparison of some demographic and clinical variables including age, gender, admission indication, antibiotic treatment, having nasogastric tube, and consciousness level between the two groups of honey 12.5% and Chlorhexidine 0.2% showed no significant statistical difference (Table 2). Analysis of the mouth and throat cultures before the intervention showed that 61.7% of total samples suffered from oropharyngeal colonization including 30% Klebsiella spp., 11.7% E. coli, 10% S. aureus and 10% P. aeruginosa. According to the results 51.7% of all participants with oropharyngeal colonization were colonized with gram-negative bacteria.

Colonized bacteria types were analyzed separately in the two groups of honey mouthwash and Chlorhexidine mouthwash. Results showed that honey mouthwash 12.5% led to the inhibition of oropharyngeal colonization of S. aureus and Pseudomonas aeruginosa on the fourth day of the intervention in all samples (Table 3). The comparison between the two groups in the oropharyngeal colonization before the intervention showed no significant statistical difference ($P = 0.79$). Also, the

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Honey group ($n = 30$)</th>
<th>Chlorhexidine group ($n = 30$)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46 ± 19</td>
<td>45 ± 18</td>
<td>0.80</td>
</tr>
<tr>
<td>Sex [$n$ (%)]</td>
<td>Female</td>
<td>8 (26.7)</td>
<td>6 (20.0)</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>22 (73.3)</td>
<td>24 (80.0)</td>
</tr>
<tr>
<td>Admission indication [$n$ (%)]</td>
<td>Internal diseases</td>
<td>6 (20.0)</td>
<td>10 (33.3)</td>
</tr>
<tr>
<td></td>
<td>Head injuries</td>
<td>24 (80)</td>
<td>20 (66.7)</td>
</tr>
<tr>
<td>Antibiotic treatment [$n$ (%)]</td>
<td>Yes</td>
<td>25 (83.3)</td>
<td>24 (80.0)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>5 (16.7)</td>
<td>6 (20.0)</td>
</tr>
<tr>
<td>Nasogastric Tube [$n$ (%)]</td>
<td>Yes</td>
<td>21 (70.0)</td>
<td>25 (83.3)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>9 (30.0)</td>
<td>5 (16.7)</td>
</tr>
<tr>
<td>GCS [$n$ (%)]</td>
<td>Light coma</td>
<td>10 (33.3)</td>
<td>7 (23.3)</td>
</tr>
<tr>
<td></td>
<td>Coma</td>
<td>16 (53.3)</td>
<td>20 (66.7)</td>
</tr>
<tr>
<td></td>
<td>Deep coma</td>
<td>4 (13.4)</td>
<td>3 (10.0)</td>
</tr>
</tbody>
</table>

Notes: $n$: number; GCS: Glasgow Coma Scale.
comparison between the two groups on the second ($P = 0.59$) and fourth ($P = 0.77$) days of the intervention was not significant. Based on the related samples Qochran’s Q test, the comparison between the oropharyngeal colonization before the intervention and on the second and fourth days of the intervention in each group showed no significant statistical difference (Table 4).

**DISCUSSION**

Since 61.7% of all samples showed oropharyngeal colonization on the first day of admission (less than 12 h after intubation), it seems that the prevalence of oropharyngeal colonization was high in our samples. Findings of previous studies on oropharyngeal colonization and its consequences such as VAP represent a high prevalence of oropharyngeal colonization (30%-60%) in mechanically-ventilated patients in the ICU.\textsuperscript{1,3,5,11} In our study, 51.7% of the patients who had oropharyngeal colonization before the intervention were contaminated with gram-negative bacteria, including Klebsiella spp., E. coli, and P. aeruginosa, respectively. Koeman et al.\textsuperscript{12} showed that the rate of colonization with gram-negative microorganisms was between 41%-52% at the admission time in the general and surgery ICUs of public and university-affiliated hospitals in the Netherlands which was consistent with our results. Also, Tanitpong et al.\textsuperscript{13} reported over 60% oropharyngeal colon- ization with gram-negative bacteria. These findings indicate that mouthwash solutions that are effective on gram-negative microorganisms should be applied more extensively. This raises the problem that Chlorhexidine mouthwash is more effective on gram positive bacteria.

According to our findings, the difference in oropharyngeal colonization rates on the 1st and 2nd two days post intervention was not significant between the honey mouthwash 12.5% and Chlorhexidine mouthwash 0.2%. Also, the comparison between the oropharyngeal colonization before the intervention and on the second and fourth days of the intervention in each study group showed no significant statistical difference. Limited clinical studies have compared the impact of these two mouthwash solutions on the oropharyngeal colonization in the intubated mechanically-ventilated patients in the ICUs specifically and the studies were mainly in vitro. Also, previous clinical studies assessed merely the effect of honey, chlorhexidine or other mouthwash solutions to reduce dental plaque or mucositis. Aparna et al.\textsuperscript{13} showed that multi-flower honey mouthwash derived from Apis mellifera honey bees spe-

<table>
<thead>
<tr>
<th>Bacterial genus</th>
<th>Pre-intervention</th>
<th>2nd days post intervention</th>
<th>4th days post intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Honey group (n = 30)</td>
<td>Chlorhexidine group (n = 30)</td>
<td>Honey group (n = 30)</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>3 (10.0)</td>
<td>2 (6.7)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Acinetobacter species</td>
<td>2 (6.7)</td>
<td>2 (6.7)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>5 (16.7)</td>
<td>6 (20.0)</td>
<td>7 (23.3)</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Klebsiella species</td>
<td>9 (30.0)</td>
<td>9 (30.0)</td>
<td>13 (43.3)</td>
</tr>
<tr>
<td>Enterobacter species</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Serratiamarcescens</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>No growth</td>
<td>11 (36.7)</td>
<td>11 (36.7)</td>
<td>9 (30.0)</td>
</tr>
</tbody>
</table>

**Table 4 Comparison of oropharyngeal colonization between the two groups (n [%])**

<table>
<thead>
<tr>
<th>Oropharyngeal colonization</th>
<th>Honey group (n = 30)</th>
<th>Chlorhexidine group (n = 30)</th>
<th>P value\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before the intervention</td>
<td>Yes</td>
<td>19 (63.3)</td>
<td>18 (60.0)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>11 (36.7)</td>
<td>12 (40.0)</td>
</tr>
<tr>
<td>First two days after the intervention</td>
<td>Yes</td>
<td>19 (63.3)</td>
<td>20 (66.7)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>11 (36.7)</td>
<td>10 (33.3)</td>
</tr>
<tr>
<td>Second two days after the intervention</td>
<td>Yes</td>
<td>21 (70.0)</td>
<td>23 (76.7)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>9 (30.0)</td>
<td>7 (23.3)</td>
</tr>
</tbody>
</table>

P value\textsuperscript{a}: Cochran’s Q test, $\chi^2$ test.
cies in comparison with chlorhexidine 0.2% and Saline normal solutions led to more growth inhibition of Eu-
bacterium nodatum, Streptococcus mutans, Campylo-
bacter rectus, Streptococcus sanginis, Aggregatibacter
actinomycetemcomitans, and Porphyromonas gingiva-
ilis, in vitro. However, their in-vivo results confirmed
the efficacy of both honey mouthwash and chlorhexi-
dine solutions in inhibiting or reducing dental plaques
as an important risk factor for oropharyngeal coloniza-
tion in mechanically-ventilated patients. Nayak et al.14
showed that there was no significant difference be-
tween the effect of Manuka honey and chlorhexidine
0.2% on plaque formation in healthy volunteers. Al-
though using these mouthwash solutions for three days
significantly reduced plaque formation compared with
the third study group, xylitol chewing gum, Jain et al.13
declared that honey mouthwash has a higher anti-
plaque effect versus chlorhexidine mouthwash 0.2%.
Although there was no significant statistical difference
between the oropharyngeal colonization in the two
study groups, the results indicated that mouthwashing
with honey solution (thyme + astragale) 12.5% twice
daily, led to the inhibition of S. aureus and P. aerugino-
sa on the fourth day in all samples. However, it was
not able to control Klebsiella spp. and E. coli coloniza-
tion, while mouthwashing with chlorhexidine 0.2%
twice daily, led to colonization of Klebsiella spp., E. co-
li, and Staphylococcus spp. in some samples. Findings
of numerous studies indicated the in vitro16-18 and in vi-
vo19 antimicrobial effect of some types of honey such as
manuka honey 10% (on oral bacteria), manuka honey
(12.5%), willow herb, and heather and buckwheat hon-
ey on microorganisms such as E. coli, P. aeruginosa,
Salmonella enteric serovar typhi, S. pneumoniae, S. pyo-
genae, S. aureus, methicillin-resistant S. aureus and
Klebsiella spp., coagulase-negative Staphylococci. How-
ever, every honey type has its own specific antimicrobi-
al effect20 probably due to the origin (geographical
place and used flowers), type and the concentration of
honey, frequency of daily mouthwash in clinical set-
tings, the application method, and other factors.
Although chlorhexidine is widely used as the gold stan-
dard oral care in the ICU21 and numerous studies have
confirmed the effectiveness of its different concentra-
tions (0.12%, 0.2% and 2%) on the prevention of orop-
apharyngeal colonization and VAP in mechanically-ven-
tilated patients in the ICU,13,15,22 the lack of efficacy or
insufficient efficacy of chlorhexidine especially on
gram negative bacteria12,21 and its side effects such as ir-
ritation of oral mucosa, allergy and cytotoxicity13 have
persuaded researchers to compare this mouthwash with
other solutions. It seems that honey used in our study
(thyme+astragale) could be an appropriate safe alterna-
tive for inhibition of oropharyngeal colonization.
In conclusion, according to our findings on the com-
mon microorganism colonization in the mouth and
throat of intubated patients under mechanical ventila-
tion in the ICUs including S. aureus, P. aeruginosa, Ke-
lebsiella pneumoniae, E. coli, Acinetobacter spp., and
Enteric spp., honey (astragalus + thyme) 12.5% was ef-
effective in the prevention of colonization of many types
of these microorganisms, it seems that using honey
mouthwash as a natural, uncomplicated and inexpen-
sive solution can be effective for preventing oropharyn-
geal colonization and the aftermath VAP. So, honey
should be considered as an effective alternative for oral
care in the ICU. Regarding the limited studies on the
efficacy of honey mouthwash in intubated mechanically-
ventilated patients hospitalized in ICU, lack of ap-
propriate evidence-based concentrations of honey
mouthwash solutions, and proper protocols for the
mouthwash, the present study results could supply use-
ful information for the nursing researchers and ICU
nurses for further investigations.
Despite our significant findings, present study had
some limitations that should be considered in future
studies including shortage of sample size and lack of a
unified standard for mouth care by honey mouthwash
in intubated mechanically-ventilated patients which
were not conclusively controllable, despite the fact that
mouthwash was randomly performed by three trained
nurses in the same way in each group.

REFERENCES
1 Kusahara DM, Friedlander LT, Petelinia MAsS, Pedreira
MLGa. Oral care and oropharyngeal and tracheal coloniza-
tion by Gram-negative pathogens in children. Nurs Crit
Care 2012; 17 (3): 115-122.
2 Khezi HD, Haidari Gori Ji MA, Morad A, Gorjii H. Com-
parison of the antibacterial effects of matrica & Persica”
and chlorhexidine gluconate mouthwashes in mechanically-
ventilated ICU patients: a double blind randomized
3 Munro CL, Grap MJ, Jones DJ, McClish DK, Sessler
CN. Chlorhexidine, toothbrushing, and preventing venti-
lator-associated pneumonia in critically ill adults. Am J
4 Labeau SO, Van de Vyver K, Brusselaers N, Vogelaers D,
Blot SI. Prevention of ventilator-associated pneumonia
with oral antiseptics: a systematic review and Meta-analy-
5 O’Reilly M. Oral care of the critically ill: a review of the
literature and guidelines for practice. Aust Crit Care 2003;
16 (3): 101-110.
6 Malhotra R, Grover V, Kapoor A, Saxena D. Comparison
of the effectiveness of a commercially available herbal
mouthrinse with chlorhexidine gluconate at the clinical
349-352.
7 Hassajee AD, YaskellT, Socransky SS. Antimicrobial effect-
viveness of an herbal mouthrinse compared with an essen-
tial oil and a chlorhexidine mouthrinse. J Am Dent Assoc
2008; 139 (5): 606-611.
8 Booker S, Murff S, Kirk L, Jablonski R. Mouth care to
reduce ventilator-associated pneumonia. AJN 2013; 113
(10): 24-30.


Jain A, Bhaskar DJ, Gupta D, et al. Comparative evaluation of honey, chlorhexidine gluconate (0.2%) and combination of xylitol and chlorhexidine mouthwash (0.2%) on the clinical level of dental plaque: a 30 days randomized control trial. Perspect Clin Res 2015; 6(1): 53-57.


