Iran Red Crescent Med J. 2017 October; 19(10):e60576. doi: 10.5812/ircmj.60576.

Published online 2017 August 27. **Research Article**

# The Effect of Ozonated Water and Chlorhexidine Gluconate on Prevention of Ventilator-Associated Pneumonia: A Double-Blind, Randomized, Clinical Trial

Nasrin Hanifi,[1,*] Masomeh Masoumi,[2] Mohammad Reza Jamshidi,[3] and Soghrat Faghihzadeh[4]

[1]Ph.D, Assistant Professor, Zanjan Nursing and Midwifery School, Zanjan University of Medical Sciences (ZUMS), IR Iran
[2]MSc. in Nursing, School of Nursing and Midwifery, Zanjan University of Medical Sciences, Zanjan, IR Iran
[3]M.D, Cardiac Anesthesiologist, Assistant Professor, School of Medicine, Zanjan University of Medical Sciences, Zanjan, IR Iran
[4]Ph.D, Professor of Biostatistics, School of Medicine, Zanjan University of Medical Sciences, Zanjan, IR Iran

[*]*Corresponding author*: Nasrin Hanifi, Ph.D, Assistant Professor, Zanjan Nursing and Midwifery School, Zanjan University of Medical Sciences (ZUMS), Sharak Karmandan Street, P.O. Box: 45154-13191, IR Iran, E-mail: nasrinhanifi@zums.ac.ir

**Received** 2016 October 15; **Revised** 2017 February 13; **Accepted** 2017 May 15.

## Abstract

**Background:** The relationship between the oral care and ventilator-associated pneumonia (VAP) was confirmed in patients undergoing ventilation.
**Objectives:** The current study aimed at investigating the effects of chlorhexidine gluconate (CHX) and ozonated water on the prevention of VAP.
**Methods:** The current double-blind, randomized, clinical trial with the experimental and control groups was conducted in Iran in 2014. In the current study, 75 inpatients undergoing ventilation were grouped through the convenience sampling method and randomly allocated into 2 groups based on the inclusion criteria. In the experimental group (39 patients), oral care provided by ozonated water, while in the control group (35 patients), oral care was provided via CHX. Clinical pulmonary infection score (CPIS) was used to examine the rate of VAP infection.
**Results:** According to the results of the current study, the occurrence rate of VAP in the experimental and control groups were 2.4% and 8.3%, respectively that was equal until the 3rd day (P value = 0.339). However, on the 4th day, the VAP occurrence rate in the experimental group (14.6%) was significantly lower than that of the control group (30.6%) (P value = 0.02).
**Conclusions:** Based on the results, ozonated water was more effective to prevent VAP than CHX. Ozone water can be used as a suitable alternative mouthwash in patients undergoing mechanical ventilation.

*Keywords:* Oral Hygiene, Chlorhexidine Gluconate, Ozone, Ventilator-associated Pneumonia, Mechanical Ventilation

## 1. Background

Ventilator-associated pneumonia (VAP) is the leading cause of infection in the intensive care unit (ICU) and the 2nd in hospital-acquired infections (1-4). VAP is a critical concern in patients admitted to ICU (5, 6). VAP occurs within 24 to 28 hours from the application of the mechanical ventilator, which is used as an artificial respiration system in patients (6, 7). Early–onset VAP is developed in patients before 96 hours of receiving the mechanical ventilation and is sensitive to antibiotic therapy, but later–onset VAP is developed after 96 hours and is resistant to antibiotic therapy (5).

As the oral cavity is in contact with the lower airways of the trachea, the oral secretion causes the transfer of colonized bacteria in the mouth to the lower airways, which results in infection and pneumonia (8, 9). The results of the current study showed that lack of adequate care of the mouth leads to dry mucosa of the mouth, reduced salivary flow, inflammation of the oral mucosa, and colonization of pathogenic bacteria in the mouth and oropharynx (10, 11). Prevention and oral care are the most significant practices to control VAP (1, 12-15), and the use of mouthwash is an important part of the oral care process (16, 17).

Nowadays, the routine oral care of patients in most ICUs in Iran and other countries is the application of 0.2% CHX by swab sticks (18). Based on various studies, different concentrations of this mouthwash are applied to patients undergoing ventilation (19, 20). However, in the studies carried out by Scannapieco and Binkley, 0.2% and 0.12% CHX were ineffective in VAP prevention (21). Zhang et al. reviewed the effectiveness of various concentrations of CHX for VAP prevention and found that the effectiveness of 0.12% and 2% CHX for VAP prevention were proved in 9 and 3 studies, respectively (22). However, other studies showed that 0.2% CHX had no toxic effect on oral cells, while the toxic effects of 2% CHX included stimulation of oral mucosa, alterations in the sense of taste, and reversible color change

of the mouth, tongue, and teeth (23-25). Thus, it is clear that there is no general consensus on the appropriate concentration of CHX in standard mouthwashes to prevent VAP (13, 26). Indeed, many of the reported results on the effectiveness of CHX for VAP prevention are contradictory.

In various studies, different mouthwashes such as honey, normal saline, Matrica and Persic™, and hydrogen peroxide are compared with CHX and the research to find an effective mouthwash with the least complications continues (27-30). The diversity of such studies further clarifies the significance of mouthwashes in oral care, specifically in patients without self-care.

Another mouthwash used in oral care is ozonated water. Ozone, an allotrope of oxygen, is a strong oxidizer (31). It is also effective to eliminate bacteria, viruses, fungi, and oral microorganisms, which accumulate in dental plaque such as *Streptococcus* spp. (23, 31). Ozone is reported to be effective in reducing dental decay, improving the immune system, and stimulating the performance of mast cells (23). The result of a study showed that the mouthwash with ozone water is more effective than compound lidocaine solution to treat chemotherapy-induced oral mucositis in patients with hematological malignancies (32).

Ozone is 1.5 times stronger and 3000 times faster than chlorine, while leaves no waste (33). Ozone is not toxic if used at concentrations of 0.05 ppm per 8 hours. In addition, liquid ozone is the most biocompatible antiseptic (23). Ozonated water is used in dentistry as a mouthwash and a disinfectant for dental instruments (31, 34). In light of this, it seems that ozone would also be an effective mouthwash for inpatients. However, the current study literature review revealed that ozonated water was not previously used as a mouthwash for ICU inpatients.

## 2. Objectives

The current study aimed at comparatively examining the effects of 0.05 ppm ozonated water and 0.2% CHX for VAP prevention in patients undergoing mechanical ventilation.

## 3. Methods

The current study was a double-blind, randomized, clinical trial with 2 groups of experimental and control. Before the study, a research license (code ZUMS.REC.1392.106) was obtained from the committee of ethics of Zanjan University of Medical Sciences. Necessary clarifications were made to the patients' guardians. Written informed consent was obtained from the patients' guardians, and both the patients and guardians were assured of the confidentiality of data.

### 3.1. Participants

The population included patients undergoing mechanical ventilation in the ICU of Mousavi Hospital in Zanjan. The research was conducted in Zanjan, Iran. Mousavi hospital, affiliated to Zanjan University of Medical Sciences, is the only trauma center in the city. ICU of the hospital has 21 beds. In this unit, patients with trauma, patients undergoing extensive surgery, and the ones with extensive burns are hospitalized. In the current study, the convenience sampling method was employed. The sampling process was performed from 17 October, 2013 to 17 March, 2014.

To determine the sample size, a pilot study was conducted. A total of 41 patients were recruited; 23 in the experimental and 18 in the control groups.

The results of the pilot study showed a reduction of VAP in the experimental group. In the experimental group, 3 out of 23 patients and in the control group 9 out of 18 patients were affected with VAP. By considering $\alpha$ = 0.01, $\beta$ = 0.1, P1 = 0.13 and P2 = 0.5 through using the following formula, the sample size of each group was estimated 40.

$$n = \frac{\left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right)^2 \left(P_1\left(1-P_1\right) + P_2\left(1-P_2\right)\right)}{\left(P_1 - P_2\right)^2} \quad (1)$$

Research samples were divided into the experimental and control groups by the 2nd author. Therefore, according to the determined size of samples, a range of numbers was randomly selected by the table of random numbers. A spot was randomly selected on the table. To achieve the desired size, non-recurring double digits were selected and recorded to provide the favorable sample. Even numbers on the table were determined by default for the experimental group and odd numbers for the control group. After admission if the patient met the inclusion criteria, by referring to the random number table and according to the pre-determined numbers for the experimental and control groups, he/she was allocated into one of the groups.

During the research, 6 out of 80 patients were excluded due to the early separation from the mechanical ventilator or receiving intermittent ventilation and T- piece, leaving a final number of 74 patients. Two of the dropped patients were in the control group and 2 in the experimental one.

Finally, 39 patients were placed in the experimental group and 35 in the control group. The power of the test was examined considering this difference, and it had no effect on the power of the test.

Inclusion criteria were as follows:

The patient should undergo mechanical ventilation for at least 12 hours, being in the age range of 18 to 70 years, no history of pneumonia and aspiration during the hospitalization, or chemotherapy, and no maxillofacial fractures,

scalds, pregnancy, anemia, alcohol intoxication, or a recent myocardial infarction.

Exclusion criteria were separation from the mechanical ventilation before the end of the study period, vomiting during the use of gastrointestinal tract, death, or being dissuaded from continuing in any part of the study either by the patients' guardians or the doctors.

### 3.2. Intervention

The intervention started as the patient was admitted to the ICU. If the patient was diagnosed with pneumonia based on the clinical pulmonary infection score (CPIS) during the first 12 hours, they were not included in the study. In the control group, the routine mouthwash was 0.2% CHX (Iran Behsa Pharmaceutical Co., Iran) and in the experimental group, it was 0.05 ppm ozonated water. The ozonated water was produced by an ozone generator (Opura Model Op-1107, Canada) installed in the ICU. The device was calibrated every time before use.

This device was attached to the faucet in ICU by a connector. Thus, the combination of oxygen and water resulted in the production of ozonated water. The concentration of ozone in the water was 0.05 ppm. The emission of a smell similar to those of sea and fish proved the proper function of the device. The maximum durability of ozone in the water was 15 minutes (35). In the experimental group, each subject was provided by a new glass of freshly ozonated water (25 mL) for each treatment.

Both of these mouthwashes were applied identically 3 times a day. Before the intervention, patients' mouth and trachea received standard suctioning. The swab impregnated with either of the mouthwashes was rubbed rotationally on the upper gums from the right to the left side of the mouth. It was, then, changed and rubbed rotationally on the lower gums from left to right. Two other swabs impregnated with the mouthwash were used to cleanse the upper and lower teeth. After using the swabs and having prepared by the suction, the cuff pressure of the ET tube was increased to 35 mmHg via a manometer specifically used to measure the ET tube cuff pressure. Then, approximately 15 mL of the mouthwash was inserted into the patient's mouth and 30 seconds later the suctioning process was conducted. Immediately after the intervention, the cuff pressure was reduced to 15 to 20 mmHg. This intervention occurred over a 4-day period.

### 3.3. Outcomes and Measurements

The demographic data were gathered via a questionnaire, which included questions addressing the patients' age, gender, diagnosis, level of consciousness, whether they were taking diuretics, the ventilation mode, and positive end-expiratory pressure (PEEP). To study the effect of CHX and ozonated water on VAP prevention, the patients were evaluated based on CPIS on the 3rd and 4th days of admission. CPIS is a standard method to diagnose pneumonia, the validity and reliability of which was demonstrated by Fartoukh et al. (36).

CPIS includes 5 variables. The scores of pneumonia diagnosis are as follows:

1. Body temperature: 36.5 to 38.4°C, score: 0; 38.5 to 39°C, score: 1; > 39°C, score: 2.

2. White blood cells (WBC) count: 4,000 to 11,000, score: 0; 11,000 to 17,000, score: 1; > 17,000, score: 2.

3. Lung secretions: no secretions (suctioning received once in 6 hours), score: 0; low secretions (suctioning needed at least 2 or 3 times in 6 hours), score: 1; high secretions (suctioning needed more than 3 times in 6 hours), score: 2.

4. $PaO_2/FiO_2$ ratio: > 200, score: 0; < 200, score: 1.

5. Infiltration in radiography: transparent, score: 0; dispersed infiltration, score: 1; localized infiltration, score: 2.

Pneumonia infection was confirmed in cases where a score of 6 or more was obtained. In the current study, body temperature was recorded as the average temperature on the 1st, 3rd, and 4th days. It should also be noted that all patients received WBC count and chest X-ray tests on a daily basis. WBC test was done in both experimental and control groups in the medical laboratory of Mousavi hospital. Chest X-ray for both groups was also taken in the ICU by an available portable X-ray device.

VAP infection checkup was carried out by the anesthesiologist (the 3rd author), the only observer blind to the intervention method. On the other hand, the Glasgow coma scale (GCS) scores of the study subjects were lower than 15, which indicated that they were not aware of the intervention method.

### 3.4. Statistical Analysis

Data were analyzed with SPSS version 22. The Kolmogorov-Smirnov test indicated the normal distribution of data. Analysis of the collected data was done by the per protocol method. To ensure the results of the analysis, the intention-to-treat method was also conducted and the results did not differ from those of the per protocol method. Chi-square and the Fisher exact tests were used to compare the demographic variables between the 2 study groups. VAP infections on the 3rd and 4th days were compared through the Cochran Q test. The VAP infection rates of the 2 groups were compared via the Fisher exact test.

## 4. Results

The obtained results showed that most of the study subjects were male. The samples consisted of 67.57% males and 32.43% females. The patients' age ranged from 18 to 68 years; 63.51% of the patients were admitted to ICU following the trauma diagnosis and 38.49% for the extensive surgeries. The mean age of the patients in the experimental and control groups were 14.42 $\pm$ 1.39 and 44.61 $\pm$ 1.78 years, respectively. According to the results, there was no significant difference between the experimental and control groups with respect to age, gender, early detection, level of consciousness, and taking diuretics (Table 1). Synchronized intermittent mandatory ventilation (SIMV) mode was applied to both groups. A PEEP of 5 cm of water was used for all patients.

None of the patients was diagnosed with pneumonia during the first 12 hours. Every single factor studied in CPIS was separately examined in the experimental and control groups, and then, compared. The WBC count rates in each group on the 3rd day were significantly different; the other examined factors were not significantly different (Table 2). However, as the total score of the variables was important to CPIS, the 2 groups were compared with respect to both their scores and the presence or absence of infection.

As the results showed, the VAP infection rate in both groups increased on the 3rd and 4th days. To determine whether there was a significant difference between the rates of VAP infection on each of these days within each group, the Cochran Q test was applied. The VAP infection rates in the experimental group on the 3rd and 4th days were not significantly different (P value = 0.421), whereas in the control group, the VAP infection rate on the 4th day significantly increased compared with that of the 3rd day (P value = 0.001) (Table 3).

To compare the results of the experimental and control groups with respect to VAP infection, the Fisher exact test was applied. The results of this test showed that on the 3rd day these groups were not significantly different. On the 4th day, however, the experimental group exhibited a significant decrease in VAP infection compared with the control group (Table 3).

## 5. Discussion

The results revealed that the VAP infection rate on the 3rd and 4th days after the patients' admission increased in both the experimental and control groups; this increase was more noticeable between the 3rd and 4th days. However, this increase between the 3rd and 4th days was not statistically significant in the experimental group. Also, the VAP infection rate in the experimental group was significantly lower than that of the control group. The results indicated that the ozonated water was more effective for VAP prevention on the 4th day.

Segers et al. reported that 0.12% CHX was effective in VAP prevention in patients undergoing heart surgery in an ICU (37). Grap et al. also reported that CHX was effective in VAP prevention, observing a VAP infection occurrence of 33.3% in the experimental group compared with 55.6% in the control group (38). In both of these studies, no mouthwash was used in the control group. In the current study, the VAP infection rate on the 4th day in the CHX group (30.6%) was close to that of reported in the study by Grap et al. (33.3%) (38). It is possible that the inclusion of a third group in the study whose oral care was not based on any mouthwash, CHX would look more effective in VAP prevention. However, considering the proven effectiveness of mouthwash in the oral care process (16), the current study considered that it is not morally possible to have a group without the administration of a mouthwash. Furthermore, the routine oral care in ICUs was based on CHX. Nevertheless, the comparison of these groups revealed that the group that received ozonated water had lower VAP infection on the 4th day compared with the CHX group. Thus, it demonstrated the effectiveness of ozonated water to prevent VAP infection.

Some studies found that CHX was ineffective to prevent VAP infection (13, 26). Munro et al. traced the effect of their intervention for 14 days and reported that CHX was effective in preventing VAP infections only up to the 3rd day (26). In the current study, it was observed that CHX had the same effect as ozonated water on the prevention of VAP infections up to the 3rd day. However, on the 4th day, a greater number of patients developed pneumonia in the CHX group than in the ozonated water group.

Laxman and Kshitish reported that ozonated water caused a greater reduction of oral microorganisms than 0.2% CHX (39). The results of the research by Huth et al. also proved greater positive effects of ozonated water on gum cells in comparison with those of other solutions (40). Although there is a lack of research on the effect of ozonated water on the prevention of VAP infection, there is literature on the relationship between VAP and oral care and the number of oral microorganisms (1, 12), and the application of ozonated water to eliminate many of such microorganisms (23, 34, 41). Thus, it can be stated that of the methods tested in the current study and other studies, the application of ozonated water had the greatest preventive effect on VAP infections.

**Table 1.** Comparison of the Demographic Variables in the Study Groups

| Demographic Variables | | Group | | | | | | The Fisher Exact Test (P Value) |
|---|---|---|---|---|---|---|---|---|
| | | Bootstrap for Percent | | | | | | |
| | | Experimental | | | Control | | | |
| | | No. (%) | 95% Confidence Interval | | No. (%) | 95% Confidence Interval | | |
| | | | Lower | Upper | | Lower | Upper | |
| Gender | Female | 14 (35.9) | 23.1 | 51 | 10 (28.6) | 14 | 42 | 0.621 |
| | Male | 25 (64.1) | 48 | 76 | 25 (71.4) | 57 | 85 | |
| Age | 18 - 28 | 10 (25.6) | 12 | 36 | 9 (25.7) | 11 | 40 | 1.000 |
| | 29 - 38 | 6 (15.4) | 5 | 28 | 9 (25.7) | 11 | 40 | |
| | 39 - 48 | 9 (23.1) | 10 | 38 | 7 (20) | 8 | 34 | |
| | 49 - 58 | 10 (25.6) | 12 | 41 | 6 (17.1) | 2 | 22 | |
| | 59 - 68 | 4 (10.3) | 2 | 23.1 | 4 (11.4) | .0 | 8 | |
| Diagnosis | Trauma | 27 (69.23) | 53 | 84 | 20 (57.14) | 40.1 | 71 | 0.486 |
| | Non traumatic (surgical) | 12 (30.7) | 15 | 46 | 15 (42.86) | 28 | 59 | |
| GCS | 3 - 5 | 2 (5.1) | .0 | 12 | 4 (11.4) | 2 | 22 | 0.529 |
| | 6 - 7 | 7 (20.5) | 10 | 33 | 10 (28.6) | 11 | 40 | |
| | 8 -9 | 15 (35.9) | 20 | 51 | 13 (37.1) | 22 | 57 | |
| | 10 - 12 | 14 (35.9) | 20 | 51 | 8 (22.8) | 8 | 37 | |
| | 13 - 15 | 1 (2.6) | .0 | 7 | 0 (0) | 0 | 0 | |
| Diuretics | Advice | 21 (53.8) | 47 | 68 | 22 (62.9) | 54 | 72 | 0.433 |
| | No advice | 18 (46.2) | 35 | 58 | 13 (37.1) | 28 | 49 | |

### 5.1. Limitations of the Study

The time limitation in the current study prevented the researchers from monitoring patients with respect to VAP infection beyond the 4th day of ventilation, and including a larger sample size. This may compromise the internal validity of the research. Hence, more studies are recommended to further investigate the use of ozonated water as a convenient and affordable solution for oral care in ICUs and generalize the results of the current study.

### 5.2. Conclusions

The results of the research clearly demonstrated that ozonated water was far more effective to prevent VAP infections than CHX. Although both methods were equally good till the 3rd day or in other words, the rates of VAP infection in each group were not significantly different, on the 4th day the number of patients diagnosed with VAP infections was significantly lower in the group treated with ozonated water.

Due to the availability of devices that produce ozone water in the ICU and the shelf life of these devices, the use of ozonated water compared with that of chlorhexidine solution is more affordable. In Iran, the current study used ozonated water as an effective mouthwash solution for the 1st time for the patients in the ICU ward, which is regarded as an innovative feature of the current study. There was no study on the use of ozone-water in mechanically ventilated patients in other countries. Therefore, the current study was the basis for further research on the use of ozone-water and different consequences in this group of patients.

## Acknowledgments

The current research was part of the MSc thesis of the 2th author, registered in the Iranian clinical trial center (IRCT2015080523520N1). The current study was financially supported by the vice-chancellery for research of Zanjan University of Medical Sciences. The authors wish to express their sincere thanks to all the patients who participated in the study, their families, and the ICU nurses who offered their great assistance.

**Table 2.** Comparison of the Studied Variables Based on CPIS in the Study Groups

| The Studied Variables Based on the CPIS Index | Scoring | Bootstrap for Percent | | | | | | | | | | | | The Fisher Exact Test (P Value) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3rd Day | | | | | | 4th Day | | | | | | 3 rd Day | 4th Day |
| | | Experimental Group | | | Control Group | | | Experimental Group | | | Control Group | | | | |
| | | No. (%) | 95% Confidence Interval | | No. (%) | %95 Confidence Interval | | No. (%) | 95% Confidence Interval | | No. (%) | %95 Confidence Interval | | | |
| | | | Lower | Upper | | Lower | Upper | | Lower | Upper | | Lower | Upper | | |
| Temprature | 36.5 - 38.4 | 7 (17.9) | 7 | 30 | 5 (49.3) | 2 | 25 | 11 (28.2) | 15 | 43 | 5 (14.3) | 2 | 28 | 0.384 | 0.228 |
| | 36.5 - 39 | 6 (15.4) | 5 | 28 | 2 (5.7) | .0 | 14 | 3 (7.7) | .0 | 17 | 1 (2.9) | .0 | 8 | | |
| | < 39 | 26 (66.7) | 51 | 79 | 28 (80) | 65 | 91 | 25 (64.1) | 48 | 79 | 29 (82.9) | 68 | 49 | | |
| WBC | 4000 - 11000 | 8 (20.5) | 7 | 33 | 7 (20) | 8 | 34 | 13 (33.3) | 17 | 48 | 5 (14.3) | 2 | 25 | 0.022 | 0.095 |
| | 11005 - 17000 | 31 (79.5) | 66 | 92 | 22 (62.9) | 48 | 77 | 22 (56.4) | 41 | 71 | 21 (60) | 42 | 77 | | |
| | < 17005 | 0 | .0 | .0 | 6 (17.1) | 5 | 31 | 4 (10.3) | 2 | 20 | 9 (25) | 11 | 40 | | |
| Lung Secretions | -No secretions | 24 (61.5) | 46 | 76 | 15 (42.9) | 25 | 60 | 10 (25.6) | 12 | 38 | 7 (20) | | | 0.241 | 0.449 |
| | +low secretion | 13 (33.3) | 20 | 48 | 19 (54.3) | 37 | 71 | 26 (66.7) | 51 | 79 | 22 (62.9) | | | | |
| | ++High secretion | 2 (5.1) | .0 | 12 | 1 (2.9) | .0 | 8 | 3 (7.7) | .0 | 17 | 6 (17.1) | | | | |
| $PaO_2/FiO_2$ | > 200 | 35 (89.7) | 79 | 97 | 29 (82.9) | 68 | 94 | 34 (87.2) | 76 | 97 | 29 (82.9) | 68 | 94 | 0.502 | 0.7 |
| | < 200 | 4 (10.3) | 2 | 20 | 6 (17.1) | 5 | 31 | 5 (12.8) | 2 | 23.1 | 6 (17.1) | 5 | 31 | | |
| Inflitration chest X-ray | Transparent | 26 (66.7) | 51 | 82.1 | 24 (68.6) | 57 | 85 | 18 (46.2) | 30 | 61 | 15 (42%) | 25 | 60 | 0.617 | 0.818 |
| | Dispersed Infiltration | 13 (33.3) | 17 | 48 | 10 (28.6) | 11 | 40 | 18 (46.2) | 30 | 61 | 18 (51) | 34 | 68 | | |
| | Localized Infiltration | .0 | .0 | .0 | 1 (2.9) | .0 | 11 | 3 (7.7) | .0 | 17 | 2 (5) | .0 | 14 | | |

**Table 3.** The VPA Infection Rate on the 3rd and 4th Days in the Study Groups

| Variable | Bootstrap for Percent | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3rd Day | | | | | | 4th Day | | | | | |
| | Experimental Group | | | Control Group | | | Experimental Group | | | Control Group | | |
| | No. (%) | 95% Confidence Interval | | No. (%) | 95% Confidence Interval | | No. (%) | 95% Confidence Interval | | No. (%) | 95% Confidence Interval | |
| | | Lower | Upper | | Lower | Upper | | Lower | Upper | | Lower | Upper |
| With VAP | 1 (2.4) | .0 | 7 | 3 (8.3) | .0 | 17 | 6 (14.6) | 5 | 28 | 14 (30.6) | 22 | 57 |
| Without VAP | 38 (97) | 92 | 100 | 32 (91.7) | 82 | 100 | 33 (85.4) | 71 | 94 | 21 (69.4) | 42 | 77 |
| The Fisher Exact Test (P Value) | 0.339 | | | | | | 0.02 | | | | | |

## Footnotes

**Authors' Contribution:** Study design: Nasrin Hanifi; data gathering: Masomeh Masoumi and Mohammad Reza Jamshidi; statistical analysis and interpretation of the data: Soghrat Faghihzadeh and Nasrin Hanifi; drafting of the manuscript: Masomeh Masoumi; critical revision of the manuscript: Nasrin Hanifi; study supervision: Nasrin Hanifi.

**Conflict of Interests:** The authors declared no conflict of interests.

**Funding/Support:** The study was granted by Zanjan University of Medical Sciences.

## References

1. Al-Tawfiq JA, Abed MS. Decreasing ventilator-associated pneumonia in adult intensive care units using the Institute for Healthcare Improvement bundle. *Am J Infect Control.* 2010;**38**(7):552–6. doi: 10.1016/j.ajic.2010.01.008. [PubMed: 20400203].
2. Heck K. Decreasing ventilator-associated pneumonia in the intensive care unit: a sustainable comprehensive quality improvement program. *Am J Infect Control.* 2012;**40**(9):877–9. doi: 10.1016/j.ajic.2011.11.010. [PubMed: 22386154].
3. Marra AR, Cal RG, Silva CV, Caserta RA, Paes AT, Moura DJ, et al. Successful prevention of ventilator-associated pneumonia in an intensive care setting. *Am J Infect Control.* 2009;**37**(8):619–25. doi: 10.1016/j.ajic.2009.03.009. [PubMed: 19559503].
4. Johnson K, Domb A, Johnson R. One evidence based protocol doesn't fit all: brushing away ventilator associated pneumonia in trauma patients. *Intensive Crit Care Nurs.* 2012;**28**(5):280–7. doi: 10.1016/j.iccn.2012.02.005. [PubMed: 22534495].
5. Barnes CM. Dental hygiene intervention to prevent nosocomial pneumonias. *J Evid Based Dent Pract.* 2014;**14 Suppl**:103–14. doi: 10.1016/j.jebdp.2014.02.002. [PubMed: 24929595].

6. Cutler LR, Sluman P. Reducing ventilator associated pneumonia in adult patients through high standards of oral care: a historical control study. *Intensive Crit Care Nurs.* 2014;**30**(2):61–8. doi: 10.1016/j.iccn.2013.08.005. [PubMed: 24314858].
7. 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-analysis. *Lancet Infect Dis.* 2011;**11**(11):845–54. doi: 10.1016/S1473-3099(11)70127-X. [PubMed: 21798809].
8. El-Rabbany M, Zaghlol N, Bhandari M, Azarpazhooh A. Prophylactic oral health procedures to prevent hospital-acquired and ventilator-associated pneumonia: a systematic review. *Int J Nurs Stud.* 2015;**52**(1):452–64. doi: 10.1016/j.ijnurstu.2014.07.010. [PubMed: 25443306].
9. Kaneoka A, Pisegna JM, Miloro KV, Lo M, Saito H, Riquelme LF, et al. Prevention of Healthcare-Associated Pneumonia with Oral Care in Individuals Without Mechanical Ventilation: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Infect Control Hosp Epidemiol.* 2015;**36**(8):899–906. doi: 10.1017/ice.2015.77. [PubMed: 25857604].
10. Adib-Hajbaghery M, Ansari A, Azizi-Fini E. Oral care in ICU patients: a review of research evidence. *KAUMS J.* 2011;**15**(3):280–93.
11. Ames NJ, Sulima P, Yates JM, McCullagh L, Gollins SL, Soeken K, et al. Effects of systematic oral care in critically ill patients: a multicenter study. *Am J Crit Care.* 2011;**20**(5):e103–14. doi: 10.4037/ajcc2011359. [PubMed: 21885453].
12. Hutchins K, Karras G, Erwin J, Sullivan KL. Ventilator-associated pneumonia and oral care: a successful quality improvement project. *Am J Infect Control.* 2009;**37**(7):590–7. doi: 10.1016/j.ajic.2008.12.007. [PubMed: 19716460].
13. Berry AM, Davidson PM, Masters J, Rolls K, Ollerton R. Effects of three approaches to standardized oral hygiene to reduce bacterial colonization and ventilator associated pneumonia in mechanically ventilated patients: a randomised control trial. *Int J Nurs Stud.* 2011;**48**(6):681–8. doi: 10.1016/j.ijnurstu.2010.11.004. [PubMed: 21185559].
14. Gallagher JA. Implementation of Ventilator-Associated Pneumonia Clinical Guideline (Bundle). *J Nurse Practition.* 2012;**8**(5):377–82. doi: 10.1016/j.nurpra.2012.02.017.
15. Jordan A, Badovinac A, Spalj S, Par M, Slaj M, Plancak D. Factors influencing intensive care nurses' knowledge and attitudes regarding ventilator-associated pneumonia and oral care practice in intubated patients in Croatia. *Am J Infect Control.* 2014;**42**(10):1115–7. doi: 10.1016/j.ajic.2014.07.008. [PubMed: 25278406].
16. Pear S, Stoessel CK, Shoemake S. The role of oral care in the prevention of hospital-acquired pneumonia. *Infect Control Today.* 2007;**11**(10):1–3.
17. Prendergast V, Kleiman C, King M. The Bedside Oral Exam and the Barrow Oral Care Protocol: translating evidence-based oral care into practice. *Intensive Crit Care Nurs.* 2013;**29**(5):282–90. doi: 10.1016/j.iccn.2013.04.001. [PubMed: 23702324].
18. Rello J, Koulenti D, Blot S, Sierra R, Diaz E, De Waele JJ, et al. Oral care practices in intensive care units: a survey of 59 European ICUs. *Intensive Care Med.* 2007;**33**(6):1066–70. doi: 10.1007/s00134-007-0605-3. [PubMed: 17384927].
19. Munro CL, Grap MJ, Elswick RJ, McKinney J, Sessler CN, Hummel R3. Oral health status and development of ventilator-associated pneumonia: a descriptive study. *Am J Crit Care.* 2006;**15**(5):453–60. [PubMed: 16926366].
20. Kim EK, Jang SH, Choi YH, Lee KS, Kim YJ, Kim SH, et al. Effect of an oral hygienic care program for stroke patients in the intensive care unit. *Yonsei Med J.* 2014;**55**(1):240–6. doi: 10.3349/ymj.2014.55.1.240. [PubMed: 24339313].
21. Scannapieco FA, Binkley CJ. Modest reduction in risk for ventilator-associated pneumonia in critically ill patients receiving mechanical ventilation following topical oral chlorhexidine. *J Evid Based Dent Pract.* 2012;**12**(2):103–6. doi: 10.1016/j.jebdp.2012.03.010. [PubMed: 22726793].
22. Zhang TT, Tang SS, Fu LJ. The effectiveness of different concentrations of chlorhexidine for prevention of ventilator-associated pneumonia: a meta-analysis. *J Clin Nurs.* 2014;**23**(11-12):1461–75. doi: 10.1111/jocn.12312. [PubMed: 23952970].
23. Anupunpisit V, Chuanchaiyakul S, Petchpiboolthai H, Jungudomjaroen S, Stapanavatr W. Characterization of infected diabetic wound after ozonated water therapy. *J Med Health Sci.* 2004;**11**(1, 2).
24. Lam OL, McGrath C, Li LS, Samaranayake LP. Effectiveness of oral hygiene interventions against oral and oropharyngeal reservoirs of aerobic and facultatively anaerobic gram-negative bacilli. *Am J Infect Control.* 2012;**40**(2):175–82. doi: 10.1016/j.ajic.2011.03.004. [PubMed: 21719150].
25. Plantinga NL, Wittekamp BHJ, Leleu K, Depuydt P, Van den Abeele AM, Brun-Buisson C, et al. Oral mucosal adverse events with chlorhexidine 2% mouthwash in ICU. *Intensive Care Med.* 2016;**42**(4):620–1. doi: 10.1007/s00134-016-4217-7. [PubMed: 26850333].
26. Munro CL, Grap MJ, Sessler CN, Elswick RJ, Mangar D, Karlnoski-Everall R, et al. Preintubation application of oral chlorhexidine does not provide additional benefit in prevention of early-onset ventilator-associated pneumonia. *Chest.* 2015;**147**(2):328–34. doi: 10.1378/chest.14-0692. [PubMed: 25317722].
27. Jain A, Bhaskar DJ, Gupta D, Agali C, Gupta V, Gupta RK, 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):53–7. doi: 10.4103/2229-3485.148819. [PubMed: 25657903].
28. Khezri HD, Gorji MAH, Morad A, Gorji H. Comparison of the antibacterial effects of matrica & Persica™ and chlorhexidine gluconate mouthwashes in mechanically ventilated ICU patients: a double blind randomized clinical trial. *Rev Chilena Infectol.* 2013;**30**(4):368–73.
29. Nobahar M, Razavi MR, Malek F, Ghorbani R. Effects of hydrogen peroxide mouthwash on preventing ventilator-associated pneumonia in patients admitted to the intensive care unit. *Braz J Infect Dis.* 2016;**20**(5):444–50. doi: 10.1016/j.bjid.2016.06.005. [PubMed: 27471125].
30. Azimi M, Jouybari L, Moghadam S, Ghaemi E, Behnampoor N, Sanagoo A, et al. Antimicrobial effects of chlorhexidine, matrica drop mouthwash (chamomile extract), and normal saline on hospitalized patients with endotracheal tubes. *Iran J Nurs Midwifery Res.* 2016;**21**(5):458–63. doi: 10.4103/1735-9066.193390. [PubMed: 27904627].
31. Cesar J, Sumita TC, Junqueira JC, Jorge AO, do Rego MA. Antimicrobial effects of ozonated water on the sanitization of dental instruments contaminated with E. coli, S. aureus, C. albicans, or the spores of B. atrophaeus. *J Infect Public Health.* 2012;**5**(4):269–74. doi: 10.1016/j.jiph.2011.12.007. [PubMed: 23021648].
32. Yan G, Sun W, Yan H. The Benefit about Ozonated Water Mouthwash for Treatment of Oral Mucositis in Patients with Hematological Malignancies. ; 2014.
33. Bezirtzoglou E, Cretoiu SM, Moldoveanu M, Alexopoulos A, Lazar V, Nakou M. A quantitative approach to the effectiveness of ozone against microbiota organisms colonizing toothbrushes. *J Dent.* 2008;**36**(8):600–5. doi: 10.1016/j.jdent.2008.04.007. [PubMed: 18502558].
34. Nagayoshi M, Fukuizumi T, Kitamura C, Yano J, Terashita M, Nishihara T. Efficacy of ozone on survival and permeability of oral microorganisms. *Oral Microbiol Immunol.* 2004;**19**(4):240–6. doi: 10.1111/j.1399-302X.2004.00146.x. [PubMed: 15209994].
35. Gupta G, Mansi B. Ozone therapy in periodontics. *J Med Life.* 2012;**5**(1):59–67. [PubMed: 22574088].
36. Fartoukh M, Maitre B, Honore S, Cerf C, Zahar JR, Brun-Buisson C. Diagnosing pneumonia during mechanical ventilation: the clinical pulmonary infection score revisited. *Am J Respir Crit Care Med.* 2003;**168**(2):173–9. doi: 10.1164/rccm.200212-1449OC. [PubMed:

12738607].
37. Segers P, Speekenbrink RG, Ubbink DT, van Ogtrop ML, de Mol BA. Prevention of nosocomial infection in cardiac surgery by decontamination of the nasopharynx and oropharynx with chlorhexidine gluconate: a randomized controlled trial. *JAMA.* 2006;**296**(20):2460–6. doi: 10.1001/jama.296.20.2460. [PubMed: 17119142].
38. Grap MJ, Munro CL, Hamilton VA, Elswick RJ, Sessler CN, Ward KR. Early, single chlorhexidine application reduces ventilator-associated pneumonia in trauma patients. *Heart Lung.* 2011;**40**(5):e115–22. doi: 10.1016/j.hrtlng.2011.01.006. [PubMed: 21411151].
39. Kshitish D, Laxman VK. The use of ozonated water and 0.2% chlorhexidine in the treatment of periodontitis patients: a clinical and microbiologic study. *Indian J Dent Res.* 2010;**21**(3):341–8. doi: 10.4103/0970-9290.70796. [PubMed: 20930341].
40. Huth KC, Jakob FM, Saugel B, Cappello C, Paschos E, Hollweck R, et al. Effect of ozone on oral cells compared with established antimicrobials. *Eur J Oral Sci.* 2006;**114**(5):435–40. doi: 10.1111/j.1600-0722.2006.00390.x. [PubMed: 17026511].
41. Azarpazhooh A, Limeback H. The application of ozone in dentistry: a systematic review of literature. *J Dent.* 2008;**36**(2):104–16. doi: 10.1016/j.jdent.2007.11.008. [PubMed: 18166260].