The influence of professional oral hygiene care on reducing ventilator-associated pneumonia in trauma intensive care unit patients

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Key points

There is a lack of evidence on the effect of professional oral hygiene care on reducing ventilator-related pneumonia for critically ill patients and a lot of clinical studies are required.

Professional oral hygiene care showed the effect of reducing the incidence of ventilator-related pneumonia in critically ill patients. In addition, there was a significant reduction in effect of *Staphylococcus aureus* and *Klebsiella pneumoniae*, the major strains of ventilator-related pneumonia.

Professional oral hygiene care improved the oral health status of critically ill patients.

Abstract

Aim This study aimed to examine the effects of professional oral hygiene care for the prevention of ventilator-associated pneumonia (VAP) and the improvement of oral hygiene among patients in the trauma intensive care unit (TICU).

Materials and methods TICU patients who underwent intubation were randomly assigned to either the experimental group (n = 29) or control group (n = 28). The developed professional oral hygiene care protocol was administered to patients in the experimental group every 24 hours. Additionally, data regarding general characteristics, medical history, oral hygiene status, Clinical Pulmonary Infection Score and quantitative polymerase chain reaction were assessed.

Results The incidence of VAP differed between the control group (10.58) and experimental group (0) post intervention. Post-admission bedside oral exam scores with significant differences in oral hygiene were observed in the experimental group (in contrast to the control group) from 48 hours onwards (10.69 ± 3.43, p = 0.06). *Staphylococcus aureus* and *Klebsiella pneumoniae* exhibited significant differences in count as professional oral hygiene care continued.

Conclusions This study suggests a model in which different health care professionals can cooperate to reduce the incidence of VAP and improve oral health conditions.

Introduction

Hospital-acquired infections are more common in patients in the intensive care unit (ICU) than in the general hospitalised population.[1] According to the Korean National Healthcare-Associated Infections Surveillance System (KONIS) data (collected from 96 South Korean hospitals and 169 ICUs), 2,524 hospital-acquired infections were confirmed between July 2014 and June 2015 and ventilator-associated pneumonia (VAP) accounted for 60.3% of 735 cases of pneumonia.[2] Although VAP incidences decreased, they still accounted for 48.8% of pneumonia cases in 2018.[3]

Mechanical ventilation is a life-sustaining treatment required for ICU patients. However, one day of mechanical ventilator use can increase the risk of VAP by 1–3%. Mechanical ventilator usage also increases the risk of VAP 6- to 21-fold compared to that in patients not receiving mechanical ventilator care.[4] The occurrence of VAP prolongs the ICU stay, resulting in increased treatment costs.[5] Moreover, since patients with VAP are twice as likely to die as other patients, VAP prevention is crucial.[6]

Trauma can affect a patient's immune system and a lack of appropriate care in the early stages of trauma may lead to increased post-trauma complications.[7,8] Complications in patients who undergo serious trauma are a major cause of late deaths. Recognising and providing active care for complications in the early stages of trauma is an important aspect of treatment for trauma patients in ICUs. Moreover, trauma patients are more likely than other surgical patients to contract hospital-acquired infections.[9] According to a US study, trauma patients exhibited higher incidences of pneumonia[10] and VAP[11] than those without trauma.

In South Korea, 1.9 million patients present to emergency departments every year with trauma. In 2017, 70,000 of those were serious trauma patients. This number increases every year. Serious trauma occurs most frequently in the working-age population (15–64 years), involving a high proportion of the financially vulnerable population; thus, long hospital admissions further increase economic and social difficulties for trauma patients.[12]

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In the US in 1974, Trukey *et al.*[13] reported that having a dedicated trauma treatment system decreases the rate of preventable deaths. As of 2014, there were 203 level-1 trauma centres in the US and 90 in Germany. After the introduction of trauma centres, the mortality of trauma patients decreased from 34% to 15% in the US and from 40% to 20% in Germany.[14]

As of 2010, the mortality rate of serious trauma patients in Korea was 35.2%, which is much higher than the 10–15% seen in the US and Japan. Regional trauma centres were introduced in 2012.[15] As of March 2019, 14 regional trauma centres existed and 3 more were being prepared. Subsequently, the preventable trauma mortality rate (trauma patients that would have survived if they had received appropriate care within an appropriate time frame) decreased from 35.2% in 2010 to 30.5% in 2015. The present goal is to decrease the nationwide rate below 20% by 2025. To achieve this goal, improving the quality of medical services provided to serious trauma patients remains an important task.[12]

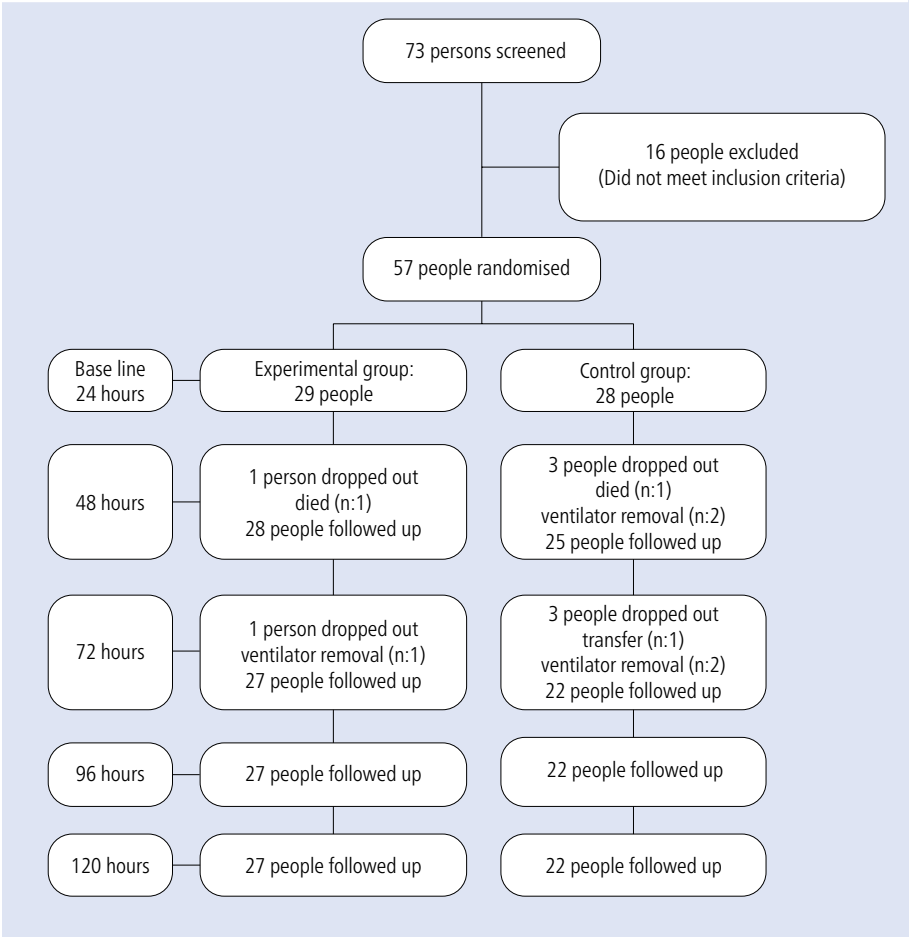
The US Institute for Healthcare Improvement has introduced the concept of a 'ventilator bundle,' which is a scientific, evidence-based 'bundle' for preventing VAP. Daily oral care with chlorhexidine (CHX) was included in the bundle as of 2010.[16] Oral care using CHX is more effective in decreasing the potential for VAP in patients on mechanical ventilators than teeth-brushing.[17,18] However, systematic reviews conducted in 2013 reported that besides the VAP-decreasing effects of CHX, evidence supporting the association between oral care and VAP is lacking.[18,19]

Some US hospitals have reported on the effects of oral care experts in reducing VAP and the cost-to-benefit ratio.[20] Similarly, in Japan, a dedicated oral care system involving dentists, nurses and dental hygienists provides oral care for admitted patients, pre- and post-operation.[21]

Systematic oral care is fundamentally important in patients requiring intensive care. As trauma patients are especially vulnerable to infection, efforts should be made to prevent hospital-acquired infections.[22] Despite extensive research on the importance of oral hygiene care, research assessing the effective strategies of oral hygiene care and its effects on trauma patients is scarce.

This study aimed to evaluate the effects of professional oral hygiene care provided by dental hygienists for improving oral hygiene and reducing VAP in trauma ICU patients on mechanical ventilators.

Fig. 1 Flowchart of the participant selection process



Materials and methods

Study design

This single-blind study, conducted from 4 March 2017 to 2 November 2017, was an interventional study that examined the effects of oral hygiene care on reducing the incidence of VAP in trauma ICU patients on mechanical ventilators.

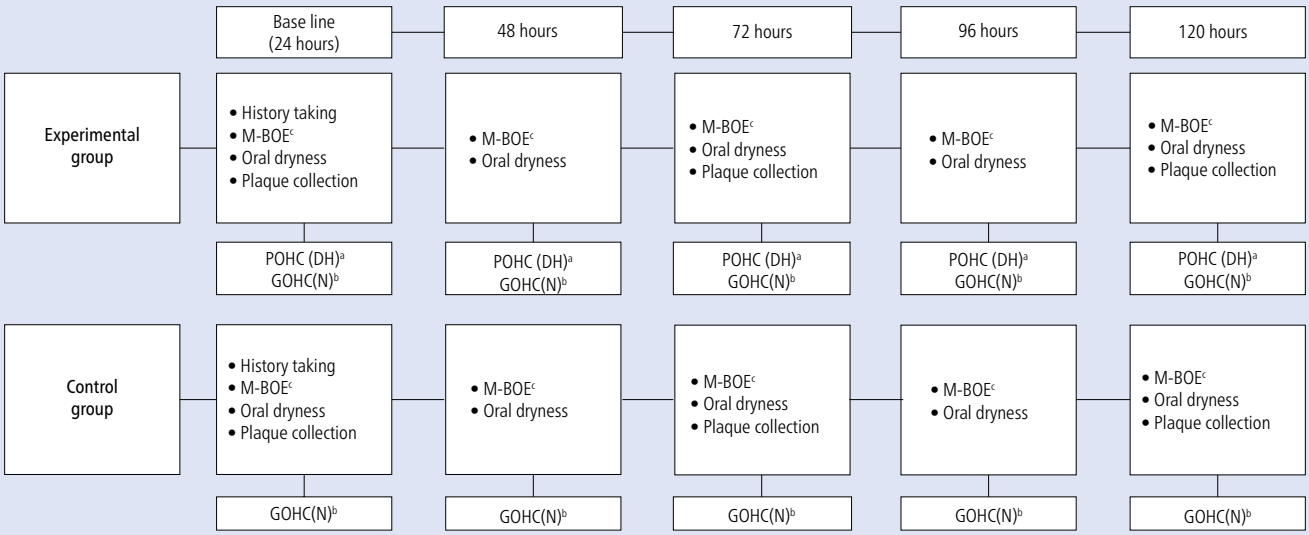
Study subjects

This study was conducted at a tertiary hospital in Wonju, Gangwon Province, among mechanically-ventilated patients admitted to a trauma ICU. The G*Power 3.1 programme developed by Heinrich-Heine-Universität Düsseldorf was used to calculate the number of necessary participants (n = 73) for this study; these participants were subsequently assigned to either the experimental or control group. On the day of admission, the charge nurse of the ICU and the attending physician determined whether the participants were appropriate for this study and assigned them to the groups accordingly. Inclusion criteria were as follows: mechanically-ventilated adult patients; aged 20 years and above; admitted to the trauma ICU; and who the attending physician found to be capable of receiving oral management. Exclusion criteria were as follows: patients (or their legal representatives) who declined to participate in the study; patients who were not well enough to receive oral care; and patients who could not receive oral care owing to severe maxillofacial injury or other reasons. We also excluded patients who were pregnant or diagnosed with pneumonia at the time of admission (Fig. 1).

Oral hygiene care protocol

The researchers prepared a protocol for professional oral hygiene care based on references from prior literature and the actual practices employed currently in ICUs. The protocol was reviewed and revised by the ICU attending physicians and charge nurses, the dentist and dental hygienist in the department of oral and maxillofacial surgery and the charge dental hygienists at Ichikawa Hospital in Japan, who were responsible for patients' oral hygiene care. A simulation was performed based on the revised protocol and a detailed manual was prepared.

Fig. 2 Experimental design of the study (note: A = POHC: professional oral hygiene care by dental hygienist; B = GOHC: general oral hygiene care by nurse; C = M-BOE: modified bedside oral exam)



Group intervention

The experimental group received oral hygiene care from nurses (using oral swabs and CHX 0.12% solution) 24 hours after mechanical ventilation initiation and every 8 hours thereafter. Dental hygienists provided additional oral hygiene care (toothbrushes, CHX 0.12% solution and cotton balls) every 24 hours. The control group only received oral hygiene care from the nurses (using oral swabs and CHX 0.12% solution) 24 hours after mechanical ventilation initiation and every 8 hours thereafter. The intervention was implemented over five days for both groups (Fig. 2).

Regarding the toothbrushing method, the bristles were placed at the gingival margin and the teeth were brushed using light vibration motions. The type of toothbrush was determined based on previous research, which revealed that it is appropriate to use a paediatric toothbrush to perform oral care for patients on a ventilator. Before conducting this study, researchers with experience in performing oral care in critically ill patients on ventilators had a discussion. Based on this, an appropriate toothbrush with a small toothbrush head, long handle length and non-coarse bristles was selected from the paediatric toothbrushes sold in the Korean market.

Before conducting this study, a preliminary review[19] was performed regarding the use of the aspirating toothbrush sold in Korea to patients on ventilators. It was concluded that this tool should not be used for these patients because it is difficult to fit it in their oral cavity due to the small opening of their mouths and the thickness of the aspirating toothbrush. A disposable suction catheter, found in the ICU where this study was conducted, was used to prevent the CHX solution from flowing into the oral cavity. The excess solution on the toothbrush and cotton balls was removed by shaking it off sufficiently on a gauze piece. The suction tube was placed as close to the head of the toothbrush as possible while brushing.

There is no evidence regarding the difference between the effect of CHX gel and CHX solution in the prevention of ventilator-associated pneumonia. Currently in Korea, the gel-type CHX has been discontinued and the hospital where this study was conducted uses 0.12% CHX solution. Therefore, 0.12% CHX solution was used in the study.

Study variables

General characteristics and medical history

Sex and age were included as general participant characteristics. Using medical records, each participant's vital signs, Acute Physiology and Chronic Health Evaluation II (APACHE II) score, antibiotic use, anticoagulant use, level of consciousness, diet and ICU admission date were assessed.

Incidence rate of VAP

This study assessed VAP cases over seven days, including two additional days following the five days of oral hygiene care, since bacterial recolonisation occurs 24–48 hours after the removal/disinfection of pathogens.[23]

VAP was calculated based on a Centre of Disease Control and Prevention report[24] by dividing the number of patients with pneumonia on mechanical ventilators by the total number of days on ventilation and multiplying the result by 1,000.

Modified bedside oral exam

To assess oral hygiene status, the bedside oral exam (BOE) developed by Prendergast *et al.*[25] for the evaluation of oral hygiene of ICU patients and the oral exam guide (OEG) developed by Beck[26] were used with minor modifications. Among the items assessed using the BOE, swallowing ability, condition of the lips, saliva and teeth (or dentures) and odour were assessed without modification. Assessments of soft tissues, including gingiva (gums), buccal mucosa and tongue, along with the OEG criteria of colour, texture and level of hydration, were adapted for use in patients with endotracheal intubation. Oral hygiene scores ranged from 0 (excellent) to 42 (very poor).

Clinical Pulmonary Infection Score

Clinical Pulmonary Infection Score (CPIS) is an index of VAP incidence, originally developed by Pugin *et al.*[27] and translated into Korean by Lee *et al.*[28] Data regarding temperature, white blood cell counts, tracheal secretions, PaO2/FiO2 ratios and chest radiographs were numerically coded and the resulting scores ranged between 0–9. A score of ≥6 indicated a very high risk of VAP.[27]

Table 1 Descriptive statistics comparing the experimental group with the control group

Characteristics	Categories	Exp group (n = 29)		Cont group (n = 28)		x^2 or t	P value
		n (%)	Mean ± SD	n (%)	Mean ± SD		
Age (year)		-	60.62 ± 15.67	-	57.43 ± 16.94	-	0.464**
Sex	Male	20 (69)	-	19 (67.9)	-	0.008	0.928†
	Female	9 (31)	-	9 (32.1)	-		
Blood pressure (T1*)	Systolic	-	134.19 ± 15.30	-	143.37 ± 52.91	-	0.775**
	Diastolic	-	73.66 ± 8.04	-	69.11 ± 7.28	-	0.192**
Temperature (T1*)		-	37.21 ± 0.99	-	37.22 ± 0.88	-	0.963**
Pulse (T1*)		-	91.69 ± 18.57	-	95.81 ± 20.25		0.430**
APACHE II		-	11.62 ± 6.34	-	12.43 ± 5.02	-	0.330**
Antibiotics	Yes	25 (86.2)	-	27(96.4)	-	1.860	0.352†
	No	4 (13.8)	-	1(3.6)	-		

Key:
* = 24 hours after initiation of mechanical ventilation
** = Analysed using independent t-test with 95% confidence interval
† = Analysed using independent chi-square test with 95% confidence interval

Laboratory method for polymerase chain reaction findings

Plaque sample collection and bacterial culture

Oral plaque bacterial samples were acquired using a sterile interdental toothbrush and an oral swab on both sides of a submandibular molar tooth (buccal). The toothbrushes and swabs were placed individually into three millilitres of distilled water after collecting the plaque. Samples were collected by a researcher to minimise errors; this was performed thrice for every two days. For culturing, one millilitre of the collected sample was transferred to a 15 millilitre tube (SPL Life Sciences, Gyeonggi-do, Korea), inoculated into nine millilitres of tryptic soy broth media (Becton, Dickinson and Company, Difco, Franklin Lakes, USA) and then placed into a 37°C aerobic incubator for 24–36 hours.

Genomic DNA preparation

Genomic DNA from plaque bacterial cultures was isolated with a genomic DNA extraction kit following the manufacturer's instructions. A water sample control was included in each analysis to ensure no bacterial contamination in the DNA extraction buffers and quantitative polymerase chain reaction (qPCR) reagents.

Examination of oral hygiene and professional oral hygiene care

Examination of oral hygiene

An oral hygiene examination was conducted daily by three dental hygienists before the professional oral hygiene care was administered. To train for the study, the dental hygienists compared all measured indices of oral hygiene using pictures of oral pathological findings from Google. Training continued until their results matched and demonstrated inter-rater consistency. Finally, 35 pictures were selected based on inter-rater consistency; Cronbach's alpha was 0.899.

Professional oral hygiene care

Professional oral hygiene care was administered by six dental hygienists after each patient's health status was confirmed by the doctor in charge. A total of three simulations were performed. First, based on the protocol prepared upon expert review, a professional oral hygiene care manual was prepared and the simulation was conducted accordingly. Following training with mannequins for endotracheal intubation, simulations and two trainings were conducted on four healthy adults without systemic diseases.

Education of nurses for oral hygiene care

To ensure that participants received equal oral hygiene care, the nurses performing oral care were educated on the research purpose and oral care methods (details on the method using the oral sponge and CHX were also provided). The education process also provided group education using printed materials and additional videos of oral care methods were available for review at any time.

Analysis

Patients' general characteristics and medical history were analysed using frequency analysis and independent t-tests. Repeated-measures analysis of variance and paired t-tests were employed to assess the oral hygiene and CPIS scores before, during and after oral care. Independent t-tests were employed to compare oral hygiene, CPIS scores and qPCR levels according to the group. All statistical analyses were performed using SPSS 21.0 software with the level of significance set to 0.05.

Ethical considerations

This study was reviewed and approved by the institutional review board of Yonsei University Wonju Severance Christian Hospital on 22 November 2017 (approval number: CR317109). If a participant was unable to directly consent to the intervention, a legally acceptable representative (patient's family) and impartial witness (nurse) provided informed consent. If such a participant recovered enough to consent directly, a re-examination was performed and written consent was obtained.

Results

General characteristics of the participants

Participants did not differ significantly in terms of age, sex, systolic blood pressure, diastolic blood pressure, or APACHE II scores, indicating homogeneity between the groups (Table 1).

Incidence rate of VAP

The following section outlines the incidence rate of VAP in the experimental and control groups. There were no cases of VAP in the experimental

group and two cases in the control group during the intervention period. After conversion to 1,000 days of mechanical ventilator use, the incidence of VAP differed between the control (10.58) and experimental (0) groups (Fig. 3).

Differences in changes in the modified BOE score of the experimental and control groups over time

When BOE scores were measured at 24 hours (T1), 48 hours (T2), 72 hours (T3), 96 hours (T4), and 120 hours (T5) after admission, the scores for the experimental group were 11.69, 11.42, 10.69, 10.65 and 9.35, respectively, while the scores for the control group were 13.47, 11.68, 13.37, 15.63 and 16, respectively. The scores did not differ significantly as a function of timing ($F = 0.963$, $p = 0.242$), but they did differ significantly as a function of the method of oral hygiene care provided ($F = 0.464$, $p = 0.000$).

According to the additional analysis of BOE scores at each time point, no significant differences were observed between the groups at T1 and T2, but significant differences were observed at T3, T4 and T5 (Table 2).

Differences in changes in the CPIS score of the experimental and control groups over time

When CPIS scores were measured at the same time points, the experimental group's CPIS score decreased from T1 to T3, while the control group's score increased from T1 to T3. However, there were no significant differences as a function of timing ($F = 0.801$, $p = 0.464$) or the method of oral hygiene care ($F = 0.651$, $p = 0.425$) (Table 3).

Fig. 3 Ventilator-associated pneumonia rate between the two groups (note: A = Standard1 is the number of VAP cases while the study subjects received professional oral hygiene care and the denominator is 7 days [5 days: intervention period, 2 days: since bacterial recolonisation occurs 24–48 hours after the removal/disinfection of pathogens] × the number of study subjects; B = Standard2 is the number of VAP cases during the study period and the denominator is the study subject's intubation days × the number of study subjects)

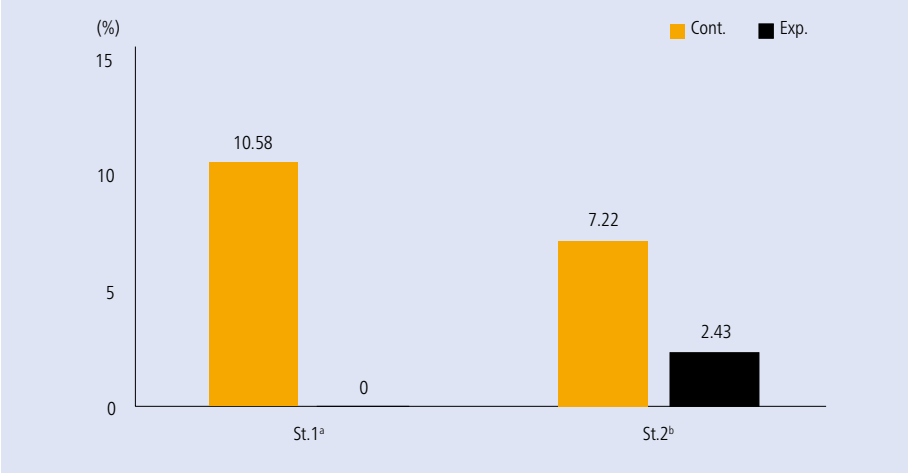


Table 2 BOE score comparison between patients who received professional oral hygiene care and those who received general oral hygiene care according to the time point

Variables	Time*	Exp group (n = 27)	Cont group (n = 22)	Effects	F	P**	t(p)†
		Mean ± SD	Mean ± SD				
BOE score	1	11.69 ± 4.52	13.47 ± 4.94	Group	0.464	0.000	t = 0.338 (p = 0.737)
	2	11.42 ± 3.88	11.68 ± 4.16	Time	0.963	0.242	t = 0.144 (p = 0.886)
	3	10.69 ± 3.43	13.37 ± 3.06	G*T	0.430	0.001	t = 2.89 (p = 0.006)
	4	10.65 ± 3.43	15.63 ± 3.47	-	-	-	t = 3.48 (p = 0.001)
	5	9.35 ± 4.70	16.00 ± 2.73	-	-	-	t = 5.01 (p = 0.000)

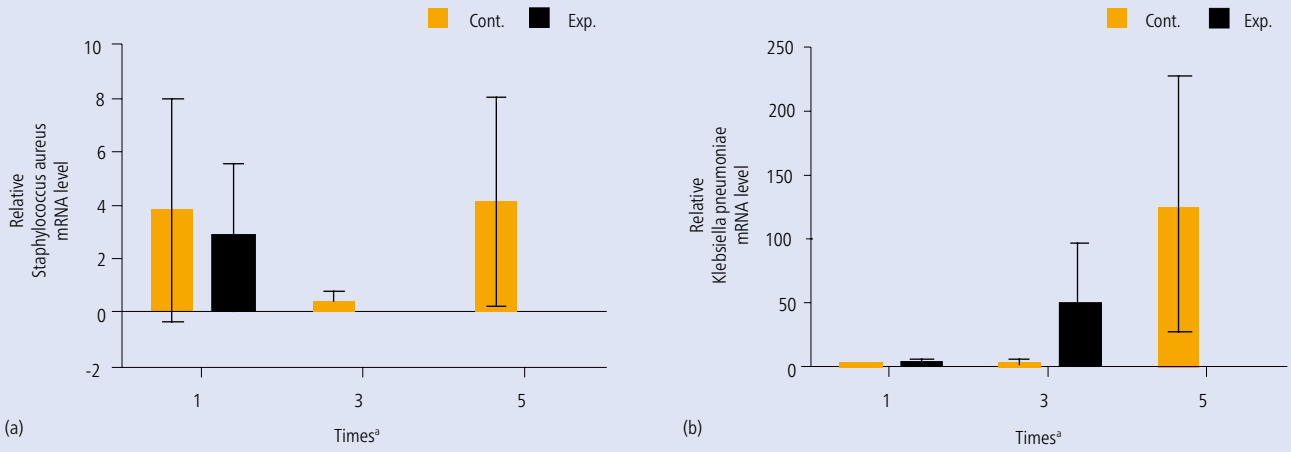
Key:
* = 1 is 24 hours; 2 is 48 hours; 3 is 72 hours; 4 is 96 hours; 5 is 120 hours
** = Analysed using repeated-measures analysis of variance
† = Analysed using independent t-test with 95% confidence interval

Table 3 CPIS score comparison between patients who received professional oral hygiene care and those who received general oral hygiene care according to the time point

Variables	Time*	Exp group (n = 19)	Cont group (n = 16)	Effects	F	P**
		Mean ± SD	Mean ± SD			
CPIS	1	1.11 ± 0.94	1.88 ± 1.31	Group	0.651	0.425
	2	1.32 ± 1.16	1.50 ± 1.21	Time	0.801	0.464
	3	1.37 ± 1.01	1.38 ± 0.96	G*T	1.399	0.253
	4	1.32 ± 1.11	1.50 ± 0.89	-	-	-
	5	1.63 ± 1.26	1.69 ± 1.20	-	-	-

Key:
* = 1 is 24 hours; 2 is 48 hours; 3 is 72 hours; 4 is 96 hours; 5 is 120 hours
** = Analysed using repeated-measures analysis of variance

Fig. 4 Differences in qPCR level of a) *S. aureus* and b) *K. pneumoniae* (note: A = 1 is 24 hours; 3 is 72 hours; 5 is 120 hours)



Differences in qPCR level of *Staphylococcus aureus* and *Klebsiella pneumoniae*

When the six microbial strains were quantified using the real-time technique, *S. aureus* and *K. pneumoniae* demonstrated significant differences in count as professional oral hygiene care was repeated (Fig. 4). Although the other strains displayed no significant differences, the number of bacteria remained constant or decreased in the second or third intervention when compared to the first.

Discussion

Within 24 hours of intubation, VAP-causing pathogens begin to colonise within the oral cavity; without proper care, the incidence rate of VAP increases.[29] Therefore, oral hygiene care of patients on mechanical ventilators is an important strategy for VAP prevention. Trauma ICU patients are particularly susceptible to infections and they exhibit higher rates of hospital-acquired infections than other ICU patients.[30] However, prevention of hospital-acquired infections in trauma patients has not been explored. Therefore, this study aimed to evaluate the effects of professional oral hygiene care for the prevention of VAP and the improvement of oral hygiene in trauma ICU patients. In this study, the experimental group received professional oral hygiene care from dental hygienists and the control group received regular oral hygiene care from nurses.

For the seven-day trial period, including the intervention period, the VAP rate of the control group was 10.58% and that of the experimental group was 0%, suggesting a positive clinical effect.

According to KONIS, the VAP incidence rate (excluding trauma ICU patients) from 2017–2018 was 3.16%.[31] In this study, the VAP incidence rate of the control group was 10.58%, which is higher than that previously reported. This indicates that trauma ICU patients are generally more sensitive to infections than other ICU patients and, thus, hospital-acquired infection rates, including VAP, are higher in trauma ICU patients than in other ICU patients.[32] The same trend was observed in our study.

The risk of VAP is reportedly higher earlier in the course of a hospital stay, increasing 3% per day during the first five days and later decreasing with time.[33] Additionally, half of the VAP cases occur within the first four days of intubation.[34,35] In our study, two of the three VAP cases occurred within two to three days of intubation in the control group, indicating a higher risk of VAP in the early days of intubation. Therefore, oral hygiene care during the early days of intubation is important for preventing VAP.

The experimental group that received professional hygiene care from dental hygienists had a significantly improved oral hygiene status; significant differences were observed after 48 hours. This shows that professional oral hygiene care within 48–72 hours of intubation is effective in preventing VAP and improving oral health.

CPIS is a complementary index that evaluates VAP risk based on temperature, white blood cell count, tracheal secretion, PaO2/FiO2 ratio and chest radiography.[30] A score ≥6 indicates a high risk of VAP. At baseline, the CPIS scores for the participants in this study were very low. The scores for the control and experimental groups were 1.11 and 1.88, respectively; the scores remained low until the last measurement, which was 1.63 for the control group and 1.69 for the experimental group. CPIS has a lower sensitivity for VAP in trauma patients because trauma patients are already at a higher risk of infection and CPIS does not consider the external state of patients.[36] This study involved severe trauma patients on mechanical ventilators for more than five days. Therefore, the risk of VAP was higher in the study population than in other ICU patients. This was not reflected in the CPIS score; hence, the number was very low. A VAP risk evaluation index for trauma ICU patients is needed.

In our study, levels of *S. aureus* and *K. pneumoniae*, the two major strains responsible for VAP, were significantly lower in the control group than in the experimental group. This suggests that the two strains cannot be chemically eliminated in the oral cavity and physical removal is necessary.[37]

Of the strains that were not significantly affected by professional oral hygiene care, *Acinetobacter baumannii* is a strain which has had a growing infection rate in recent years. It is more resistant to antibiotics than *S. aureus* and is difficult to control.[38] As it is becoming one of the major pathogenic strains causing VAP, ways to control *A. baumannii* growth/infection need to be discovered.

Previous studies have reported that 0.12% CHX effectively reduces VAP.[19] In this study, 0.12% CHX was used for oral care alongside toothbrushing and soft tissue cleaning. It has been reported that toothbrushing does not have a significant effect in reducing VAP[19] but a significant difference was observed in our study.

This study differs from previous studies in that a professional oral hygiene care protocol

was created and trained dental hygienists performed professional oral hygiene care, including toothbrushing. Furthermore, dental hygienists and nurses cooperated to provide oral care to the experimental group. These approaches led to different results in our study than the findings of studies.

One limitation of this study is that it is difficult to generalise its results owing to the high attrition rate (approximately 34%). The subjects were ICU patients with different health conditions, drastic health status changes and variables (death, extubation and room changes). Notably, however, this study suggested a model in which health care professionals can cooperate to reduce VAP and it evaluated the effects of professional oral hygiene care in trauma ICU patients. Based on our results, competency development programmes and educational training programmes should be developed, wherein dental hygienists and other health care professionals can work together to provide oral hygiene care to ICU patients.

Conclusion

For proper oral hygiene care, an accurate evaluation of a patient's oral hygiene status is needed.[39] Many tools exist for assessing the oral hygiene status worldwide;[25,26,39] however, their uses differ depending on the institution and individuals[9] and there are no standards or evidence for frequency or timing of their usage. Therefore, an evaluation index assessing the oral hygiene status of patients is urgently needed for providing proper oral hygiene care to ICU patients on mechanical ventilators. Further research must be conducted to study the long-term effects of oral hygiene care on the incidence of VAP and to establish detailed guidelines.

Ethics declaration

This trial was registered in Clinical Research Information Service, Osong (Chungcheongbuk-do) (KCT0005275), which is affiliated with the WHO International Clinical Trial Registry Platform.

The authors declare no other conflicts of interest.

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Author contributions

So-Jung Mun and Ma-I Choi designed the study, analysed data and drafted the manuscript. Sun-Young Han and Hyun-Sun Jeon collected and analysed data. Eun-Sil Choi, Seung-Eun Won, Ye-Ji Lee and Ji-Hye Yang collected data. Chi-Yun Baek and Hongjin Shim helped review the manuscript.

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