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Chlorhexidine decreases the risk of ventilator-associated pneumonia in intensive care unit patients: a randomized clinical trial

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Background and Objective: The aim was to evaluate whether oral swabbing with 0.2% chlorhexidine gluconate (CHX) decreases the risk of ventilator-associated pneumonia (VAP) in intensive care unit (ICU) patients.

Material and Methods: Sixty-one dentate patients scheduled for invasive mechanical ventilation for at least 48 h were included in this randomized, double-blind, controlled study. As these patients were variably incapacitated, oral care was provided by swabbing the oral mucosa four times/d with CHX in the CHX group (29 patients) and with saline in the control group (32 patients). Clinical periodontal measurements were recorded, and lower-respiratory-tract specimens were obtained for microbiological analysis on admission and when VAP was suspected. Pathogens were identified by quantifying colonies using standard culture techniques.

Results: Ventilator-associated pneumonia developed in 34/61 patients (55.7%) within 6.8 d. The VAP development rate was significantly higher in the control group than in the CHX group (68.8% vs. 41.4%, respectively; $p = 0.03$) with an odds ratio of 3.12 (95% confidence interval = 1.09–8.91). *Acinetobacter baumannii* was the most common pathogen (64.7%) of all species identified. There were no significant differences between the two groups in clinical periodontal measurements, VAP development time, pathogens detected or mortality rate.

Conclusion: The finding of the present study, that oral care with CHX swabbing reduces the risk of VAP development in mechanically ventilated patients, strongly supports its use in ICUs and indeed the importance of adequate oral hygiene in preventing medical complications.

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Ventilator-associated pneumonia (VAP) continues to be one of the major causes of morbidity and mortality among patients hospitalized in intensive care units (ICUs) (1). VAP develops in at least 48 h endotracheal intubation. VAP is defined as early onset when it develops in the first 4 d of mechanical ventilation and as late onset when it develops later (2). The pathogenesis of VAP involves aspiration of bacteria from the oropharynx

into the lung and subsequent failure of the host defence systems to clear the bacteria, resulting in the development of lung infection (1,3). In mechanically ventilated ICU (MV-ICU) patients, the major potential respiratory bacterial pathogens include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter* species and enteric species. Previous studies have shown that dental plaque and oral mucosa are often colonized by pulmonary respiratory pathogens (PRPs) (4,5). These findings suggest that dental plaque may be an important reservoir of the PRPs that cause VAP. On the other hand, dental status may be a risk factor for pneumonia and respiratory tract infections, and dentate patients develop aspiration pneumonia more often than edentulous subjects (6,7). Thus, improving oral hygiene in MV-ICU patients to reduce dental plaque has the potential to reduce the risk of VAP.

Different antimicrobial agents have been studied for their plaque-inhibitory effects and antiplaque efficacy (8,9). From these studies, chlorhexidine digluconate (CHX) has become regarded as the gold standard for oral antiseptics as a result of its superior clinical and microbiological effects (10–14). The ability of topical oral applications of CHX to prevent VAP has been evaluated. CHX is of particular interest as an oral disinfectant in MV-ICU patients because of its substantivity. Several recently published clinical trials of intra-oral disinfection with topical CHX (15–22) or povidone-iodine gargle and toothbrushing (23) and meta-analyses (24–26) have demonstrated a reduction in the prevalence of oropharyngeal colonization by PRPs, as well as a reduction in the rate of VAP in MV-ICU patients. Based on these results, improvement of oral hygiene in MV-ICU patients was recommended to prevent VAP (27,28). However, some studies investigating the utility of CHX failed to show a reduction in the incidence of pneumonia (26,29,30). We hypothesized that lack of adequate oral care may be related to a higher risk of VAP in ICU patients and that oral care involving swabbing with 0.2% CHX four times a day can reduce the risk of VAP development in this patient population. Therefore, the aim of the present study was to compare the incidence of VAP between CHX-treated and control MV-ICU patients.

Material and methods

Patient population

The study was designed as a randomized, double-blind, controlled clinical trial. Eligible patients were enrolled in the study between November 2007 and November 2009 in Ege University, School of Medicine, Department of Chest Diseases, Respiratory ICU (Izmir/Turkey). The average length of stay in this unit in the year before the start of the study was approximately 10 d. Incidence density of VAP in the last 3 years ranged from 30 to 60 per thousand ventilator-days. The study was approved by the local Ethics Committee, and the study protocol was conducted in full accordance with the Declaration of Helsinki. Once participant eligibility had been established, written informed consent was obtained from each patient's next of kin or healthcare proxy.

Inclusion/exclusion criteria

Eligible dentate patients were those admitted to the respiratory ICU and expected to be intubated and mechanically ventilated for at least 48 h after admission. The exclusion criteria were as follows: a witnessed aspiration (to eliminate patients with chemical pneumonitis); confirmed diagnosis of post-obstructive pneumonia (e.g. advanced lung cancer); known hypersensitivity to CHX; absence of consent; a diagnosed thrombocytopenia (platelet count $< 25 \times 10^6/mm^3$ and/or an international normalized ratio of > 1.8 or other coagulopathies); a ''do not intubate'' order; age under 18 years; pregnancy; presence of oral mucositis; and readmission to the same ICU. Secondary exclusion criteria were survival expectation < 1 wk and edentulism.

Study design

Eligible patients were randomly assigned to either the CHX group or the control group (Fig. 1). Oral care was provided by swabbing the oral mucosa with either CHX or saline on sponge pellets, four times a day (at 6 AM, 12 AM, 6 PM and 12 PM). Applications lasted for 1 min, and approximately 30 mL of 0.2% CHX or saline was used. The control group received the routine oral care provided by saline applications in this ICU. The ICU staff nurses performed all applications. All teeth present, and the intra-oral soft tissues (including buccal mucosa, vestibule, gingiva, and the floor of the mouth and tongue dorsum) were swabbed. Excess rinse was suctioned out of the subject's mouth after 1 min. In addition, deep suctioning was performed every 6 h and following position changes, to assist in the removal of oropharyngeal secretions pooled on top of the cuff of the endotracheal tube.

The study nurses trained all ICU staff nurses to perform the standardized technique for application of the rinse. Only experienced nurses (i.e. those with at least 5 years of ICU experience) were included in the study, and all were trained in a similar manner. The head nurse periodically checked that ICU staff nurses were adhering to the study protocol. All patients were followed for up to 14 d or until discharge from the ICU, extubation or death.

Randomization

The randomization prepared a set of subject identification numbers (SIDs) that identified individual treatment assignments. The study nurse obtained the SID number based on the randomization when the patient was enrolled. The SID number was sent to the head nurse of the ICU who then assigned the appropriate treatment. Assignment of treatment was blinded to patients and to all investigators, including the periodontist recording the clinical periodontal measurements, the respiratory ICU physicians involved in the study and outcome statisticians.

Airway sampling

Lower respiratory tract specimens were obtained at each sampling time-point–on

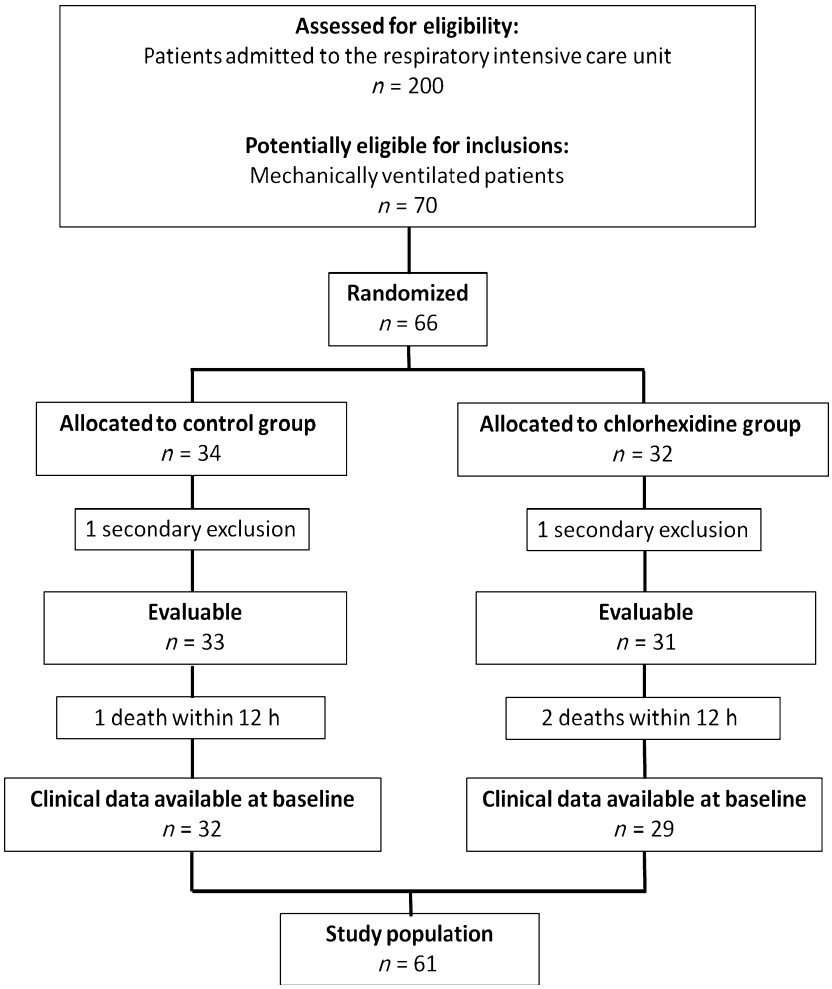

Fig. 1. Flow chart of the study.

admission and on day 7 of intubation, or when suspected VAP occurred – using a minibronchoalveolar lavage (mini-BAL) technique (rather than bronchoscopy) that involves sample collection through placement of a sterile suction catheter in the endotracheal tube (Combicath™; Plastimed, Saint-Leu-La Foret, France). Briefly, this technique involves insertion of a catheter through the endotracheal tube and its advancement until resistance is encountered (31). The catheter is then withdrawn by 1 cm, the inner portion of the co-axial catheter advanced to dislodge the poly-ethylene glycol plug and then 20 mL of normal saline is instilled through the catheter. After 30 s, the specimen is withdrawn and sent for quantitative bacteriology. The presence of $\geq 10^4$ colony-forming units/mL of a target PRP in mini-BAL fluid, or a positive pleural fluid culture in the absence of previous pleural instrumentation, was considered as positive evidence for the diagnosis of pneumonia (32).

Outcome variables and potential confounding variables

The selected primary outcome variables were incidence of VAP and mortality. Secondary outcomes assessed were length of mechanical ventilation, length of stay in the ICU and the presence in mini-BAL of potential respiratory pathogens (*S. aureus, P. aeruginosa, Acinetobacter* species, and the enteric species *Klebsiella pneumoniae, Serratia marcescens, Escherichia* species, *Proteus mirabilis* and *Escherichia coli*) determined by quantifying colonies using standard culture at each evaluation time-point. Clinical periodontal status, age, gender, diagnosis on admission and co-morbid diseases were recorded as explanatory or confounder variables.

The severity of illness score utilizing the Acute Physiology and Chronic Health Evaluation (APACHE) II system was determined for each patient at baseline (33). This index utilizes information present in the patient's hospital records, including physiologic information (temperature, mean arterial pressure, heart rate, respiratory rate, oxygenation, arterial pH, serum levels of sodium, potassium and creatinine, hematocrit and white blood cell count) and age.

Clinical periodontal measurements

Intra-oral examination was carried out with the subject lying flat on the bed, to

facilitate a reproducible examination position for the clinician. Clinical examination of all participating subjects was carried out using mouth mirrors, a head light, and dental and periodontal probes. Clinical periodontal recordings comprising dichotomous plaque index (present or absent), probing depth and bleeding on probing (present or absent within 15 s after periodontal probing) were performed at six sites (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual) on each tooth present, except for third molars, using a Williams periodontal probe. All measurements were performed by a single calibrated examiner (ÖÖ). The intra-examiner reliability was high, as revealed by intraclass correlation coefficients of 0.87 and 0.85 for probing depth and clinical attachment level measurements, respectively. The hard palate, soft palate, buccal mucosa, tongue and gingiva were examined for abnormalities, including inflammation, ulceration or other signs of inflammatory irritation that might be expected to be secondary to exposure to CHX. All patients were monitored for potential intra-oral (mucositis, thrush, tooth staining, alterations in taste and tooth hypersensitivity) and systemic (mortality) adverse events.

Statistical analysis

Based on the incidence of VAP during the last 5 years in the respiratory ICU, it was conservatively estimated that approximately 70% of all subjects admitted to the ICU would develop VAP. In order to have a power of 81% to detect a difference of 0.40 between the null hypothesis (that both CHX and control group proportions are 0.70) and the alternative hypothesis (that the proportion in the CHX group is 0.30), using a two-sided chi-square test with continuity correction and with a significance level of 0.05, it was determined that a minimum group size of 28 participants per treatment group was required.

Baseline between-group comparisons were performed using the chi-square test. The Mann–Whitney *U*-test was performed to analyze clinical and demographic data of the study groups. Logistic regression analysis was performed and the odds ratio of VAP occurring in the CHX group vs. the control group was calculated. All tests were performed at a significance level of $\alpha = 0.05$. All statistical calculations were performed using the SPSS version 17.0 statistical software package.

Results

A total of 200 patients were admitted to the ICU during the recruitment period. Seventy of these patients were mechanically ventilated and therefore potentially eligible for inclusion in the present study. Of the 66 patients who gave informed consent and were randomly assigned to the CHX or control groups, two were excluded before sampling for secondary reasons (topical antibiotic usage). Another three patients were excluded from the study because they died within 12 h after placement of mechanical ventilation, leaving 61 patients for whom baseline clinical data were available ($n = 29$ in the CHX group and $n = 32$ in the control group). Thus, analysis of the primary outcomes (incidence of VAP and mortality) was performed on the data from 61 subjects (Fig. 1). No significant differences were found for demographic, clinical and laboratory characteristics between the CHX and control groups on admission Table 1. The most frequent co-morbid disease was cardiac diseases (48.9%) followed by endocrine (19.2%) and renal (10.63%) disorders. No significant correlations were observed between co-morbidities and group assignment.

ICU stay (12.17 ± 11.3 d in the CHX group and 15.44 ± 13.5 d in the control group) and number of days of invasive mechanical ventilation (IMV) (9.00 ± 8.3 d in the CHX group and 12.28 ± 11.9 d in the control group) were similar in the CHX and control groups.

The clinical periodontal measurements and the numbers of teeth present were similar in the study groups ($p > 0.05$) (Tables 2 and 3). No intra-oral adverse events, such as mucositis or tooth staining, were noted during the study period. The distribution of probing depth thresholds was similar in the study groups ($p > 0.05$).

The study groups were subdivided as VAP positive [VAP (+)] or VAP negative [VAP (−)] and the clinical periodontal measurements of these groups are given in Table 4. Twenty-two patients (68.8%) in the control group and 12 patients (41.4%) in the CHX group were diagnosed with VAP. The rate of VAP occurrence in the control group was significantly higher than in the CHX group with an odds ratio of 3.12 (95% confidence interval = 1.09–8.91, $p = 0.03$). Most cases of VAP (27 out of 34) were defined as late onset. *Acinetobacter baumannii* was the most common (64.7%) pathogen isolated in the 34 cases of VAP in the two groups. The APACHE II score was not significantly different between the VAP (+) and VAP (−) control groups ($p = 0.14$). The APACHE II score of the VAP (−) CHX group was significantly lower than that of the VAP (+) CHX patients ($p = 0.039$) (Table 4).

There were no significant differences with regards to duration of VAP development between the CHX and control groups. Significantly shorter ICU stay was found for VAP (−) patients in both control and CHX groups than in the VAP (+) patients of the same groups ($p < 0.0001$ and $p = 0.013$, respectively). The duration of mechanical ventilation was significantly longer in the VAP (+) CHX and control groups ($p = 0.002$ and $p < 0.0001$, respectively). The VAP (+) control group had a significantly longer hospitalization period than the VAP (−) counterparts ($p = 0.03$) (Table 4). The mortality rates were similar in the CHX (17/29) and control (19/32) groups ($p > 0.05$).

Crude logistic regression analysis indicated that the odds ratio of VAP development in the control group was 3.12 (Table 5). Age itself increased this odds ratio of VAP development to 5.05 with adjusted logistic regression analysis. When assessed together with the APACHE II score, the odds ratio was 7.98 in the control group.

Discussion

As VAP continues to be a common complication of critical care, development of preventive approaches are

Table 1. Baseline characteristics of the chlorhexidine gluconate (CHX) and control groups

	CHX group (n = 29)	Control group (n = 32)	p-value
Clinical or biochemical variable			
Age (years)	60.5 ± 14.7	56.0 ± 18.2	0.301
Co-morbidities (n) (%)	21 (72.2)	26 (81.25)	0.545
APACHE II	23.9 ± 5.7	24.7 ± 6.2	0.693
PaO_2/FiO_2	157.4 ± 82.6	166.4 ± 74.1	0.995
Leucocytes (mm^3)	14,444 ± 7660	14,430 ± 11,664	0.312
PCT (ng/dL)	8.3 ± 11.9	7.6 ± 18.9	0.592
Albumin (g/dL)	3.0 ± 0.8	3.1 ± 0.7	0.592
Antibiotic usage before admission	11	18	0.116
Secondary outcome variables			
Hospitalization (d)	16.7 ± 11.9	17.7 ± 13.3	0.638
ICU stay (d)	12.2 ± 11.3	15.4 ± 13.5	0.279
IMV (d)	9.0 ± 8.3	12.3 ± 11.9	0.363

Data are presented as n, n (%) or mean ± standard deviation.
APACHE, Acute Physiology and Chronic Health Evaluation; ICU, intensive care unit; IMV, invasive mechanical ventilation; PCT, procalcitonin; VAP, ventilator-associated pneumonia.

Table 2. Clinical periodontal measurements of the chlorhexidine gluconate (CHX) and control groups

Variable	CHX group (n = 29)	Control group (n = 32)	p-value
Teeth present (n)	13.4 ± 7.8	13.9 ± 8.7	0.881
Probing depth (mm)	3.8 ± 1.1	3.7 ± 1.0	0.92
Plaque index (%)	86.6 ± 21.6	84.7 ± 19.3	0.692
Bleeding on probing (%)	48.4 ± 29.2	48.9 ± 30.2	0.916

Data are presented as mean ± standard deviation.

Table 3. Clinical periodontal measurements of the chlorhexidine gluconate (CHX) and control groups according to the state of being ventilator-associated pneumonia (VAP) positive [VAP (+)] or VAP negative [VAP (–)]

	CHX group (n = 29)			Control group (n = 32)		
	VAP (+) (n = 12)	VAP (–) (n = 17)	p-value	VAP (+) (n = 22)	VAP (–) (n = 10)	p-value
Teeth present (n)	13.6 ± 7.4	13.2 ± 8.8	0.807	14.5 ± 8.7	12.9 ± 8.9	0.60
Probing depth (mm)	3.6 ± 1.1	3.9 ± 0.9	0.773	3.7 ± 1.1	3.6 ± 1.0	0.919
Plaque index (%)	86.8 ± 24.4	83.2 ± 15.5	0.212	82.1 ± 15.2	96.5 ± 7.9	0.067
Bleeding on probing (%)	51.1 ± 33.2	47.3 ± 28.7	0.807	45.3 ± 30.1	55.7 ± 26.9	0.186

Data are given as mean ± standard deviation.

essential to reduce the incidence of this costly infection. The present study was performed (i) to evaluate whether oral care with swabbing four times daily with CHX reduces VAP development in intubated MV-ICU patients admitted to the respiratory ICU and (ii) to determine whether worse clinical periodontal status is related to a higher VAP development risk in this particular patient group. The rate of pneumonia development was 68.8% and 41.4%, respectively in the control and CHX groups. More importantly, there was no significant difference in mortality between the study groups. The odds ratio of VAP development in the control group was 3.12-fold higher than in the CHX group. The present findings indicated no significant differences in clinical periodontal measurements comprising number of teeth present, probing depth, plaque index and bleeding on probing between patients developing and not developing VAP. The probing-depth thresholds were also similar in the study groups, suggesting no significant relationship between clinical periodontal status and VAP development risk in ICU patients. One may expect that age and/or APACHE II score, which are known risk factors for VAP development, caused this odds ratio. However, the present study groups were similar with regard to age distribution and APACHE II score. This fact provides further support for the hypothesis that oral care with CHX decreases the risk of VAP development in ICU patients.

The average length of stay in this unit in the year before the start of the study was approximately 10 d. Incidence

Table 4. Baseline characteristics and secondary outcome variables of the chlorhexidine gluconate (CHX) and control groups according to the state of being ventilator-associated pneumonia (VAP) positive [VAP (+)] or VAP negative [VAP (−)]

	CHX group (n = 29)			Control group (n = 32)		
	VAP (+) (n = 12)	VAP (−) (n = 17)	p-value	VAP (+) (n = 22)	VAP (−) (n = 10)	p-value
Clinical or biochemical parameter						
Age (years)	67.5 (47–86)	55.0 (23–77)	0.088	65.0 (26–83)	46.5 (20–68)	0.016
APACHE II	29.0 (21–36)	22.0 (14–35)	0.039	24.0 (11–35)	26.5 (20–39)	0.142
PaO_2/FiO_2	130.0 (51–318)	164.0 (60–310)	0.654	177.0 (48–300)	140.0 (45 –304)	0.389
Leucocytes (n/mm^3)	11,675 (1220–20,720)	12,430 (7700–32,210)	0.223	12,130 (6630–60,300)	10,245 (60–42,000)	0.655
PCT (ng/dL)	3.5 (0.4–15.3)	1.01 (0.14–32.39)	1.00	1.1 (0.1–10.8)	1.4 (0.6–64.1)	0.307
Albumin (g/dL)	2.9 (2.0–3.7)	2.90 (1.8–5.0)	0.690	3.1 (1.4–4.7)	3.3 (1.8–3.8)	0.822
Secondary outcome variables						
Hospitalization (d)	19.0 ± 14.8	15.1 ± 9.5	0.399	21.4 ± 13.9	9.4 ± 6.9	0.003
ICU stay (d)	17.6 ± 15.2	8.4 ± 5.1	0.013	19.7 ± 14.2	6.0 ± 3.2	< 0.0001
IMV (d)	14.1 ± 10.7	5.4 ± 3.1	0.02	15.5 ± 13.1	5.2 ± 3.1	< 0.0001
VAP development (d)	6.4 ± 3.4	–	–	7.0 ± 2.7	–	–
Antibiotic usage after admission (n)	12	17	–	22	10	–
Mortality (n) (%)	10 (83.3)	7 (41.2)	0.422	12 (54.5)	7 (70)	0.467

Data are given as median (range).
APACHE, Acute Physiology and Chronic Health Evaluation; ICU, intensive care unit; IMV, invasive mechanical ventilation; PCT, procalcitonin.

Table 5. Crude and adjusted odds ratios and 95% confidence interval (95% CI) of ventilator-associated pneumonia (VAP) development

Groups	Odds ratio	95% CI	
		Lower	Upper
Crude			
CHX (reference)	–	–	–
Control	3.12	1.09	8.91
Adjusted			
Control	7.98	2.04	31.21
Age	1.07	1.02	1.12
APACHE II	0.96	0.86	1.08

APACHE, Acute Physiology and Chronic Health Evaluation; CHX, chlorhexidine gluconate.

density of VAP in the last 3 years ranged from 30 to 60 per thousand ventilator-days. The rate of infection with VAP in the respiratory ICU was reported to be 28–85% in Turkey and the incidence of pneumonia among residents of ICUs ranges from 16.4 to 26.5 per 1000 patient-days (34).

The previously published studies (15,16,35) investigating the effect of oral antiseptics on prevention of nosocomial infections have been carried out in general ICUs. In contrast, our study was carried out in the respiratory ICU of a Chest Disease Department. In the present study, CHX was applied by means of swabbing. This was mainly because none of the patients was conscious and also to make sure that CHX was applied directly onto each and every tooth surface. Therefore, swabbing was the most effective way of consistently applying CHX to all of the study population and indeed swabbing in this way is a useful addition to the application of antiseptics by the periodontal community, particularly to very ill and hospitalized patients (36). In the present study, oral care was provided by trained nurses with at least 5 years of experience in ICU and those nurses were periodically checked by the head nurse to ensure that they were adhering to the study protocol. Nurse effectiveness may be regarded as a confounder, and assuring consistency by means of a numerical evaluation would increase the reliability. Recording plaque index on a daily basis could be a way of numerical evaluation of nurse consistency and these may be considered as limitations of the present study. However, the difficulty of such clinical work in mechanically ventilated unconscious patients in the ICU should be borne in mind.

Previous studies analyzed BAL samples, whereas our study evaluated mini-BAL samples. Analysis of BAL material is well known to be highly sensitive and specific in terms of VAP diagnosis. However the invasive nature of BAL sampling and high risk of complications need to be weighed against the high sensitivity and specificity of the data. On the other hand, mini-BAL is as reliable as BAL in terms of sensitivity and specificity of the data and the sampling technique has the advantages of being less invasive and having a lower risk of complications (31,37). Therefore, the present study is based on analysis of mini-BAL samples rather than BAL samples. To the best of our knowledge, this is the first study correlating mini-BAL data and clinical periodontal findings. The difference in methodology may explain the differences between our findings and those of previous studies, and the present study suggests that analysis of mini-BAL may be appropriate in future studies.

Topical application of CHX two to four times a day was suggested to reduce the risk of development of VAP (17–21,35). Scannapieco *et al.* (38). reported that there were no significant differences between one or two

applications of 0.12% CHX rinse with regard to the reduction of VAP development. Furthermore, genetic similarities between bacteria isolated from the lung and dental plaque have been demonstrated and it was suggested that dental biofilms are important reservoirs for these respiratory pathogens (39,40). Thus, mechanisms other than reduction of PRPs in dental biofilm must be considered to help explain the apparent efficacy of CHX in preventing VAP. One possible explanation is that CHX inhibits the viability of the planktonic bacteria in the oral secretions. The subsequent reduction in the number of viable PRPs in the secretions eventually reduces the number of viable organisms aspirated into the lower airway and therefore prevents subsequent infection. Alternatively, the virulence potential of the bacteria may be reduced by CHX. Recently, it was suggested that CHX is able to bind to bacterial components such as lipopolysaccharide and proteases (25,41).

A recent meta-analysis of trials concluded that CHX is effective in preventing VAP (25). These analyses revealed, however, that there was a great variation in the populations studied as well as in the concentration, preparation and dosing schedule of CHX. Clinical trials of CHX have tested concentrations of 0.12% and 0.2% applied two to four times a day, in the form of a rinse or gel. Topical application of CHX to the oral cavity of MV-ICU patients in some cases appears to prevent VAP, but neither the optimal concentration nor the frequency of application of this agent has been clarified so far. Studies validating the utility of CHX on reducing pneumonia are not unanimous. Fourrier *et al.* (35). reported that gingival decontamination with 0.2% CHX gel significantly decreased the oropharyngeal colonization by bacteria in ventilated patients but was not sufficient to reduce the incidence of respiratory infections. Previously, Houston *et al.* (18). obtained a greater reduction in pneumonia development with 0.12% CHX rinse than with an essential oils rinse in ICU patients. Scannapieco *et al.* (38). compared the effect of once-daily vs. twice-daily application of 0.12% CHX and reported no significant difference in terms of reducing oral colonization by PRPs. Although CHX usage either once daily or twice daily reduced the oral colonization of PRPs by a similar extent, the number of patients with VAP was not significantly different between the once-daily and twice-daily CHX usage groups. In the present study, swabbing four times daily with 0.2% CHX reduced significantly the number of patients with VAP compared with the control (41.4% and 68.8%, respectively). The significant difference between the CHX and control groups in the present study might be explained by the use of not only a higher concentration of CHX but also the treatment schedule (four times daily) compared with the previous studies (30,35,38). Chan *et al.* (24). stated that unpublished small studies with negative findings exist, which suggests the possibility of publication bias. Scannapieco *et al.* (38) stated that the standardized oral-care regime used in the ICU reduced the number of organisms in dental plaque to a level where additional reductions by CHX were not detectable or suctioning excess fluid at the time of application could have reduced the effect of CHX. Our present findings are promising in that the addition of oral swabbing with 0.2% CHX, four times a day, to the standard oral-care regime may be more effective in reducing pathogenic bacteria in oral biofilms, eventually significantly reducing VAP development.

Although oral swabbing with 0.2% CHX reduced the risk of VAP development in mechanically ventilated patients, no significant differences could be demonstrated in the length of ICU stay, duration of hospitalization and mechanical ventilation between the CHX and control groups. The VAP (+) CHX group presented slightly better results than the VAP (+) control group. One possible limitation of the present study is the rather low patient numbers in the study groups. The present study was conducted in a respiratory ICU and most of the patients had severe respiratory deficiencies such as acute respiratory distress syndrome, and many were suffering from other co-morbid diseases, eventually increasing the VAP rate in spite of the active surveillance. This fact may be regarded as another limitation of the present study.

Adverse events have rarely been reported in clinical trials using CHX in ICU patients. A meta-analysis of seven clinical trials indicated that no adverse effects were reported in any of these studies (25). However, Tantipong *et al.* (22) reported that 9.8% of patients who received 0.2% CHX developed irritation of the oral mucosa. Our present findings are in line with that of the meta-analysis (25) as 0.2% CHX resulted in no adverse effects such as mucosal irritation or tooth staining.

Oral colonization with potential respiratory pathogens appears to contribute to pulmonary infections. The relationship between periodontitis and risk of pneumonia is presently unknown. The limited access to the oral cavity of ICU patients and the rather short stay of these patients in hospital present logistical challenges to research in this field. Therefore, it is rather difficult to determine whether periodontitis is related to pneumonia in MV-ICU subjects.

In conclusion, within the limits of the present study, it may be suggested that oral care in ICU patients, of application of 0.2% CHX four times a day, reduces the risk of VAP development. The present study did not reveal significant differences in clinical periodontal measurements between the study groups, suggesting no evidence of a significant effect of clinical periodontal status on VAP development risk. Oral hygiene practices seem to be needed before intubation of patients in ICUs and the existing clinical periodontal condition does not seem to be closely related to the risk of pneumonia. This means that the association between nosocomial pneumonia may be practical in terms of oral hygiene and not related to periodontal systemic disease interactions. However, larger-scale intervention studies are required to address this issue in greater detail. Therefore, adoption of adequate oral hygiene measures, which may include supplementation with oral CHX swabbing, is warranted to reduce the VAP development rate.

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Conflict of interest and Source of Funding

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References

1. Raghavendran K, Mylotte JM, Scannapieco FA. Nursing home-associated pneumonia, hospital-acquired pneumonia and ventilator-associated pneumonia: the contribution of dental biofilms and periodontal inflammation. *Periodontol 2000* 2007;**44:**164–177.
2. American Thoracic Society. Infectious Diseases Society of America Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. *Am J Respir Crit Care Med* 2005;**171:**388–416.
3. Verghese A, Berk SL. Bacterial pneumonia in the elderly. *Medicine (Baltimore)* 1983;**62:**271–285.
4. Scannapieco FA, Stewart EM, Mylotte JM. Colonization of dental plaque by respiratory pathogens in medical intensive care patients. *Crit Care Med* 1992;**20:**740–745.
5. Fourrier F, Duvivier B, Boutigny H, Roussel-Delvallez M, Chopin C. Colonization of dental plaque: a source of nosocomial infections in intensive care unit patients. *Crit Care Med* 1998;**26:**301–308.
6. Terpenning M, Bretz W, Lopatin D, Langmore S, Dominguez B, Loesche W. Bacterial colonization of saliva and plaque in the elderly. *Clin Infect Dis* 1993;**4:**S314–S316.
7. Mojon P, Budtz-Jorgensen E, Michel JP, Limeback H. Oral health and history of respiratory tract infection in frail institutionalised elders. *Gerodontology* 1997; **14:**9–16.
8. Mandel ID. Chemotherapeutic agents for controlling plaque and gingivitis. *J Clin Periodontol* 1988;**15:**488–498.
9. Wu CD, Savitt ED. Evaluation of the safety and efficacy of over-the-counter oral hygiene products for the reduction and control of plaque and gingivitis. *Periodontol 2000* 2002;**28:**91–105.
10. Lang NP, Catalanotto FA, Knopfli RU, Antczak AA. Quality-specific taste impairment following the application of chlorhexidine digluconate mouthrinses. *J Clin Periodontol* 1988;**15:**43–48.
11. Brecx M, Netuschil L, Reichert B, Schreil G. Efficacy of Listerine, Meridol and chlorhexidine mouthrinses on plaque, gingivitis and plaque bacteria vitality. *J Clin Periodontol* 1990;**17:**292–297.
12. Brecx M, Brownstone E, MacDonald L, Gelskey S, Cheang M. Efficacy of Listerine, Meridol and chlorhexidine mouthrinses as supplements to regular tooth cleaning measures. *J Clin Periodontol* 1992;**19:**202–207.
13. Quirynen M, Avontroodt P, Peeters W, Pauwels M, Coucke W, van Steenberghe D. Effect of different chlorhexidine formulations in mouthrinses on de novo plaque formation. *J Clin Periodontol* 2001;**28:**1127–1136.
14. Herrera D, Roldan S, Santacruz I, Santos S, Masdevall M, Sanz M. Differences in antimicrobial activity of four commercial 0.12% chlorhexidine mouthrinse formulations: an in vitro contact test and salivary bacterial counts study. *J Clin Periodontol* 2003;**30:**307–314.
15. DeRiso AJ 2nd, Ladowski JS, Dillon TA, Justice JW, Peterson AC. Chlorhexidine gluconate 0.12% oral rinse reduces the incidence of total nosocomial respiratory infection and nonprophylactic systemic antibiotic use in patients undergoing heart surgery. *Chest* 1996;**109:**1556–1561.
16. Fourrier F, Cau-Pottier E, Boutigny H, Roussel-Delvallez M, Jourdain M, Chopin C. Effects of dental plaque antiseptic decontamination on bacterial colonization and nosocomial infections in critically ill patients. *Intensive Care Med* 2000;**26:**1239–1247.
17. Genuit T, Bochicchio G, Napolitano LM, McCarter RJ, Roghman MC. Prophylactic chlorhexidine oral rinse decreases ventilator-associated pneumonia in surgical ICU patients. *Surg Infect* 2001;**2:**5–18.
18. Houston S, Hougland P, Anderson JJ, LaRocco M, Kennedy V, Gentry LO. Effectiveness of 0.12% chlorhexidine gluconate oral rinse in reducing prevalence of nosocomial pneumonia in patients undergoing heart surgery. *Am J Crit Care* 2002;**11:**567–570.
19. Koeman M, van der Ven AJ, Hak E *et al*. Oral decontamination with chlorhexidine reduces the incidence of ventilator-associated pneumonia. *Am J Respir Crit Care Med* 2006;**173:**1348–1355.
20. 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:**2460–2466.
21. Lansford T, Moncure M, Carlton E *et al*. Efficacy of a pneumonia prevention protocol in the reduction of ventilator-associated pneumonia in trauma patients. *Surg Infec* 2007;**8:**505–510.
22. Tantipong H, Morkchareonpong C, Jaiyindee S, Thamlikitkul V. Randomized controlled trial and meta-analysis of oral decontamination with 2% chlorhexidine solution for the prevention of ventilator-associated pneumonia. *Infect Control Hosp Epidemiol* 2008;**29:**131–136.
23. Mori H, Hirasawa H, Oda S, Shiga H, Matsuda K, Nakamura M. Oral care reduces incidence of ventilator-associated pneumonia in ICU populations. *Intensive Care Med* 2006;**32:**230–236.
24. Chan EY, Ruest A, Meade MO, Cook DJ. Oral decontamination for prevention of pneumonia in mechanically ventilated adults: systemic review and meta-analysis. *BMJ* 2007;**334:**889.
25. Chlebicki MP, Safdar N. Topical chlorhexidine for prevention of ventilator-associated pneumonia: a meta-analysis. *Crit Care Med* 2007;**35:**595–602.
26. Pineda LA, Saliba RG, El Solh AA. Effect of oral decontamination with chlorhexidine on the incidence of nosocomial pneumonia: a meta-analysis. *Crit Care* 2006;**10:**R35.
27. Tablan OC, Anderson LJ, Besser R, Bridges C, Hajjeh R. Guidelines for preventing health-care – associated pneumonia, 2003: recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee. *MMWR Recomm Rep* 2004;**53:**1–36.
28. Craven DE. Preventing ventilator-associated pneumonia in adults: sowing seeds of change. *Chest* 2006;**130:**251–260.
29. Panchabhai TS, Dangayach NS, Krishnan A, Kothari VM, Karnad DR. Oropharyngeal cleansing with 0.2% chlorhexidine for prevention of nosocomial pneumonia in critically ill patients: an open-label randomized trial with 0.01% potassium permanganate as control. *Chest* 2009;**135:** 1150–1156.
30. Bellissimo-Rodrigues F, Bellissimo-Rodrigues WT, Viana JM *et al*. Effectiveness of oral rinse with chlorhexidine in preventing nosocomial respiratory tract infections among intensive care unit patients. *Infect Control Hosp Epidemiol* 2009;**30:**952–958.
31. Kollef MH, Bock KR, Richards RD, Hearns ML. The safety and diagnostic accuracy of minibronchoalveolar lavage in patients with suspected ventilator-associated pneumonia. *Ann Intern Med* 1995;**122:**743–748.
32. Ioanas M, Ferrer R, Angrill J, Ferrer M, Torres A. Microbial investigation in

ventilator-associated pneumonia. *Eur Respir J* 2001;**17:**791–801.
33. Knaus WA, Zimmerman JE, Wagner DP, Draper EA, Lawrence DE. APACHE-acute physiology and chronic health evaluation: a physiologically based classification system. *Crit Care Med* 1981;**9:** 591–597.
34. Turkish Thoracic Society. The consensus report on diagnosis and treatment hospital-acquired pneumonia in adults. *Turk Toraks Derg* 2009;**10:**3–24. (Article in Turkish)
35. Fourrier F, Dubois D, Pronnier P *et al*. PIRAD Study Group: effect of gingival and dental plaque antiseptic decontamination on nosocomial infections acquired in the intensive care unit: a double-blind placebocontrolled multicenter study. *Crit Care Med* 2005;**33:**1728–1735.
36. Lorente L, Blot S, Rello J. Evidence on measures for the prevention of ventilator-associated pneumonia. *Eur Respir J* 2007; **30:**1193–1207.
37. Tasbakan SM, Gurgun A, Basoglu OK, Ekren PK, Pullukcu H, Bacakoglu F. Comparison of bronchoalveolar lavage and mini-bronchoalveolar lavage in the diagnosis of pneumonia in immunocompromised patients. *Respiration* 2011;**81:** 229–235.
38. Scannapieco FA, Yu J, Raghavendran K *et al*. A randomized trial of chlorhexidine gluconate on oral bacterial pathogens in mechanically ventilated patients. *Crit Care* 2009;**13:**R117.
39. El-Solh AA, Pietrantoni C, Bhat A *et al*. Colonization of dental plaques: a reservoir of respiratory pathogens for hospital-acquired pneumonia in institutionalized elders. *Chest* 2004;**126:**1575–1582.
40. Heo SM, Haase EM, Lesse AJ, Gill SR, Scannapieco FA. Genetic relationships between respiratory pathogens isolated from dental plaque and bronchoalveolar lavage fluid from patients in the intensive care unit undergoing mechanical ventilation. *Clin Infec Dis* 2008;**47:**1562–1570.
41. Cronan CA, Potempa J, Travis J, Mayo JA. Inhibition of *Porphyromonas gingivalis* proteinases (gingipains) by chlorhexidine: synergistic effect of Zn(II). *Oral Microbiol Immunol* 2006;**21:**212–217.