EFFECTIVENESS OF SUCTION ABOVE CUFF ENDOTRACHEAL TUBE (SACETT) IN PREVENTING VENTILATOR ASSOCIATED PNEUMONIA IN CRITICAL PATIENTS IN INTENSIVE CARE UNIT

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Abstract

Background: The mechanical ventilator is an indispensable breathing tool in the Intensive Care Unit (ICU). But the mechanical ventilator is associated with the risk of Ventilator Associated Pneumonia (VAP). VAP occurs due to poor hygiene of the endotracheal tube (ETT). ETT hygiene should be maintained to inhibit bacterial development in the lungs using suction above cuff endotracheal tube (SACETT) to prevent VAP.

Objective: To analyze the effectiveness of SACETT in preventing Ventilator Associated Pneumonia (VAP) in critical patients in the ICU.

Methods: This was a quasi-experimental study with posttest only with control group design with 15 samples in intervention group (SACETT and Chlorhexidine 0.2%) and 15 in control group (ETT, Open Suction, and Chlorhexidine 0.2%) with purposive technique sampling. The Simplified Clinical Pulmonary Infection Score (CPIS) was used to measure VAP.

Results: This study illustrates that there was no VAP incidence in the intervention group, and as much as 13.3% VAP in the control group. SACETT was more effective in preventing VAP than standard ETT on day 4 (p = 0.001).

Conclusion: SACETT is more effective in preventing VAP than standard ETT in the fourth day in patients with neurological, cardiovascular, urinary, digestive, and immune system disorders.

Keywords: suction above cuff endotracheal tube; standard endotracheal tube; associated pneumonia ventilator; critical patient

INTRODUCTION

A mechanical ventilator is an indispensable respiratory aid in an Intensive Care Unit (ICU) of hospital. However, mechanical ventilation is also strongly associated with the risk of Ventilator Associated Pneumonia (VAP) (Bouza et al., 2001). VAP is one of the causes of morbidity and mortality in ICU. Pneumonia due to ventilator use is a nosocomial pneumonia that is very common in patients who have mechanically ventilated (with endotracheal tube or tracheostomy) for at least 48 hours (Patricia, Dorrie, & Barbara, 2012).

Pneumonia is an infectious disease that occurs with very high frequency, with the incidence of 9-24% after 48 hours from the beginning of artificial respiratory management (Chastre & Fagon, 2002).

The incidence of VAP in the world is quite high, varying between 9 and 27% and the mortality rate exceeding 50%. The VAP problem ranks 2nd as a nosocomial infection...
in hospitals in the United States. The incidence of nosocomial pneumonia ranges from 5 to 10 cases per 1000 patients, the incidence rate increases 6 to 20 times in ventilator-installed patients, the mortality rate ranges from 20 to 50% (Perhimpunan Dokter Paru Indonesia, 2005). Studies on the Global prospective epidemiologic and surveillance study of ventilator-associated pneumonia in ICU in 56 locations in 11 countries in four regions: The United States, Europe, Latin America and Asia Pacific showed a total of 1873 incidents. VAP incidence in Indonesia based on VAP research at Cipto Mangunkusumo Hospital has found 27.4% of cases during January 2003 until December 2012 (Saragih, Amin, Sedono, Pitoyo, & Rumende, 2014). VAP incidence data at Fatmawati General Hospital (RSUP) found that there was an increasing trend, the highest incidence occurred in July 2014 which was 21.2 % and the lowest of September 2014 was 5.53 % (RSUP Fatmawati, 2014). While based on research at Central Hospital Force Land (RSPAD) Gatot Soebroto, VAP incidence during the year 2014 as much as 21.8% of patients (Handari, 2015).

The use of standard ETT commonly used in various hospitals illustrates the condition that the secretions accumulated in the upper respiratory tract of the patient are removed by using a suction tube inserted into the oral cavity, nasal cavity and / or throat cavity but not reaching into the area above the balloon cuff. This allows the contamination of the suction tube into the respiratory tract, which increases the risk of infection in the lung so that it can cause VAP, which can affect the increasing length of day care and patient care costs in the ICU room. SACETT aims to clean up the secretions above the ETT cuff balloon with the aim of preventing the microbialization and colonization of pathogenic germs in the lung where the accumulated secretions are removed by connecting the vacuum suction machine with suction cuff to SACETT, so there is no need to enter the suction tube into the canal upper respiration with reach to the area just above the balloon cuff. Thus, the use of SACETT is expected to minimize the risk of VAP occurring in the ICU chamber.

A study compared Suction Above Cuff Endotracheal Tube (SACETT) with standard ETT in preventing VAP in patients with neurologic disease ventilator using randomized controlled pilot study method, conducted in 24 months with 54 respondents in which 4 of them dropped out and the results found differences microbiological incidence VAP on day 5 between the two ETT models in which SACETT was lower than standard ETT but not statistically significant with effect size of 0.14 (medium category) and not specific in the use of oral hygiene antiseptic (Jena et al., 2016). Oral hygiene as one of the independent measures of nursing services is essential to be
carried out routinely in order to maintain the health of the patient's oral cavity and prevent the risk of lung infection from the upper gastrointestinal tract. Chlorhexidine has been widely demonstrated, and used as an effective prophylactic antiseptic in preventing the occurrence of VAP in patients with integrated intravenous ventilators. Previous study of the effects of endotracheal tubes with polyurethane cuff (PUC) and subglottic secretion drainage (SSD) using open suction system (OSS) and Chlorhexidine as oral hygiene in pneumonia found no significant difference between the two groups studied (conventional ETT and ETT PUC-SSD) after day 4, but the incidence of VAP in the PUC-SSD ETT group was lower than in the conventional ETT group with an effect size of 0.186 (medium category) (Lorente, Lecuona, Jiménez, Mora, & Sierra, 2007).

VAP diagnostic examination is done through Clinical Pulmonary Infection Score (CPIS). CPIS is most effectively used to identify patients who require antibiotic therapy, minimize exposure, and patients who require treatment therapy longer. The classical CPIS contains 7 examination variables consisting of body temperature, leucocytes, tracheal secretions, PaO2 / FiO2, piston photographs, and development of pulmonary infiltrate and culture examination (Luyt, Chastre, Fagon, & Group, 2004). Previous study modified CPIS by removing lung infiltrates development component and culture examination to minimize unnecessary use of antibiotics but still providing flexibility in the management of patients with pneumonia (da Silva, de Aguiar, & Fonseca, 2014). Another study suggested that modified CPIS without culture examination is easier to do in patients with pneumonia after 72 hours (day 4), which is modified CPIS has a good correlation with the results of the classical CPIS examination (Balanchivadze, Dy, & Boyce, 2015). This study aims to reveal the possibility of the influence of the type and design of Endotracheal Tube (ETT): Suction Above Cuff Endotracheal Tube (SACETT) and Endotracheal Tube standard (ETT) with Open Suction System (OSS) and Chlorhexidine 0.2% as oral hygiene in preventing VAP in patients installed mechanical ventilator.

METHODS

Study design
This was a quasi-experimental study with posttest only with control group design, which the cause and effect variable that occurs in the research object is measured or collected simultaneously, momentarily or once at one time (at the same time), and no follow up (Sastroasmoro & Ismael, 2002). In this study there are two groups, namely SACETT group and OSS-ETT group which both groups are using Chlorhexidine 0.2% as oral hygiene twice daily.

Sample
Thirty respondents were selected using purposive sampling, which 15 were assigned in each group. Determination and division of SACETT and OSS-ETT groups were based on ETT installed groove, which respondents indicated the installation of mechanical ventilator from Emergency Installation (IGD) installed standard ETT in IGD (OSS-ETT group). While the respondent indicated the installation of mechanical ventilator from the inpatient room and or ICU installed ETT with suction above cuff in ICU (SACETT group). The inclusion criteria of the samples were: patients with mechanical ventilator and treated in ICU for at least 72 hours, adult males and females (> 18 years old), patients with GCS <8 (decreased function of coughing and swallowing reflexes), patients with body temperature in the normal range (36.5 - 37.5°C). The exclusion criteria were: patients with prior history of pneumonia, patients with malignant disease, patients with HIV / AIDS, and patients who are taking a corticosteroid

Instrument
SACETT was attached to patients who met the inclusion criteria for subsequent intervention groups and OSS-ETT was attached to the control group, which both of them have oral hygiene using Chlorhexidine 0.2% twice daily. VAP examination uses simplified CPIS (body
temperature, leucocytes, tracheal secretions, PaO2 / FiO2, and Photo thorax) after 72 hours of installed intubated ventilator.

Data analysis
Data were analyzed using IBM SPSS 21. Data were evaluated using percentage and frequency as well as parametric tests to compare CPIs values after 72 hours of mechanical ventilator installation in the SACETT and OSS-ETT groups. The data normality test uses Shapiro-Wilk, and Independent t-Test is used to determine the effect of further use of SACETT and OSS-ETT on VAP.

Ethical consideration
This research has obtained permission from the Health Research Commission of Health Polytechnic of Kemenkes Semarang with approval number: 019 / KEPK / Poltekkes-SMG / EC / 2017, which stated that this research was ethically qualified. Prior to the research, prospective respondents were given informed consent which contains information about the purpose, benefits, and research procedures.

RESULTS
Table 1 shows that the average age of respondents in the SACETT group was 54.27 years with the maximum age of 77 years and minimum of 74 years. While the average age of the group of OSS-ETT was 52.80 years, with the maximum age of 74 years and minimum of 30 years. The statistical test obtained p-value = 0.5819 showed the age range data of respondents were homogeneous.

Table 1 Homogeneity test and frequency distribution of age of respondents (n=30)

| Variable | Group   | Mean | Median | SD     | Min | Max | p value |
|----------|---------|------|--------|--------|-----|-----|---------|
| Age      | SACETT  | 54.27 | 55.00  | 13.237 | 27  | 77  | 0.819   |
| OSS-ETT  | 52.80   | 54.00 | 10.591 | 30     | 74  |     |         |

*) Mann Whitney U Test (p<0.05)

Table 2 shows that the frequency and percentage of gender of respondents consisted of 18 (60%) of males in which 8 (26.7%) males in the SACETT group and 10 (33.3%) in the OSS-ETT group, while the female respondents were 12 (40%) which 7 (23.3%) in the SACETT group and 5 (16.7%) in the OSS-ETT group. Chi Square shows p = 0.456, which indicated that gender data were homogeneous.
Table 2Homogeneity test and frequency distribution of gender and medical diagnosis of respondents \( (n=30) \)

| Variable | Category | Group | Total | \( p \) value |
|----------|----------|-------|-------|-------------|
|         |          | SACETT| OSS-ETT|             |
| Gender  | Male     | 8     | 26.7  | 10          | 33.3        | 18 | 60.0 | *0.456 |
|         | Female   | 7     | 23.3  | 5          | 16.7        | 12 | 40.0 |
| Medical diagnosis | Nervous | 4     | 13.3  | 4           | 13.3        | 8 | 26.7 |
|         | Cardiovascular | 4  | 13.3  | 8           | 26.7        | 12 | 40.0 |
|         | Digestive | 2     | 6.7   | 0           | 0.00        | 2 | 6.70  | **0.660 |
|         | Immune   | 2     | 6.7   | 2           | 6.70        | 4 | 13.3 |
|         | Urinary  | 3     | 10.0  | 1           | 3.30        | 4 | 13.3 |

*) Chi Square Test \( (p<0.05) \) **) Kolmogorov Smirnov \( (p<0.05) \)

Frequency distribution and percentage of respondent characteristics based on medical diagnoses show that 12 (40%) respondents with cardiovascular disease as the highest percentage, which 4 (13.3%) respondents were in SACETT group and 8 (26.7%) respondents in the OSS-ETT group. The lowest percentage was in digestive system as many as 2 (6.70%) respondents. Kolmogorov Smirnov test showed \( p \)-value = 0.730, which indicated that the medical diagnostic data of respondents were homogeneous.

Table 3 Homogeneity test and frequency distribution of gender and medical diagnosis of confounding variables \( (n=30) \)

| Variable | Category | Group | Total | \( p \) value |
|----------|----------|-------|-------|-------------|
|         |          | SACETT| OSS-ETT|             |
| Antibiotic | Carapenem | 4     | 13.3  | 2           | 6.7         | 6 | 20.0 |
|         | Cephalosporin | 5  | 16.7  | 4          | 13.3        | 9 | 30.0 |
|         | Quinolone  | 2     | 6.7   | 5          | 16.7        | 7 | 23.3 |
|         | Nitroimidazole | 4  | 13.3  | 4          | 13.3        | 8 | 26.7 |
| Head position | 30° | 7     | 23.3  | 8          | 26.7        | 15 | 50.0 | *1.000 |
|         | 45°      | 8     | 26.7  | 7          | 23.3        | 15 | 50.0 |
| Diet    | 600 cc   | 4     | 13.3  | 4          | 13.3        | 8 | 26.7 |
|         | 800 cc   | 11    | 36.7  | 8          | 26.7        | 19 | 63.3 | *0.438 |
|         | 1000 cc  | 0     | 0.00  | 3          | 10.0        | 3 | 10.0 |
| Circuit type | Disposable | 8   | 26.7  | 8          | 26.7        | 16 | 53.3 | *1.000 |
|         | Reusable | 7     | 23.3  | 7          | 23.3        | 14 | 46.7 |

*) Levene Test \( (p>0.05) \)

Table 3 shows that most antibiotic use is cephalosporin group (30.0%), average head position is 30-45° (50.0%), diet with the highest frequency is 800 cc (63.3%), use of circuit type with the highest frequency is disposable circuit (53.3%). Levene test results showed probability values for antibiotic variables \( p = 0.425 \), head position \( p = 1.000 \), diet \( p = 0.438 \), circuit type \( p = 1.000 \), which indicated that all confounding variables can be controlled.

Table 4 Frequency distribution and percentage of VAP incidents in patients with mechanical ventilator in the ICU \( (n=30) \)

| Variable | Category | Group | Total | \( p \) value |
|----------|----------|-------|-------|-------------|
|         |          | SACETT| OSS-ETT|             |
| VAP incident | No VAP | 15    | 50.0  | 11          | 36.7        | 26 | 86.7 |
|         | VAP     | 0     | 0.00  | 4          | 13.3        | 4 | 13.3 |
| Total   |         | 15    | 50    | 15          | 50          | 30 | 100 |

Table 4 shows that there was no VAP incidence in the SACETT group whereas in the OSS-ETT group there were 13.3% of respondents who experienced VAP.

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The Independent t-test as shown in the table 5 obtained p-value = 0.001, which indicated that there was a significant difference between the use of SACETT and OSS-ETT on the incidence of Ventilator Associated Pneumonia in patients with mechanical ventilator with reference to Clinical Pulmonary Infection Score, which the mean of SACETT group (2.93 SD = 0.799) was smaller than the mean of OSS-ETT group (5.07 SD = 1.668). It can be concluded that CPIS value was better on SACETT than SETT.

### DISCUSSION

**Description of Respondent’s Characteristics**

The mean age of respondents in this study was 54.27 years in the SACETT group and 52.80 in the SETT group, which the age range of 46-65 years was categorized as elderly (Depkes, 2009). This was consistent with the previous study with the most respondents at the age below 60 years (Saragih et al., 2014). Similar with another study with the use of suction above cuff tracheotomy in reducing the average incidence of VAP in ICU chambers with most respondents in the range of 43-55 years (Ledgerwood et al., 2013). The other comparative study of polyurethane and polyvinyl cuff on VAP showed the most average respondents was 54 years old (Mahmoodpoor et al., 2013). Another research about the age relationship of VAP patients with length of stay in ICU shows that the average age of respondents installed mechanical ventilator was 49 years (Dewi & Harahap, 2014).

Risk factors that can trigger the occurrence of VAP include age over 60 years, disease severity, acute or chronic lung disease, excessive sedation, enteral nutrition, and severe burns (Rab, 2010). A study states that the age and degree of disease redness are two factors which play a role in patient outcomes in the ICU, but these relationships may be affected by acute physiological disorders, physiologic changes with age, and different treatment in each ICU (Boumendil et al., 2004).

The highest number of genders of respondent was males. This was not in accordance with a previous study that most of respondents were females (Ledgerwood et al., 2013; Yagmurdur, Tezcan, Karakurt, & Leblebici, 2016). However, patients critically ill in the ICU are basically not always dominated by one gender whether male or female. During the study, we found more male patients treated in the ICU with an indication of mechanical ventilator installation. The frequency of most respondents' medical diagnoses is a disease of the cardiovascular system followed by a disease of the nervous system. A research by The Canadian Critical Care Trials Group suggests that respondents with the most mechanical ventilators in trauma disease, followed by cardiovascular disease, respiratory disease, and neurological diseases (Group, 2006). Another study stated that most ventilator-installed patients are in cardiovascular system disease (Harde et al., 2013). Another also showed that the most respondents are with cardiovascular system disease (Saragih et al., 2014). Conditions in the cardiovascular system are closely related to the respiratory system so that when there is a disorder of the heart it will affect the respiratory system function. When the heart is unable to pump enough blood to maintain a smooth circulation, the result will be a buildup of blood and extra pressure that causes fluid to accumulate into the lungs. When the lung has lost its function, it requires a breathing apparatus such as a mechanical ventilator (Terry & Weaver, 2014). The use of mechanical ventilator is a risk factor in the occurrence of pneumonia because the airway

**Table 5** Effectiveness of the use of SACETT and OSS-ETT on VAP (n=30)

| Variable | SACETT Mean ± SD | OSS-ETT Mean ± SD | p value |
|----------|------------------|------------------|--------|
| CPIS     | 2.93 ± 0.799     | 5.07 ± 1.668     | 0.001  |

*) Independent t-Test (p <0.05)
is intubated, so that it can be the entrance of germs in the oral cavity if it is not kept clean.

The most antibiotic use in this study was cephalosporin (30.0%). Cephalosporin is an antibiotic capable of passing penicillin constraints. The drug is a semisynthetic preparation of the Acremonium Chepalosporium that is effective for fungus for both positive and negative gram (Azwar & Onk, 2004). The use of cephalosporin class antibiotics in this study is for prophylaxis in preventing pulmonary parenchymal infections due to the installation of an intubated ventilator. According to a study, 60% of antibiotics use is cephalosporin. This is because the majority of respondents are with low economy i.e. patients with health insurance. Another factor that supports the high use of cephalosporin class antibiotics is the very easy way of administration, once daily in an IV or bolus (Fauziyah, Radji, & Nurgani, 2011).

Fifty-percent of respondents have 30° and 45° head up position. The head-up position in the ventilator-mounted patient is essential to prevent aspiration of the oropharyngeal tract. A study suggests that a 15-30° back-rest elevation is insufficient to prevent VAP, while patients with elevated 45° semi recumbent positions have significantly lower incidences in VAP events (Alexiou, Ierodiakonou, Dimopoulos, & Falagas, 2009).

Administration of enteral nutrition in this study with the highest volume was 800 cc / 24 hours (63.3%). Patients with intubated mechanical ventilation should be controlled in the volumes of enteral nutrition administered to minimize residual volume to prevent aspiration from aerodigestive channels. This is in accordance with a study indicated that the tolerance limit of intubated mechanical ventilators in enteral feeding was not greater than 250 cc / 6 h (not more than 1000 cc / 24 h). This is because if the provision of nutrients above the tolerable volume of the patient increases the likelihood of vomiting and aspiration risk in patients which also increases the risk of VAP (Poulard et al., 2010).

The use of disposable circuits in this study (53.3%) is more than the use of reusable circuits (46.7%). During the course of the study, patients with intubated mechanical ventilators used more disposable circuits in order to maintain the sterility of oxygen and air entering the lungs during the use of mechanical ventilators to prevent VAP.

**Effectiveness Between the Use of SACETT and OSS-ETT on VAP**

VAP incidence was found in 4 (13.3%) respondents in the OSS-ETT group and no VAP was found in the SACETT group. In accordance with the study by Ledgerwood et al with the result that the incidence of VAP in the use of suction above cuff is lower than the use of standard tubes. This occurs because the use of ETT model with suction port over balloon cuff can control oral secretion in ventilator-installed patients in order not to enter the lungs. In contrast to the use of standard ETT that use open suction catheters to control secretions in the oral cavity but are unable to reach the upper respiratory to prevent secretion from entering the lungs so that microbe may occur in lung tissue.

Comparative analysis is performed on the CPIS score as a measure of incidence of VAP with grouping of SACETT and OSS-ETT variables in which there was significant difference between the two groups. The results of this study contrasted with a study by Jena et al, in which there was no significant difference in clinical and microbiological incidence of VAP between the use of SACETT and standard ETT with the conclusion that other strategies for VAP prevention were similar (Jena et al., 2016). Differences were suggested by Mao et al that subglottic secretion suction reduces VAP incidence and duration of mechanical ventilator use and delayed the onset of VAP (Mao et al., 2016). Another study for the incidence of VAP using endotracheal tubes with polyurethane cuff and subglottic secretion drainage (ETT-PUC-SSD) and conventional endotracheal tube by Lorente.
et al found that decreased incidence of VAP either early onset or late onset in treated patients using ETT-PUC-SSD (Lorente et al., 2007).

Microbial agents that because pneumonia have three forms of primary transmission, namely: (1) secretive aspiration containing pathogenic microorganisms that have colonized the oropharynx, (2) infectious aerosol inhalation, and (3) hematogenous spread of the extrapulmonary portion. The most common way is aspiration and inhalation of infectious agents. The colonization of microorganisms in the oropharynx is common in 48 hours after hospitalization, oropharyngeal secretions lead to pathological consequences whereby normal persons take fewer oropharyngeal secretions during sleep and will become worse when oral hygiene or when oral hygiene are not good. Suction mechanism in subglottic secretion drainage can decrease the incidence of VAP with continuous secret aspiration mechanisms to prevent micro aspiration from the upper airway into the lungs and minimize injury to the tracheal mucosa in order to avoid colonization of microorganisms (Lorente et al., 2007).

VAP is closely related to the upper respiratory hygiene level. The action in maintaining the hygiene of the oral cavity of the patient is installed by a mechanical ventilator intubated by doing two important things, namely suctioning and oral hygiene. Standard ETT intubation with open suction is an old method but is maintained because of the policy of most hospitals in the area despite the risks of contamination during suctioning. Oral hygiene using Chlorhexidine 0.2% has been extensively studied and has a good effect on maintaining oral hygiene. This study combines intubation using SACETT + Chlorhexidine 0.2% in an effort to prevent VAP.

This study has an effect size of 1.668 with a very strong category, meaning that this study is able to provide better effects in preventing VAP with the location of differences in the combination of intubation using SACETT and oral hygiene using Chlorhexidine 0.2% twice a day. According to previous study, Chlorhexidine 0.2% is an effective antiseptic to decrease VAP incidence compared with 0.19% NaCl usage. Thus, it can be concluded that our study is in accordance with the results of the previous study (Tantipong, Morkchareonpong, Jaivindee, & Thamlikitkul, 2008).

Previously, the Closed Suction System (CSS) method was considered capable of reducing contamination of the upper and upper respiratory tracts in infection prevention efforts, but some studies did not show any difference in the use of CSS over Open Suction Method (OSS) methods in the treatment of installed patients with intubated mechanical ventilators prevention of VAP. Based on a meta-analysis study shows that the comparison of CSS and OSS use in VAP prevention was not significant with CI = 1.50-5.52, which means the use of CSS did not provide benefit in preventing VAP compared with OSS use (Siempos, Vardakas, & Falagas, 2008). Another also stated that there was no difference use of CSS and OSS in the prevention of VAP nosocomial infections in ventilator-installed patients (Badriyah, 2016). Meanwhile, the use of 0.2% Chlorhexidine in OSS and CSS have the same impact in ventilator-installed patients (Debora, Leksana, & Sutiyono, 2012). A new method of preventing VAP is the incorporation of a closed suction system with ETT in a single tool, SACETT, which minimizes the risk of micro aspiration by suction that directly reaches the area above the ETT cuff balloon. The combination of SACETT and oral hygiene intubation using Chlorhexidine 0.2% twice daily can prevent VAP than standard ETT intubation with an open suction system with Chlorhexidine 0.2% twice daily as oral hygiene.

**CONCLUSION**

VAP incidence based on CPIS assessment on the fourth day was as much as 13.3% in the use of OSS-ETT. This study showed that the combination of intubation using SACETT and
oral hygiene using Chlorhexidine 0.2% twice daily was more effective than standard ETT intubation with open suction system in preventing VAP, with p-value = 0.001. The use of SACETT and oral hygiene using Chlorhexidine 0.2% proved to prevent VAP in patients <60 years old and with indications of intubation in cardiovascular system disease, nervous system, immune system and urinary system and digestive system. Therefore, in the prevention of VAP in critical patients, it is recommended to combine SACETT and oral hygiene with Chlorhexidine 0.2%.

Declaration of Conflicting Interest
None declared.

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Author Contribution
All authors contributed equally in this study.

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