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Research Article

Comparison Effects of Chlorhexidine 0.12%, Povidone Iodine 1%, and Fluoride-Based Toothpaste as Oral Hygiene Agents on the Growth of Tracheal Bacterial Colonization and Antibiotic Resistance in Mechanical Ventilation Patients

Muhammad Fajrin Sulaeman[1], Faisal Muchtar[1,2]*, Hisbullah[1,2], Syafri Kamsul Arif[1,2], Nur Surya Wirawan[1,2], Madonna Damayanthie Datu[1,2]

[1]*Department of Anaesthesia, Intensive Care and Pain Management, Hasanuddin University, Makassar, Indonesia.*

[2]*Department of Anaesthesia, Intensive Care and Pain Management, Dr. Wahidin Sudirohusodo General Hospital, Makassar, Indonesia*.

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ABSTRACT

Background: Ventilator-Associated Pneumonia (VAP) is a common complication in mechanically ventilated ICU patients and is associated with bacterial colonization in the oral cavity. Poor oral hygiene can increase the risk of bacterial aspiration into the lower airway. Various oral hygiene agents such as chlorhexidine, povidone iodine 1%, and fluoride toothpaste are used to prevent this colonization, but their effectiveness is still variable. This study aimed to compare these three agents on tracheal microorganism growth and antibiotic resistance profile in mechanically ventilated patients.

Methods: This single-blind randomized clinical trial included 45 ICU patients on mechanical ventilation at Dr. Wahidin Sudirohusodo Hospital, Makassar. Subjects were allocated into three groups to receive oral hygiene using chlorhexidine 0.12%, povidone iodine 1%, or fluoride toothpaste twice daily for five days. Tracheal aspirates were collected at baseline (before intervention), Day 3, and Day 5 to assess microbial colony counts and antibiotic susceptibility profiles.

Results: The chlorhexidine group showed the most significant reduction in tracheal microorganism colony counts from 1833.33± 566.5 to 1226.7± 461.7 CFU/mL on day 5 ($p < 0.001$), compared to povidone iodine and fluoride. The highest antibiotic resistance was found in *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. The chlorhexidine group also had the lowest number of multiresistant isolates.

Conclusion: Among the evaluated oral hygiene agents, chlorhexidine 0.12% was the most effective in reducing tracheal microbial colonization and showed a lower tendency for antibiotic resistance development. It is recommended as a superior oral care agent for preventing VAP in mechanically ventilated ICU patients.

Introduction

Ventilator-associated pneumonia (VAP) is the most common nosocomial infection in intensive care unit (ICU) patients undergoing mechanical ventilation. VAP accounts for approximately 50% of nosocomial pneumonia cases and is estimated to occur in 5-40% of patients who are on a ventilator for more than 2 days [1].

Despite rapid developments in the field of therapy and intensive care, the incidence and mortality rate of VAP

The authors declare no conflicts of interest.

*Corresponding author.
E-mail address: faisal_kedok@yahoo.com

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remain high. Data from the International Nosocomial Infection Control Consortium (INICC) shows that the incidence of VAP is approximately 14.1 per 1000 ventilator-days, with mortality reaching 36.6% [2]. VAP also causes prolongation of hospitalization and intubation duration, increased antibiotic use, and increased medical costs [3].

One of the main risk factors for VAP is poor oral health during ventilator use. Patients in the ICU are particularly susceptible to changes in oral flora due to decreased salivary production, tube insertion, and other systemic conditions. Within the first 48 hours of hospitalization, the oral flora can change to be dominated by Gram-negative bacteria, which form plaque and have the potential to migrate to the lungs via aspiration, triggering VAP [4]. Therefore, consistent implementation of oral hygiene is one of the important strategies in the prevention of VAP, as recommended by the CDC [2,5].

Various agents have been used in oral hygiene practice, including 0.12% chlorhexidine solution, 1% povidone iodine, and fluoride toothpaste. Chlorhexidine has broad antimicrobial effects and is able to inhibit bacterial colonization and prevent microbial adhesion to tooth enamel [5-6]. Meanwhile, povidone iodine 1% as a pre-procedural mouthwash is also known to be bactericidal against salivary microorganisms [7]. Fluoride toothpaste has been demonstrated to have antibacterial properties against oral pathogens like Streptococcus mutans and to strengthen enamel [8-9].

The combination of mechanical plaque removal (e.g., brushing) and chemical agents has been shown to be more effective than single methods [2,10]. However, previous studies have shown mixed findings. Vidal et al. reported that the combination of tooth brushing and 0.12% chlorhexidine gel reduced the incidence of VAP, although it was not significant compared to chlorhexidine without tooth brushing [11]. Estaji et al. found that toothpaste was more effective in reducing oral lesions than chlorhexidine, while Chacko et al. stated that there was no significant difference between mechanical and chemical debridement [12-13]. Research on the comparative effects of chlorhexidine, povidone iodine 1%, and fluoride toothpaste on the colonization of tracheal microorganisms is still limited. Therefore, this study aims to compare the three agents as part of oral hygiene against the growth of tracheal microorganism colonies and their antibiotic sensitivity and resistance in patients with mechanical ventilation.

Methods

This study used a single-blind randomized clinical trial conducted at Dr. Wahidin Sudirohusodo Hospital Makassar starting in November 2024 until the sample size was met. The study population included patients undergoing mechanical ventilation, and samples were taken by consecutive sampling according to the inclusion criteria. The minimum sample size was determined as 30 people, who were randomly divided into three groups with 10 patients each. The three groups received oral hygiene treatment twice a day for five days using one of three materials: chlorhexidine 0.12%, povidone iodine 1%, or fluoride toothpaste.

Subjects were selected based on the inclusion criteria, namely patients aged ≥ 18 years who underwent mechanical ventilation for more than 48 hours and had a Glasgow Coma Scale≤ score of 8. Exclusion criteria included lower airway infection before ventilator insertion, allergy to intervention materials, oropharyngeal anatomical abnormalities, and very poor or no dental condition. Patients were excluded from the study if they had severe complications that required discontinuation of the intervention, died before day 5, were discharged from the ventilator before the final sampling time, or withdrew from the study.

Oral hygiene procedures were performed in the supine position with the head-up 30°, using sterile materials and tools according to the treatment group. In addition to antibiotic resistance and sensitivity testing, tracheal microorganism colony analysis was conducted prior to intervention, 48 hours later (day 3), and 96 hours later (day 5). Age, body mass index, length of mechanical ventilation, length of oral hygiene, and microbiology results were among the information gathered. With a significance level of 5% ($p < 0.05$), the ShapiroWilk test for normality was used to analyse the data, followed by the independent t-test or MannWhitney U-test based on the data distribution. The Hasanuddin University Health and Dr. Wahidin Sudirohusodo Hospital Research Ethics Committee gave its approval to this study under protocol number 1039/UN4.6.4.5.31/PP36/2024.

Results

This study included 45 mechanically ventilated ICU patients at Dr. Wahidin Sudirohusodo Hospital were split equally into three groups and given fluoride toothpaste, povidone iodine 1%, and chlorhexidine 0.12% for dental hygiene. Age, gender, and BMI were baseline characteristics that were similar across groups and did not differ statistically significantly ($p > 0.05$) (Table 1). On day 3 and day 5, there were notable differences in the number of tracheal microorganism colonies between the groups ($p < 0.001$). The group that used chlorhexidine had the lowest mean colony counts on both days, followed by those that used fluoride toothpaste and povidone iodine (Table 2, Figure 1).

The Chlorhexidine group showed the biggest decrease in colony count from day 3 to day 5. The percentage of patients with bacterial growth on day five was lower in the chlorhexidine group (33.3%) than in the povidone iodine (53.3%) and fluoride (73.3%) groups, albeit not

statistically significant (p = 0.090) (Table 3). The most common microorganism found in all groups was Pseudomonas aeruginosa, which was less common in the Chlorhexidine group (Table 4).

Table 1- Characteristics of the research sample

Characteristics		Chlorhexidine	Povidone Iodine	Fluoride	P value
Age[(a)] (years)		43.0± 13.8	42.5± 14.2	36.5± 15.4	0.397[ns]
Gender[c]	L	9 (60)	9 (60)	11 (73,3)	0.678[ns]
	P	6 (40)	6 (40)	4 (26,7)	
BMI[(a)] (kg/m^2)		23.1± 2.3	24.1± 2.5	23.5± 2.4	0.523[ns]

Numerical data are shown as mean± SD, and categorical data are shown as n (%). [a]One Way Anova, [b]Kruskal Wallis, [c]Chi-Square test, ns: not significant, *: significant

Table 2- Comparison of tracheal microorganism colonies between groups and each group

Variables	Chlorhexidine	Povidone Iodine	Fluoride	P value
Colony H3[a]	1833.3± 566.5	2973.3± 593.4	3560.0± 1162.4	0,000*
Colony H5[b]	1226.7± 461.7	2286.7± 771.7	3313.3± 1158.7	0,000*
$\Delta_{(H5-H3)}$ [(b)]	-606.7± 147.4	-686.7± 341.9	-246.7± 63.99	0,000*
P value	0,001*	0,000*	0,000*	

Data are shown with mean± SD. [a]One Way Anova, [b]Kruskal Wallis, [c]Chi-Square test, ns: not significant, *: significant

Table 3- Comparison of bacteria types between groups

Bacteria Type[c]	Chlorhexidine	Povidone Iodine	Fluoride	P value
Found	5 (33,3)	8 (53,3)	11 (73,3)	0.090[ns]
Not found	10 (66,7)	7 (46,7)	4 (26,7)	

Data are shown with mean± SD. [a]One Way Anova, [b]Kruskal Wallis, [c]Chi-Square test, ns: not significant, *: significant

Table 4- Distribution of Antibiotic Resistance and Sensitivity to Bacteria

Group	Bacteria	Sensitive	Intermediate	Resistance
Chlorhexidine	Eschercia coli	6	1	7
	Staphylococcus aureus	8	0	1
	Pseudomonas aeruginosa	10	0	0
	Acinobater baumani	11	1	0
Povidone Iodine	Eschercia coli	5	1	5
	Staphylococcus aureus	8	0	1
	Pseudomonas aeruginosa	10	0	0
	Acinobater baumani	1	0	11
Fluoride	Eschercia coli	5	1	5
	Staphylococcus aureus	8	0	1
	Pseudomonas aeruginosa	9	1	0
	Acinobater baumani	0	0	12
	Staphylococcus haemolyticus	3	0	4
	Staphylococcus saprophyticus	6	0	1

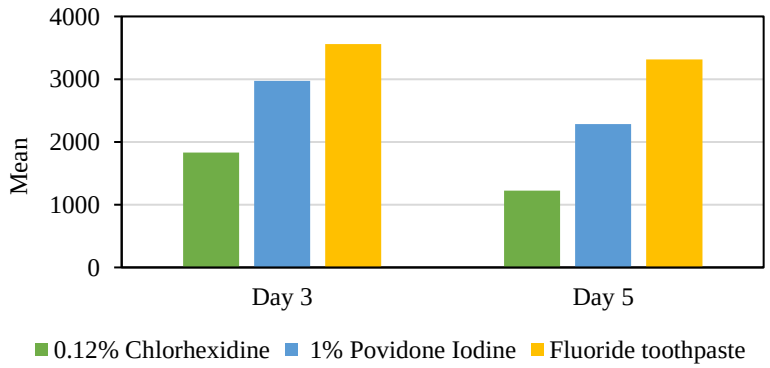

Figure 1- Comparison of tracheal microorganism colonies in the three groups.

Discussion

This study involved 45 patients undergoing mechanical ventilation in the ICU of Dr. Wahidin Sudirohusodo Hospital who were divided into three treatment groups with 15 patients each based on the type of oral hygiene given, namely chlorhexidine 0.12%, povidone iodine 1%, and fluoride toothpaste. Baseline characteristics such as age, gender, and body mass index showed a homogeneous distribution, with no significant differences between groups. This ensures that the results obtained can be attributed more to the effect of the oral hygiene intervention rather than the influence of confounding factors.

The effectiveness of each oral hygiene agent was assessed by the change in the number of tracheal microorganism colonies on days 3 and 5 after the intervention. The analysis showed that there was a significant difference in colony counts between groups ($p < 0.001$). Colony counts were highest in the fluoride group, lowest in the chlorhexidine group, and lowest in the povidone iodine group. From day three to day five, colony counts significantly decreased in each group, but the largest decline was seen in the chlorhexidine group. This indicates that chlorhexidine is the most effective agent in suppressing the growth of microorganisms in the airway.

The mean number of tracheal microorganism colonies was visibly lowest in the chlorhexidine group across both Day 3 and Day 5, with a notable reduction over time. Conversely, the fluoride toothpaste group showed the highest colony counts on both days, with a relatively smaller decline. This provides additional support for the statistical findings showing chlorhexidine's superior efficacy in reducing microbial colonisation.

Tracheal cultures showed that the chlorhexidine group had the lowest percentage of bacterial growth (33.3%) compared to fluoride (73.3%) and povidone iodine (53.3%). Although this difference was not significant ($p > 0.05$), descriptively it appears that chlorhexidine has greater potential in preventing bacterial colonization. The active components of chlorhexidine work by dissolving the bacterial cell wall and inhibiting the production of biofilms, which serve as the substrate for the development of microbial colonies in patients receiving mechanical ventilation. Pseudomonas aeruginosa was the most prevalent microorganism across all groups. This bacterium is known to be the main pathogen causing Ventilator-Associated Pneumonia (VAP) and has the ability to be resistant to various antibiotics. However, compared to the other groups, the Chlorhexidine group had fewer patients with pseudomonas growth and bacterial colonisation. This implies that chlorhexidine use may aid in halting the colonisation of the primary pathogens responsible for VAP. In addition to suppressing the number of microbial colonies, chlorhexidine was also associated with lower incidence of bacterial growth and antibiotic resistance. These findings are consistent with the study by Li et al., which showed that the use of chlorhexidine as an oral hygiene agent has an important role in the prevention of nosocomial infections, especially VAP [3]. Therefore, chlorhexidine is recommended as the main agent in oral care procedures in the ICU to reduce the risk of VAP.

On the other hand, although fluoride toothpaste is known to have antimicrobial effects, its use in the context of mechanically ventilated patients has not shown optimal results. Brailsford et al. showed that fluoride can affect oral flora, but the agent is not effective enough in preventing colonization of tracheal microorganisms [14]. Therefore, its use in ICU patients requires further evaluation, especially in relation to clinical effectiveness and the risk of antimicrobial resistance. The relative efficacy of different oral hygiene products in preventing microbial colonisation in patients on mechanical ventilation is first assessed in this study. Although there are some limitations, such as small sample size and limited duration of observation, these results provide a basis for further research. Further studies with larger sample sizes and longer-term observations are needed to strengthen the results and provide more accurate clinical recommendations regarding the selection of effective oral hygiene agents in the prevention of nosocomial infections in the ICU.

Conclusion

The use of chlorhexidine 0.12% as an oral hygiene agent in mechanically ventilated patients in the ICU proved to be more effective in reducing the number of tracheal microorganism colonies than povidone iodine 1% and fluoride toothpaste. In addition, the chlorhexidine group also showed a lower proportion of bacterial growth as well as less tendency of antibiotic resistance in isolated pathogenic bacteria, thus potentially providing better protection against ventilator-associated infections.

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