

Effectiveness of Tongue Cleaning Plus Chemical Mouthwash Agents into the Number of Oral Microorganisms Caused Aspiration Pneumonia in Bedridden Elderly Patients: A Crossover Study

Nilobon Aiemyen^{1,2*}, Chaipat Luangnam², Songsak Suksan², Pastraporn Payukaparp², Janpen Kwansirikul³, Patcharaphol Samnieng¹

1. Department of Preventive Dentistry, Faculty of Dentistry, Naresuan University, Thailand.
2. Sirindhorn College of Public Health, Phitsanulok, Thailand.
3. Department of Psychiatry, Faculty of Medicine, Naresuan University, Thailand.

Abstract

To evaluate the effectiveness of tongue cleaning plus mouthwash on the number of microorganisms caused pneumonia in bedridden elderly patients.

Twelve bedridden elderly participated in this crossover design. The participants were randomly divided into three groups of tongue cleaning methods as follows: group 1, chlorhexidine gluconate (CHX); group 2, water (W); group 3, povidone iodine (PVP-I). The number of microorganisms caused aspiration pneumonia (total anaerobic bacteria, total aerobic bacteria, *Streptococci*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*) and *Candida spp.* were measured on the dorsal of tongue surface at baseline, rapidly and at 3 hours after tongue cleaning.

The percentage of the number of microorganisms reduction for tongue cleaned with CHX (> 60 % for all) were higher than PVP-I and W group, and trended to continue effect for 3 hours. The percentage of reduction for tongue cleaned plus CHX was significant differences in the number of total anaerobic bacteria, total aerobic bacteria, *Streptococci*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* after cleaning and 3 hours after cleaning compared to W group. The effectiveness of tongue cleaning plus CHX to reduce the number of bacteria was no difference the tongue cleaning plus PVP-I. For the number of *Candida spp.*, the CHX was significant differences in rapidly after cleaning but only the CHX group was significantly in 3 hours after cleaning compared to PVP-I and W.

CHX might be recommended for clinical practice guideline of tongue cleaning in the bedridden elderly patients.

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Introduction

One of the leading causes of death in the elderly is pneumonia.¹ In the United States, the rate of deaths from pneumonia in the elderly is doubled in people aged 65 and older compared with less than or equal to 65.² In Asia, the first super-aged society is Japan. It was found that pneumonia is the third leading cause of death in

the elderly, with inhalation of aspiration pneumonia causing the death of up to 80% of the elderly.³ The data from disease surveillance in Thailand 2015 found that the incidence of pneumonia has tripled in the age group 65 years and older, compared to the age group 55-64 years. The mortality rate from pneumonia in the elderly group is 0.23%.⁴ The bedridden elderly group are completely dependent on others, requiring long term care from their caregivers. These things create a burden and emotional impact on the caregiver. Especially if they have more health problems and their bodies have infection that causing bedridden elderly to come back to get hospitalized more often or ultimately causing the bedridden elderly to death.⁵ One factor that causes inhalation of aspiration

*Corresponding author:

Nilobon Aiemyen
Department of Preventive Dentistry, Faculty of Dentistry,
Naresuan University, Thailand.
Sirindhorn College of Public Health, Phitsanulok, Thailand.
E-mail: nilobon@scphpl.ac.th

secretions or food from the inside of the mouth into the lungs become more severe until the infection are microbes that caused dental caries and periodontal disease.⁶ They are found the most in the mouth and the respiratory system, including Anaerobic bacteria; *Bacteroides*, *Prevotella*, *Fusobacterium*, *Peptostreptococcus*. Aerobic bacteria; *Streptococcus*, *Staphylococcus aureus*, *Haemophilus influenza*, *Pseudomonas aeruginosa*, and *Candida Spp.*⁷⁻⁹ Most of the microbes that causes of pneumonia are anaerobic bacteria which are found up to 93% and they stay on the dorsal of tongue surface.¹⁰⁻¹² The tongue may be a source of accumulation of microbes in the mouth that may the result of Pneumonia due to inhalation.

The tongue has papillae covering the dorsal of surface. They help to accumulate bacteria and dead cells in the mouth, including the epithelial cells (desquamated epithelial cells), forming a white coating covering the tongue (coated tongue).¹³ So, cleaning the mouth in the bedridden elderly group by mechanical methods may not be effective enough to reduce the amount of microbes immediately. Including the inability to control the amount of bacteria in the mouth for a long time. The use of mouthwash with antimicrobial agents is an alternative to enhance the effectiveness of reducing or controlling the amount of microbes in the mouth for a longer period.^{1, 14} The mouthwash that prevent and treat infection such as Chlorhexidine gluconate (CHX), Povidone-iodine (PVP-I).

Therefore, the researcher is interested to study the effectiveness of tongue cleaning using CHX and PVP-I mouthwash on the changes in microorganisms that cause aspiration pneumonia in bedridden elderly patients and we hope this research will be part of the search for practical guidelines for cleaning the tongue in the bedridden elderly patients in Thailand to reduce the risk of aspiration pneumonia, reduce the cost, shorten the time of hospitalization, reduce the frequency of returning to treatment and reduce the death rate from pneumonia infections.

Materials and methods

Participant: In this cross-over study were 12 people who live in a community within a radius of 20 kilometers from Naresuan University at Phitsanulok, Thailand. They must be people who aged 60 years and over, both males and

females according to the calendar year as of the research date, diagnosed by medical personnel or screened by the Barthel Activities of Daily Living assessment and confirmed that they were the bedridden elderly who can feel but can't move and use hands to clean the mouth and live with a caregiver. Exclusion criteria are people with pathological conditions at the tongue, such as ulcers (traumatic ulceration, recurrent aphthous ulceration, oral mucositis) or disorders of the tongue that may interfere with the process of collecting samples of microbes that accumulate on the tongue. People who use mouthwash with antibacterial during a period of 1 week before the research. People who have a history of allergic reactions such as people who have a history of allergic reaction to CHX, PVP-I and people with thyroid disorders and people with obstacles in the research includes people who sensitive to gagging or vomiting.

This research has been approved by the ethics committee in human research of Naresuan University, project number 0121/62 and has passed the Thai Clinical Trials Registry (TCTR), number TCTR20190614001. The samples group will be explained the details of the research project, then sign to consent and participate in the research project. Before we start to collect the data, the teeth, tongue and epithelium of the oral cavity in the bedridden elderly patients will be cleaned by brushing teeth with W (W) daily by a caregiver for a period of 7 days after eating breakfast between 07.00 - 08.00 a.m.

Procedure of research

Each of samples will receive the tongue cleaning with all 3 types of mouthwash by setting a washout period for 1 week before changing the tongue cleaning with other mouthwash. Before beginning the procedure of research, the researchers evaluated the appearance of white coating and collected microbes on the tongue before beginning to clean the tongue with each type of mouthwash.

Oral cleaning Protocol

Tooth brushing: For volunteers who having teeth, dip a soft bristled toothbrush (Systema[®] tooth brush Extra soft, Thailand) in W. Then divide the teeth in the oral into 4 parts: upper right, upper left, lower left and lower right. Brushing each part with a horizontal scrub technique. The duration of brushing depends on the amount of teeth remaining in the oral follow by Tajima et al., 2017.

Brushing time = amount of teeth remaining in the oral (Including retained root) / 32 teeth X 5 minutes

For volunteers without teeth, cleaning the edentulous with a sterile gauze that moistened with W.

Tongue cleaning: The researcher has passed the standardization of tongue cleaning from all the dental cleaning experts of the samples. The samples were divided into 3 groups. All samples will clean their tongue with all 3 types of chemicals. Group I: clean the tongue by using a soft bristled toothbrush (Systema® tooth brush Extra soft, Thailand), dip in CHX (Chlorhexidine digluconate solution 20% in H₂O, Sigma-Aldrich®, USA) with 0.12% concentration, 15 milliliters. Group II: clean the tongue by using a soft bristled toothbrush, dip in 15 ml. of W (control group). Group III: clean the tongue by using a soft bristled toothbrush, dip in Povidone iodine (Polyvinylpyrrolidone - Iodine complex 100 mg / ml, Sigma-Aldrich®, USA) with 1% concentration, 15 milliliters.

The researchers held the samples tongue with sterile gauze as far away from the mouth as possible. Divide the tongue into 3 parts (Fig. 1). Clean each part of the tongue 10 times with a force of 100 gf from the front of the sulcus terminalis to the tip of the tongue. Modifying from Juyeon et al. tongue cleaning method (Juyeon et al., 2013)

Moisturizing their lips after cleaning with a water-based gel (Q-C® Lubricating Jelly Water Soluble, Thailand) to prevent the corner of the lips to dry, broken and ulceration.

Note: for all phases of the research, the samples were prescribed preventive measures for suffocation and adverse reactions from the research.

Data collection

The researchers evaluated the white coating and collected microbes on the tongue after cleaning the tongue. The researchers will divide the collection for 3 times; before cleaning the oral cavity, immediately after oral cleaning and 3 hours after cleaning.

Assessment of white coating on the tongue. White coating index assessed by vision, divide the tongue into 9 parts and calculate score follow by Kobayashi et al., 2017.

Collecting microbes on the tongue. The researcher swabs the oral microorganisms on the tongue with a sterile cotton (Longmed®, size M,

Thailand) 5 times from the front of the sulcus terminalis to the tip of the tongue with a force of 20 gf in one direction. Then soaked a cotton swab in phosphate-buffered saline solution (2 ml.) and then immersed in a cool box at 4 °C and sent to the laboratory. Microbes that distributed in the solution were diluted in a serial dilution. Then suction 20 microliters of solution and dissolve on a petri dish, repeated 8 times, then incubated in the incubator.

Aerobic bacteria were cultured on the Brain Heart Infusion Agar (Himedia®, India), incubated in a culture incubator at 37 °C under 5% CO₂ for 24-48 hours. Anaerobic bacteria were cultured on Tryptic Soy Agar in anaerobic condition, incubated at 37 °C for 5-7 days. *Streptococcus* were cultured on the Mitis Salivarius Agar, incubated in the incubator at 37 °C for 48 hours in a 5% CO₂. *Staphylococcus aureus* were cultured on Mannitol Salt Agar, incubated in an incubator at 37 °C for 24-48 hours. *Pseudomonas aeruginosa* were cultured on Cetrimide Agar Base, incubated in a culture incubator at 37 °C with O₂ for 24 hours. *Candida spp.* were cultured on Sabouraud Dextrose Agar, incubated in an incubator at 37 °C with O₂ for 24-48 hours.

Then counting the amount of microbes that appear on the media in colonies / milliliters.

Data analysis

1. Descriptive statistics: it consists of the average, percentage, standard deviation of the amount of microorganisms that cause aspiration pneumonia. Percentage of reduction in the amount of microbes that cause aspiration pneumonia and white coating index on the tongue before and after cleaning the tongue with CHX, W and PVP-I immediately and after cleaning for 3 hours calculated by:

$$\text{Percentage of reduction} = \frac{\text{(The beginning's average microbial counts - average microbial counts after cleaning)}}{\text{The beginning's average microbial counts}} \times 100$$

2. Inferential statistics: to compare the differences of the bacterial, fungal and white coating index and comparing the effectiveness of CHX, W and PVP-I by using the One-Way ANOVA statistics. After testing the result showed that there are statistically significant differences at the level of 0.05. Therefore, the testing of the differences of each pair by means of multiple

comparison using Post hoc test with SPSS version 22.

Results

Participants were 12 bedridden elderly, 3 males (25%) and 9 females (75%). An average age was 79.16 ± 6.75 years.

Table 1 shows the percentage of reduction microorganisms on the tongue compared with the differences of the effectiveness for CHX, W and PVP-I, respectively. Significant difference were observed the percentage of reduction in amount of total aerobic bacteria, total anaerobic bacteria, *Streptococcus* and *Staphylococcus aureus* for CHX and PVP-I compared with W after cleaning immediately and after cleaning 3 hours. However, there were no significant difference for CHX compared with PVP-I after cleaning immediately and after cleaning 3 hours.

The percentage of reduction the number of *Pseudomonas aeruginosa* decreases in all 3 groups after cleaning immediately and after cleaning 3 hours, but there were not significantly different for CHX, W and PVP-I.

The significantly decreasing in this results were observed the amount of *Candida spp* for CHX compared with W after tongue cleaning immediately and after cleaning 3 hours. There were no significant difference for PVP-I compared with W after tongue cleaning immediately, but there was significantly different for PVP-I compared with CHX and W at 3 hours after tongue cleaning.

The percentage of reduction in the amount of white coating decrease in all 3 groups after cleaning immediately and after cleaning 3 hours (Table 2). There were not significant differences for CHX, W and PVP-I, but the percentage of reduction in the amount of white coating for CHX is lower than PVP-I and W at 3 hours after tongue cleaning, respectively.

Discussion

According to the report of the situation, the elderly in Thailand suffer from pneumonia up to 1,058.59 people per 100,000 population.⁴ Each illness of the bedridden elderly brings burden to the caregivers, relatives, health personnel in providing care, moving including the cost of hospitalization. The reducing one risk factor that may cause aspiration pneumonia infection were reducing and controlling the

amount of microbes in saliva. The amount of microbes found in the saliva related to the amount of microbes on the tongue. Tongue cleaning is therefore a necessity for the bedridden elderly. The cleaning with brushing the tongue from the back to the front of the tongue, in addition to being able to reduce the amount of white coating on the tongue and reduce bad breath, it also reduce the amount of microbes on the tongue. Cleaning the tongue by using a soft toothbrush to clean the tongue can reduce bad breath level by 45% that is considered to have good performance.¹⁵ Cleaning the tongue by using the toothbrush with W can reduce the volatile sulfur compound by 42% but is effective in just 25 minutes.¹⁶ The use of mouthwash is an alternative agents to enhance the effectiveness of reducing or controlling the amount of microbes in the mouth for longer periods.^{1, 14} It is the origin of this research that uses liquid substances to clean the tongue plus mechanical cleaning technique. In addition, the liquid can spread into the deep groove between the papillae on the tongue and can reduce the number of microbes that cause aspiration pneumonia. This research uses a soft bristled toothbrush with CHX, PVP-I and W can reduce the bacteria that cause aspiration pneumonia and can reduce the *Candida spp*. as well as the Tajima et al. which has to clean the tongue by using a tongue brush moistened with mouthwash in the elderly after and can reduced the amount of all microbes covered on the tongue.¹⁷

Most of the bedridden elderly have dry mouth characteristics that causing the white coating dry covering the tongue and has a thick amount covering the middle and back of the tongue which is difficult to wipe off. Cleaning the tongue with a liquid solution, CHX and PVP-I that combined with mechanical cleaning and makes it easier to wipe off tongue white coating. The decrease in saliva increases the amount of epithelial cells from the oral mucosa that causing the tongue coating formation.¹⁸ This is consistent with Shimizu et al. research, they found that the white coating index will decrease if the amount of microbes on the tongue decreases.¹⁹

Many previous studies have conducted clinical studies on the use of CHX or PVP-I to reduce the prevalence of oral and pharyngeal microbes, to reduce the rate of aspiration pneumonia in admitted patients who wearing ventilator.^{20, 21} In this study, 0.12% concentration

of CHX was chosen, which has extensive antiseptic activity, stable and long lasting²² and choose to use 1% concentration of PVP-I due to 0.35% to 1.0% concentration of PVP-I that used for the elderly at risk of pneumonia.^{23,24}

According to a study by Ishikawa et al., it was found that a 0.35% concentration of PVP-I can reduce all microbes with statistical significance but could not significantly reduce *Streptococci*, *Staphylococcus* and the black bacteria group.²³ But according to a study by Yoneyama et al., who cleaned the mouth with 1% concentration of PVP-I can reduce the incidence of aspiration pneumonia.²⁴ The results of this study show that the use of CHX is more effective in reducing microbes that cause aspiration pneumonia than PVP-I and W. Cleaning the tongue with W can reduce the microbes that cause aspiration pneumonia but the use of chemical mouthwash agents combined with mechanical cleaning technique can increase the effectiveness of the microbes.

Tongue cleaning with CHX was found to have the average percentage of the reduction of the amount of microbes after immediate cleaning and 3 hours between 60-90%, close to previous the studies, that was conducted in a sample group aged 22-65 years by giving the sample to rinse the mouth with 0.12% concentration of CHX 10 ml. for 30 seconds. It was found to be able to reduce all anaerobic bacteria on the tongue as much as 81–90% after 3 hours.²⁵ This study was conducted in the bedridden elderly with physical limitations which may cause aspiration of chemicals into the lungs. Therefore, the design of the research method uses the amount of mouthwash and less than previous studies which clean the tongue by using mouthwash containing irrigation on the tongue more than 15 ml. in combination with the use of high speed vacuum ejector.²⁶ In addition, cleaning the tongue with CHX can reduce the amount of aerobic bacteria and all anaerobic bacteria. That are significantly different from the groups that are cleaned with W because CHX is a solution that allows the substance to spread into the sulcus, consistent with the studies of Herrera et al. They discussed this study that after giving the sample to rinse CHX for 1 minute and measure the amount of bacteria, the amount of aerobic bacteria and anaerobic bacteria in 5 hours, it was found that the amount of the bacteria decreased significantly different from the control group that

received a saline solution²⁷ and in this study, it was also found that CHX can reduce the amount of *Streptococci*, *Staphylococcus aureus* which is Gram-positive bacteria and *Candida spp.*, differences from the control group were statistically significant. CHX can reduce the amount of *Pseudomonas aeruginosa* has no difference with the W group, consistent with the study of Scannapieco et al. They found that CHX can significantly reduce Gram positive bacteria due to CHX is more sensitive to bind to gram positive bacteria than gram negative bacteria.²⁸ In addition, CHX can kill a wide range of microbes, so can also kill *Candida spp.*

After cleaning the tongue for 3 hours with mechanical cleaning technique with CHX can reduce the amount of microbes. (All aerobic bacteria, All anaerobic bacteria, *Streptococci*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida spp.*) While the samples that cleaning the tongue with W increased the number of microbes in 3 hours. Due to the ability of CHX that attaches to the tissues within the mouth, teeth, tongue and epithelium. That is the result about the release of the effect all the time. Consistent with previous studies that found that using CHX can reduce the amount of aerobic and anaerobic bacteria 85% while the control group that used W increased the microbes to 300% after 24 hours of oral cleaning.²⁹

PVP-I is a broad antimicrobial agent like CHX, also can inhibit gram positive bacteria, gram negative bacteria, spores of bacteria, *Candida spp.*, protozoa and viruses. From the results of this study, it was found that cleaning the tongue with 1% concentration of PVP-I can decrease all of aerobic and anaerobic bacteria, *Streptococci* and *Staphylococcus aureus*, significant differences with the group that cleaning the tongue with W after immediate cleaning and is effective for 3 hours ($p < 0.05$). This is consistent with Kunisada et al. laboratory studies, it shows that PVP-I is highly resistant to *Streptococci* bacteria. The use of 1% concentration of PVP-I can reduce the amount of microbes in saliva for at least 4 hours before the procedure.³⁰ Tongue cleaning with CHX is effective to reducing *Candida spp.* which is statistically different with tongue cleaning with PVP-I after cleaning for 3 hours ($p < 0.05$). The laboratory studied that compared 4%, 2%, 1%, 0.8%, and 0.5% concentration of CHX and 10% concentration of PVP-I to inhibition of *Candida*

albicans, that was found CHX was more effective than PVP-I in all concentrations.³¹

The results of this research found that cleaning the tongue with CHX was affected with microbial changes that cause of aspiration pneumonia rather than cleaning the tongue with PVP-I and W. The important thing to clean the oral of this elderly group is the position that the elderly lay on the weak side or adjust the bed so that the head can be raised to about 30-60 degrees to prevent suffocation while cleaning the oral. Then, cleaning teeth, wipe the cheeks, palate and cleaning the tongue with CHX to reduce and control the amount of microbes in the mouth that cause aspiration pneumonia.

From previous study showed that CHX that has an effect on suppressing microbes should be 0.12% concentration of CHX at least 15 milliliters, the duration of inhibition of microbes within 30 seconds.³² For 15% concentration of PVP-I, 15-20 ml, spit out for 30 seconds can reduce microbes in the mouth for more than 4 hours before oral procedures³². The limitation for this study, the volume of mouthwash not equal the previous one. For bedridden elderly in this research studied the effectiveness of mechanical cleaning with CHX and PVP-I, which affects only the microbes on the tongue and the samples were bedridden elderly who cannot restrict the muscles in different areas of the body so they cannot spit. Therefore, a soft bristled toothbrush is dipped in each chemical substance, then cleaned the tongue 10 times in each part for a total of 30 times and the chemicals contact with the tongue for 30 seconds to prevent the sample

choking mouthwash and causing severe allergic reactions.

For Recommendation, clinical practice guideline should be brushing tongue for bedridden elderly at least with W and for more effective should plus with CHX or PVP-I with good position, good method and carefully protection.

Conclusions

Tongue cleaning with CHX can reduce and control the amount of microbes that cause aspiration pneumonia for bedridden elderly (all aerobic and anaerobic bacteria, *Streptococci*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*) is not different from cleaning the tongue with PVP-I. Cleaning the tongue with CHX was effective in reducing *Candida spp.* which was statistically different with cleaning the tongue with PVP-I after cleaning for 3 hours. CHX might be recommended for clinical practice guideline of tongue cleaning in the bedridden elderly patients.

Declaration of Interest

The authors report that there is no conflict of interest.

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Tongue cleaning methods	Percent reductions					
	Total aerobic		Total anaerobic		<i>Streptococci</i>	
	Immediately after cleaning	3 hrs.	Immediately after cleaning	3 hrs.	Immediately after cleaning	3 hrs.
CHX	63.33 ± 17.33	77.46 ± 17.27	88.01 ± 3.99	91.03 ± 9.72	81.33 ± 13.09	82.44 ± 15.96
W	39.63 ± 24.71	11.25 ± 51.52	37.08 ± 6.06	24.06 ± 5.93	43.36 ± 28.83	24.29 ± 26.36
PVP-I	59.08 ± 15.75	68.42 ± 23.39	86.41 ± 0.72	4.57 ± 22.41	67.84 ± 24.79	71.22 ± 28.08

Tongue cleaning methods	Percent reductions					
	<i>Staphylococcus aureus</i>		<i>Pseudomonas aeruginosa</i>		<i>Candida spp.</i>	
	Immediately after cleaning	3 hrs.	Immediately after cleaning	3 hrs.	Immediately after cleaning	3 hrs.
CHX	76.40 ± 15.39	89.50 ± 9.86	62.15 ± 2.88	70.78 ± 1.19	76.74 ± 17.81	84.00 ± 14.24
W	43.11 ± 20.43	37.45 ± 34.02	31.94 ± 12.40	11.29 ± 17.57	49.96 ± 22.76	22.31 ± 32.01
PVP-I	66.04 ± 23.93	75.59 ± 18.16	44.16 ± 44.60	63.45 ± 48.55	64.36 ± 28.08	51.34 ± 38.42

Table 1. shows the percentage of reduction microorganisms on the dorsal tongue surface compared with the differences of the effectiveness for CHX, W and PVP-I by using the One-Way ANOVA statistics. * < 0.05 CHX: tongue cleaning plus chlorhexidine gluconate, W: tongue cleaning plus water (control group), PVP-I: tongue cleaning plus povidone-iodine.

Tongue cleaning methods	Percent reductions of white coating	
	Immediately after cleaning	3 hrs.
CHX	31.79 ± 30.32	29.14 ± 18.23
W	24.60 ± 19.71	16.51 ± 19.47
PVP-I	32.41 ± 22.19	25.87 ± 21.95

Table 2. Shows the percentage of reduction in the amount of white coating on the dorsal tongue surface compared the differences of the effectiveness for CHX, W and PVP-I by using the One-Way ANOVA statistics.

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