

The Effect of Immersing Post Hemimaxillectomy Patients' Alginate Impression In Sodium Hypochlorite 0.5% on *Klebsiella pneumoniae* Count Over Gypsum Cast

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Abstract: Pneumonia is a major problem in health that can lead to death which one of the cause is *Klebsiella pneumoniae*. Post hemimaxillectomy cause communication between the nasal cavity and nasopharynx antrum opens, pathogenic bacteria such as *K. pneumoniae* can easily penetrate into the oral cavity. Post hemimaxillectomy requires rehabilitation by fabricating obturator on gypsum cast. The purpose of this study is to determine the effect of immersion post hemimaxillectomy patients' alginate impression in sodium hypochlorite 0.5% on *K. pneumoniae* count over gypsum cast. This is an experimental study. Sixteen sample were divided into eight groups consisting of control group (two minutes), control group (four minutes), disinfection group (two minutes), disinfection group (four minutes) of the alginate impression obtained from four post hemimaxillectomy patients'. The bacteria *K. pneumoniae* were obtained from the swab on surface of the impression and cast, cultured on MacConkey's agar, then counting the number of colonies was conducted. Study results showed there was no growth of *K. pneumoniae* on MacConkey's agar after immersing post hemimaxillectomy patients' alginate impression in sodium hypochlorite 0.5% for four minutes. There was an effect of immersing post hemimaxillectomy patients' alginate impression in sodium hypochlorite 0.5% for two and four minutes on *K. pneumoniae*. Immersion alginate impression in sodium hypochlorite 0.5% for four minutes can be used to disinfect the impression of post hemimaxillectomy patients' because sodium hypochlorite 0.5% is effective to eliminate *K. pneumoniae* on impression and cast.

Keywords: alginate impression, *Klebsiella pneumoniae*, post hemimaxillectomy.

Date of Submission: 20-02-2018 Date of acceptance: 06-03-2018

I. Introduction

Pneumonia or lower respiratory tract infection is a major problem in the health field. Pneumonia can cause death. One of the bacteria that cause pneumonia are *Klebsiella pneumoniae*. Post hemimaxillectomy cause communication between nasal cavity and nasopharynx antrum to be open, pathogenic bacteria such as *K. pneumoniae* can easily penetrate into the oral cavity. The defect can cause a person not confident, difficulty during speech, chewing, and swallowing. The defect requires prosthetic rehabilitation by fabricating obturator. Making impression was using alginate impression material to obtain cast for fabricating obturator. Alginate impression material is often used in dentistry because it is easily manipulated, convenient for the patient and economic.[1,2] Alginate impression could be a medium of microorganisms transmission from patient to dentist, assistant, nurse and dental technician. Alginate impression has hydrophilic properties that it will blend with saliva and blood so it could potentially contain pathogenic microorganisms.[3,4] When alginate impression which is contain pathogenic microorganisms poured with gypsum, we will get a cast contain pathogenic microorganism.[5,6] One of pathogenic microorganisms contained in alginate impression and cast are *K. pneumoniae*. *K. pneumoniae* isolated from the oropharynx or gastrointestinal tract of about 5% of healthy people and higher rate of isolation in the hospitalized. *K. pneumoniae* may cause severe destructive pneumonia and nosocomial urinary tract infection.[7] British Dental Association (BDA)[8] published guidelines for disinfecting impression with a disinfectant. Alginate impression after removed from the oral cavity, it should be rinsed with water to remove blood, saliva, or debris.[9] Rinsing alginate impression with water will reduce the number of microorganisms by 48.5%. [10] There are two methods of disinfection impression, by spraying and immersing. Dentists in Hong Kong who disinfected their impressions for study casts, 77% used immersion in disinfectant and 19% used a spray method.[11] Sodium hypochlorite is easily found in every household with a concentration of 5.25% and could be an option to disinfect alginate impression. CDC [12] recommends the use of sodium hypochlorite with a 1:10 dilution (0.525%) for 10 minutes. Sodium hypochlorite is recommended by

the Environmental Protection Agency (EPA). It is good for surfaces and efficient against broad spectrum of microorganisms. But it has an unpleasant odor and chemical instability.[13] Immersion the impression in sodium hypochlorite 0.5% for 5 minutes effectively decontaminate alginate impression and reduce immersion time can minimize changes in the dimensional stability.[14] Immersion in sodium hypochlorite for 2 minutes can eliminate the number of colonies of *Pseudomonas aeruginosa*. [15] Immersion impression in a sodium hypochlorite concentration 0,5-1% for 10-15 minutes to inhibit the growth of microorganisms such as *Streptococcus sanguis*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Staphylococcus aureus*, *Staphylococcusepidermidis*, and *Pseudomonas aeruginosa*. [16] Disinfection of the impression can be a challenge. Disinfectants should effectively kill microorganisms without damaging the impression or reducing accuracy. The purpose of this study is to determine the effect of immersing post hemimaxillectomy patients' alginate impression in sodium hypochlorite 0.5% on *K. pneumoniae* count over gypsum cast.

II. Materials and Methods

Subjects and Materials

The study was conducted in accordance with a protocol approved by the Ethical Committee of Faculty of Medicine University of Sumatera Utara, and informed consent was obtained from all subjects. The subject included 4 adults, randomly selected from the patients of the Department of Prosthodontics at Dental Hospital University of Sumatera Utara, with the following inclusion criteria: (1) post hemimaxillectomy minimum of 2 weeks, (2) defect in the maxillary arch (Figure 1), (3) good general health, and (4) consist of *Klebsiella pneumoniae* on impression. Exclusion criteria were (1) swelling and bleeding, (2) received antibiotic treatment and (3) radiation therapy.



Figure 1. Defect in maxillary arch of post hemimaxillectomy patients

An irreversible hydrocolloid impression material (Alginoplast, Heraus Kulzer, Germany) were used to take the impression of the patients and poured with gypsum type III (Hera Moldano, Heraus Kulzer, Germany). The immerse disinfectant used in the study was sodium hypochlorite 0,5%. Suitable stock tray was used for the impression. Total 16 impressions of maxillary was taken out in 4 post hemimaxillectomy patients every week for 4 weeks, which were divided into 4 group of each containing 4 impression: group A-4 impression which control group for 2 minutes, group B-4 impression which disinfection for 2 minutes, group C-4 impression which control group for 4 minutes, and group D-4 impression which disinfection for 4 minutes.

Impression and Cast

After alginate impression setting for 90 seconds in the subject's mouth, the impression was removed, rinsed with distilled water for 15 seconds to remove excess saliva. Group A- included 4 alginate impression, kept in air tight polyethene bag for 2 minutes, then each of the impression was removed and rinsed with distilled water for 15 seconds. Group B- included 4 alginate impression, immersed in sodium hypochlorite 0.5% for 2 minutes, then each of the impression was rinsed with distilled water for 15 seconds. Group C- included 4 alginate impression, kept in air tight polyethene bag for 4 minutes, then each of the impression was removed and rinsed with distilled water for 15 seconds. Group D- included 4 alginate impression, immersed in sodium hypochlorite 0.5% for 4 minutes, then each of the impression was rinsed with distilled water for 15 seconds. The surface of impression on defect areas was swabbed with dry sterile cotton swab for 30 seconds (Figure 2). The swab was immediately applied to MacConkey agar culture media for microbiological sampling (Figure 3). Group A, B, C, D impression were poured with gypsum type III. The cast was removed from the impression

after 60 minutes. The surface of cast on defect area was swabbed with dry sterile cotton swab for 30 seconds (Figure 4). The swab was immediately applied to MacConkey agar culture media for microbiological sampling. The swab of the samples was incubated aerobically at 37°C for 24 hours. After 24 hours of culturing, one colony among this culture was selected for the staining purpose and biochemical testing in identification process.



Figure 2. The surface of impression on defect area was swabbed with dry sterile cotton swab for 30 seconds.



Figure 3. The swab was immediately applied to MacConkey agar culture media.

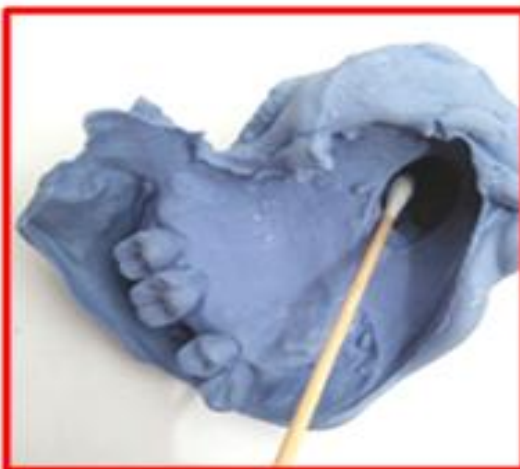


Figure 4. The surface of cast on defect area was swabbed with dry sterile cotton swab for 30 seconds.
Quantification *K. pneumoniae* colonies

The number of *K. pneumoniae* colonies on MacConkey agar culture media (Figure 5) are calculated and derived data in units of CFU (Colony Forming Unit). *K. pneumoniae* colony count of each sample was used to determine the percentage reduction number of *K. pneumoniae* colony.

The percentage reduction number of *K. pneumoniae* colonies:

$$\frac{\text{The number of colonies (after treatment)} - \text{The number of colonies (before treatment)}}{\text{The number of colonies (before treatment)}} \times 100\%$$



Figure 5. *K. pneumoniae* colonies on MacConkey agar culture media.

The results were recorded, analyzed and compared with the control and were subjected to statistical analysis.

III. Result

Results of independent t-test showed there is the effect of immersing alginate impression with sodium hypochlorite 0.5% for 2 minutes to decrease the number of *K. pneumoniae* on the surface of impression $p=0.013$ ($p<0.05$) and Mann Whitney test showed that there is the effect of immersing alginate impression with sodium hypochlorite 0.5% for 4 minutes to decrease in the number of *K. pneumoniae* on the surface of impression $p=0.014$ ($p<0.05$) (Table 1 and 2).

Table 1. Effect of immersing alginate impression with sodium hypochlorite 0.5% for 2 minutes to decrease the number of *K. pneumoniae* on the surface of impression with an independent t-test.

Group	n	$\bar{x} (\%) \pm SD$	<i>p</i>
A=Control 2 minutes	4	14.83 ± 5.90	0.013*
B=Disinfection 2 minutes	4	35.22 ± 23.90	

Description : * Significant ($p<0.05$)

Table 2. Effect of immersing alginate impression with sodium hypochlorite 0.5% for 4 minutes to decrease of *K. pneumoniae* on the surface of impression with Mann Whitney test.

Group	n	$\bar{x} (\%) \pm SD$	<i>p</i>
C=Control 4 minutes	4	17.85 ± 20.13	0.014*
D=Disinfection 4 minutes	4	100 ± 0	

Description : * Significant ($p<0.05$)

Results of independent t-test showed no effect of immersing alginate impression with 0.5% sodium hypochlorite for 2 minutes to decrease the number of *K. pneumoniae* on the cast value of $p=0.142$ ($p>0.05$), Mann Whitney test showed there is the effect of immersing alginate impression with sodium hypochlorite 0.5% for 4 minutes to the decline in the number of *K. pneumoniae* on the cast value of $p=0.014$ ($p<0.05$) (Table 3 and 4).

Table 3.Effect of immersing alginate impression with sodium hypochlorite 0.5% for 2 minutes on the number of *K. pneumoniae* on cast with independent t-test.

Group	n	\bar{x} (%) \pm SD	p
A=Control 2 minutes	4	54.54 \pm 19.98	0.142
B=Disinfection 2 minutes	4	67.73 \pm 16.15	

Description: * Significant ($p < 0.05$)

Table 4.Effect of immersing alginate impression with sodium hypochlorite 0.5% for 4 minutes on the number of *K. pneumoniae* on cast with Mann Whitney test

Group	n	\bar{x} (%) \pm SD	p
C=Control 4 minutes	4	61.72 \pm 6.42	0.014*
D=Disinfection 4 minutes	4	0	

Description: * Significant ($p < 0.05$)

IV. Discussion

Based on the findings of *K. pneumoniae* decrease in the number of colonies in the control group was 2 minutes and 4 minutes were just rinsed with distilled water showed that the mean number of *K. pneumoniae* was only reduced 14.83% and 17.85%. It showed that alginate impression was not enough rinsed with water. Rinsing impression with water was not effective in removing pathogens bacterial and the bacteria will remain on the impression surface. Therefore, it is inadequate simply rinse the impression without the use of disinfectant.[4] This results are consistent study conducted by Correia-Sousa alginate impression rinsing with water will reduce the number of microorganisms on the surface of the alginate impression.[10] *K. pneumoniae* was not growing in the impression so that the number of colonies *K. pneumoniae* decreased although not disinfected because the best temperature growth of *K. pneumoniae* colonies is 37°C.[17] Alginate impression material is contaminated with bacteria due to the composition, structure, and the mechanism of setting hydrophilic.[18] Based on data, there were *K. pneumoniae* on the surface of impression and need to be disinfected to prevent cross infection from patients to dentists, assistants, nurses and laboratory technicians. Haralur studied the alginate impression with water rinsing showed decrease mean number of aerobic bacteria become 74.82 CFU when compared without water rinsing with mean 105.64 CFU.[6] British Dental Association (BDA) [8], Centers for Disease Control and Prevention (CDC) [12], and American Dental Association (ADA)[19] published guidelines for disinfecting impression. The guidelines consist of cleaning and disinfection of the impression using a disinfectant. The mean number of *K. pneumoniae* colonies on impression in the group disinfection 2 minutes was 39.96% and in the group disinfection 4 minutes was 100%. ADA[19] recommends the use of sodium hypochlorite 0.525% with immersing method for disinfecting alginate impression. Sodium hypochlorite 0.525% has powerful antimicrobial and fast acting, prices are relatively cheap and accessible. Immersion was better than spraying because all the parts are exposed to the disinfectant solution.[20] Alginate impression disinfected with sodium hypochlorite 0.5% for 2 minutes showed a decrease in the number of *K. pneumoniae* but not enough to eliminate *K. pneumoniae* colonies. Immersion alginate impression in sodium hypochlorite 0.5% for 4 minutes can eliminate all colonies present on alginate impression. Mechanism action of sodium hypochlorite against microorganisms is based on the ability to penetrate the cells of microorganisms through the cell wall and plasma membrane then inhibit the activity of enzymes that are important for the growth of microorganisms and damage plasma membrane.[11] Duration of immersion is one of the factors that influence the effectiveness of disinfectant to eliminate *K. pneumoniae* colonies. Alginate impression materials should be handled with care to prevent distortion during disinfection procedures. Alginate impression with a minimum contact time will cause less distortion during disinfection.[7] Results of independent t-test showed there is the effect of immersing post hemimaxillectomy patients' alginate impression with sodium hypochlorite 0.5% for 2 minutes on the number of *K. pneumoniae* with $p = 0.013$ ($p < 0.05$). This indicates that the immersion alginate impression using sodium hypochlorite 0.5% for 2 minutes better and can reduce the number of *K. pneumoniae* on the surface of impression compared with controls group. Alginate impression after being removed from the oral cavity, it should be rinsed with water to remove blood, saliva, or debris that may disturb the surface of impression to expose the disinfectant.[9] Alginate impression which is not adequately disinfected will be contaminated the cast with bacteria. The colonies bacteria will be found on gypsum cast made from these impressions.[7] Mann Whitney test showed that there is the effect of immersing post hemimaxillectomy patient's alginate impression in sodium hypochlorite 0.5% for 4 minutes on the number of *K. pneumoniae* with $p = 0.014$ ($p < 0.05$). This indicates that immersion in sodium hypochlorite 0.5% for 4 minutes better and it can effectively eliminate the number of *K. pneumoniae* on the impression when compared with the control group 4 minutes. Sodium hypochlorite is an intermediate level disinfectant which can not be the inactivation of spores, but it can kill Gram positive and Gram negative bacteria, *Mycobacterium tuberculosis*, HIV and HBV.[4,11] The mean number of *K. pneumoniae* colonies on the cast of the control group 2 minutes was 54.54% and disinfectant group 2

minutes was 67.73%. The mean number of *K. pneumoniae* colonies on the cast of the control group 4 minutes was 61.72% and disinfectant group 4 minutes was 0. Cast was not a suitable medium for the multiplication of colonies of microorganisms because pH of gypsum type III was 6.1 to decrease the number of bacteria.[5] Disinfection procedures of impression should be done to reduce the risk of contamination to the cast. Cast derived from alginate impression will carry microorganisms of the oral cavity and can survive for long periods of time. *K. pneumoniae* is microorganisms on maxillary cast that obtained from post hemimaxillectomy patients alginate impression. *K. pneumoniae* can survive the longest in the cast up to 4 days, compared *Staphylococcus aureus* (survive up to 3 days), *Escherichia coli* and *Candida albicans* (survive up to 2 days). *K. pneumoniae* are gram-negative bacteria which have a thick layer which is made of a polysaccharide capsule forming.[5] In patients with oral mucosa injuries, *K. pneumoniae* can cause suppurative inflammatory process.[6] Results of independent t-test showed no effect of immersing post hemimaxillectomy patients' alginate impression in sodium hypochlorite 0.5% for 2 minutes on the number of *K. pneumoniae* on the cast with $p=0.142$ ($p>0.05$). Inadequate disinfection on the impression will produce a cast which still contained *K. pneumoniae* colonies. This is according to Haralur studied, bacteria still contained in the cast even after disinfection and the bacteria will carry over to the cast.[6] Mann Whitney test results showed there is the effect of immersing post hemimaxillectomy patients' alginate impression in sodium hypochlorite 0.5% for 4 minutes on the number of *K. pneumoniae* on the cast with $p=0.014$ ($p<0.05$). Disinfecting alginate impression for 4 minutes effectively eliminate *K. pneumoniae*. There is no *K. pneumoniae* on the cast when impression poured with gypsum type III. It is expected that not occur cross-infection from patient to technicians who touched the cast with their hand is often injured while working. Individual protection (gloves, protective goggles and masks) should be used to prevent possible contamination from impression and cast with microorganisms, prevent of transmission and distribution to the dental laboratory.[5]

V. Conclusion

Immersion alginate impression in sodium hypochlorite 0.5% for four minutes can be used to disinfect the impression of post hemimaxillectomy patients' because sodium hypochlorite 0.5% is effective to eliminate *K. pneumoniae* on impression and cast.

Acknowledgements

The authors wish to thank Prof Dr Ismet Danial Nasution, Dr Syafrinani, Dr Putri Welda Utami Ritonga from Department of Prosthodontics, and Dr Ameta Primasari from Department of Oral Biology Faculty of Dentistry, University of Sumatera Utara for their support and suggestion for the study.

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Veronica Angelia."The Effect of Immersing Post Hemimaxillectomy Patients' Alginate Impression In Sodium Hypochlorite 0.5% on Klebsiella Pneumoniae Count Over Gypsum Cast" IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), vol. 17, no. 3, 2018, pp. 75-81.