Randomised Controlled Trial

Role of chlorhexidine on tracheostomy cannula decontamination in relation to the growth of Biofilm-Forming Bacteria Colony- a randomized controlled trial study

Syahrial Marsinta Hutauruk, Bambang Hermani, Putri Monasari *

ENT-Head and Neck Department, Faculty of Medicine, Universitas Indonesia-Cipto Mangunkusumo National Hospital, Jalan Diponegoro No. 71, Jakarta Pusat, Jakarta, 10430, Indonesia

ARTICLE INFO

Keywords:
Biofilms
Chlorhexidine
Bacteria colony
Tracheostomy cannulae

ABSTRACT

Background: Regular cleaning of the cannula in the trachea is very important for infection prevention. How to wash the tracheal cannula which is good to reduce the possibility of colonies of biofilm-forming bacteria and the growth of bacterial and the pattern of bacterial on the tracheal cannula is still unknown. This study aims to evaluate the efficacy of decontamination of the tracheal cannula using chlorhexidine and NaCl 0.9% in patients using the tracheal cannula to decrease biofilm-forming bacterial colony.

Methods: 40 subjects were grouped into 20 subjects in the control group washing the cannula using 0.9% NaCl and the interventional group washing cannula using 2.5% chlorhexidine solution and 0.9% NaCl. This study used a parallel randomized controlled trial of 2 groups with a single blinded.

Results: 40 subjects studied, 17 subjects (85%) each group produced biofilm-forming bacteria prior to intervention. After intervention in the study group, 15 subjects were biofilm negative and 5 biofilm positive subjects $p = 0.001$. The most common bacteria found in the control group is *Pseudomonas aeruginosa*, while in the study group some bacteria such as *Acinetobacter* sp. and *Proteus mirabilis*. Amoxicilin-Clavulanate had the highest resistance to biofilm forming bacteria in both groups. Piperacillin, ceftazidime, ciprofloxacin and meropenem have the highest sensitivity to biofilm-forming bacteria.

Conclusion: There was a significant decrease in the number of colonies that produced biofilm in the tracheal cannula in the study group compared to the control group in tracheal cannula washing.

1. Introduction

In tracheostomy patients, the defensive role of the nose, nasopharynx and upper respiratory mucosa is absent and allows bacteria to have direct access from the stoma to the respiratory tract. Gram-negative enteric bacteria are organisms that frequently colonize and easily infect the lower airway. Infections in tracheostomy stoma, tracheobronchitis, and pneumonia are common infections that occur after tracheostomy. The risk factor for infection in patients with tracheostomy occurs due to exposure to large amounts of bacteria because they do not pass through the upper airway defense system. In patients with tracheostomy, normal airway flora is at risk of being replaced by virulent pathogens such as gram-negative enteric bacteria [1]. Knowledge of the microbacterial patterns in the tracheal cannula is essential for standardizing empiric antibiotic strategies or for initiating antibiotic therapy after the patient has acquired an infection [2].

The tracheal cannula will often be exposed to enzymes, antioxidants and bacteria found in the tracheal mucosa, thereby accelerating aggregation, increasing colonization of microorganisms and forming biofilms on the surface of the cannula. Biofilm is a three-dimensional complex structure consisting of living bacteria in an extracellular matrix or excreted polymeric substance containing polysaccharides, nucleic acids and proteins. Infections caused by biofilms are difficult to eradicate because the excreted polymeric substance in biofilms can increase bacterial resistance and prevent antibiotics from reaching these bacteria. [3, 4]. Research conducted by Puspito et al., obtained results on tracheostomy cannula in patients in the ENT polyclinic, Laryngeal-Pharyngeal Division, ENT Department, National Central Hospital Cipto

* Corresponding author. ENT-Head and Neck Departement, Faculty of Medicine, Universitas Indonesia, Dr. Cipto Mangunkusumo Hospital Jl., Diponegoro no. 71, Jakarta, 10430, Indonesia.
E-mail address: putrimonasari@gmail.com (P. Monasari).

https://doi.org/10.1016/j.amsu.2021.102491
Received 9 April 2021; Received in revised form 31 May 2021; Accepted 5 June 2021
Available online 10 June 2021
2049-0801/© 2021 The Authors. Published by Elsevier Ltd on behalf of IJS Publishing Group Ltd. This is an open access article under the CC BY-NC-ND license
Mangunkusumo, namely the formation of positive biofilms from 23 swabs of cannula with a total of 10 (50%) on the inner cannula and 13 (65%) on the outer cannula by washing using only 0.9% NaCl [5].

A regularly cleaned tracheostomy cannula is essential for infection prevention. Several methods for cleaning the cannula in the trachea include rinsing with 0.9% NaCl, soaking with a hydrogen peroxide solution or using a chlorhexidine solution to reduce the number of colonies found in the inner cannula [5,6]. Chlorhexidine solution is an option for cleaning internal cannula regularly [2].

Until now, there has been no research report on a tracheostomy cannula washing method that can reduce biofilm-forming bacterial colonies using 0.9% NaCl and chlorhexidine. The choice of chlorhexidine is due to a cationic biguanide, with a very wide antimicrobial spectrum. The antimicrobial effect of chlorhexidine is related to the negative interaction between chlorhexidine (cation) and the bacterial cell surface. After chlorhexidine is absorbed in the surface of the bacterial cell wall, chlorhexidine will decrease the resistance of the cell membrane and cause the release of intracellular materials. Chlorhexidine is a strong antiseptic that is widely used in hospitals for hand sanitation, disinfection of surgical environments, and sterilization of instruments used in invasive procedures. The antimicrobial effect of chlorhexidine is that it can absorb cationic molecules into the cell surface of microorganisms, accelerating changes in cell membrane permeability, resulting in loss of intracellular components and osmotic imbalance in cells [7].

2. Material and method

This study is a two-group, single-blinded, randomized controlled clinical trial to compare the method of cleaning the cannula in the trachea in tracheostomy patients who were given the addition of 0.9% chlorhexidine and NaCl and a control group of tracheostomy cannula cleansing in tracheostomy patients without chlorhexidine. Evaluation of biofilms, characteristics of microorganisms, the risk of developing antibiotic resistance in the patients who had a tracheostomy during the period June to August 2020 at RSUPN dr. Cipto Mangunkusumo (RSCM). Subjects were patients who had a tracheal cannula for at least 7 days in the ENT-Pharyngeal Clinic RSCM who had met all the study inclusion criteria.

Each subject who had a tracheostomy cannula and was included in the research criteria came from the outpatient unit of the ENT poly Laryngeal division, Cipto Mangunkusumo National Central Hospital was given an explanation of the purpose and objectives of the study, the stages, and the examination technique. Subjects willing to be research subjects signed informed consent. Anaesthesia and medical records were recorded by looking for secondary data which included tracheostomy surgery data, time of last cannula change, history of intubation, use of antibiotics, periodic cannula treatment, and lower respiratory tract infections. The patient underwent an ENT physical examination of the stoma tracheostomy, examined the biocarriers through the tracheostomy lumen by inserting a flexible tube with a diameter of 3 mm. The examination was carried out by researchers accompanied by a supervisor, staff of the Laryngeal Division of the ENT Department of the Faculty of Medicine, University of Indonesia - National Central General Hospital Cipto Mangunkusumo until an appropriate assessment is obtained. Assessment of the results of the examination in order to rule out a misplaced tracheostomy pathway, blockage in the lumen of the tracheal cannula. The results of the assessment were recorded in the study medical records.

The patients were then divided into 2 groups. Patients in the first group were washed using 0.9% NaCl, by flowing it and brushing it on the inner cannula and then immersing it with 0.9% 100 mL NaCl for 10 min, while the second group was washing the cannula using 0.9% NaCl. Flow and carried out brushing on the inner cannula then soaked with NaCl 0.9% 100 mL for 10 min then soaked with 2.5% chlorhexidine solution at least 20 mL evenly for 10 min. Isolate the bacteria from the swabs that are taken and grown in the blood agar media inoculated into four 5 mL polystyrene tubes containing 5 mL tryptic soy broth (TSB) and four polystyrene tubes containing TSB, the tube containing the suspension of the bacterial isolate is incubated at 37 °C for 24 h. The tube was washed with phosphate buffer saline (PBS) pH 7.2-7.4 twice each 5 mL to remove planktonic cells and then dried in an inverted position, then the tube was stained with 5 mL 0.1% crystal violet for 30 min, the remaining dye was discarded and then washed with distilled water twice with 5 mL each. After washing, the tube was dried in the air in an inverted position, then the biofilm layer was observed on the walls and bottom of the tube. To avoid subjectivity, observations were made by three people separately. A positive result is determined by the formation of a purple layer adhering to the inner surface to the bottom of the tube wall if a ring-like layer is formed around the tube but not to the bottom of the tube, it is considered that no biofilm or negative result is formed. In this study, the test was carried out in 4 tubes and tested positive if all isolates were detected as biofilm producers. This research paper is fully compliant with the CONSORT criteria [27], parameter measurements were analyzed by IBM Statistical Package for Social Science version 20. A comparison of parameters was analyzed using the McNemar test.

3. Result

This study was conducted on 40 patient subjects who had tracheostomy cannula. This study succeeded in determining the role of chlorhexidine addition in tracheal cannula washing on the growth of biofilm-forming bacterial colonies. Characteristics in the study and control groups there were no significant differences in terms of gender, age group, mean age or level of education.

Table 1 shows that in the control group, there were 14 subjects without comorbidities and 6 subjects with comorbidities, including 3 subjects with hypertension, 2 subjects with diabetes mellitus and 1 subject with malnutrition. In the study group, there were 16 subjects without comorbidities and 4 subjects with comorbidities including 1 subject with hypertension, 3 subjects with diabetes mellitus. There were no subjects with fever and breathlessness in both groups.

In this study (Table 2), 17 subjects in the control group with positive biofilms before washing consisted of 2 subjects with positive strong intensity biofilms, 9 medium intensity subjects and 6 weak intensity subjects. In the study group, there were 17 subjects with positive biofilms before washing consisting of 6 subjects with positive strong intensity biofilms, 6 subjects with medium intensity and 5 subjects with weak intensity.

Table 3 shows that the most biofilm-forming bacteria found in the trachea cannula before washing are Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumonia. Data were obtained from 17 subjects (85%) who produced biofilm-forming bacteria in the study group and the control group bacteria that formed positive biofilms.

Table 4 shows that Amoxicillin-clavulanic acid has the highest resistance to biofilm-forming bacteria in both groups while the second order is Ampicillin sulbactam. Piperacillin, cefazidime, ciprofloxacin and meropenem have the highest sensitivity to biofilm-forming bacteria.

| Characteristic | Group | P Value |
|---------------|-------|---------|
| Sex           | Study | Control |
| Laki-laki     | 11    | 11      |
| Perempuan     | 9     | 9       |
| Age group     |       | 0,744   |
| 20-60 yrs     | 13    | 12      |
| 61-80 yrs     | 7     | 8       |
| Mean Age      | 51,1  | 57,5    |
| +/-           | 13,2  | 12,4    |
| Characteristic | Group | P Value |
|---------------|-------|---------|
| Sex           | Study | Control |
| Laki-laki     | 11    | 11      |
| Perempuan     | 9     | 9       |
| Age group     |       | 0,744   |
| 20-60 yrs     | 13    | 12      |
| 61-80 yrs     | 7     | 8       |
| Mean Age      | 51,1  | 57,5    |
| +/-           | 13,2  | 12,4    |
or absence of biofilms before and after washing the inner cannula with chlorhexidine. In the table, it can be concluded that there is a decrease in biofilm after washing the cannula with chlorhexidine from 17 from 20 subjects to 5 out of 20 subjects. The table above shows that there are significant differences in positive biofilms before and after washing the cannula with a combination of 0.9% NaCl and chlorhexidine.

In the table below (Table 7), it is concluded that there is no change in the number of subjects with positive biofilms before and after washing the cannula with 0.9% NaCl.

The test results in the Table 8 show that the p-value is 0.000 (p < 0.005), it can be concluded that there is a significant difference in the number of bacteria before and after washing the cannula with chlorhexidine. Where there is a decrease in the number of bacteria after washing.

In the Table 9, it is concluded that there is no significant difference in the number of bacteria before and after washing the cannula with 0.9% NaCl. There was a decrease in the number of double bacteria after washing, but the decrease was considered insignificant.

In Table 10, it can be seen that the most biofilm-forming microbes found in the cannula in the trachea after washing in the control group were Pseudomonas aeruginosa whereas in the study group almost no bacteria were found but some bacteria such as Acinetobacter sp. and Proteus mirabilis.

Table 11 shows that tracheostomy cannula washing has a significant relationship (p = 0.003) to biofilms, where there is a tendency for the more routine washing of biofilms to be formed.

4. Discussion

The age distribution of subjects in this study had a mean age of 51.1 years for the study group and 57.5 years for the control group. These characteristics are similar to the study by Kawale et al., where the greatest prevalence of tracheostomy was in young adults, with a range of 15–50 years. Solomon et al. also reported that the mean age of their study subjects was 60 years [17].

After the intervention, many bacteria were eradicated in the study group. Bacteria that were still found in the cannula in the trachea after washing with chlorhexidine and 0.9% NaCl were Acinetobacter and Proteus mirabilis (n = 2), and Pseudomonas aeruginosa (n = 1). In addition, new bacteria Staphylococcus aureus and Staphylococcus saprophyticus (n = 1) were found which were not previously found before the intervention. Meanwhile, in the control group, there was a decrease or increase in the number of bacteria found after washing with 0.9% NaCl. The most bacteria found after washing were Pseudomonas aeruginosa (n = 7), Klebsiella pneumoniae (n = 5), Escherichia coli and Proteus mirabilis (n = 4). These data indicate that although not entirely eradicated, the addition of chlorhexidine can eradicate most bacteria, especially Pseudomonas aeruginosa. Similar findings were reported by Silva, where there was a significant reduction of Pseudomonas aeruginosa bacteria in tracheal cannula with PVC material [18]. Based on biofilm status before washing, only 10 patients (25%) subjects had comorbidities, 7 patients with positive biofilms and 3 patients with negative biofilms (p = 0.153). The comorbidities found in the subjects were hypertension and diabetes mellitus. Lastra et al. stated that hypertension and diabetes mellitus can increase the inflammatory response in the respiratory tract mucosa, which in turn leads to microbial colonization [19]. Microbial colonization of the tracheal mucosa in a patient using a tracheostomy can increase biofilm formation [20]. Hypertension can increase oxidative stress resulting in tissue damage, impaired endothelial function, and decreased nitric oxide. Diabetes mellitus with hyperinsulinemia or insulin resistance can cause tissue damage and impaired blood vessel function as well [19].

All study subjects were not experiencing fever and shortness of breath. This indicates that the subject is not having a respiratory tract infection, which could be a risk factor for the formation of bacteria and biofilms in the tracheostomy cannula. In our study we found all of the
subjects with decontamination ≤5 times every month it found positive biofilm 93% (n = 31) and all the subject with decontamination >5 times every month it found negative biofilm 57% (n = 4). Eroglu et al. stated that bacterial colonization in the tracheal mucosa after insertion of the endotracheal tube was formed 2 days after insertion. Biofilm formation occurred 3 days after insertion. The length of time using the

| Antibiotics                     | Study groups     | Control groups                  |
|---------------------------------|------------------|----------------------------------|
|                                 | Resistant        | Intermediate                     | Sensitive |
| Chloramphenicol                 | 10               | 0                                | 6         |
| Gentamicin                      | 2                | 0                                | 26        |
| Kanamycin                       | 7                | 4                                | 17        |
| Amikacin                        | 2                | 0                                | 22        |
| Aztreonam                       | 4                | 2                                | 23        |
| Sulbactam/Ampicillin            | 12               | 0                                | 17        |
| Cefotaxime/Cefazolin            | 11               | 1                                | 15        |
| Amoxicillin/Clavulanic          | 16               | 1                                | 13        |
| Ceferaxone                      | 9                | 1                                | 19        |
| Ceftazidime                     | 2                | 0                                | 27        |
| Cefoperazone                    | 3                | 0                                | 22        |
| Ciprofloxacin                   | 4                | 2                                | 20        |
| Piperacillin/Taxo                | 1                | 3                                | 26        |
| Ceferazone/Sulbactam            | 0                | 3                                | 11        |
| Cefapime                        | 1                | 1                                | 18        |
| Tigecyclin                      | 6                | 3                                | 5         |
| Meropenem                       | 2                | 2                                | 23        |
| Imipenem                        | 5                | 1                                | 21        |
| Levofloxacin                    | 3                | 0                                | 15        |
| Quinolone                       | 1                | 1                                | 10        |

**Table 5**

Distribution of subjects according to intervention and group results.

| Intervention results               | Group P value |
|-----------------------------------|---------------|
| Study                             | Control       |
| Biofilm post decontamination      |               |
| strong                            |               |
| Moderate                          |               |
| weak                              |               |
| none                              |               |
| Amount of microbacteria after decontamination | 0.001         |
| One type                          |               |
| Two type’s                        |               |
| None                              |               |

**Table 6**

Distribution of subjects according to biofilms before and after decontamination using chlorhexidine.

| Biofilm                       | Post decontamination | Total |
|--------------------------------|----------------------|-------|
| (+) biofilm                   | 5                    | 12    |
| (-) biofilm                   | 0                    | 3     |
| Total                         | 5                    | 15    |

Mc Nemar; p = 0.000.

**Table 7**

Distribution of subjects according to biofilms before & after decontamination using 0.9% NaCl.

| Biofilm                       | Post decontamination | Total |
|--------------------------------|----------------------|-------|
| (+) biofilm                   | 15                   | 2     |
| (-) biofilm                   | 2                    | 1     |
| Total                         | 17                   | 3     |

Mc Nemar p = 1000.

**Table 8**

Distribution of study subjects according to the number of bacterial at baseline and after decontamination using chlorhexidine.

| Amount of bacterial type | After decontamination | Total |
|--------------------------|-----------------------|-------|
| Double                   | 0                     | 5     |
| single                   | 1                     | 2     |
| none                     | 0                     | 0     |
| Total                    | 1                     | 7     |

Mc Nemar p = 0.002.

**Table 9**

Distribution of study subjects according to the number of bacterial at baseline and after decontamination using NaCl 0.9%.

| Amount of microbacterial type | After decontamination | Total |
|------------------------------|-----------------------|-------|
| double type                  | 7                     | 3     |
| single type                  | 5                     | 5     |
| none                         | 0                     | 0     |
| Total                        | 12                    | 8     |

Mc Nemar p = 0.727.

**Table 10**

List of bacterial post decontamination.

| Type of bacterial              | Group |
|--------------------------------|-------|
| Study                          | Control |
| Acinetobacter sp.              | 2     | 1    |
| Escherichia coli               | 0     | 4    |
| Enterobacter sp.               | 0     | 1    |
| Klebsiella oxytoca             | 0     | 2    |
| Klebsiella pneumoniae          | 0     | 5    |
| Proteus mirabilis              | 2     | 4    |
| Proteus vulgaris               | 1     | 2    |
| Pseudomonas aeruginosa         | 1     | 7    |
| Serratia marcescens            | 0     | 1    |
| Staphylococcus aureus          | 1     | 0    |
| Staphylococcus saprophyticus   | 1     | 0    |
endotracheal tube was statistically significant in influencing the colonization of biofilm-producing bacteria in the lumen of the endotracheal tube ($p = 0.006$) [21]. Puspito et al. stated that positive biofilms were found in the tracheal cannula worn by patients as early as 15 days after insertion of the tracheal cannula [8]. Silva et al. stated that cannula washing was not effective if the biofilm had developed 7 days after exposure to plasma. Silva et al. investigated the effect of cannula washing with evidence of *Staphylococcus aureus* and *Pseudomonas aeruginosa* infections in several types of cannula. Most ENT specialists recommend replacement of the tracheostomy tube in pediatric patients every 7 days. In patients with complications, replacement is recommended every 4 days [18].

Solomon et al. conducted a study on the relationship between tracheostomy treatment and bacterial growth, which was calculated in colony forming units (CFU). Twenty-one patients who had a tracheostomy in place reported tracheostomy treatment by changing the inner colony forming units (CFU). Twenty-one patients who had a tracheostomy had sputum culture results of *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, *Escherichia coli*, and *Klebsiella pneumoniae*. The sensitivity test of *Pseudomonas aeruginosa* to various antibiotics showed that there was no single antibiotic sensitivity. 100%. Mina et al. reported that *Pseudomonas aeruginosa* has resistance to meropenem (49.27%), imipenem (41.94%), ciprofloxacin (67.5%), levofloxacin (68.02%), ceftazidime (55.86%), amikacin (55.48%), piperaclillin (61.58%), gentamicin (67.41%), cefixime (70.98%), and aztreonam (69.42%). The study also stated that the strains with the highest level of antibiotic resistance were the biofilm-producing *Pseudomonas aeruginosa* strains [24]. However, this study did not research specifically for each microbe, so it could not further analyzed the pattern of resistance of each microbe to antibiotics.

After intervention, in the study group, the number of bacteria resistant to antibiotics decreased significantly, such as amoxicillin/clavulanic acid (81%), ampicillin/sublactam (75%), cefotaxim/cephazoline (81%), and chloramphenicol (70%). The decline in resistance in the control group was not much found, only one reduction in amoxicillin/clavulanic acid (6%) and cefotaxim/cephazoline (10%), three in ampicillin/sublactam (25%), and there was no change to chloramphenicol. Based on these data, chlorhexidine can affect antibiotic resistance. It is not known the specifics of each bacterium, and no further analysis of changes in antibiotic resistance after chlorhexidine washing could be carried out for each bacterium. Köjalg et al. conducted a study related to antibiotic resistance as an indicator of bacterial susceptibility to chlorhexidine. Non-fermenting bacteria tolerate high concentrations of chlorhexidine, especially the most susceptible Gram-positive cocci. This study succeeded in finding an association between chlorhexidine and susceptibility to antibiotics at the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) among Gram negative bacteria and especially on MBC in Gram positive bacteria. Resistance to ciprofloxacin, imipenem, cefotaxime, ceftazidime, gentamicin, and aztreonam indicates increased resistance to chlorhexidine in Gram negative bacteria. This study helps clinicians to predict susceptibility to chlorhexidine [22]. Kampf also found that with the use of chlorhexidine, new antibiotic resistance was found, including ceftazidime, sulfamethoxazole, imipenem, cefotaxime, and tetracycline [25]. These findings are consistent with research, which in the study group, after leaching with the addition of chlorhexidine, the amount of resistance to ceftazidime and gentamicin remained, and ciprofloxacin and imipenem were only halved.

Based on the results of data analysis, it was found that washing the tracheal cannula with chlorhexidine and 0.9% NaCl significantly reduced the number of bacterial ($p = 0.000$) in vitro compared to only 0.9% NaCl alone. Through further analysis, in the study group, there were 10 patients with multiple microorganisms before washing, who then became single in 5 patients and eradicated in 5 patients. In a single microorganism before washing, it was found to be single in 2 patients and eradicated in 7 patients ($p = 0.002$). Meanwhile, in the control group, there were 10 patients each with multiple and single microorganism before washing. In the dual microorganism group before washing, 7 patients still showed multiple results and 3 patients became single. In the single microorganism group, 5 patients became multiple and 5 patients remained single. However, the results of the analysis in the control group were not significant with $p = 0.727$.

| Clinical characteristics | Biofilm before decontamination | P value |
|--------------------------|--------------------------------|---------|
|                          | Positive | Negative |         |
| Comorbidity              |          |          |         |
| Hypertension             | 7        | 3        | 0.153   |
| Type 2 Diabetes malnourishment | 27      | 3        |         |
| Fever                    | 0        | 0        |         |
| present                  | 34       | 6        |         |
| Dyspnea                  | 0        | 0        |         |
| present                  | 34       | 6        |         |
| Cannule changing         |          |          |         |
| ≤6 weeks                 | 8        | 5        | 0.075   |
| >6 weeks                 | 12       | 15       |         |
| Routine cannule decontamination | 31      | 2        | 0.003   |
| ≤5 times                 | 3        | 4        |         |
| >5 times                 |          |          |         |
| Antibiotics              | 1        | 1        | 0.281   |
| present                  | 33       | 5        |         |
| Lower respiratory tract infection | 1      | 0        | 1000    |
| present                  | 33       | 6        |         |
| Granulation tissue       | 1        | 0        | 1000    |
| present                  | 33       | 6        |         |
From the data in the result section, it can be concluded that biofilm is more eradicated in the study group than in the control group (60% vs 0%). Based on the results of data analysis, it was found that washing the tracheal cannula with chlorhexidine and 0.9% NaCl significantly washed and even eliminated the biofilm ($p = 0.001$) in vitro compared to only 0.9% NaCl. After the intervention, more biofilms were eradicated in the study group than in the control group (70.5% vs 11.8%). In the study group, 6 out of 7 Pseudomonas aeruginosa bacteria were eradicated by washing using chlorhexidine and 0.9% NaCl. This finding is inconsistent with previous studies, which stated that P aeruginosa biofilms exposed to chlorhexidine only decreased biofilm viability by 40%, and the P. aeruginosa bacterial strains on biofilms were resistant to antiseptics, where chlorhexidine with a minimum inhibitory concentration (MIC) could not destroy the biofilm. [10,26]

The use of chlorhexidine as an antimicrobial also does not damage the cannula. Many techniques of cannula decontamination in the trachea have been used, such as hydrogen peroxide and boiling water. Hydrogen peroxide, as an example of a hypochlorite solution, is not recommended for decontamination of tracheal cannula because it can damage the cannula material [5]. To minimize the risk of bias from the cannula cleansing procedure, the investigators provided a checklist to the patient so that the steps for treating tracheal cannula performed independently could be evaluated. Cleaning the cannula is a relatively easy procedure to perform, however, their implementation must be carried out with care and hygiene. Besides the cannula treatment factor, another factor that could influence the outcome of this study was the difference in the risk of intra-operative infection during tracheostomy surgery.

5. Conclusion

The most biofilm-producing bacteria before intervention were Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae. There was a significant decrease in the number of colonies that produced biofilms in the tracheal cannula in the study group decontamination using 0.9% chlorhexidine and NaCl compared to 0.9% NaCl alone in tracheal cannula washing ($p = 0.001$). Amoxicillin-clavulanic acid, Ampicillin-sulbactam and Cephalozin had the highest resistance to biofilm-forming bacteria in both groups while meropenem, cefazidime and ciprofloxacin still had a fairly good sensitivity.

Sources of funding

The authors report an external source of funding of this article from International Indexed Publications of health sciences 2020 (Publikasi terindeks internasional sains Kesehatan) Universitas Indonesia Grant program with letter agreement number 2248.

Ethical approval

Ethical approval clearance from ethics committee board, Faculty of Medicine, Universitas Indonesia with protocol number 20-03-0261.

Trial registration number

Name of the registry: researchregistry
Unique identifying number or registration ID: researchregistry6732
Hyperlink registration: https://www.researchregistry.com/browse-the-registry#home/registrationdetails/607021292e1791001c499f47.

Consent

Written informed consent was obtained from the patient for publication of this cross-sectional study and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

Author contributions

Syahrial Marsinta Hutauruk contributes in the study concept or design, data collection, analysis and interpretation, oversight and leadership responsibility for the research activity planning and execution, including mentorship external to the core team.

Bambang Hermani contributes in the study concept or design, data collection, analysis and interpretation.

Putri Monasari contributes to the study concept or design, data collection and writing the paper.

Guarantor

Syahrial Marsinta Hutauruk is the sole guarantor of this submitted article.

Provenance and peer review

Not commissioned, externally peer-reviewed.

Declaration of competing interest

None.

References

[1] E.R. Oliver, A. Gist, M.B. Gillespie, Percutaneous versus surgical tracheostomy: an updated meta-analysis, Laryngoscope 117 (2007) 1570–1575.

[2] G. Bjørling, A.L. Belin, C. Helstrom, et al., Tracheostomy inner cannula care: a randomized crossover study of two decontamination procedures, Am. J. Infect. Contr. (2006) 600–5.

[3] J. Rodnye, C.P. Ojano-Dirain, P.J. Antonelli, R.C. Silva, Effect of repeated tracheostomy tube reprocessing on biofilm formation, Laryngoscope 126 (4) (2016) 996–999.

[4] P.C. Bogino, M. Oliva M de las, F.G. Sorroco, W. Giordano, The role of bacterial biofilms and surface components in plant-bacterial associations, Int. J. Mol. Sci. 14 (8) (2013) 15838–15859.

[5] D. Pupito, Analisis Biofilm Dan Mikroba Pembentuknya Pada Pasien Terpapang Kanal Trakeostomi. Tesis, Universitas Indonesia, Jakarta, 2018 h.42

[6] L. McGill, Policy for the Care of Patients with Tracheostomy Tubes, 2012, pp. 1–49.

[7] P.C. Bonez, C.F. Alves, T.V. Dalmonlin, et al., Chlorhexidine activity against bacterial biofilms, Am. J. Infect. Contr. 41 (2013) e19–e22.

[8] M.R. El Cheikh, J.M. Barbosa, J.A.S. Caixeta, M.A.G. Avelino, Microbiology of tracheal secretions: what to expect with children and adolescents with tracheostomies, Int. Arch. Otorhinolaryngol. 22 (1) (2018) 50–54.

[9] J.M. Cline, C.R. Woods, S.E. Ervin, B.K. Rubin, D.J. Kinse, Surveillance tracheal aspirate cultures do not reliably predict bacteria cultured at the time of an acute respiratory infection in children with tracheostomy tubes, Chest 141 (3) (2012) 625–631.

[10] J. Perkins, J. Mouzakes, R. Pereira, S. Manning, Bacterial biofilm presence in pediatric tracheostomy tubes, Arch. Otolaryngol. Head Neck Surg. 130 (3) (2004) 339–343.

[11] D.H. Solomon, J. Wubb, R.A. Buttaro, A. Truant, A.M.S. Soliman, Characterization of bacterial biofilms on tracheostomy tubes, Laryngoscope 119 (8) (2009) 1633–1638.

[12] R.C. Silva, C.P. Ojano-Dirain, P.J. Antonelli, Effectiveness of pediatric tracheostomy tube cleaning, Arch. Otolaryngol. Head Neck Surg. 138 (2) (2012) 251.

[13] G. Lastra, S. Syed, I.R. Kurukulasuriya, C. Manrique, J.R. Sowers, Type 2 diabetes mellitus and hypertension: an update, Endocrinol Metab. Clin. N. Am. 43 (1) (2014) 103–122.

[14] A. Mukundan, M.P. Kamaht, S. Baliga, V.S.,S. K.M. Bhojwani, V. Prasad, Bacterial flora of the lower respiratory tract during and after a week of tracheostomy 4 (January) (2017) 92–98.

[15] S. Eric, S. Oerup, O. Urgan, E. MD Ozge, M.D. Onur, M.D. Arzu Denizbasi, M. Haldun Akoglu, M.D. Cigdem Ozpolat, M. Ebru Akoglu, Endotracheal tube biofilm and its relationship to ventilator associated pneumonia in a neonatal ICU, Nat. Sci. 10 (12) (2012), 132–140.

[16] C. Russell, Providing the nurse with a guide to tracheostomy care and management, Br. J. Nurs. 14 (8) (2005) 428–433.

[17] M. Leonhard, O. Assadian, M. Zumtobel, S.B. Schneider, Microbiological evaluation of different represencing methods for cuffed and uncuffed tracheostomy tubes in homecare and hospital setting, GMS Hyg. Infect. Contr. 11 (2016).

[18] S. Backman, G. Björland, U.B. Johansson, M. Lysdahl, A. Markström, U. Schedin, et al., Material wear of Polymeric tracheostomy tubes: a six-month study, Laryngoscope 119 (4) (2009) 657–664.
[25] Y. Mina, M. Mohammad Yousef, A. Naser, S. Nasser, R. Ghotaslou, Antibiotic resistance patterns of biofilm-forming Pseudomonas aeruginosa isolates from mechanically ventilated patients, Int. J. Sci. Stud. 5 (5) (2017) 1–6.

[26] M.E. Wand, L.J. Bock, L.C. Bonney, J.M. Sutton, Mechanism of increased resistance to chlorhexidine and cross-resistance to colistin following exposure of Klebsiella pneumoniae clinical isolates to chlorhexidine, Antimicrob. Agents Chemother. 61 (1) (2017) e01162–16.

[27] R. Agha, A. Abdall-Razak, E. Cronley, N. Dowlat, C. Iosifidis, G. Mathew, for the STROCSS Group, The STROCSS 2019 guideline: strengthening the reporting of cohort studies in surgery, Int. J. Surg. 72 (2019) 156–165.