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The difference in biofilms formations on duration less than 90 d and more than 90 d of tracheotomy cannula usage

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ABSTRACT

Currently, prevention of local and systemic infections caused by implantable devices is increasingly improved. Tracheostomy is a surgical action followed by an implantable device called tracheotomy cannula into a trachea to maintain upper airway patenting. The incidence of biofilm-related complications and infections is associated with the length of duration of the attached tracheostomy. The formation and spread of biofilms from distal cannula increase the infection incidence in stoma, tracheitis, and even peripheral pneumonia. However, until now there has been no consensus on when the tracheostomy replacement supposedly conducted. Some manufacturers recommend that cannula replacement supposedly conducted within 30 d, but the data are not yet in agreement and need further study. This study aimed to determine the difference in biofilms formations in a duration of less than 90 d and more than 90 d of tracheotomy cannula usage. It was a cross-sectional study involving patients who underwent a tracheostomy at the Department of Otorhinolaryngology of Dr. Sardjito General Hospital, Yogyakarta. Fisher exact test was applied to analyze the biofilms formations of the two different duration of tracheostomy cannula usage. A total of 20 patients were involved in this study. Durations of more than 90 d had more biofilms formations compared to less than 90 d, although it was not significantly different ($p>0.05$). However, the PR value of 6 indicated that subjects who have attached cannula more than 90 d clinically have 6 times higher risk for developing biofilms formations than those less than 90. In conclusion, there is no significant differences in biofilms formations between the less than 90 d and more than 90 d of tracheostomy cannula usage. However, clinically subjects with longer duration of tracheostomy cannula usage have higher risk for developing biofilms formations.

Keywords:
bacterial biofilm;
duration of tracheotomy cannula;
complication;
risk factor;
tracheostomy care

ABSTRAK

Saat ini, pencegahan infeksi lokal dan sistemik yang disebabkan oleh alat implan semakin berkembang. Trakeostomi adalah tindakan pembedahan yang diikuti dengan implan kanula trakeostomi ke dalam trakea untuk mempertahankan patensi jalan napas atas. Insiden komplikasi dan infeksi terkait biofilm dikaitkan dengan lamanya durasi kanul trakeostomi yang terpasang. Pembentukan dan penyebaran biofilm dari kanula distal meningkatkan kejadian infeksi pada stoma, trakeitis, dan bahkan pneumonia perifer. Namun demikian, sampai saat ini belum ada konsensus mengenai kapan seharusnya penggantian trakeostomi dilakukan. Beberapa industri merekomendasikan agar penggantian kanula seharusnya dilakukan dalam 30 hari, namun data tersebut belum sesuai dan perlu kajian lebih lanjut. Penelitian ini bertujuan untuk mengetahui perbedaan pembentukan biofilm pada durasi kurang dari 90 hari dan lebih dari 90 hari penggunaan kanula trakeotomi. Penelitian ini merupakan studi potong lintang yang melibatkan pasien yang menjalani trakeostomi di Departemen THT RSUP Dr. Sardjito Yogyakarta. Uji eksak Fisher digunakan untuk menganalisis pembentukan biofilm dari dua durasi

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penggunaan kanula trakeostomi yang berbeda. Sebanyak 20 pasien dilibatkan dalam penelitian ini. Durasi lebih dari 90 hari memiliki pembentukan biofilm yang lebih banyak dibandingkan kurang dari 90 hari, meskipun tidak berbeda nyata (p>0,05). Namun, nilai PR 6 menunjukkan bahwa subjek yang dipasang kanula lebih dari 90 hari secara klinis memiliki risiko 6 kali lebih tinggi untuk terbentuknya biofilm dibandingkan mereka yang kurang dari 90. Kesimpulannya, tidak ada perbedaan nyata dalam pembentukan biofilm antara kurang dari 90 d dan lebih dari 90 hari penggunaan kanula trakeostomi. Namun, secara klinik subjek dengan durasi penggunaan kanula trakeostomi yang lebih lama memiliki risiko lebih tinggi untuk terbentuknya biofilm.

INTRODUCTION

Historically, a tracheostomy represented the only treatment available for upper airway obstruction. Today, tracheotomy remains an important indication for tracheostomy, although numerous others intervention are available. A tracheostomy may be required in an emergent setting to bypass an obstructed airway, or more commonly, may be placed electively to facilitate mechanical ventilation, to wean from a ventilator, or to allow more efficient management of secretions referred to as pulmonary toilet, among other reasons. Although tracheostomy is mostly temporary, there are special conditions such as impaired airway function or unresolved conditions in which long-term or even permanent tracheostomy should be performed.[1,2] Due to long-term tracheostomy, it can cause some local or systemic infections. Infection can occur due to the accumulation of various kinds of microorganisms on the surface of the cannula which could form a biofilm.[2,3]

The biofilm formation on medical devices such as tracheostomy can lead to a chronic infection in the surrounding area as well as systemic infection. The biofilm formation has also been reported on other medical devices such as in venous catheters, urinary catheters, prosthetic heart valves, contact lenses, and intrauterine devices (IUDs). The Centers for Disease and Prevention estimated that more than 65% of chronic bacterial infections in humans are related to biofilms, and according to the National Institutes of Health it is as much as 80%.[4]

The duration of tracheostomy use has been related to tracheitis which is associated with the thickness of the biofilm that becomes attached to the inner cannula and can increase the risk of plugging, local lesions of the stoma including granulation, and peripheral pneumonia due to the release and spread of biofilms into the lungs.[5,6]

There is no consensus or universal guideline on when to replace a tracheostomy cannula. Most tracheostomy manufacturers mentioned that 30 d is the limit for replacing the cannula even though no agreement has been reached.[7] One author mentioned that degradation of the cannula will occur in 3 mo after the cannula has been inserted.[8] This event can be one of the indicators of the presence of biofilms.[9]

The purpose of this study was to determine the difference in bacterial biofilms with a duration of less than 90 d and more than 90 d of tracheostomy cannula usage at the Dr. Sardjito General Hospital, Yogyakarta, Indonesia.

MATERIALS AND METHODS

Design of study

This study used a cross-sectional research design conducted at the Departement of Otorhinolaryngology (ENT), Dr. Sardjito General Hospital, Yogyakarta, from August 2020 to January 2021. The protocol of the study was approved by the Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta (KE/FK/0843/EC/2020).

Procedure

Twenty patients who meet the inclusion and exclusion were involved in this study. The inclusion criteria were patients who underwent tracheostomy procedures at Dr. Sardjito General Hospital, Yogyakarta conducted by the Department of ENT and willing to participate in the study. Patients with an abscess around the stoma were excluded in this study.

The dependent variable was assessed by the presence or absence of a biofilm and the main independent variable was the duration of the insertion of the cannula divided by less than 90 d and more than 90 d. The course of the study was to collect patients who have undergone tracheostomy procedures by the Department of ENT at Dr. Sardjito General Hospital, Yogyakarta and sample selection was based on inclusion and exclusion criteria. Subjects' samples of tracheostomy were sent to the Department of Microbiology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada for biofilm tests of culture isolates.

Biofilm testing

The cannula that has been removed from the patients would be put in a special container. The sampling was conducted on the part of the cannula that looks wet and dirty, and then it would be planted on blood agar and McConkey media for 48 h at 37 ^{o}C. The used cannula would be put on autoclave machine and it would be destroyed early. Each colony was taken with a sterile ose needle and inoculated with a microplate which added by 200 µL acid isopropanol 5%. The microplate formed the biofilm would be dark in color and the absorption was read at a λ 595 nm. To obtain quantitative data, readings were carried out with a microplate reader.

The results of biofilm formation were seen from the value of ODc and OD of growth control. The ODc value was obtained from the mean OD of negative control + 3x standard deviation (SD) of negative control. The results of biofilms obtained varying results: OD ≤ ODC means no biofilm, OD bacteria > ODc and ≤ 2x ODc values means a weak biofilm, OD bacteria > 2x ODc values and ≤ 4x ODC values means a moderate biofilm, OD bacteria > 4x ODc values means a strong biofilm. However, this study only aimed to see whether there was a biofilm or not, so the limitation of variation was not analyzed further.

Statistical analysis

The results of the study were presented in frequency distribution tables of the characteristics of the research subjects and an overview of each research variable with prevalence ratio (PR) using a 95% confidence interval (CI). Statistical analysis used Fisher exact tests with $p < 0.05$ was considered significant.

RESULTS

A total of 20 subjects were involved in this study. The sample size calculation obtained 20 subjects for each group with a minimum of 10 subjects in a duration of less than 90 d and 10 subjects in a duration of more than 90 d. The characteristics of subjects are presented in TABLE 1. There were 13 (65%) males compared to only 7 (35%) females. Adults with age 20 to >60 y were 11 (55%) subjects, and children aged 2 to 19 y were 9 (45%) subjects. For educational background, most of the subjects have attended school with as many as 17 (85%) subjects, compared to only 3 (15%) who have not attended school.

As many as 15 (75%) subjects performed routine daily washing of the cannula 3-4 times, while 5 (25%) subjects washed the cannula twice. The subjects with the duration of the tracheostomy cannula less than 90 d were 10 (50%), while those with more than 90 d were 10 (50%). Biofilms were obtained from

as many as 13 (65%) samples, and for those that did not contain biofilms were 7 (35%) samples.

There were 18 (100%) subjects with no complications of pneumonia, and 0 (0.0%) had no data from X-ray thoracic imaging. There were 7 (33.3%) subjects who had stoma granulation, and 13 (66.7%) without stoma granulation. Subjects with a history of active smoking were 10 (50%) subjects, while 10 (50%) subjects were not active smokers.

The most common indications for tracheostomy were due to airway obstruction in 19 (95%) subjects and prolonged endotracheal tube in 1 (5%) subject. Most subjects had gram-negative bacteria when the bacterial culture was performed in as many as 14 (70%) subjects compared to gram-positive bacteria in 6 (30%) subjects.

TABLE 1. Characteristics of research subjects

Characteristics	Frequency [n (%)]
Gender	
• Female	7 (35.0)
• Male	13 (65.0)
Age	
• Child (2 s/d 19 y.o.)	9 (45.0)
• Adult (20 s/d >60 y.o.)	11 (55.0)
Education	
• Not in school yet	3 (15.0)
• Elementary-High School	17 (85.0)
Wash times	
• 2x/day	5 (25.0)
• 3-4x/day	15 (75.0)
Duration	
• <90 day	10 (50.0)
• >90 day	10 (50.0)
Biofilm	
• No biofilm	7 (35.0)
• Yes biofilm	13 (65.0)
Pneumonia	
• No	18 (100.0)
• Yes	0 (0.0)
Stoma granulation	
• No	13 (65.0)
• Yes	7 (35.0)
Smoking	
• No	10 (10.0)
• Yes	10 (10.0)
Tracheotomy indication	
• Upper airway obstruction	19 (95.0)
• Prolonged endotracheal intubation	1 (5.0)
Bacteria type	
• Gram (-)	14 (70.0)
• Gram (+)	6 (30.0)

To evaluate the difference in biofilm formations in less than 90 d and more than 90 d of tracheostomy cannula usage, Chi-square tests was performed. However, due to it did not meet the feasibility of the Chi-square test, additionally performed Fisher's exact tests was performed (TABLE 2).

TABLE 2. Fisher's exact test to determine biofilm differences in several durations of tracheostomy cannula usage

Duration	No biofilm [n (%)]	Biofilm [n (%)]	p	PR	CI 95%
• <90 d	6 (60.0)	4 (40.0)	0.029*	6.0	1.07-41.21
• >90 d	1 (10.0)	9 (90.0)			

TABLE 3. Other variables tested for association with biofilm occurrence

Characteristics	Non biofilm [n (%)]	Biofilm [n (%)]	p	PR	95% CI
Gender					
• Female	2 (28.6)	5 (71.4)	0.526	0.74	0.19-2.89
• Male	5 (38.5)	8 (61.5)			
Age					
• Children (2 - 19 y.o.)	4 (44.4)	5 (55.6)	0.370	1.63	0.49-5.47
• Adult (20 - >60 y.o.)	3 (27.3)	8 (72.7)			
Education					
• Not in school yet	2 (66.7)	1 (33.3)	0.270	2.27	0.76-6.73
• Elementary – High School	5 (29.4)	12 (70.6)			
Wash time					
• 2x/day	2 (40.0)	3 (60.0)	0.594	1.20	0.33-4.36
• 3-4x/day	5 (33.3)	10 (66.7)			
Smoking					
• No	3 (30.0)	7 (70.0)	0.500	0.75	0.22-2.52
• Yes	4 (40.0)	6 (60.0)			
Tracheotomy indication					
• Upper airway obstruction	7 (36.8)	12 (63.2)	0.650	0.63	0.45-0.89
• Prolonged ET	0 (0.0)	1 (100.0)			
Bacterial type					
• Gram (-)	4 (28.6)	10 (71.4)	0.336	0.57	0.18-1.81
• Gram (+)	3 (50.0)	3 (50.0)			
Biofilm strength					
• Weak - moderate	2 (13.3)	13 (86.7)	-	-	-
• Strong	0 (0.0)	0 (0.0)			
Pneumonia					
• No	6 (31.6)	13 (68.4)	-	-	-
• Yes	1 (100.0)	0 (0.0)			
Stoma granulation					
• No	3 (25.0)	9 (75.0)	0.561	0.75	0.17-3.35
• Yes	2 (33.3)	4 (66.7)			

There were significant differences in the results of biofilm cultures in several durations of using a tracheostomy cannula (p=0.029). In the cannula duration of more than 90 d, more biofilm was found than in less than 90 d. Clinically, the PR value was 6, meaning that subjects who had a cannula for more than 90 d had a 6 times higher risk for developing biofilms formation than subjects with less than 90 d.

TABLE 3 shows the relationship of other variables as risk factors for the presence or absence of biofilm formation. The results showed that there were no significant differences in all tested groups (p> 0.05).

DISCUSSION

The processes of biofilm formation on the cannula occurs gradually over time. Shortly after the cannula is inserted into the body, the surface of the biomaterial is rapidly covered by a layer of protein, fibrin, platelets, and other elements known as a film, which changes the surface properties of the biomaterial.[10] Ravendraa *et al*.,[11] reported that biofilms began to appear on the tracheostomy cannula as early as 7 d after insertion.

At this initial stage, the actual attachment of microorganisms can easily be removed by routine cleaning. However, the cannula is always exposed to fluid from the stoma and trachea which is rich in bacteria causing significant changes in biomaterials. The changes of the surface of the cannula cause bacterial adhesion to be easier, so that the bacterial attachment will be irreversible. Costerton *et al*.,[12] reported that biomaterial change in the cannula caused a substratum effect that could increase the attachment of bacteria to a rougher surface. Backman *et al*.,[7] described the greatest severity of biomaterial degradation of the tracheostomy cannula occurring at 3 - 6 mo, when the cannula may become brittle and discolored.

This fragility and degradation can serve as indicators of the presence of a biofilm. The National Respiratory Center (NRC) reported that the cannula can still be used if there is no visible color and no broken cannula fragments are found. The NRC conducted a further study and determined that if the cannula is made of silicone it can be used for up to 85 d, while PVC can last up to 56 d and polyurethane (PU) for up to 51 d. Kumarasinghe *et al*.,[14] explained that the interval of cannula replacement should be 4 wk until 3 mo after insertion in order to prevent any further degradation and complications. Norkahfi *et al*.,[15] also recommended that the tracheostomy cannula should be replaced in less than 3 mo because the damage to the cannula reaches its peak within 3 mo due to various causes, especially damage to the surface properties of the cannula due to biofilm.

Some limitations of this study including this study was conducted not in serial form, but taking sample at one time only. Therefore, the independent variable that mostly influenced on biofilm formation could not be controlled. Furthermore, this study only described in general the presence or absence of a biofilm on the cannula because from the results of the study. Only one type of biofilm classified as strong biofilm was observed. Further study is needed to evaluate the microorganisms that involved in the biofilm formation.

CONCLUSION

In conclusion, there is no significant differences in bacterial biofilms formation between the less than 90 d and more than 90 d of tracheostomy cannula usage. More biofilms are found in cannula with durations of more than 90 d compared to cannula with durations of less than 90 d.

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REFERENCES

1. Raimonde AJ, Westhoven N, Winters R. (2021, December 29). Tracheostomy - StatPearls - NCBI Bookshelf. Tracheostomy. Retrieved July 3 2022. https://www.ncbi.nlm.nih.gov/books/NBK559124/
2. Wolcott R, Dowd S. The role of biofilms: are we hitting the right target? Plast Reconstr Surg 2011; 127(Suppl 1):28s-35. https://doi.org/10.1097/prs.0b013e3181fca244
3. Kinnari TJ. The role of biofilm in chronic laryngitis and in head and neck cancer. Curr Opin Otolaryngol Head Neck Surg 2015; 23(6):448-53. https://doi.org/10.1097/moo.0000000000000200
4. Roberts CG. The role of biofilms in reprocessing medical devices. Am J Infect Control 2013; 41(5 Suppl):77-8. https://doi.org/10.1016/j.ajic.2012.12.008
5. Rodney J, Ojano Dirain CP, Antoneli PJ, Silva RC. Effect of repeated tracheostomy tube reprocessing on biofilm formation. Laryngoscope 2016; 126(4):996-9. https://dx.doi.org/10.1002%2Flary.25473
6. David SE, Ho B, Kent SM, Hughes CA, Mattenburg D, Rueggeberg FA, *et al*. Changes in pediatric tracheostomy tubes exposed to home dishwashing. Int J Pediatr Otorhinolaryngol 2017; 100:96-102. https://doi.org/10.1016/j.ijporl.2017.06.020
7. Backman S, Bjorling G, Johansson UB, Lysdahl M, Markström A, Schedin U, *et al*. Material wear of polymeric tracheostomy tubes: a six-month study. Laryngoscope 2009; 119(4):657-64. https://doi.org/10.1002/lary.20048
8. Perkins J, Mouzakes J, Pereira R, Manning S. Bacterial biofilm presence in pediatric tracheostomy tubes. Arch Otolaryngol Head Neck Surg 2004; 130(3):339-43. https://doi.org/10.1001/archotol.130.3.339
9. Zhu H, Das P, Brereton J, Roberson D, Shah RK. Surveillance and management practices in tracheotomy patients. Laryngoscope 2012; 122(1):46-50. https://doi.org/10.1002/lary.22375
10. Cipriano A, Mao ML, Hon HH, Vazquez D, Stawicki SP, Sharpe RP, *et al*. An overview of complication associated with open and percutaneous tracheotomy procedures. Int J Crit IIIn Inj Sci 2015; 5(3):179-88. https://doi.org/10.4103/2229-5151.164994
11. Raveendra N, Rathnakara SH, Haswani N, Subramaniam V. Bacterial biofilms on tracheostomy tubes. Indian J Otolaryngol Head Neck Surg 2021; 6:1-5. htpps://doi.org/10.1007/s12070-021-02598-6
12. Costerton JW, Stewart PS. Battling biofilms. Sci Am 2001; 285(1):74-81. https://doi.org/10.1038/scientificamerican0701-74
13. Wood TK. Insights on *Escherichia coli* biofilm formation and inhibition from whole-transcriptome profiling. Environ Microbiol 2009; 11(1):1-15. https://doi.org/10.1111/j.1462-2920.2008.01768.x
14. Kumarasinghe D, Wong EH, Duvnjak M, Smith MC, Palme CE, Riffat F. Colonization rates of tracheostomy tubes associated with the frequency of tube changes. ANZ J Surg 2020; 90(11):2310-4. https://doi.org/10.1111/ans.15970
15. Norkahfi MR, Marina MB, Wawaddah A, Abdullah SM. Prolonged placement of trachestomy tube causing unusual complication. Med & Health 2016; 11(2):298-302. https://doi.org/10.17576/MH.2016.1102.19