

RESEARCH ARTICLES

THE EFFECT OF DURATION OF USE AND HYGIENITY ON MICROBIAL PROFILE OF USED MASK SURFACE USED BY CHILDREN SCHOOL IN JAKARTA

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ABSTRACT

Background: Coronavirus Disease 2019 (COVID-19) is a respiratory infectious disease caused by the SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) virus. The main medium of transmission of COVID-19 is droplets from the respiratory tract. One way to prevent this is to wear a mask. In the context of good and correct mask use, the duration of mask use is one of the concerns, because a study shows that microbes in the form of potentially harmful bacteria and fungi can be found in the use of masks for a long duration. In addition, dental hygiene is also a factor in the development of microorganisms after wearing a mask. Children aged 0-18 years are vulnerable to contracting Covid-19, especially from family. However, until now there have been no studies conducted to identify the hygiene of masks used by children.

The aim of this study is to determine the hygiene profile of masks used by school children by identifying the type, number of microbes and knowing the factors that affect the hygiene of the use of masks by elementary, junior high and high school age children.

Method: This study is a cross-sectional study by conducting interviews and collecting masks that have been worn by elementary school students in Jakarta. The surface of the face side mask was then swabbed and cultured on Nutrient Agar media. Data were analyzed using the Mann Whitney

test.

Results: The subjects in this study were 62 children with an age range of 7-13 years with the majority being female as many as 34 children (54.84%). This study found that there was no significant difference between the number of colonies based on tooth brushing activities before using a mask and the duration of mask use (p 0.396; p 0.268), while there was a significant difference with dental caries (p 0.041). In addition, there was a significant relationship between the presence of pathogens in the mask and the activity of brushing teeth before using the mask (p 0.027) with an OR value of 3.709 (95% CI 1.288- 10.685), meaning that respondents who did not brush their teeth before using the mask had a 3.709 times chance of having pathogens in the mask they used. Meanwhile, in terms of the presence of pathogens, there was no significant relationship between the presence of pathogens in the mask with dental caries and duration of mask use (p 0.166; p 0.516). The types of bacteria identified were dominated by non-pathogenic bacteria in humans, such as *Staphylococcus epidermidis* and *Staphylococcus aureus*. This could have occurred because it was affected by the pH of the skin surface which tends to be acidic, due to the free fatty acids contained in the skin.

Conclusion: Dental and oral hygiene using masks is related to the presence of pathogenic microbes in the use of masks to prevent Covid-19 in children.

Keywords: Covid-19, children, microbes, masks, hygiene

INTRODUCTION

The Covid-19 pandemic has spread throughout the world, including Indonesia since December 2019. Indonesia experienced two peaks of daily case waves in October 2021, reaching more than 56.000 additional cases every day. Transmission occurs through splashing of liquid droplets when talking, coughing or sneezing, both from individuals

with symptoms and without symptoms. The government is trying to prevent transmission by implementing health protocols, including using masks properly.¹⁻³

Children aged 0-18 years are vulnerable to contracting Covid-19, especially from their families. However, it is difficult for children to get used to wearing masks.^{4,6} Based on a study conducted by Park in 2022, it was found that the number of colonies on the face mask surface was 13.4 times

higher than the outer side with an average number of each colonie are 168.6 ± 24.7 and 36.0 ± 7.0 . Based on these studies, colonies formed mostly consisting of *Staphylococcus epidermidis* and/or *Staphylococcus aureus*.⁷

When face-to-face schools were opened, children always wore masks so that the growth of microbes on masks could potentially cause disease in children. Studies have been conducted on adults, but have not identified microbes in children's masks. There are several characteristics that can have an impact on the composition of microorganisms in the oral cavity, including age, diet, gender and dental and oral hygiene.⁸ Therefore, it is necessary to identify microbes in children's masks for safe use. Until information is obtained to provide recommendations for the proper duration of wearing masks and maintaining oral and dental hygiene before using masks, especially in children

METHODS

This research is a cross-sectional study to determine the microbial profile on the surface of masks used by school-age children in Jakarta. Interviews were conducted with each respondent to find out the duration of wearing a mask, dental and oral hygiene, and the health condition of the respiratory tract. The masks that have been used are then collected to be swabbed on the masks.

The target population in this study were elementary school-age children who attended face- to-face schooling at school and wore masks for a certain period of time, taking into account the level of knowledge and compliance which was greatly influenced by supervisors (parents and teachers). The reachable population in this study were elementary school children in Jakarta. Estimated sample size in cross-sectional research is obtained using $n = N Z^2 [p (1-p)] : NE^2 + Z^2 [p (1-p)]$. The information about this formula is $n =$ Size or number of sample respondents, $Z =$ reliability coefficient or standard normal variable value of 1.65 with a confidence level of 90%, $E =$ tolerable error rate (margin of error) of 10%, $P =$ Total proportion = $P_1 + P_2 = 0.5$, $N =$ The size of the population in elementary school Pondok Pinang 10 Pagi Jakarta, which is around 250-300 children. Based on that formula, the minimum sample size for this study was 53-55 subjects. To minimize errors, there was an additional sample of 10% so that the total sample was 62 subjects. Sampling of the research at primary schools in Jakarta was carried out using stratified cluster sampling. In the cluster sampling method, the sample is randomly selected from groups in the population that occur naturally, for example in sub-districts, cities, provinces, and so on. Then stratified sampling was carried out on strata of a group of related subjects in class group divisions. The selection of elementary school is also based on the willingness of the institution to be involved in the research, the ability of the

researcher, and the compatibility of the schedules of both parties.⁹

Before data collection, teachers and students were given an explanation about the research to be conducted as well as informed consent. Data collection was carried out by interviews to determine the samples that fell within the criteria as well as making observations to see whether there were cavities or not. At the same time, researchers also collected the masks used and put each used mask into a sterile bag.

Samples of used masks were taken to the microbiology laboratory at the Faculty of Medicine, UIN Jakarta. Then, the surface of the face mask is swabbed using a sterile cotton swab. Next, bacterial culture was carried out using the Streak Plate Method on Nutrient Agar media using a calibrated sterile tube measuring 10. Incubation was carried out for 24 hours at 37°C. After that, the number of colonies was calculated manually in media in which bacterial growth occurs, one point counts as one colony.

ETHICAL APPROVAL

This research was approved by ethical approval of Islamic State University Syarif Hidayatullah Jakarta. The number of ethical approval is B-061/F12/KEPK/TL.00/10/2022

RESULTS

A. Respondent characteristics

This study involved 62 children who were students. The characteristics of respondents in this study consisted of gender and age.

Based on Table 1, it is known that based on gender, the majority of subjects were women, namely 34 children (54.84%) and the remaining 28 children (45.16%). The percentage between women and men is quite balanced. In addition, the number of subjects in this study were 62 children with an age range of 7-13 years. The results of univariate analysis of the age of the children showed that the highest percentage of children was in the 11 years old group (25.81%) and the lowest percentage was in the 13 years old group (1.61%).

B. Profile of Mask Use and Dental Hygiene

This study found that 54.80% of students did not brush their teeth before using masks and 54.80% of students had dental caries. Fifty six point fifty percent of students use masks for 4 hours and 43.50% use masks for 2 hours. Fifty six point fifty percent of students found pathogens in the masks used, while the average number of colonies found in student masks was 93.19 with a standard deviation of 120.87.

C. The relationship between the profile of mask use and dental brushing habits with the number of colonies

Based on the behavior of brushing their teeth before using a mask, the average number of colonies in respondents who brushed their teeth was 100.89 with a standard deviation of 147.77, while the average number of colonies in respondents who did not brush their teeth was 86.85 with a standard deviation of 95.12. The statistical test results showed a value of 0.396 at a value of 0.05 which indicated that there was no significant change in the number of colonies based on tooth brushing activity before and after using the mask.

Based on dental caries, the average number of colonies in respondents who had dental caries was 65.06 with a standard deviation of 92.22 while the average number of colonies in respondents who did not have dental caries was 127.36 with a standard deviation of 142.84. The statistical test results showed a value of 0.041, at an α value of 0.05, meaning that there was a significant difference between the number of colonies between students with dental caries and students who did not have dental caries.

Based on the length of time using the mask, the average number of colonies in respondents who used a mask for 2 hours was 66.00 with a standard deviation of 83.49 while the average number of colonies in respondents who used a mask for 4 hours was 114.17 with a standard deviation of 140.85. The statistical test results showed a value of 0.268, at an α value of 0.05, meaning that there was no significant difference between the number of colonies between students who wore masks for 2 hours and 4 hours.

D. The effect of the profile of mask use and oral hygiene on the presence of pathogens in masks

Based on the table 4, the majority of respondents masks in the group that brushed their teeth did not contain pathogens were 60.70%, while the majority of respondents' masks in the group that did not brush their teeth contained pathogens, were 70.60%. The statistical test results showed that there was a significant relationship between the presence of pathogens on the mask and the activity of brushing your teeth before using the mask with an OR value of 3.709 (95% CI 1.288-10.685) and p-value of 0.05. This means that respondents who did not brush their teeth before using a mask had a 3.709 times chance of having pathogens in the mask that they used compared to respondents who brushed their teeth before using a mask.

Based on the presence of dental caries, the majority of respondents masks in the dental caries group did not contain pathogens, namely 52.90%, but the majority of respondents masks in the non-dental caries group contained pathogens, namely 67.90%. The statistical test results showed a p-value of 0.166 which indicated that there was no significant relationship between the presence of microorganisms on masks and dental caries at a value of 0.05. The majority of

respondents masks in the 2-hour mask wearing group contained pathogens, namely 63.00%, while the majority of respondents masks in the 4-hour mask wearing group contained pathogens, namely 51.40%. The results of the statistical test showed a p-value of 0.516 which indicated that there was no significant relationship between the presence of pathogens on masks and the duration of wearing masks at a value of 0.05.

In the multivariate analysis, the variables analyzed were those with a p-value on the bivariate <0.25 . Based on this, 4 variables (brushing teeth before wearing a mask, dental caries, length of time using a mask, and frequency of brushing teeth) were included in the multivariate analysis. The results of the analysis show that the variable that has a significant influence after adjusting other variables is brushing their teeth before using a mask with an AOR value of 3.410 (95% CI) meaning that after adjusting for other variables, respondents who do not brush their teeth before using a mask have a 3.41 chance of having masks that contain pathogens compared to respondents who brush their teeth before using a mask.

Table 1. General Characteristics of Respondents

Characteristics	Amount (n)	Percentage (%)
Gender		
Man	28	45.16
Woman	34	54.84
Age (years)		
7	2	3.23
8	11	17.74
9	13	20.97
10	15	24.19
11	16	25.81
12	4	6.45
13	1	1.61

Table 2. Profile of Mask Use and Dental Hygiene

Variables	n	%
Brush Teeth Before Wearing Mask	Yes	28 45.20
	No	34 54.80
Dental caries	Yes	34 54.80
	No	28 45.20
Long Using Mask	2 hours	27 43.50
	4 hours	35 56.50
Presence of Pathogens in Masks	Yes	35 56.50
	No	27 43.50
Number of Colonies In Mask (Mean \pm SD)	93.19 \pm 120.87	

Table 3. The relationship between the profile of mask use and oral hygiene with the number of colonies

Variable	Number of Colonies		p-Value
	Means	SD	
Brush Teeth Before Wearing Mask	Yes	100.89	0.396
	No	86.85	
Dental caries	Yes	65.06	0.041
	No	127.36	
Long Using Mask	2 hours	66.00	0.268
	4 hours	114.17	

Table 4. The effect of the profile of mask use and oral hygiene on the presence of pathogens in masks

Independent Variable	Presence of Pathogens in Masks				P-Value	OR (95% Ci)	AOR (95% Ci)
	Yes		No				
	N	%	N	%			
Brush Teeth Before Wearing Mask	Yes	11	39.30	17	60.70	0.027	3,709 (1.288-10.685)
	No	24	70.60	10	29.40		
Dental caries	Yes	16	47.10	18	52.90	0.166	0.421 (0.149-1.192)
	No	19	67.90	9	32.10		
Long Using Mask	2 hours	17	63.00	10	37.00	0.516	0.623 (0.224-1.735)
	4 hours	18	51.40	17	48.60		

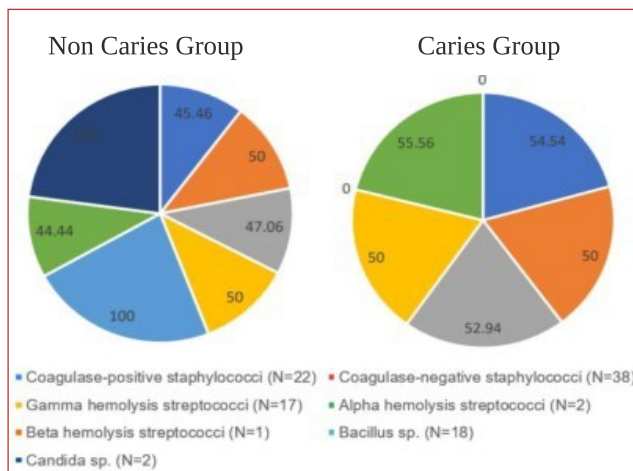


Figure 1. Identification of Bacterial Types in Caries and Non-Caries Groups

DISCUSSION

A. Brush Teeth Before Wearing a Mask with Number of Microbial Colonies and Presence of Pathogens on the Surface of the Used Face Mask

This study found no statistically significant change in the number of colonies based on the behavior of brushing teeth before using a mask. This is actually contrary to previous research on this matter. In 2022, Park found a link between the practice of consuming natto (a traditional Japanese food made from soybeans fermented by *Bacillus subtilis*) and the number of *B. subtilis* colonies on the surface

of face masks. According to research findings, those who consumed natto had a much larger percentage of *B. subtilis* colonies (76%) than those who did not consume natto (28%)⁷ This shows that the microbial conditions found in the oral cavity which are influenced by certain foods can affect the number of microbial colonies on the surface of the face mask. In addition, microbial growth on the surface of the face of the mask is also influenced by high humidity in the space between the mask and around the face, which is 80% as a result of the lining material in the mask that absorbs water.¹⁰ Likewise with research conducted by Sahoo on in 2022 examined the comparison of the number of *Streptococcus mutans* bacterial colonies in the group that brushed their teeth before and after breakfast. In this study, it was found that the group that brushed their teeth after breakfast experienced a decrease in the number of colonies by 29% (from 108.4 ± 18.5 CFU/ml to 77.0 ± 16.9 CFU/ml).

Another study conducted by Fauzi in 2018 also tested the effect of giving sucrose and fructose on the number of bacterial colonies in the oral cavity. Based on this study, there was an increase in the number of bacterial colonies in the oral cavity when given fructose, were 87×10^3 CFU/ml to 137.72×10^3 CFU/ml and when given sucrose, were 71.60×10^3 to 91.76×10^3 CFU/ml. This study shows that the content in food that is usually consumed daily including at breakfast can be a good substrate for the growth of bacteria in the oral cavity.¹² Based other study, brushing teeth after breakfast with a rinse or without a rinse before breakfast reduced

overall levels of the cariogenic bacteria *Streptococcus mutans* more effectively than brushing teeth before breakfast.¹¹

The results obtained in this study are not in accordance with previous studies. In this study, a higher number of colonies on the face mask surface was found in children who brushed their teeth. This is likely to occur due to other factors that can affect contamination on the surface of the face mask such as microorganisms found on the skin. In addition, children's compliance with wearing masks is still lacking, thus allowing contamination to occur both on the outer and inner surfaces of the masks due to the behavior of frequent touching, repeatedly removing and wearing the mask, and lowering the mask under the nose or chin.

However, this study found a significant relationship between the presence of pathogens in the mask and the activity of brushing their teeth before using the mask with an OR value of 3.709 (95% CI 1.288-10.685) meaning that respondents who did not brush their teeth before using a mask had a 3.709 times chance of having pathogens in the mask they used. compared to respondents who brushed their teeth before using a mask. Therefore, it is important to brush your teeth after breakfast and before wearing a mask to prevent excess bacterial growth in the oral cavity.

B. Dental Caries with Number of Microbial Colonies and Presence of Pathogens on the Surface of Used Face Masks

This study found a significant difference in the number of colonies but no difference in the presence of pathogens between students with dental caries and students without dental caries. Cariogenic diet and poor oral hygiene can lead to dysbiosis and biofilm-mediated oral diseases such as dental caries.^{13,14} Dental caries is a serious public health problem worldwide, and is the most common disease identified in the 2015 Global Burden of Disease study (2.3 billion people).¹⁵ According to RISKESDAS statistics for 2018, 79% of children aged 3-15 years have dental caries.¹⁶

There are no studies regarding the relationship between dental caries and pollution on the surface of face masks. A study conducted by Elianora in 2019 found an increase in the number of bacterial colonies from the saliva of children with caries compared to children who did not have caries.¹⁷ Meanwhile, the results of Eriksson's study in 2017, which divided subjects into groups free of caries and caries, found that the caries-free group had 44.0% *Streptococcus mutans* colonization in saliva, while the caries group had 83.8% *Streptococcus mutans* colonization in saliva.¹⁸ This research is in line with Sounah's 2020 study using subjects aged 24 years which were also divided into caries-free groups and caries groups. In the caries-free subject group, there was *Streptococcus mutans* colonization in the saliva of 4.4 CFU/mL, while the caries subject group had more *Streptococcus mutans* colonization in saliva, namely 4.6 x 106 CFU/mL.¹⁹ However, the results obtained in this study is

not in line with previous research. In this study, the number of colonies on the face mask surface was higher in children who were not carious. This is likely to occur due to other factors that can affect such as the normal flora on the skin. the results obtained in this study were not in line with previous studies. In this study, the number of colonies on the face mask surface was higher in children who were not carious. This is likely to occur due to other factors that can affect such as the normal flora on the skin. the results obtained in this study were not in line with previous studies. In this study, the number of colonies on the face mask surface was higher in children who were not carious. This is likely to occur due to other factors that can affect such as the normal flora on the skin.

Based on this figure, the types of bacteria identified are dominated by non-pathogenic bacteria in humans, such as *Staphylococcus epidermidis* and *Staphylococcus aureus*. Whereas in a study conducted by Eisenstein stated that some common bacteria, such as coagulase- positive staphylococci (*Staphylococcus aureus*) and β hemolytic streptococci (*Streptococcus pyogenes*), were inhibited by acidic pH, resulting in the growth of coagulase-negative staphylococci and γ hemolytic streptococci (*Streptococcus mutans*) more dominating. In addition, coagulase-negative staphylococci have also been considered as the main bacterial colonizers of the skin.²⁰

Although some bacteria were found on the surface of masks in Mardaneh's study on children in 2016, most of these bacteria were not pathogenic, but there are also a number of bacteria that have the potential to become pathogenic in humans, including *Staphylococcus aureus* (a commensal bacteria, but when grown separately). excess can cause various diseases); *B. cereus* (intestinal bacteria, can cause food poisoning); and *Staphylococcus saprophyticus* (a bacterium that causes urinary tract infections); and *Pseudomonas luteola* (opportunistic pathogenic bacteria).²¹

C. Duration of Mask Wear with Number of Microbial Colonies and Presence of Pathogens on the Surface of Used Face Masks

This study found no significant difference between the number of colonies and the presence of pathogens between students who wore masks for 2 hours and 4 hours. This is related to a laboratory experimental study conducted in 2005 by testing the number of bacteria in wearing surgical masks in adults for 1, 2, 4, and 6 hours, it was found that the effectiveness of disposable surgical masks in protecting against bacteria was 95% in Bacteria Filtration Efficacy (BFE) just showed a significant decrease after 4 hours of use.²²

Meanwhile, Madion's study from 2023 found that there were 123 different bacterial isolates in total (1-5 isolates per mask), including *Staphylococcus aureus* from 11 masks (15.9%) and clinically important gram-negative bacteria

from 22 masks (31.9%). There was also no statistically significant difference in the number of clinically important bacteria between masks worn for more than or less than 6 hours, according to Madion's study.²³ An investigation conducted by Chughtai in 2019 of 148 doctors and nurses in 3 homes hospital in Beijing showed respiratory virus contamination on the outside of a medical mask worn during a shift that lasted 6-8 hours. The wearer can become self-contaminated if microorganisms are present on the outside of the mask. By handling and using a contaminated mask again, a person can become infected themselves. Research shows that healthcare worker's masks are consistently contaminated with germs if bacteria are present. This study showed decreased numbers when concentrating only on clinically significant bacteria while these bacteria were still present (15.9% for *S. aureus* and 31.9% for gram- negative organisms).²⁴

Bacteria, especially pathogenic bacteria, accumulated on surgical masks and more on cotton face masks after 4 hours of wearing, according to research conducted by Lize Dalanghe in 2021.²⁵ This is consistent with Zhiqing's study in 2018, which found that when doctors' masks the same surgery was analyzed for bacteria, there was a substantial increase in the 2- hour group. As a result, this study recommends that surgeons change masks after every operation, especially those lasting more than 2 hours.²⁶ The study also detected a significant accumulation of bacteria based on the type of mask, cloth masks (average 1.48×10^5 CFU/mask) and surgical masks (average 1.98×10^4 CFU/mask). This is thought to occur because cloth masks are a good substrate for bacterial growth due to their ability to hold moisture very well.²⁶

In addition, other studies have found that cotton masks have more bacterial colonies than surgical masks, with *Staphylococcus* and *Streptococcus* being the most common germs found in surgical masks. The masks should be replaced in four hours because it can cause mask filtration to not function optimally and can increase the potential for transmission of Covid- 19, so when individuals are active outside the home that they need to carry spare masks.²⁸⁻²⁹ According to Yudhastuti's research from 2020, cloth masks can be cleaned with detergent and must be worn for four hours to prevent contamination by other microorganisms such as bacteria because these microorganisms prefer damp cloth masks with an average temperature of 270C and a relative humidity of 75%.³⁰

Based on these results, it was found that the difference in the number of colonies or the presence of pathogens in the use of masks did not only depend on the length or duration of wearing the mask, but also related to the type of mask, the use of masks by individuals at risk of contamination such as health workers, and the environment using masks.

Research Limitations

- In this study there were respondents who were experiencing have an upper respiratory tract infection, and pathological bacteria were found in the swab results of the respondents, so further research was needed regarding sick conditions such as ISPA that can affect microbial colonies on wearing masks
- There are many confounding factors that influence the variable number of bacterial colonies on the surface of the face mask that cannot be controlled, such as not being given the same mask, mask wearing behavior, eating habits/dietary patterns which we did not examine.
- Calculation of the number of bacterial colonies growing on the media was counted manually without using a colony counter device
- The data distribution is not normal and the variants are not the same.
- Respondents involved in this study were elementary school-age children, so it is necessary to consider the ability and understanding of children in answering questions.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare

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Non declared.

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No funding was received

CONCLUSION

Dental and oral hygiene using masks is related to the presence of pathogenic microbes in the use of masks to prevent Covid-19 in children.

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