ORIGINAL RESEARCH

Identification of bacterial contaminants on glasses used by students of Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia class of 2016

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Article Info

Article history:
Received Jul 21, 2020
Revised Oct 15, 2020
Accepted Nov 8, 2020
Published Jan 1, 2021

Keywords:
Glasses
Contamination
Bacillus sp.
Pseudomonas sp.
Staphylococcus aureus
Healthy lifestyle

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ABSTRACT

Background: People nowadays suffer from refractive disorder that declines the vision. The most common therapy to overcome this problem is to use glasses. The continuous use of glasses and the difficulties on disinfecting the entire surface can cause bacterial contamination. Those bacteria can cause various eye diseases. This fact pushed the researcher to find out if there were bacterial contaminants on glasses used by students of Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, class of 2016. **Objective**: To prove the species of bacterial contaminants and pathogenic bacteria on glasses used by students of Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia class of 2016. **Materials and Methods**: Cotton swab moistened in sterile distilled water was swabbed to 30 glasses on its inside lens and the part touching the nose. The sample was then inoculated on blood agar and Mac Conkey, and incubated for 24 hours in 37ºC. Then, the isolates were identified macroscopically and microscopically. **Results**: The whole sample that had been examined was contaminated with pathogenic bacteria with risk of causing eye diseases. Those bacteria were identified as *Bacillus sp.* (50%), *Pseudomonas sp.* (46%), *Staphylococcus aureus* (20%), and *Staphylococcus epidermidis* (16%). **Conclusion**: The result of this research showed that there was contamination by bacteria on glasses used by students of Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia class of 2016. All bacteria found were pathogenic to the eye. This showed that glasses could become a reservoir of the pathogenic bacteria that could potentially cause recurring eye infection.

How to cite:
Anjani, H.L., Purwanta, M., Rochmanti, M. 2021. Identification of bacterial contaminants on glasses used by students of Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia class of 2016. Majalah Biomorfologi, 31(1): 18-23.

Majalah Biomorfologi (Biomorphology Journal) p.ISSN:0215-8833, e.ISSN: 2716-0920
doi: 10.20473/mbiom.v31i1.2021.18-23

BACKGROUND

According to Mireku and Ebenezer (2017), approximately 2.3 billion people in the world suffer from refractive disorder that causes decline in vision. Glasses are chosen to be the most common and the cheapest therapy to overcome this problem (Osaro-Matthew et al., 2015).

According to Lange (2013), there is a risk of bacterial contamination in glasses due to their continuous use and the difficulty of disinfection on the entire surface of the glasses. A study conducted by Ruth et al (2015) showed that there was bacterial contamination by *Eschericia coli, Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella sp., Bacillus sp., Streptococcus sp.*, and negative coagulase Staphylococcus on the lens and the frame used by workers and students in Michael University of Agriculture Umudike, Nigeria (Osaro-Matthew et al., 2015). The lack of awareness of workers and students about personal hygiene, for example not washing their hands after rubbing their nose contributes to bacterial contamination of the glasses they use. In addition to hand washing habits, bacterial transmission can occur through handshakes with other people. The habit of cleaning glasses only with water or even just with a cloth is also a cause of bacterial contamination. The results also showed that the cleaning fluid specifically formulated to clean the lens cannot stop bacterial growth (Osaro-Matthew et al., 2015).

Contamination of glasses by bacteria can cause various diseases on the eye. *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* are known to cause corneal ulcers. *Staphylococcus aureus* can cause acute dacryocystitis and exposure to *Streptococcus hemolyticus* can cause acute conjunctivitis (Kim et al., 2013).

This fact had driven the researchers to find out if there were bacterial contaminants on glasses used by students of Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia class of 2016. If the results of the study indicate bacterial contamination, the researchers want to identify the types of bacteria that can grow and develop on the lens of glasses and identify pathogenic bacteria that can increase the risk of eye infections. These two things will be tried to be answered in this study.

OBJECTIVE

This research was conducted to identify the species of bacterial contaminants and the species of pathogenic bacteria on glasses used by students of Faculty of Medicine Universitas Airlangga, Surabaya, Indonesia class of 2016.

MATERIALS AND METHODS

This research was a descriptive study conducted in Department of Microbiology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia from March to June 2019. The variables in this study were glasses, cleaning behavior of glasses, and bacteria on glasses. The research sample consisted of 30 glasses selected through simple random sampling in a population of 118 glasses used by students of Faculty of Medicine Universitas Airlangga, Surabaya, Indonesia class of 2016. Samples were taken by using sterile cotton swabs that had been moistened with sterile distilled water. The cotton swab was swabbed to the inner lens of the glasses including the part that touched the nose, then it was inoculated to the blood Agar and MacConkey Agar by streaking and incubated for 24 hours at 37°C. After incubation for 24 hours at 37°C, isolates that grew on the blood Agar and MacConkey Agar were examined macroscopically and microscopically to distinguish between Gram positive and Gram negative bacteria.

All isolates on agar media which showed the growth of Gram positive bacteria were tested biochemically through catalase test. If the catalase test result was positive, the next test was the mannitol fermentation test. This test was conducted with the aim of identifying the Staphylococcus bacteria. *Staphylococcus aureus* was identified if the result of the mannitol fermentation test was positive. If the mannitol fermentation results were negative, the novobiosin test was then conducted. *Staphylococcus saprophyticus* would show resistance to novobiosin, while *Staphylococcus epidermidis* was still sensitive to novobiosin. Isolates with negative results on the catalase test would be observed on blood Agar of its haemolytic type. Optochin test was carried out on isolates that showed alpha-type haemolytic (partial haemolytic), if the bacteria was sensitive to Optochin test, it was identified as *Streptococcus*

viridans. In beta hemolytic type, bacitracin test was conducted. Sensitive results showed isolates as *Streptococcus pyogenes* and resistant results showed isolates as *Streptococcus agalactiae*.

Isolates that showed the characteristics of gram-negative rod bacteria would be identified by biochemical tests of Triple Sugar Iron, indole, methyl red, voges proskauer, citrate, motility, urea, and oxidase test.

RESULTS

Identification of bacterial contaminants on glasses was conducted on 30 students in Faculty of Medicine Universitas Airlangga, Surabaya, Indonesia class of 2016, consisting of 24 women and 6 men, by using the simple random sampling method. From 30 glasses, 40 bacterial isolates were identified as 4 types of bacteria, namely *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus sp.*, and *Pseudomonas sp.*, as listed in Table 1.

Table 1. Results of biochemical test of swab sample on glasses

No	1	2	3	4
Gram Staining	Coccus, Gram +	Coccus, Gram +	Rod, Gram + (formed spore)	Coccobacillus, Gram -
Catalase	+	+	N/A	N/A
Mannitol	+ (yellow)	- (red)	N/A	N/A
Novobiosin	N/A	Sensitive	N/A	N/A
TSI	N/A	N/A	N/A	Alk/Alk
Gas	N/A	N/A	N/A	-
H_2S	N/A	N/A	N/A	-
Indole	N/A	N/A	N/A	-
MR	N/A	N/A	N/A	-
VP	N/A	N/A	N/A	-
Citrate	N/A	N/A	N/A	+
Motility	N/A	N/A	N/A	+
Urea	N/A	N/A	N/A	-
Bacteri	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Bacillus* sp.	*Pseudomonas* sp.

Notes : N/A : Not applicable
TSI : Triple Sugar Iron
MR : Methyl Red
VP : Voges-Proskauer Sensitive : ≥ 16 mm
Alk : Alkaline
H_2S : Hydrogen sulphide

Based on Table 1, there are 4 species of bacteria identified through Gram staining and biochemical tests. In column number 1, *Staphylococcus aureus* could be identified through the positive result on Gram staining in the form of cocci, positive reaction on catalase test, and positive reaction on mannitol test. In column number 2, *Staphylococcus epidermidis* could be identified through the positive result on Gram staining in the form of cocci and positive reaction on the catalase test, but it showed negative reaction on the mannitol test. Then, identification was continued with the novobiosin test and a sensitive result was obtained with the measured diameter of ≥16 mm. In column number 3, the result was also positive on Gram staining and spores were seen. This bacteria was identified as *Bacillus sp.*

In column number 4, *Pseudomonas sp.* was identified through negative result on Gram staining in the form of coccobacillus. Bacteria identification was continued with biochemical tests in the form of TSI, indole, MR, VP, citrate, motility, and urea. The TSI test result showed alkali/alkali (Alk/Alk), without gas, and without H2S. Indole, MR, VP, and urea test results were negative, while the citrate and bacterial motility tests were positive.

Along with sampling, researchers conducted interviews with the owner of the glasses to find out the habit of cleaning the glasses. The frequency of cleaning the glasses can be seen in Table 2.

Table 2 contains data on the frequency or how often they clean the glasses they use. The frequency of cleaning the glasses was grouped into three, namely the group that cleaned their glasses at least once per day (43% or 13 samples), at least three times per week (43% or 13 samples), and the group that cleaned their glasses less than once per week (13% or 4 samples).

Table 2. Frequency of cleaning the glasses

No	Frequency	Total
1.	≥ 1 time/ day	13
2	≤3 times/ week	13
3	<1 time/ week	4
	Total	30

Table 3. Instruments used for cleaning the glasses

No	Instruments	Total
1.	Tissue	4
2.	Cloth	11
3.	Lens Cleaning Solution	9
4.	Water	4
5.	Soap	2
	Total	30

In Table 3, the most used instrument is cloth with a percentage of 37% or 11 people. Then 30% or 9 glasses owners use lens cleaning solution, 13% or 4 people use water, 13% or 4 others use tissue, and the remaining, 7% or 2 people use soap.

DISCUSSION

The bacteria with the highest number were *Bacillus sp.*, which were found in 15 samples (50%). Bacillus species are bacteria that are spread in nature with high concentrations in soil, water, and plants-derived food (Schultz et al., 2017). According to research conducted by Ruth et al. (2015), *Bacillus sp.* appear on the surface of the glasses because these bacteria are bacteria that can form spores (Osaro-Matthew et al., 2015). This causes them to survive in all types of environments and can colonize various types of surface objects. *Bacillus sp.*, except *Bacillus anthracis*, is considered to have a less significant role in the context of human infection. However, in the eye, *Bacillus sp.* are known to cause infections such as conjunctivitis, dacryocystitis, and endophthalmitis (Dave et al., 2018). Endophthalmitis caused by *Bacillus cereus* can cause vision loss in just a few days (Callegan et al., 2017).

The second highest number of bacteria in this study were *Pseudomonas sp.* which were found in 14 samples (46.667%). *Pseudomonas sp.* is the bacteria that can grow well in a variety of environments. This is related to the easy transmission of *Pseudomonas sp. Pseudomonas sp.* can be transmitted through direct contact with water, and these bacteria can even be found in disinfectant fluids in hospitals (Murray et al., 2016). In this study, the transmission of these bacteria can be related to the way the glasses owners use water to clean their glasses. In the clinical environment, *Pseudomonas sp.* are known to cause opportunistic infections (Fritz et al., 2020). As a contaminant on glasses, *Pseudomonas sp.* can cause various diseases of the eye, such as corneal ulcers (Kim et al., 2013). One of the species of Pseudomonas, *Pseudomonas aeruginosa*, is the most common bacteria found to be the cause of keratitis in contact lens wearers (Watson et al., 2018).

In this study, *Staphylococcus aureus* was identified in 6 samples (20%). *Staphylococcus aureus* is the bacteria that can grow well on human skin (Willey et al., 2014). The part of the human body that often becomes the site of S. aureus is the front nasal cavity or anterior nares. These bacteria are often found in the area around the eyes (Tacconelli et al., 2011). *Staphylococcus aureus* can cause various kinds of infections in human, including infections of the eye. The study suspected that *Staphylococcus aureus* contaminate glasses through handkerchief used to clean the nose. In addition, transmission of *Staphylococcus aureus* can also occur if someone does not wash his hands and shakes hands with others (Osaro-Matthew et al., 2015).

The fourth bacteria identified in this study were *Staphylococcus epidermidis* which were found in 5 samples (16.667%). In this study, *Staphylococcus epidermidis* was the least common bacterial contaminant found in glasses. This was different from the research conducted by Birgit et al on the glasses used by students and nurses in Germany. Research conducted in 2015 found that *Staphylococcus epidermidis* was the most common bacterial contaminant in glasses (Fritz et al., 2018). *Staphylococcus epidermidis* is a common flora of the skin and is one of the pathogenic bacteria that is often found in

nosocomial infections (Willey et al., 2014). According to research conducted by Heung-Soo et al, this bacteria is a contaminant on glasses that can cause keratitis (Kim et al., 2013). In addition, *Staphylococcus epidermidis* can also cause conjunctivitis, blepharitis, corneal ulcer, and endophthalmitis (Flores-Páez et al., 2015).

In this study, all of the samples (100%) were contaminated by bacteria that can increase the risk of eye diseases. It also shows glasses as the site for the growth of pathogenic bacteria that can transmit disease, especially in the clinical environment (Fritz et al., 2018). However, in this study, there was not any subject that has eye infection. Under normal circumstances, the eye has a natural defense system on the ocular surface and tear fluid that are antibacterial (Zierhut et al., 2013). This shows that in normal and healthy eyes that are not traumatized or injured, eye infections by bacteria can be prevented.

Through interviews conducted by researchers with the glasses owners, data were obtained about the frequency of cleaning the glasses and the instruments used by the owners to clean their glasses. The frequency and efficiency in cleaning glasses are assumed to be related to microbial load or the number of colony counts on glasses that are frequently used (Fritz et al., 2018). Ruth et al (2015) stated in their research that the instruments used to clean the glasses played a role in the contamination of the glasses (Osaro-Matthew et al., 2015). Clothes or veils that are used as instruments for cleaning glasses can carry bacterial contaminants. The cloth that is rarely washed can also be a site for bacteria to grow and transfer bacteria to the surface of the glasses. In addition, unsterile water can also be the agent for bacterial transmission.

CONCLUSION

The results of this study indicated that there was bacterial contamination on glasses used by students of Faculty of Medicine Universitas Airlangga, Surabaya, Indonesia class of 2016. In this study, there were 4 identified species of bacteria, the *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus sp.*, and *Pseudomonas sp.* All bacteria found in this study were pathogenic to the eye. This shows that glasses can be the reservoir of pathogenic bacteria that can potentially cause repeated infections in the eye. To obtain bacterial isolates that are more diverse and specific, it is suggested for future researchers to use the PCR as the method and it is recommended to do the colony count.

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