

Identification and Counting of Bacterial Colonies on Mobile Phones Before and After 70% Alcohol Disinfection

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ABSTRACT

Cell phones have become a very important communication tool in everyday life. Considering that many people are in the habit of using their mobile phones while doing other activities, most people even have the habit of taking their phones to the toilet. Cell phones become inanimate objects that play an important role in the transmission of microorganisms. Therefore, we need to maintain hand hygiene by keeping our hands clean after handling the mobile phone before performing activities and disinfecting the mobile phone regularly, considering that this object cannot be washed while the mobile phone is a potential vector for infectious diseases. Mobile phones can effectively eliminate hand hygiene due to their compulsive use despite hand washing. The purpose of this study was to determine the role of 70% alcohol cotton on the surface of mobile phones to reduce the potential spread of bacteria. Based on the results of the research conducted, there is a difference in the number of bacterial colonies on mobile phones before and after disinfection with 70% alcohol cotton, as evidenced by the obtained p-value of 0.0001 ($p < 0.05$).

Keywords: cellphone, swab alcohol 70%, counting of bacterial

INTRODUCTION

Along with the increasing development of Science and Technology (IPTEK) in the field of communication, namely mobile phones (pose) cell phones have become a modern communication tool that almost all people have and use, not only as a communication tool but also as a medium for learning and entertainment, and media in developing economic businesses. So it is not surprising that currently, various levels of society, from business people, teachers/lecturers, paramedics/health workers, school children, and students have cell phones and cell phones are one of the mandatory items that must always be carried.

The widespread use of cell phones is a potential source of transmission of pathogenic bacteria among users, considering that many people have the

habit of operating their cell phones while carrying out other activities such as taking cell phones to the toilet, using cell phones while eating, and the same goes for health workers who also use cell phones a lot. while serving patients and health workers on duty in the laboratory but paying little attention to personal hygiene (Kakade *et al.*, 2020).

According to Chitturi, et al., (2019), cell phones can be a health hazard that has proximity to our limbs such as the face, ears, lips, and hands of its users can be a reservoir of pathogens that can cause infection. Lack of awareness of regular handwashing with soap or poor handwashing habits and lack of disinfection of the surface of handheld mobile phones make them vulnerable to exposing them to microorganisms and resulting in the spread of different

microorganisms from one user to another (Sayed Selim and Farouk Abaza, 2015).

Recommendations related to personal hygiene that should start from the individual level, namely to routinely wash hands with soap (CTPS) and routinely clean the surface of cellphones, are optimal efforts in killing microorganisms and breaking the chain of transmission of microorganisms (Elisanti et al., 2020). However, the practice of CTPS is not commonly carried out in certain conditions, and considering that cellphones are electronic materials that cannot be cleaned using water and soap to replace the CTPS function, there are antiseptic and hand sanitizer products that can reduce and even dispel microorganisms such as bacteria and viruses.

The ideal antiseptic can inhibit growth and damage microbial cells, and spores without damaging body tissue. Alcohol is an antiseptic material and has proven safe as a basic ingredient for sterilization products (Silakhudin and Fatmawati, 2015). According to Yunanto (2020), alcohol derivatives, especially those used for antiseptics such as 70% alcohol, are proven to kill gram-positive and gram-negative bacteria, including multi-drug resistant pathogens, Mycobacterium tuberculosis, viruses, and fungi. safe and able to prevent infection of the umbilical cord of newborns (Elisanti et al., 2020). Other studies have also shown that 70% alcohol solution that has been used all day still has an inhibition zone of 4.00 mm (good) (Purbosari, 2021).

Based on research by Chitturi & Lakshmi (2019), cell phones can be a health hazard that has proximity to our limbs such as the face, ears, lips, and hands of its users can be a reservoir of pathogens that can cause infection. Lack of awareness of regular handwashing with soap or poor handwashing habits

and lack of disinfection of the surface of handheld cell phones cause the vulnerability of making cell phones exposed to microorganisms and resulting in the spread of different microorganisms from one user to another. (Kumari Chitturi and Jeevana Lakshmi, 2018).

Recommendations related to personal hygiene should start from the individual level, namely to routinely wash your hands with soap (CTPS) and routinely clean the surface of your cell phone to be an optimal effort to kill microorganisms and break the chain of transmission of microorganisms (Elisanti et al., 2020). However, the practice of CTPS is not usually carried out in certain conditions considering that cellphones are electronic materials that cannot be cleaned using water and soap. To replace the function of CTPS, there are antiseptic and hand sanitizer products that can reduce or even eliminate microorganisms such as bacteria and viruses.

The ideal antiseptic is an antiseptic that can inhibit the growth and damage microbial cells and spores without damaging body tissue. Alcohol is an antiseptic ingredient and has been proven safe as a basic ingredient for sterilization products (Silakhudin and Fatmawati, 2015). Alcohol 70% has been proven to be safe and able to prevent infections in the umbilical cord of newborn babies (Elisanti *et al.*, 2020). Other research also shows that a 70% alcohol solution that has been used all day still has an inhibition zone of 4.00 mm (good) (Purbosari, 2021)

Transmission of bacteria that can cause infection can be through several things, one of which is through hands holding objects contaminated with microorganisms (Ya'aba et al., 2021). The transmission that is feared comes from places that have the potential to

become infectious sources such as hospital treatment rooms, clinical pathology laboratory rooms, or microbiology laboratories both in health care institutions and in pension institutions that are vulnerable to being exposed to potential hazards of microorganisms, as well as the Microbiology Laboratory in one of the educational institutions in Palembang City.

During the practicum, students must interact with examination materials that contain microorganisms. So there is a possibility of microorganism pollution at the location where students do practicum plus the lack of student awareness of cleanliness makes students continue to use cellphones extensively, in addition to helping them document and also to find information related to what they are studying. Based on the description above, the purpose of this study is to see the bacterial distribution on the surface of cell phones that have not been cleaned and those that have been cleaned with 70% alcohol and identify whether pathogenic bacterial genus is found on cell phones of DIV Medical Laboratory Technology Study Program students who have done practicum in the Microbiology Laboratory.

METHOD

Design, place, and time

The type of research used in this research is analytical descriptive. The research location was carried out at the Microbiology Laboratory, Faculty of Health Sciences, Musi Charitas Catholic University. On March 15 – 25 2022.

Population, Sample, and Sampling Technique

The sample population used in this research is an affordable population that meets the criteria for the research to be conducted, namely swab samples

from cell phones. The samples taken from this research were cellphone swab samples from all students of the DIV TLM Study Program who had carried out practicums in the microbiology laboratory.

Tools and materials

The materials used in this research were physiological NaCl, and sterile cotton swabs which were used to take samples. *Plate Count Agar* (PCA) and *Blood Agar Plate* (BAP) media were used to isolate and count the number of colonies from swabs. Gram staining reagent to see the type and shape of bacteria isolated from cellphone swabs, plasma citrate media to carry out coagulation tests, H₂O₂ 30% to carry out catalase tests. Biochemical media such as carbohydrates (glucose, lactose, mannitol, sucrose, and maltose), Methyl red, Voges Proskauer, SIM (Sulfur Indole Motility), Urea, Simon citrate, TSIA (Triple Sugar Iron Agar), decarboxylation media, and phenylalanine and mueller hinton agar (MHA) media and novobiocin antibiotic disk. All media used, both isolation media and biochemical media, are first subjected to Quality Tests and Sterility Tests, namely 10% of the test media made.

The tools used in this research include test tubes, Petri dishes, tweezers, tubes, Bunsen lamps, autoclaves, dry heat ovens (DHO), incubators, vortexes, microscopes, and Colony Counters.

Procedures

In this study, the research sample of student cellphones was labeled with the initials HB 1- HB 45 for cellphones that were swabbed before the respondents washed their hands, and the cellphones were cleaned with 70% alcohol cotton. HS 1-HS 45 initials for samples whose cellphones have been cleaned with

alcohol cotton and the respondent has washed their hands with soap (Sadiq *et al.*, 2021).

1. Pre Analytics

Pre-analytical preparation includes preparing instruments and sampling materials to process examination materials.

The examination material is taken using a swab technique, namely wiping a sterile cotton swab that has been moistened with sterile physiological NaCl thoroughly and evenly on the surface of the cellphone screen (sample) before cleaning, then inserting the cotton swab into a test tube containing sterile physiological NaCl with the initials code HB and the sterile cotton swab used to wipe the surface of the cell phone after cleaning it with a 70% alcohol cotton swab are put into a test tube containing sterile physiological NaCl with the initials code HS.

Analytic

a. Isolate the inspection material on isolation media

The samples that have been obtained are planted in isolation (culture) media, namely PCA and BAP media to compare the number of colonies that grow from the samples before and after cleaning using 70% alcohol. Then the media was incubated at 37 °C for 18-24 hours.

b. Gram staining

The isolation results or colonies that grew on PCA media before being cleaned with 70% alcohol cotton were first identified microscopically by making preparations of the growing colonies and then stained with gram stain to see the type and shape of bacteria through a microscope 1000 times magnification (Connie R. Mahon, 2019).

c. Biochemical test

After knowing the type of bacteria and its shape, whether the type of bacteria isolated is gram negative or gram positive and the shape is a rod or coccus, then proceed with making a suspension and carrying out biochemical tests on the isolated bacterial colonies.

For gram-positive coccus bacteria, the catalase test is first carried out, then if the catalase test is positive, the sugar test is continued (mannitol, glucose, sucrose), coagulase test, and susceptibility test using the Kirby Bauer method.

Against gram-negative bacteria, bacilli are continued by isolating them in biochemical media such as Indol, Methyl red, Voges Proskauer, and Simon Citrate (IMVIC).

All bacteria that have been inoculated in biochemical media are continued by incubation at 37 °C for 18-37 hours (Brooks *et al.*, 1991).

2. Post Analytics

All bacterial isolation results on media that had been incubated at 37 °C for 37 °C were observed for growth, then the growth of the number of colonies that grew on PCA HB 1-45 and HS 1-45 media was calculated, then microscopic observations were made on the colonies that grew on PCA HB and PCA HS media. Microscopic observations were carried out on bacterial colonies growing on PCA HB to see the overall picture of the bacteria on the surface of the cellphone before cleaning and changes in biochemical reactions according to the color produced. The results are then compared with the characteristics of the bacteria and their biochemistry table (Goodfellow *et al.*, 2012).

Processing and analysis of data

1. Data processing

This research uses primary data, namely data obtained directly by examining the number of colonies growing on PCA media using the *Colony Counter tool*.

2. Data analysis

The data analysis used in this research was the Wilcoxon test by looked at the differences in data on the number of colony growth that came from cellphone swab results.

The Wilcoxon test is a comparative or difference test if the

data scale for the two variables is quantitative (interval or ratio) and the data is not normally distributed $P < 0.05$.

RESULTS

The examination material that has been cultured and incubated is then identified using microscopic, cultural, and biochemical tests to identify bacteria that grow on isolated cellphones that have not been disinfected using 70% alcohol. The observation results can be seen in figure 1, table 1, and table 2.

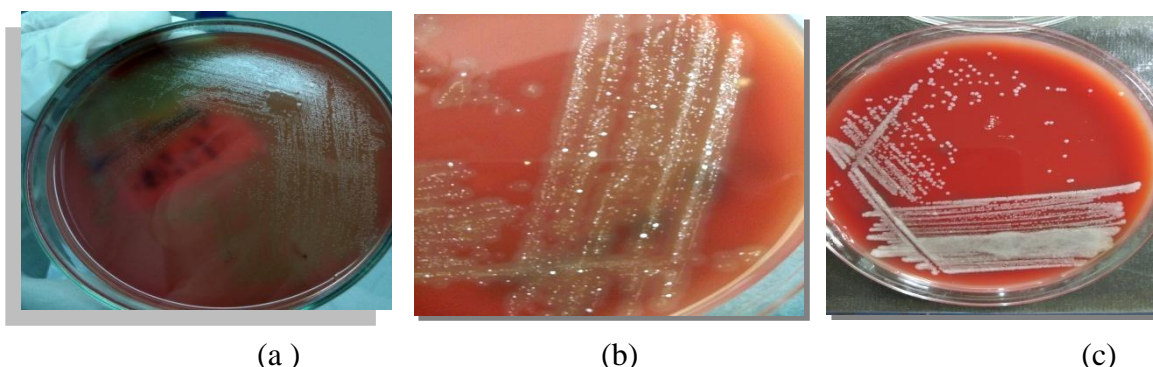


Figure 1. Bacterial hemolysis activity on BAP media (a) alpha hemolysis (b) beta hemolysis (c) gamma hemolysis

Table 1 . Results of macroscopic observations of bacterial growth on BAP media

No	Media	n	%
1	Alpha Hemolysis	18	40.1
2	Beta Hemolysis	11	24.4
3	Gamma Hemolysis	16	35.5
Total		45	100

Table 2. Results of bacterial identification based on microscopic morphology

No	Type	n	%
1	Gram (+) coccus	32	71.1
2	Gram (+) bacilli	10	22.2
3	Gram (-) bacilli	3	6,8
Total		45	100

Macroscopic observations in Table 1 by looking at the morphology of bacterial growth and hemolysis activity

in BAP media showed that 18 (40.1%) alpha hemolysis, 11 (24.4%) beta hemolysis, 16 (35.5%) gamma

hemolysis while observations microscopically, according to Table 2, it can be seen that 32 (71.0%) of the isolated samples were gram-positive coccus bacteria, the appearance of the field of view in the microscope was round purple, 10 (22.2%) were gram-positive rod bacteria (purple rod colored bacteria), and 3 (6.8%) of them were gram-negative bacilli (red rod colored bacteria).

After microscopic identification, biochemical tests are continued to see the genus of the bacteria that has been isolated. Based on the results of microscopic identification, 32 specimens were gram (+) coccus bacteria, then the catalase test was continued to differentiate whether these bacteria were *Micrococaceae* or *Streptococcus*. A total of 32 samples were obtained with positive catalase, indicating that 32 isolates belonged to the *Micrococaceae* family and continued with the *Tryptone Yeast Extract Agar* test. This test was used to differentiate between the *Micrococy* and *Stahphlococcy* genera. A total of 32 samples produced positive TYEA showing that 32 isolates were *Staphylococcus sp*. Identification was carried out only until determining the genus for gram-positive coccus bacteria, due to limited funds to continue until

knowing the species of bacteria isolated from cellphone swabs.

Against gram-positive bacteria, bacilli are followed by biochemical tests using carbohydrate media (glucose, lactose, mannitol, sucrose, and maltose), Methyl red, Voges Proskauer, SIM (Sulfur Indole Motility), Urea, Simon citrate, TSIA (Triple Sugar Iron Agar), decarboxylation media, and phenylalanine. Gram-negative bacilli were identified by biochemical tests using IMVIC media (Indol, Motility, Voges Proskauer, Indol, and Simon citrate). The IMVIC test is a simple biochemical test used to identify *Escherichia coli*.

Based on the results of the biochemical tests carried out, a description of the microorganisms obtained from cellphone swabs that had not been disinfected with 70% alcohol was obtained in Table 3. There were 32 isolates (71.1%) of gram-positive coccus bacteria, namely *Staphylococcus* spp. There were 10 isolates of gram-positive bacteria, namely *Bacillus sp* (22.2%), and the remaining 2 isolates (4.4%) were gram-negative bacteria, namely *Escherichia coli* obtained from cellphone codes 12 and 15, and 1 isolate of *Klebsiella pneumonia* (2,3%) isolated from mobile phone code HB-5.

Table 3. Results of bacterial identification from cellphone swabs

No	Type	n	%
1	<i>Staphylococcus spp</i>	32	71.1
2	<i>Bacillus sp</i>	10	22.2
3	<i>Escherichia coli</i>	2	4.4
4	<i>Klebsiella pneumoniae</i>	1	2,3
Total		45	100

In this study, colony growth was observed from cellphone swabs that had not been disinfected using 70% alcohol, and on cellphone swabs that had been disinfected, colonies were then counted using a *Colony counter*. From the calculation results, it was found that the average number of bacteria from cellphone isolates that had not been disinfected was 60 CFU/ml, while the number of colonies that grew from Pondel isolates that had been disinfected with 70% alcohol was 4 CFU. The data from bacterial colony counts was then analyzed using statistics.

The normality test is carried out at the beginning so that researchers know what statistical tests will be used. The normality test using the *Kolmogorov-Smirnov test* obtained a sig value. which shows a P value <0.05, this indicates that the data is not normally distributed. After the Normality Test is carried out, the researcher then describes the data that has been processed.

Based on the descriptive data results obtained, the concentration of data on the number of bacteria isolated from cellphones that had not been disinfected using 70% alcohol cotton was 60 and the data distribution was a minimum of 25 and a maximum of 155. Meanwhile, for cellphones that had been disinfected with 70% alcohol cotton, the data concentration was 4. The minimum data distribution was 0, and the maximum was 12.

The hypothesis test used was the Wilcoxon Signed Rank Test and obtained a Z value of -5.843 with a p-value of 0.0001 where this p-value is less than the critical value limit (Asymp. Sig 2 tailed = 0.05) so that the hypothesis H1 is accepted and H0 is rejected. These results state that there is a significant difference between the number of bacterial colonies that grow from cellphone swabs that have not been disinfected with 70% alcohol and the number of colonies that grow

from cellphone swabs that have been disinfected with 70% alcohol.

DISCUSSION

The microorganisms on cell phones are very diverse, one of which is often found in *S. aureus*. *S. aureus* is a pathogenic bacteria that is often found in humans both from the community and in nosocomial infections. According to Karkee et al., (2017) *S. aureus* is one of the normal flora bacteria that is often found on the skin, making it susceptible to becoming a pathogenic bacteria.

Normal flora in parts of the body is not always beneficial, under certain conditions normal flora can cause disease. Based on the type, normal flora is divided into 2, namely resident flora and transient flora (contaminated flora). Resident flora are microorganisms that are consistently present on human hands and are not easily removed by mechanical friction because they have adapted to human hands, while transient flora are transit flora or contamination flora whose type depends on the environment where you work, these germs are easily removed by washing your hands thoroughly. effective, for example; *Staphylococcus aureus*, *Streptococci*, *Pseudomonas*, *E. Coli*, and *Bacillus sp* (Sanders et al., 2016).

The moist and warm surface of a cellphone is a good area for the growth of bacteria, based on previous research, it is stated that several bacteria have been isolated from cellphones, namely: *S. aureus* (positive coagulation), *Staphylococcus* coagulase-negative, *Bacillus sp*, *Micrococcus sp*, *Streptococcus sp*, *E. coli*, *Klebsiella sp*, *Pseudomonas sp* (Rahman et al., 2018). A study at one of the South Korean State Teaching Hospitals stated that the use of touch screen cellphones found more pathogenic bacteria compared to cellphones with buttons.

From the results of this research in table 3 it can be seen that the identification results taken from cellphone samples that had not been cleaned with 70% alcohol, namely that the most bacteria were found to be *S.aureus* 32 (71.1%), then *Bacillus* sp 10 (22.2%) was found.), *E.coli* 2 (4.4%), *K.pneumoniae* 1 (2.3%). These results are in line with research conducted by Karkee et al., (2017) and research by Rahman et al., (2018) that the most common bacteria found was *S.aureus* (Karkee *et al.*, 2017). Based on the results of this study, it shows that cellphones have the potential to be a vector or medium for transmitting microorganisms if the user does not regularly wash their hands, clean the cellphone and does not avoid using cellphones at the same time (Onwubiko et al., 2015).

Although no prevalence shows the morbidity or mortality rate due to disease transmission through cell phones, the discovery of the above bacteria on cell phones allows the transfer of disease-causing bacteria to humans (KUMAR et al., 2021). As reported by the CDC in Connie R. Mahon's research (2019), *S.aureus* is the cause of post-surgical wound nosocomial infections and bloodstream nosocomial infections in North America, Latin America, and Europe. *S. aureus* is also the cause of toxic shock syndrome, pneumonia, thrombophlebitis, abscesses internal organ infections, and many others (Mohammed et al., 2019). *Bacillus* itself is a bacterium that can form spores even though it is included in normal flora bacteria, but in conditions or by pathogenic bacillus it can cause diseases such as *Bacillus anthracis* and *Bacillus cereus* (Campista-León et al., 2020). *E.coli*, which is also included in the normal flora bacteria in the colon and provides benefits to humans in preventing the colonization of pathogenic bacteria in

human digestion, can also cause disease, namely diarrhea also known as diarrheogenic *E. coli* (Shah et al., 2013).

Hands are a part of the body that often comes into contact with various objects. Therefore, hand hygiene is something that needs to be considered to prevent the spread of microorganisms. Hand hygiene is the most important basic technique in infection prevention and control. Based on research washing hands can reduce the number of germs by 90% and the number of bacteria will return a lot after 8 hours where the number of normal flora bacteria on the skin will increase (Allegranzi et al., 2010).

CONCLUSION

There were 10 isolates of gram-positive bacteria, namely *Bacillus* sp (22.2%), and the remaining 2 isolates (4.4%) were gram-negative bacteria, namely *Escherichia coli*, which were obtained from cell phones code 12 and 15. *Klebsiella pneumonia* was 1 isolate (2.3 %) isolated from cell phone code 05 found in cell phone swab isolates that were not disinfected with alcohol and there were differences in the number of bacterial colonies on cell phones that were not disinfected using 70% alcohol with the disinfected 70% alcohol.

SUGGESTION

Further research will examine the bacteria found in the officers' hands laboratory to determine the potential for bacterial transmission from cell phones and research

MDR (multi-drug resistant) bacteria that contaminate cellphones among laboratory personnel.

ACKNOWLEDGMENTS

Thanks are given to Musi Charitas Catholic University, Faculty of Health Sciences, students, and students of the DIV Medical Laboratory Technology study program who have provided the opportunity so that research can be

carried out and to other parties who have contributed to this research.

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