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Identification of *Staphylococcus aureus* Bacteria on the Palms of Visitors to Panumbangan Health Center

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ABSTRACT

Background & Objective: Hand hygiene is one of the efforts to prevent and control the spread of bacteria that can cause infectious diseases. Nosocomial infections can be caused by gram-positive bacteria, such as *Staphylococcus aureus* which is the most dominant bacterium causing skin and soft tissue infections. The purpose of this study was to determine the presence of *Staphylococcus aureus* bacteria in the palms of visitors to the Panumbangan Health Center.
Method: This study is a descriptive observational study with a simple random sampling technique conducted from May to June 2022. Respondents in this study were 20 health center visitors. Sampling was carried out at the Panumbangan Health Center and the examination was carried out at the STIKes Muhammadiyah Ciamis Bacteriology Laboratory. The measurement methods used were gram staining, media culture, and biochemical tests. The instruments used were a microscope, TSB media, MSA media, blood agar media, $H2O2$, and citrate plasma. The data obtained from the examination results were presented descriptively in tabular form and explained in narrative.
Result: The results showed that 6 samples (30%) identified *Staphylococcus aureus*, 11 samples (55%) *Staphylococcus* epidermidis, and 3 samples (15%) suspected *Streptococcus sp*.
Conclusion: It can be concluded that 30% of *Staphylococcus aureus*, 55% of *Staphylococcus epidermidis*, and 15% of *Streptococcus sp*. were identified.

Keywords: Hands; Healthcare; *Staphylococcus aureus*.

Introduction

The Community Health Center is one of the agencies engaged in seeking health services for the community (Luthfia & Alkhajar, 2019). Everyone has the right to obtain health services from various agencies that provide

health services. Health service standards The Community Health Center (Puskesmas) is one of the health service facilities that the community trusts as a step toward getting first aid (Sanah, 2017).

Apart from being a place for treatment, health centers or other healthcare facilities can also be a source of disease originating from patients or visitors who have carrier status. The source of the disease can be caused by microorganisms, one of which is bacteria. Disease bacteria can live and develop in the health care environment, such as air, water, medical equipment, or non-medical objects. The main cause of death in some countries today is caused by infectious diseases related to health services, referred to as nosocomial infections (Irdan, 2018).

The health service becomes a meeting place for some people in it, interacting either directly or indirectly with patients, or visitors who visit patients who are being treated. This condition can be a risk factor for disease transmission, because patients and visitors carry the bacteria so that it spreads in the air, for example through sneezing, coughing, and talking. The incidence of infectious diseases obtained from health services is still a problem, where the mortality and morbidity rates are still relatively high (Fajriyah, 2015).

Healthcare-acquired infections are referred to as nosocomial infections, which are acquired in healthcare settings. The incidence of nosocomial infections worldwide is still increasing. According to the World Health Organization (WHO), 8.7% of patients experience healthcare-acquired infectious diseases, covering fifty-five hospitals in Southeast Asia, Europe, the Mediterranean, and the Pacific (Wahyuni, 2017).

In addition to medical staff, visitors can potentially become vectors of infection spread in health services. Other researchers stated that the spread of infection in health services can be through direct touch from the hands of visitors because pathogenic bacteria obtained from patients or visitors are higher at 39.6%. This shows that visitors have the potential to spread pathogens of nosocomial infections (Randan & Sihombing, 2020).

Hand hygiene is one of the steps taken to prevent and control the spread of infection (Iswati, 2015). Hand washing is the most effective way to reduce the incidence of nosocomial infections. Washing hands using water (without soap) can reduce the number of bacteria by 23% while washing hands with water and soap can reduce the number of bacteria to 8% (Hertina et al., 2019).

Nosocomial infections can be caused by gram-positive bacteria, such as *Staphylococcus aureus* which is the most dominant cause. *Staphylococcus aureus* can cause skin and soft tissue infections to deeper infections such as sepsis, pneumonia, endocarditis, and bacteremia. *Staphylococcus aureus* can be found in the community and is always present in the healthcare environment which is the cause of nosocomial infections (Erlin et al., 2020).

Panumbangan Health Center receives health services related to infectious diseases, such as HIV, syphilis, and pneumonia. With these conditions, it is necessary to be vigilant for themselves and related agencies to prevent transmission to medical staff, and visitors. The health center already has a place to wash hands, and an appeal to wash hands in the form of a poster before entering the health center.

The presence of visitors in the health center can be one of the factors causing disease transmission and the risk of contracting diseases obtained from patients, or the health center environment. Hand hygiene by washing hands with soap is one method of controlling the spread of infection. In the

Puskesmas environment, hand washing behavior is still not going well. After observing several visitors to the Panumbangan Health Center, it was found that visitors did not wash their hands properly, where hands as a transmission transfer medium are very influential in increasing disease transmission.

For every healthcare institution, the implementation of an infection prevention and control system is expected to be able to maintain and protect people with low immune systems and healthy people. Everyone is expected to have self-awareness and behavior changes about the importance of maintaining hand hygiene by washing hands to prevent and protect people from exposure to pathogenic bacteria.

Objective

The purpose of this study was to determine the *Staphylococcus aureus* bacteria on the palms of visitors to the Panumbangan Health Center.

Method

This study is a descriptive observational study with a simple random sampling technique conducted from May to June 2022. Respondents in this study were 20 health center visitors. Sampling was carried out at the Panumbangan Health Center and the examination was carried out at the STIKes Muhammadiyah Ciamis Bacteriology Laboratory.

The measurement methods used were gram staining, media culture, and biochemical tests.

The instruments used were a microscope, TSB media, MSA media, blood agar media, $H202$, and citrate plasma.

The implementation procedure consists of pre-analytical, analytical, and post-analytical stages. The pre-analytical stage is (1) sterilization of tools and materials, and (2) making media (TSB media, MSA, blood agar, control, and 3% hydrogen peroxide). The analytical stage includes: (1) the first day, namely taking specimens and planting on TSB media; (2) the second day, namely reading TSB media, planting on MSA media; (3) day three, namely reading MSA media, gram staining, Catalase Test, Coagulase Test and planting on Blood Agar media; and (4) on day four, namely reading the results of growth on Blood Agar media.

Results

The results of the study can be seen in Table 1 below:

TABLE 1 Identification Results on Various Media

Sample Code	TSB	Macroscopic (MSA)	Microscopic (Gram)	Catalase/Coagulase Test	Suspected Bacteria
A	(+) Cloudy	Round, convex, white, NMF.	Coccus, chains, purple, gram (+)	(-)/(-)	*Streptococcus. sp*
B	(+) Cloudy	Round, convex, yellow-gold, MF.	Coccus, wine, purple, gram (+)	(+)/(+)	*Staphylococcus aureus*
C	(+) Cloudy	Round, convex, white, NMF.	Coccus, chains, purple, gram (+)	(-)/(-)	*Streptococcus. sp*
D	(+) Cloudy	Round, convex, white, NMF.	Coccus, chains, purple, gram (+)	(-)/(-)	*Streptococcus. sp*
E	(+) Cloudy	Round, convex, yellow-gold, MF.	Coccus, wine, purple, gram (+)	(+)/(+)	*Staphylococcus aureus*
F	(+)	Round, convex,	Coccus, wine,	(+)/(-)	*Staphylococcus*

Sample Code	TSB	Macroscopic (MSA)	Microscopic (Gram)	Catalase/Coagulase Test	Suspected Bacteria
	Cloudy	white-gray, NMF.	purple, gram (+)		*epidermis*
G	(+)	Round, convex,	Coccus, wine,	(+)/(+)	*Staphylococcus*
	Cloudy	yellow-gold, MF.	purple, gram (+)		*aureus*
H	(+)	Round, convex,	Coccus, wine,	(+)/(+)	*Staphylococcus*
	Cloudy	yellow-gold, MF.	purple, gram (+)		*aureus*
I	(+)	Round, convex,	Coccus, wine,	(+)/(-)	*Staphylococcus*
	Cloudy	white, NMF.	purple, gram (+)		*epidermis*
J	(+)	Round, convex,	Coccus, wine,	(+)/(-)	*Staphylococcus*
	Cloudy	white-gray, NMF.	purple, gram (+)		*epidermis*
K	(+)	Round, convex,	Coccus, wine,	(+)/(+)	*Staphylococcus*
	Cloudy	yellow-gold, MF.	purple, gram (+)		*aureus*
L	(+)	Round, convex,	Coccus, wine,	(+)/(-)	*Staphylococcus*
	Cloudy	white-gray, NMF.	purple, gram (+)		*epidermis*
M	(+)	Round, convex,	Coccus, wine,	(+)/(-)	*Staphylococcus*
	Cloudy	white-gray, NMF.	purple, gram (+)		*epidermis*
N	(+)	Round, convex,	Coccus, wine,	(+)/(-)	*Staphylococcus*
	Cloudy	gray, NMF.	purple, gram (+)		*epidermis*
P	(+)	Round, convex,	Coccus, wine,	(+)/(+)	*Staphylococcus*
	Cloudy	yellow-gold, MF.	purple, gram (+)		*aureus*
Q	(+)	Round, convex,	Coccus, wine,	(+)/(-)	*Staphylococcus*
	Cloudy	white, NMF.	purple, gram (+)		*epidermis*
R	(+)	Round, convex,	Coccus, wine,	(+)/(-)	*Staphylococcus*
	Cloudy	white-gray, NMF.	purple, gram (+)		*epidermis*
S	(+)	Round, convex,	Coccus, wine,	(+)/(-)	*Staphylococcus*
	Cloudy	white, NMF.	purple, gram (+)		*epidermis*
T	(+)	Round, convex,	Coccus, wine,	(+)/(-)	*Staphylococcus*
	Cloudy	white, NMF.	purple, gram (+)		*epidermis*
C (+)	(+)	Round, convex,	Coccus, wine,	(+)/(+)	*Staphylococcus*
	Cloudy	yellow-gold, MF.	purple, gram (+)		*aureus*
C (-)	(-)	No growth	(-)	(-)/(-)	(-)
	Clear	colonies, and no change in the media			

Note:
(+) = Positive Gas, Lumps
(-) = Negative Gas, Lumps
MSA = *Mannitol Salt Agar*

Based on Table 1 after incubation at 37 ° C for 18-24 hours, the results of planting on TSB media all samples turned cloudy, then planting on MSA media and gram staining. In this study, out of 20 samples planted on MSA media, 6 samples were suspected positive for *Staphylococcus aureus* bacteria and experienced a change in color from pink to yellow, with round, golden yellow colonies, and 14 other samples did not experience a change in color on the media with round, white-gray colonies.

Gram staining was carried out with the result that 17 samples were gram-positive with predominantly round colonies (coccus) clustered like grapes and purple, and 3 samples were gram-positive with round

chain-shaped colonies. Furthermore, the catalase test was carried out on the colonies, the results obtained from 20 samples as many as 17 samples were positive for the catalase test characterized by forming gas bubbles, and the other 3 samples did not produce gas bubbles.

A coagulase test was performed to determine the bacterial species, 6 samples were positive for the coagulase test by producing clots on citrate plasma and 14 other samples did not produce clots. On MSA media that is positive, there is a yellow color change, then planting is carried out on Blood Agar media with the results obtained in the following table:

TABLE 2 Macroscopic Results of Planting on Blood Agar Media

Sample Code	Macroscopic Blood Agar Media (Shape, color)	Type
A	-	-
B	Round, ash-yellow	*α hemolytic*
C	-	-
D	-	-
E	Round, ash-yellow	*α hemolytic*
F	-	-
G	Round, ash-yellow	*α hemolytic*
H	Round, ash-yellow gold	*β hemolytic*
I	-	-
J	-	-
K	Round, ash-yellow	*α hemolytic*
L	-	-
M	-	-
N	-	-
O	-	-
P	Round, white- grayish	*α hemolytic*
Q	-	-
R	-	-
S	-	-
T	-	-

Note:
α = Alpha *β = Betha* - = no planting

Planting on blood agar media is done to determine the ability of *Staphylococcus aureus* bacteria to hemolyzes blood. In Table 2 of the 20 samples, 6 samples that were suspected positive for *Staphylococcus aureus* bacteria were planted on AD media, and the other 14 samples were not planted. Then the results obtained from planting on Blood Agar media showed that the bacterial colonies that grew were round, with the color of the colonies dominated by a gray-yellowish color. The nature of bacteria in the media from the 6 samples studied, 5 samples were α hemolytic, namely the ability of bacteria to partially lyse erythrocytes in the media, characterized by a partial clear zone around the colony, and 1 other sample was β hemolytic, namely bacteria can lyse complete erythrocytes with a clear zone around the colony.

Discussion

Based on the results of the research that has been carried out, on 20 samples of palm swabs of visitors to the Panumbangan Health Center and cultured in various media, the results show the presence of bacterial growth. This is based on direct observation, that the growth of *Staphylococcus aureus* bacteria occurs due to poor personal hygiene of visitors, along with the condition of hands and nails that are not maintained healthily so the results of the study obtained 6 samples that were positive for Staphylococcus aureus bacteria, 11 samples suspected of growing *Staphylococcus epidermidis* bacteria, and 3 other samples suspected of *Streptococcus sp*. bacteria.

The sample results showed positive *Staphylococcus aureus* bacteria, this can occur because visitors who come to the Panumbangan Health Center come from various villages, along with their information in one day rarely do hand hygiene. Lack of personal hygiene can be a factor in the ease of hand contamination by bacteria. Hands as a medium for spreading various microorganisms, can be easily contaminated by bacteria that can be obtained from several

factors such as holding items whose conditions are less sterile, and can also be from the air.

Normally, bacteria develop according to their habitat, for example, Staphylococcus aureus is a normal flora bacteria in the nasal mucosa and perineum. The transfer of these bacteria from their place of origin to the palm can occur due to their habit of holding in the area. This is probably why *Staphylococcus aureus* was found in this study. This bacterium is one of the species that can cause infectious diseases and is the most pathogenic bacteria in humans.

Based on Table 1 after inoculation on various media obtained results, the presence of bacteria is indicated by changes in TSB media to become more turbid. This turbidity occurs because bacteria can ferment carbohydrates in the media. After culturing on TSB media for 24 hours, the samples were then cultured on MSA (Mannitol Salt Agar) media. Planting on MSA media resulted in all samples from palm swabs showing colony growth.

The results of Table 1 obtained the results of 6 samples out of 20 samples that showed the growth of colonies surrounded by yellow zones, and 14 other samples did not show any yellow zones around the colonies. *Staphylococcus aureus* can form lipochrome pigments that produce yellow colonies. On MSA media, colony growth is surrounded by a yellow zone, due to the ability of bacteria to ferment mannitol (mannitol fermenter) and produce acid, which turns phenol red on agar yellow. Bacteria that are unable to ferment mannitol will show a red or pink zone around the colony (Dewi, 2013).

Based on Table 4.1, the gram-staining results of all samples from palm swabs are gram-positive. Bacterial colonies that grow on MSA media, 17 samples show gram-positive bacteria that are purple and round clustered like grapes, and 3 other samples are gram-positive purple with round chain-shaped colonies. In gram staining *Staphylococcus aureus* is a gram-positive bacterium and is coccus-shaped, purple in color, and arranged in clusters like grapes. This purple color is because the bacteria retain the first dye, namely crystal violet (Sabudi & Hendrayana, 2017).

Positive catalase test results are indicated by the presence of gas bubbles (O_2) produced by *Staphylococcus sp*. The results of the catalase test in Table 2 as many as 17 samples out of 20 samples showed positive results by forming gas bubbles. A positive catalase test occurs because *Staphylococcus sp*. bacteria produce the enzyme catalase and can hydrolyze hydrogen peroxide (H_2O_2) into water (H_2O) and gas bubbles (O_2) (Toelle, 2014).

The coagulase test obtained positive results indicating the formation of clots on the glass object. The results obtained in this study showed positive results for *Staphylococcus aureus* in as many as 6 samples. The coagulase test aims to determine the ability of bacteria to produce coagulase enzymes and can coagulate plasma. Positive coagulase reaction to distinguish *Staphylococcus aureus* from other species of *Staphylococcus sp*. (Dewi, 2013).

On blood agar media at the time of 24-hour incubation, there was no visible hemolysis zone, after incubation for 48 hours a hemolysis zone was formed around the colony. The hemolysis zone is formed due to the hemolysin toxin produced by *Staphylococcus aureus*. In the research that has been done, positive samples of *Staphylococcus aureus* are planted on blood agar media and the results of growing colonies appear ash to yellowish, convex, shiny surfaces and are alpha-hemolysin, which produces a partially clear hemolysis zone around the colony.

Maintaining hand hygiene by washing hands is a form of prevention from being

contaminated by bacteria on the palms of the hands, so it is recommended to always maintain hand hygiene before and after doing something. Hand washing is an activity to remove dirt and reduce bacteria on the hands by rubbing hands using water and soap, as well as alcohol-based materials (hand sanitizers) (Hasanah & Mahardika, 2021). Thus, washing hands with soap, in addition to being able to avoid various bacterial contaminants that stick to the hands, the content in soap that has a bond between sodium and potassium with high fatty acids that are germicidal can inhibit bacterial growth and easily detach from the skin.

In this study, hands as an extrinsic factor that can cause nosocomial infections in the Panumbangan Health Center health service where the majority of patients have low immunity. The results of the study obtained from palm swabs of visitors to the Panumbangan Health Center were positive for *Staphylococcus aureus* bacteria in as many as 6 samples (30%), suspected *Staphylococcus epidermidis* bacteria in as many as 11 samples (55%), and 3 other samples (15%) suspected *Streptococcus sp*. The number of bacteria obtained, from bacterial isolation on TSB media, and then identified by culture on MSA media, Blood Agar, and Catalase-coagulase test.

Conclusion

It can be concluded that as many as 30% identified *Staphylococcus aureus* bacteria, 55% *Staphylococcus epidermidis*, and as many as 15% suspected *Streptococcus sp*. For further research, it is hoped that conducting bacterial examinations of other species in health services before washing hands and after washing hands and conducting bacterial resistance tests on the palm.

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Conflict of Interest

There is no conflict of interest in this study.

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