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Research Article

Antagonism Test Of Isolate Normal Flora On Palms Against Staphylococcus Aureus And Staphylococcus Epidermis

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ABSTRACT

Background: The body's normal flora is a form of the body's defense against initial infection, namely the attachment and multiplication of pathogenic bacteria. This research aims to examine the antagonistic of the normal flora in human palm against Staphylococcus aureus and Staphylococcus epidermis.
Methods: This research consisted of two steps. Step I. Isolation of normal flora bacteria on human palms and Step II is Antagonistic test between normal flora isolates against Staphylococcus aureus and Staphylococcus epidermis which carried out separately using disc diffusion to determine the diameter of the inhibition zone formed.
Results: A total of 8 isolates were successfully isolated and 4 of them were able to form an inhibition zone against the test bacteria.
Conclusion: MB1.2 isolate is the most potential for further research as an antagonism antibacterial agent against Staphylococcus aureus and Staphylococcus epidermis.

Key words: Antagonistic, normal flora, Staphylococcus aureus, Staphylococcus epidermis

INTRODUCTION

Infectious diseases in humans are caused by the entry of a pathogenic microbe into the body and multiplies causing illness and even death (1). Many pathogenic microorganisms are found in food, drinks, the environment or on the surfaces of objects around us. Examples of pathogenic microbes that cause infections and diseases that are commonly found in Indonesia are Staphylococcus aureus which causes skin diseases such as blains or impetigo and Staphylococcus epidermis which causes acne.

Patients with skin diseases in Indonesia are still relatively high because not many people are aware of personal hygiene. As with acne sufferers in Indonesia, it shows an increase from year to year. 60% of acne vulgaris sufferers in 2014, 80% in 2015 and 90% in 2016. Staphylococcus aureus and Staphylococcus epidermis are pus-forming microbes which are responsible for the development of various acne and skin abscesses. Although acne vulgaris is not life threatening, it can cause serious social and psychological problems for sufferers (2).

The mechanism for pathogenic microbes to cause disease begins with attachment, multiplication, avoiding host immunity, entry into the target tissue/cell then destruction of the target cell causing disease (3). If an infection or disease has occurred, antibiotics are usually given starting from topical to oral depending on the level disease severity and cells/tissues affected.

However, the current use of antibiotics is less than optimal due to antibiotic resistance in bacteria. Normal flora are microorganisms that naturally live in the human body to balance and filter other harmful microorganisms (4).So that in this study the natural mechanisms of self-defense from the presence of transient flora and normal flora of the human palm will be investigated to prevent the growth of pathogenic bacteria that these pathogenic bacteria cannot cause infection and for future can utilize compounds present in normal flora as candidates for antibiotic compound.

METHOD

This study consisted of two stages: Stage I. It consisted of 2 series of bacterial isolates namely Staphylococcus aureus and Staphylococcus epidermisvand series 2 was by isolate of bacteria normal flora on human palms. Stage II. Antagonistic test between normal flora isolates and Staphylococcus aureus and Staphylococcus epidermis which were carried out separately.

The source of sample came from female and male humans with an age range of 17-30 years with characteristics of being healthy or not in a sick state (measured by body temperature parameters) in the STIKes Ngudia Husada Madura Health Analyst Study Program. The number of samples used is 30 samples. In the test, a positive control was used for the antibiotic ciproflaxim, The treatment was repeated 2x repetitions.

Isolation of normal flora bacteria of human palm

A participant who has been selected is asked to wear a lab coat, mask and remove all accessories in the finger area and then wash his hands according to the correct hand washing rules. This is done to select transient bacterial flora on the palms of the hands. After washing their hands, participants were asked to dip their hands into a basin and sterile aquadest located in Enkas. The results of the participants' soaking hands would then be homogenized using a vortex and taken as much as 10 ml which was diluted to 10-7(5). Then duplo planting was carried out on

sodium agar media by pour plate method and incubation for 24 hours. Observation of morphological characteristics including colony shape, edges and colony color (6). Each different colony was recruited on NA media that had been prepared in a petri dish.

Bacterial Antagonistic Test

Screening for antagonistic activity of isolates of normal flora of the human palm flora was carried out by testing the normal flora of the human palm against two bacteria Staphylococcus Aureus and Staphylococcus Epidermis. Antibacterial activity screening method was carried out based on the method from Wismayanti et al (2019) (7), namely through the agar overlay method and confirmed by the agar diffusion method.

Antibacterial Activity Test of Human Palm Normal Flora Isolates

Antibacterial activity was carried out after the potential isolate screening method. Potential isolates were cultured on Mannitol Salt Agar (MSA) and incubated for 24 hours. After incubation, centrifugation was carried out at 3,500 rpm for 10 minutes. The formed supernatant was transferred to a sterile tube to test the antibacterial activity (7).

As much as 100 microliters of pathogenic bacterial culture which had previously been incubated for 24 hours was dripped and spread over a petri dish filled with MSA media with a spreader. 5 ml of the bacterial supernatant was taken and dripped onto a paper disc, then the paper disk was placed over the MSA, each of which contained Staphylococcus aureus and Staphylococcus epidermis and incubated for 24 hours. The results obtained were analyzed descriptively.

DISCUSSION

The results obtained were carried out by observing the morphology of each isolated colony. Each colony has a different shape and growth. This is because each bacterium has its own morphological characteristics so that characteristics can be distinguished between individuals and between species (8). Morphological characteristics observed included colony shape, color, margins, cell surface shape and cell coupling type. Based on observations, bacterial colonies were obtained with curved, raised and irregular characteristics. While the shape of the edges of the bacteria varies from irregular, wavy and notched.

Isolation of the most dominant normal flora of the human palms resulted in 8 dominant isolates. The antagonistic results against Staphylococcus aureus and Staphylococcus epidermis showed that only 4 isolates had activity against the tested bacteria which was indicated by the appearance of an inhibition zone around the paper disk area (Table 1). Just as the shape of cells and bacterial colonies varied, the adaptability of bacteria also varied, so that this was the reason why only 4 isolates were able to show growth and resistance to antagonistic bacteria (5)

Tabel 1. Screening of antagonistic activity of normal palm flora isolates against Staphylococcus Aureus and Staphylococcus Epidermis bacteria

No	Kode Isolat	*Staphylococcus Aureus*	*Staphylococcus Epidermis*
1	MB1.1	-	+
2	MB4.2	-	+
3	MB3.1	+	+
4	MB2.1	-	-
5	MB1.2	+	+
6	MB6.1	+	+
7	MB3.2	-	-
8	MB7.2	+	+

Notes: (+) there is antibacterial activity; (-) There is no antibacterial activity.

The effectiveness of the normal bacteria found in the palm flora was seen through the antagonistic bacteria that were successfully isolated from pathogenic bacteria. The method used is cross scratch method. Bacteria that give a positive response will form an inhibition zone (8) (Table 2).

No	Isolate Code	Inhibition zone diameter (mm)	
		Staphylococcus aureus	***Staphylococcus epidermis***
1	Control	20,56	15,38
2	MB3.1	3,22	6,89
3	MB1.2	5,77	9,33
4	MB6.1	4,93	2,31
5	MB7.2	1,43	4,83

RESULTS

Control using Ciprofloxacin resulted in the largest diameter of the inhibition zone. This is because ciprofloxacin is indeed an antibiotic that is bacteriostatic. The mechanism of action of Ciprofloxacin is to inhibit the bacterial DNA replication process.

The four isolates showed a good response with the formation of clear zones due to the growth of these isolates. This is due to the fact that the metabolites produced are strong enough to inhibit Staphylococcus aureus and Staphylococcus epidermis.

While the ability of the four bacterial isolates is unknown whether their antibiotic properties are bacteriostatic or bacteriocid or just inhibit or interrupt pathogenic bacteria. It is known that there are two kinds of antagonism, namely true antagonism and vector antagonism. True antagonism occurs when an organism actually inhibits other organisms, while vector antagonism occurs when a type of bacteria actively inhibits other bacteria due to limited food and life (8). However, in this study the nature of the antagonism of the four isolates could not be confirmed.

The inhibition zone formed by normal flora bacteria was much smaller than the positive control of Ciprofloxain. This shows that the sensitivity of Staphylococcus aureus bacteria and Staphylococcus epidermis to antibacterial compounds has decreased due to the presence of antibacterial content in Ciprofloxain.

Inhibition of microbial growth is influenced by several factors including microbial count, concentration of antimicrobial compounds, microbial species, temperature, and the presence of other microbes (9). In addition, the activity of bacteria in inhibiting other bacteria is also influenced by the pH of the environment, the size of the inoculum (10). The speed of bacterial growth, the nature of the media, and the incubation conditions (11). Based on the description of Melia et al. (2019), there is a category of bacterial inhibition based on the inhibition zones formed around the wells, namely the inhibition zone of 1-3 mm has a weak inhibition potential, the inhibition zone of 3-6 mm is in the moderate category, and the inhibition zone > 6 mm is in the category of strong inhibition (12). It can be concluded that the results of the inhibition zones of the four bacteria were moderate except for isolates MB6.1 and MB6.2 which were weak.

From the results of the analysis of the inhibition zones formed, it can be concluded that MB1.2 isolate has the most potential for further research as an antigosim antibacterial agent against Staphylococcus aureus and Staphylococcus epidermis which requires further testing of the bacterial isolate.

The existence of antibiotics, in this case Ciprofloxain, cannot be replaced by normal flora defense mechanisms. Microorganism infections require antibacterial/antimicrobial compounds to be able to weaken/kill infections from microorganisms and we need to screen another potential compounds as new antibacterials.

CONCLUSION

A total of 8 isolates were successfully isolated and 4 of them were able to form an inhibition zone against the test bacteria. MB1.2 isolate is the most potential for further research as an antigosim antibacterial agent against Staphylococcus aureus and Staphylococcus epidermis which requires further testing of the bacterial isolate. The existence of antibiotics, in this case Ciprofloxain, cannot be replaced by normal flora defense mechanisms

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