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The growth of Staphylococcus aureus in the blood agar plate media of sheep blood and human blood groups A, B, AB, and O

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HIGHLIGHTS

Human blood can be used as a substitute for sheep blood to make BAP media to see the hemolysis type of *S. aureus* bacteria.

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A B S T R A C T / A B S T R A K

Blood agar plate media is a medium used to distinguish pathogenic bacteria based on their hemolytic power on red blood cells. *Staphylococcus aureus* is a bacterium that can emulate red blood cells with three types of hemolysis, namely α, β, γ, and δ. Usually, BAP media is made by adding 5-10% of sheep blood. Human blood has a substance similar to sheep's blood. The use of human blood as a substitute for sheep blood in making BAP media may be a solution, but it is not yet known whether there are differences in the growth and hemolysis of *S. aureus* bacteria on BAP media in sheep's blood and human blood. This research is an experimental study with a completely randomized design (CRD) of 3 replications which aims to determine whether there are differences in growth and hemolysis of bacteria *S. aureus* in BAP media of sheep blood and human blood groups A, B, AB, and O and using ANOVA for statistical analysis. The results showed that *S. aureus* bacteria could grow and show hemolysis in BAP media in sheep blood and human blood in groups A, B, AB, and O. The results of subsequent studies show a significant level of 0.05 it can be concluded that *S. aureus* bacteria can grow and show hemolysis in BAP media of sheep blood and human blood groups A, B, AB, and O, there are significant differences in the number of *S. aureus* bacteria colonies grown in BAP media of sheep's blood and human blood groups A, B, AB and O.

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1. INTRODUCTION

Staphylococcus aureus is one of the coccus gram-positive pathogenic bacteria. *S. aureus* can cause infection in humans or animals.[1] Almost all humans have been infected with *S. aureus* with varying degrees of severity. *S. aureus* is one of the most

important members of the group of potentially pathogenic bacteria (PPB).[2] These bacteria can also cause mild skin infections to severe life-threatening infections.[3] *S. aureus* causes disease in mammals because it produces several types of exoprotein.[4] *S. aureus* is also able to emulate red blood cells with four kinds of hemolysis, namely α, β, γ, and δ. The character of hemolysis displayed by *S. aureus* identified from broilers experiencing Bumblefoot and Arthritis showed 80% α-hemolysis, 10% β-hemolysis, and 10% γ-hemolysis. The *S. aureus* hemolysis type can be identified by culturing it in the Blood Agar Plate (BAP) media.[5]

BAP is one example of general, enriched and differential solid media because in the manufacturing process addition of defibrinated blood is carried out. Blood is a substance that is rich in nutrients so that most bacteria can grow in media containing blood. BAP media is used to distinguish pathogenic bacteria based on their hemolytic power on red blood cells. This enriched media supports the growth of many pathogenic organisms but at the same time allows for differential characterization of bacteria based on their hemolytic patterns.[6]

Generally, BAP media is made by adding defibrinated sheep blood. Blood must be defibrated or placed in a container containing anticoagulants to prevent freezing.[7] The blood agar media is made from the basal medium with the addition of 5-10% blood (defibrination) at a temperature of 50-60^{o}C.[8] For sheep's blood to be standard media as growth to identify the type of bacteria and as a medium for testing hemolysis from various pathogenic bacteria.

Sheep blood contains proteins, fats, and carbohydrates. Nutritional levels are influenced by nutritious supply. Glucose, protein, and triglyceride levels before and after eating experience different concentrations.[9] The results research showed that protein levels in serum blood of sheep had a significant difference in some feeding variations, whereas glucose levels in serum blood of sheep did not differ significantly in some feeding variations.[10] Normal adult sheep blood contains 9.0 - 11.1 erythrocytes, 11.6 13.0 hemoglobin, and 32.0 - 37.0 hematocrit.[9] The number of erythrocytes is also influenced by nutrition. The results of the study showed that vitamin E supplementation had a significant effect on the number of erythrocytes.[11] The presence of erythrocytes causes sheep's blood to be used as an additional material for BAP media which serves to see hemolysis.

Human blood also contains protein, fat, and carbohydrates from the absorption of human digestive results. Carbohydrates in the blood are obtained from the digestive process. After going through the mouth, stomach, and small intestine, carbohydrates enter the lymphatic fluid then into the capillary arteries and flow through the portae vein to the liver and which partially enters the large intestine.[12] Blood plasma is a solution containing albumin, anticoagulants, hormones, various types of proteins, and multiple types of salt.[13]

Human blood has a substance similar to sheep's blood. The use of human blood as a substitute for sheep blood in making BAP media may be a solution for health education laboratories which generally have difficulties in procuring sheep's blood. Human blood group O which has expired for five days and five days before expiration is proven to be used as a substitute for sheep's blood in making BAP media to grow *S. aureus*, *Streptococcus β-hemolytic, Streptococcus γ-hemoliticus, Streptococcus pneumonia, Vibrio El Tor and Clostridium perfringens* bacteria.[8] Blood that has been stored as a different quality with fresh blood, the longer the shelf life of blood, the quality of blood will decrease. Blood stored for longer will have fewer platelet counts.[14] The shelf life of blood also affects the number of erythrocytes, the longer the shelf life of blood, the lower the number of erythrocytes.[15] The use of stored blood or expired blood as a substitute for sheep blood in making BAP media can give different results to bacterial growth or the hemolysis.

Fresh human blood group O and AB have also been proven to be used as a substitute for sheep blood in making BAP media to grow *S. aureus* bacteria.[16] The use of fresh human blood groups A, B, AB, and O which are used as a substitute for sheep blood in making BAP media to grow *S. aureus* bacteria has never been reported, so it is not yet known whether there are differences in growth and hemolysis of *S. aureus* in BAP media in sheep blood and human blood groups A, B, AB, and O. Based on the background described, it is necessary to do research on the growth of *S. aureus* bacteria in BAP media of sheep blood and human blood groups A, B, AB, and O.

2. MATERIALS AND METHOD

This research is an experimental study that uses Completely Randomized Design (CRD) with three replications. The samples in this study were BAP media of sheep blood and human blood groups A, B, AB, and O used in research. The BAP media of sheep blood used in this study is BAP media purchased in the form of each plate, and the BAP media of human blood used in this study is BAP media that are made by themselves in the laboratory. Human blood is obtained from venous blood sampling of students participating in the practice of bacteriology. The results of the study were analyzed using ANOVA with a significance level of 0.05.

Research materials in this research are BAP media base (Merck), sheep blood, human blood groups A, B, AB, and O, pure culture of *S. aureus* bacteria, and standard solution Mc. Farland 0.05. This research containing three steps are a) Preparation of Bacterial Suspensions: a pure culture of S. aureus inoculated on sterile PZ was incubated for 1 x 24 hours. After the incubation period is complete, bacterial suspense is synchronized with MC Farland solution 0.05; b) Bacterial Culture: Bacterial suspense was inoculated on BAP media using the T streak method and then incubated 37^{o}C for 24 to 48 hours, and' c) Observation: after 24 hours the number of colonies was calculated, then the number of colonies on average. After 48 hours the calculation of the number of colonies and measurement of the hemolysis zone were calculated, then averaged.

3. RESULTS AND DISCUSSION

In this study, two outcomes were obtained, the number of bacterial colonies and hemolysis of *S.aureus* bacteria on BAP media of sheep blood and human blood of groups A, B, AB, and O.

3.1. Number of bacterial colonies

The growth of *S. aureus* bacteria on BAP media in sheep and human blood groups A, B, AB, and O after 24 hours incubation showed that the colonies looked smooth, round, small, separate, and white. At 48 hours incubation, the colony looks bigger. *S. aureus* colonies on BAP media with the addition of sheep blood appeared smaller and finer than *S. aureus* colonies grown on BAP media with the addition of human blood groups A, B, AB, and O. Summary of data on *S. aureus bacteria* colonies in BAP media with the addition of sheep blood and human blood groups A, B, AB, and O are presented in Table 1.

Table 1. Number of Colonies of *S. aureus* Bacteria in BAP Media with Addition of Sheep Blood and Human Blood Groups A, B, AB, and O

Blood Type in BAP Media	Amount of *S.aureus* Growth (Colony)			Average of *S. aureus* Growth (Colony)
	1	2	3	
Sheep Blood	128	130	127	128
A blood type	47	42	45	45
B blood type	30	31	33	31
AB blood type	34	49	43	42
O blood type	67	95	79	80

Table 1 shows that all BAP media of sheep blood and human blood in group A and B, AB, and O can be overgrown by *S. aureus*. This is because these bacteria get enough nutrients to support their growth. Carbohydrates and proteins as the main elements needed for bacterial growth are contained in various blood groups.[16] *S. aureus* does not require blood for its growth, but by adding blood to the media, it can add nutrients so that the bacteria will thrive and be able to show the hemolityc ability.[8]

Table 1 also shows the difference in the number of bacterial colonies that grow on BAP media in sheep blood and human blood in groups A, B, AB, and O. The number of bacterial colonies in BAP media in sheep blood has the highest number, with an average of 128 colonies. Summary of the results of data analysis using ANOVA test is presented in table 2.

Table 2. Summary of The Results of Data Analysis Using Anova

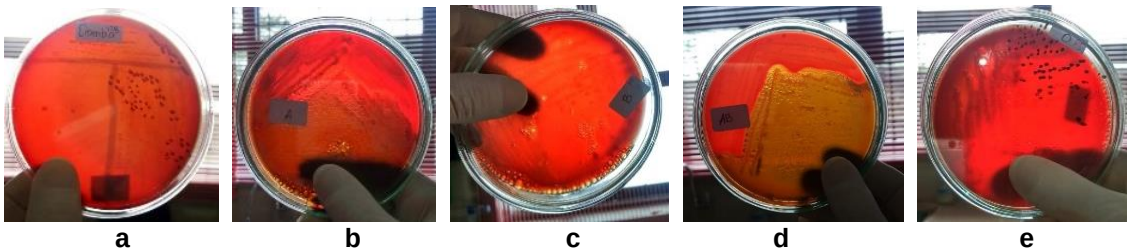
Test	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	18964.667	4	4741.167	89.344	0.0001
Within Groups	530.667	10	53.067		
Total	19495.333	14			

Based on the Table 2 shows that ANOVA test results obtained a significance value of 0.00 < 0.05 which means that there are significant differences in the number of *S.aureus* colonies grown on BAP media in sheep blood and human blood groups A, B, AB, and O. Variability in appearance, colony size, and hemolytic activity depends on blood agar media. Sheep blood is the blood standard used for making BAP media.[17] Sheep blood is an essential compound that is used to make blood agar media, and this media is a standard medium for isolating bacteria that can emit blood.[16] Human blood is not recommended for the culture of bacteria because it contains antibacterial and antibodies that can inhibit bacterial growth or cause false hemolysis.[18] Blood type O has the second highest number of *S. aureus* colonies. In its implementation, blood type O is often used as a substitute for sheep blood in making BAP media.

The results of this study prove that human blood can be used as a substitute for sheep blood as a material for making BAP media used to grow *S. aureus* bacteria. Health education laboratories need not be confused if they do not have stock of sheep blood, because they can use human blood instead.

3.2. Hemolysis

At the time after 24 hours incubation, there has not been seen a hemolysis zone. In the incubation period of 48 hours, a zone of hemolysis is formed, but the diameter of the hemolysis zone cannot be measured because it is too wide and joins one another. The hemolysis type of *S. aureus* in BAP media of sheep blood and human blood groups A, B, AB, and O all show β-hemolysis. They are shown in Picture 1.



a **b** **c** **d** **e**

Picture 1. Hemolysis of *S. aureus* in BAP media: a) sheep blood; b) A blood type; c) B blood type; d) AB blood type; and e) O blood type

Source: Turista and Puspitasari (2018)

In this study, the zone of hemolysis was apparent after 48 hours of incubation. The results of hemolysis obtained from all media are β-hemolysis where a clear zone forms around the colony. Sometimes the β hemolysis zone is not visible after a 24-hour incubation period and requires a more extended incubation period.[18] Hemolysis zone is formed in all BAP media, both in BAP media of sheep blood and BAP media in human blood. The hemolysis zone was formed due to the presence of hemolysin toxins produced by *S. aureus.*[19] Hemolysin is an exoprotein that has enzymatic activity and toxins.[20] *S. aureus* bacteria have four types of hemolysin. *S. aureus* produced four types of hemolytic toxins, namely α, β, γ, and δ.[21] α and β hemolysin are most important in determining the pathogenicity of infections.[22] β toxin is neutral sphingomyelinase produced by certain strains of *S. aureus.*[23] β hemolysin is produced in large quantity by several *S. aureus* and secreted into the culture medium as an exotoxin with a molecular weight of 35.000.[4]

The hemolysis zone appears most clearly and has the largest diameter found in BAP media in AB blood type of human, and is followed by human blood group A, B, O, and the zone of hemolysis at least seen in BAP media in sheep blood. The most significant hemolytic activity is seen in rabbit blood agar media, which is followed by human blood agar, and then the blood agar of other animals, including sheep.[17] The higher hemolysis zone in BAP media of human blood, when compared with BAP sheep blood, is related to the size of sheep blood erythrocytes, where sheep blood has erythrocyte size that is smaller than human blood. Substantially, the differences in morphology and composition of red blood sheep erythrocytes and human blood are different. Morphology of human erythrocytes has a diameter of 6 - 8 µm, whereas sheep blood erythrocytes have a diameter of 1 - 2.6 µm.[16] Hemolysis is a process of ruptured erythrocytes, so that the size of the erythrocytes affects the area of the hemolysis zone. The higher the size of the erythrocytes, the greater the hemolysis zone.

The results of this study prove that human blood can be used as a substitute for sheep blood to make BAP media used to see the hemolysis type of *S. aureus* bacteria. *S. aureus* cultured on BAP media that uses human blood, in general, has a clearer and larger hemolysis zone. Health education laboratories do not need to be confused if they do not have stock of sheep blood, because they can use human blood instead.

4. CONCLUSION

S. aureus bacteria can grow and show hemolysis in BAP media of sheep blood and human blood groups A, B, AB, and O. There are significant differences in the number of *S. aureus* colonies grown in BAP media of sheep's blood and human blood groups A, B, AB and O. This shows that human blood can be used as a substitute for sheep blood to grow and to know the hemolysis type of *S. aureus* bacteria.

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REFERENCE

1. Karlina, C. Y., Ibrahim, M., & Trimulyono, G. Aktivitas Antibakteri Ekstrak Herba Krokot (*Portulaca oleraceae* L) Terhadap *Staphylococcus aureus* dan *Escherichia coli*. *Lentera Bio.* 2013; 2 (1).
2. Gluck, U & Gebbers, J.O. Ingested Probiotics Reduce nasal Colonization With Pathogenic Bacteria (*Staphylococcus aureus, Streptococcus pneumoniae,* and β-hemolytic streptococci). *The American Journal of Clinical Nutrition*. 2003; 77 (2).
3. Lutpiatina, L. Cemaran *Staphylococcus aureus* dan *Pseudomonas aerogenosa* pada Stetoskop di Rumah Sakit. *Jurnal Teknologi Laboratorium*. 2017; 6 (2).

4. Dinges, M. M., Orwin, P. M., Schlievert, P. M., Biology, S., & The, O. F. Exotoxins of *Staphylococcus aureus*. *Clinical Microbiology Reviews*. 2000; 13 (1).
5. Khusnan, W. P. Identifikasi dan Karakterisasi Fenotipe *Staphylococcus aureus* Asal Kasus Bumblefoot dan Arthritis pada Broiler. *Jurnal Kedokteran Hewan*. 2012; 6 (2).
6. Yeh, E., Pinsky, B. A., Banaei, N., & Baron, E. J. Hair Sheep Blood, Citrated or Defibrinated, Fulfills All Requirements of Blood Agar for Diagnostic Microbiology Laboratory Tests. *Journal Plos One*. 2009; 4 (7).
7. Russell, F. M., Biribo, S. S. N., Selvaraj, G., Oppedisano, F., Warren, S., Seduadua, A.Carapetis, J. R. As Bacterial Culture Medium, Citrated Sheep Blood Agar is a Practical Alternative to Citrated Human Blood Agar in Laboratories of Developing Countries. *Journal of Clinical Microbiology*. 2006;44 (9).
8. Djannatun, T., Rochani, J. T., Wikaningrum, R., & Widiyanti, D. Pemanfaatan Darah Manusia yang Kadaluarsa Sebagai Pengganti Darah Domba dalam Pembuatan Media Agar Darah Plat (ADP). *Yarsi Medical Journal*. 2008; 16 (2).
9. Astuti, D. A., Ekastuti, D. R., Sugiarti, Y., & Marwah, M. Profil Darah dan Nilai Hematologi Domba Lokal yang Dipelihara di Hutan Pendidikan Gunung Walat Sukabumi. *Agripet*. 2008; 8 (2).
10. Retno Sri Wahjuni, Retno Bijanti, R. S. Profile Total Protein dan Glukosa Darah Domba yang Diberi Starter Bakteri Asam Laktat dan Yeast pada Rumput Gajah dan Jerami Padi. *Jurnal Ilmiah Kedokteran Hewan*. 2011; 4 (1).
11. Fassah, D.M & Khotijah, L. Pengimbuhan Vitamin E dalam Ransum Kaya Asam Lemak Tidak Jenuh Terhadap Profil Darah Induk Domba Lantasi. *Jurnal Veteriner*. 2016; 17 (3).
12. Siregar, N. S. Karbohidrat. *Jurnal Ilmu Keolahragaan*. 2014; 13 (2).
13. Mallo, P. Y., Sompie, S. R. U., Narasiang, B. S., & Bahrun. Rancang Bangun Alat Ukur kadar Hemoglobin dan Oksigen Dalam Darah dengan Sensor Oximeter Secara Non-Invasive. *Jurnal Teknik Elektro dan Komputer*. 2012; 1 (1).
14. Naim, Nurlia. Pengaruh lama Penyimpanan Darah Donor Terhadap Hasil Pemeriksaan Trombosit, Eritrosit dan Hemoglobin Pada Unit Transfusi Darah Rumah Sakit Umum Lasinrang Kabupaten Pinrang. *Media Analis.* 2016; V (1).
15. Tadjuddin Naid, Dzikra Arwie, dan F. M. Pengaruh Waktu Penyimpanan Terhadap Jumlah Eritrosit Darah Donor. *As-Syifaa*. 2012; 4 (1).
16. Woelansari, Evy Diah. Pola Pertumbuhan *Staphylococcus aureus* Pada Media Agar Darah Manusia Golongan O, AB, dan Darah Domba Sebagai Kontrol. *Jurnal Ilmu Dan Teknologi Kesehatan*. 2016; 3 (2).
17. Fabiano, M., Boriollo, G., Francisco, M., Netto, R., Júnior, J., Tadeu, C., & Höfling, J. F.. 2017. Performance Evaluation of Oxacillin-resistant Staphylococcus aureus Genotypes and Taxa on Human and Animal Blood Agar Culture Media. *African Journal of Microbiology Research.* 2017; 11(21).
18. Goldman, Emanuel & Green, Lorrence H. *Practical Handbook of Microbiology.* CRC Press: United States of America: 2009.
19. Herlina, Nina. Isolasi dan Identifikasi *Staphylococcus aureus* Dari Susu Mastitis Subklinis di Tasikmalaya, Jawa Barat. *Pros Sem Nas Masy Biodiv Indon.* 2015; 1 (3).
20. Ward, J. M., Williams, R. J., Henderson, B., Nair, S. P., Poole, S., Wilson, M., & O'Hara, B. P. Identification of a Novel Gene Cluster Encoding Staphylococcal Exotoxin-Like Proteins: Characterization of the Prototypic Gene and Its Protein Product, SET1. *American Society for Microbiology.* 2000; 68 (8).
21. Purnomo, A., Hartatik, Salasia, S. I. O., & Soegiyono. Isolasi dan Karakterisasi *Staphylococcus aureus* Asal Susu Kambing Peranakan Ettawa. *Media Kedokteran Hewan.* 2006; 22 (3).

22. Khusnan, W.P. Karakterisasi Faktor-Faktor Virulensi *Staphylococcus aureus* Asal Susu Kambing Peranakan Etawa Secara Fenotip dan Genotip. *Jurnal Sain Veteriner*. 2016; 34 (1).
23. Huseby, M., Shi, K., Kent Brown, C., Digre, J., Mengistu, F., Keun, S. S., Earhart, C. A. Structure and Biological Activities of Beta Toxin from Staphylococcus aureus. *Journal of Bacteriology*. 2007; 189 (23).