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LAMPIRAN

English Version

ISOLATION OF *Staphylococcus aureus* BACTERIA IN THE LEGS OF CHICKENS SUSPECTED OF STAPHYLOLOCOCCOSIS

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ABSTRACT. *Staphylococcus aureus (S. aureus)* is a major bacterial cause of bumblefoot and arthritis that causes movement disorders and joint paralysis in broiler chickens. Lesions, swelling, and lameness of the foot pads are common clinical signs. Bacterial isolation aims to determine the presence of *S. aureus* bacteria in chicken legs suspected of staphylococcosis. Bacterial isolation is carried out on nutrient agar (NA) media, then the growing colonies are transferred one smear loop at a time to mannitol salt agar (MSA) media, which is then cultured again at 37°C for 48 hours. It was then followed by Gram staining and the identification of microorganisms using a 100 X 10 magnification microscope. The results of bacterial isolation and Gram staining showed the presence of *S. aureus* bacteria, so it was known that the chicken leg sample was positive for staphylococcosis.

Kata kunci: *bumblefoot,* chicken, MSA, *Staphyloccus aureus*, staphylococcosis,

I. INTRODUCTION

Staphylococcus aureus (S. aureus) is the most prevalent bacterial species causing bumblefoot and arthritis in broiler chickens, which can lead to movement problems and paralysis of the joints. More than 90% of bumblefoot cases and more than 50% of arthritis cases in broiler chickens are caused by *S. aureus* bacteria (Rasheed, 2011).

Bumblefoot commonly occurs in poultry that have a large body size. The usual clinical signs are swollen foot pads and limping. This condition is usually caused by an injury that causes an infection of the subcutaneous tissue of the foot pad. This infection causes acute inflammation that is necrotic. At the microscopic level, lesions on the feet of birds with bumblefoot may show edema, necrosis, and granulomas infected by bacteria. This condition causes pain in chickens and can interfere with perching and walking activities, as well as limiting access to feed and drinking water, resulting in weight loss in chickens, and causing losses to the farming industry (Khusnan et al., 2012).

S. aureus infection is one of the most serious poultry diseases affecting commercial broilers and layers. It causes mortality (0-15%) and reduces poultry production performance. *S. aureus* is a normal flora of the skin and upper respiratory tract in chickens (Youssef et al., 2019).

Staphylococcus aureus can be identified through bacterial isolation methods by utilizing microorganism growth media. Microorganism growth media is a material consisting of a mixture of nutrients needed by microorganisms for their growth. Microorganisms utilize nutrient media in the form of small molecules assembled to compose cell components. Isolation of microorganisms to become pure cultures can be done using growth media (Krihariyani *et al.*, 2016).

II. MATERIALS AND METHODS

II.1 MATERIAL

Sample was tested out at the diagnostic laboratory of the Educational Veterinary Clinic of Hasanuddin University, Makassar in June, 2022.

a. Equipments

The tools used include beakers, bunsen, ose, erlenmeyer, petri dishes, horn spoons, digital scales, ovens, water baths, incubators, microscopes, pipettes, tongs, matches, pens, tube clamps, trays.

b. Materials

The materials used include nutrient agar powder, tryptic soy agar powder, parchment paper, distilled water, aluminum foil, object

glass, methylene blue, 95% ethanol, safranin, lugol, immersion oil, labels, spirits.

II.2 METHODS

a. Sampling

The chickens that were examined were broiler chickens aged approximately 1 month from the chicken sales place, with the condition of the foot pads with crusts and the position of the chicken that was more often seen sitting. The samples used were broiler chicken legs suspected of being infected with *S. aureus*, which was noted by the presence of lesions and crusts on the pads of the chicken's feet (Figure 1). The bacterial culture of chicken bumblefoot wounds was carried out by the direct swab method.

Figure 1. Staphylococcosis suspect chicken leg (personal documentation, 2022)

b. Preparation of Agar Media

Turn on the bunsen, prepare the scales, heat the stirring rod and the rim of the nutrient agar jar. Weigh 2.8 g of NA powder and 22.2 g of MSA powder on parchment paper alternately. Heat the rim of the measuring cup and Erlenmeyer then add 100 ml of distilled water into the Erlenmeyer which has been measured in the measuring cup and then cover with aluminum foil. Enter the instant medium then homogenize by rotating slowly and better homogenize in a waterbath for 15 minutes. sterilize in the oven at 120°C until boiling. The media is removed from the oven, kept at room temperature (its characteristic feels warm on the skin) then poured into a sterilized petri dish.

c. Bacterial Isolation

Samples were cultured on nutrient agar (NA) media, and the culture method performed was the streak plate method. Furthermore, the cultured media was incubated at 37°C for 24 hours by turning it over. Identification of S. aureus was carried out by moving one smear loop of bacteria from NA media to mannitol salt agar (MSA) media in a petri dish and then incubating at 37°C for 18–24 hours. Bacterial colonies that grew yellow on MSA media were suspected to be *S. aureus*, and a Gram stain was performed for identification.

d. Gram Stain

The Gram staining method is used to observe the Gram and morphological properties of bacteria. The method of manufacture is to make a review preparation on a glass object and fix it on a Bunsen burner. Then, the crystal violet solution is dripped and left for 1-2 minutes. The remaining dye is removed and rinsed with running water. The entire preparation was then dripped with Lugol solution and left for 30 seconds. After that, the Lugol solution was discarded, and the preparation was washed with running water. The preparation is then diluted with 96% alcohol until all the dye is washed off, and it is immediately washed with running water. Next, safranin dye was dripped and left for 2 minutes, then rinsed with running water and allowed to dry. To observe the results, the preparations were examined under a 100 x 10 magnification microscope using emersion oil (Sarudji et al., 2017).

III. RESULTS AND DISCUSSION

Isolation on NA media obtained results showing that there is colony growth in the direction of the scratches made before. The colonies that formed seemed to be white (Figure 2).

Figure 2. Colonies on NA media (personal documentation, 2022).

NA media is used as a culture medium for bacterial isolates in general. Beef extract and peptone in NA are used as basic ingredients because they are sources of protein, nitrogen, vitamins, and carbohydrates that are needed by microorganisms to grow and develop (Fatnariza et al., 2017).

The process of making a pure culture of S. aureus was carried out on MSA media. The colonies formed are yellow in color, following the direction of the scratch (Figure 3). The yellowish color of the S. aureus bacterial colonies is due to the lipochrome pigment produced by *S. aureus* itself (Wenas et al., 2020).

Staphylococcus aureus has the ability to ferment mannitol. It can be proven when *Staphylococcus aureus* is cultured in mannitol agar, where there is a change in color from red to yellow (Arif, 2017).

MSA is a selective and differential medium for the identification of Staphylococcus sp. This medium contains 7.5% sodium chloride salt, so it becomes a selective medium because most bacteria cannot grow at a salt concentration of 7.5% except Staphylococcus (Aryal, 2016). The results of bacterial isolation from MSA media that are positive for mannitol fermentation are then Gram-stained and then identified under a microscope.The results of Gram staining and identification of bacteria under a microscope derived from MSA media show bacteria that match the morphology of *S. aureus*. *Staphylococcus aureus* is a Gram-positive and coccus-shaped bacterium that produces a purple color in Gram staining (Figure 4).

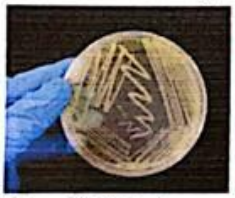

Figure 3. Colonies on MSA media (personal documentation, 2022)

Figure 4. *S. aureus* magnification 100 x 10 (personal documentation, 2022).

The method of bacterial classification is through Gram staining, where bacteria are categorized into two groups, namely Gram-positive bacteria that are purple and Gram-negative bacteria that are red (Misbach and Yuniarty, 2016). Gram-negative bacteria have cell walls that contain higher lipids and a thinner peptidoglycan layer compared to the cell walls of Gram-positive bacteria (Leboffe and Pierce, 2010). The usage of alcohol in the staining process will cause the lipids in the cell wall of Gram-negative bacteria to dissolve, so that the pores will open and cause the crystal violet and iodine complex to escape from the cell. As a result, the bacterial cell wall becomes colorless. Furthermore, bacterial cells will absorb safranin dye so that it appears red when observed under a microscope (Pelczar and Chan, 2007).

Bacteria retain the purple color because the crystal violet is still attached to the bacterial cells after the Gram staining process. The Gram nature of a bacterium is influenced by the content of the cell wall, with Gram-positive bacteria having a thicker peptidoglycan content compared to Gram-negative bacteria (Dewi, 2013). The outer cell wall of Gram-positive bacteria consists of thick peptidoglycan (Retnowati et al., 2011). When tested with alcohol, the cell wall of Gram-positive bacteria is dehydrated so that the pores shrink and the permeability of the cell wall and membrane decreases. This causes the crystal violet complex with iodine to not be able to leave the cell, so the safranin dye cannot enter the cell wall (Pelczar and Chan, 2007).

CONCLUSION

Based on the identification of bacteria in chickens with foot pad lesions that grew on MSA media, Gram-positive bacteria were found with a round bacterial shape resembling grapes. Bacteria that are similar to the identification results are *Staphylococcus aureus*, which is one of the bacteria commonly found in the skin area and upper respiratory system in poultry.

SUGGESTION

As a result of a rise in germs on the surface of the chickens' fleg, staphylococcosis in

chickens is highly correlated with the cleanliness of the chicken house. Therefore, staphylococcosis should be avoided by routinely cleaning the floor of the house.

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