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# The Characteristic of *Staphylococcus aureus* Isolated from Dairy Cow in the Milk Collecting Line of in Malang

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## ABSTRACT

This research was aimed was to know the effect subclinical mastitis stage for Friesian Holstein dairy milk quality in all months of lactation in the milk collecting line in Malang. *Staphylococcus aureus* (S. aureus) is one of the major bacterial causes of mastitis and subclinical mastitis in dairy cows. This disease is caused economic losses due to falling milk production and the addition of production costs. One of the criteria for the differentiation of *S. aureus* is the formation of pigment. Phenotype characteristics of *S. aureus* in vitro as a cause of subclinical mastitis in dairy cows are using 30 isolates. The level of mastitis subclinical is used by reagent IPB-l. Isolation and identification of *S. aureus* carried by several biochemical tests, namely: culture on Agar Blood Frame (ABF) media, observation of cell morphology, catalase test, coagulates test, and fermentation of mannitol test. Character is done by observing colony pigment on ABF media. The incidence rate of subclinical mastitis milk samples are from 198 dairy cattle, 107subclinical mastitis positive samples (54%) and 91 (46%) negative subclinical mastitis. From 30 isolates of *S. aureus* isolates subclinical mastitis caused 43.33% and 56.67% negative subclinical mastitis. Based on pigment production of the *S. aureus* isolated, 11 isolates (36.67%) produces a yellow pigment, 17 isolates (56.67%) white pigment, 1 isolates (3.33%) and an orange pigment isolates (3.33%) are not pigmented.
**KEYWORDS**: Milk, *Staphylococcus aureus*, subclinical mastitis

## INTRODUCTION

Mastitis (udder inflammation of the internal network) generated a lot of losses. This results in decreased milk production which reached to 70%, the presence of antibiotic residues in milk, the high cost of treatment and culling of milk. Besides an increase in the cost of replacement dairy cows, waste the milk, and death in cattle [1]. Staphylococcus aureus (S. aureus) is the main bacteria that cause many losses and a major cause of subclinical mastitis. It is reported that the outbreak of mastitis in the USA ever caused by a single agent S. aureus [2]. Generally, Mastitis caused by the entry of bacterial pathogens into the nipple hole into the udder and breed in it, rise inflammation reaction. The occurrence of mastitis is always associated with three factors, namely beef cattle, causing inflammation (80-90 % are caused by microorganisms) and the environment [3]. The usual causative agent that attacks the udder besides bacteria, are fungi, mycoplasma, algae and virus.

Tranter [4] said the mastitis causal is divided into three parts: the main power (prevalent infection), sporadic movement (sporadic infection) and the movement is not common (uncommon infection). The main movement in the mastitis incidence is *Streptococcus agalactiae*, *Streptococcus dvsgalactiae*, *Streptococcus uberis* and *Staphylococcus aureus*. Sporadic causal includes *foliform* groups *(Escherichia coli*, *Klebsiella pneumoniae* and *Enterobacter aerogenes*). Uncommon causal consists of *Corynebacterium pyogenes*, *Pseudomonas aeroginosa*, *Mycoplasma sp.*, *Leptospira spp*., and *fungi*. Udder infected by *Streptococcus agalactiae* often found on dairy cow farms that has no mastitis supervision. Wibawan *et al*. [5] have results, *Streptococcus agalactiae* isolated on subclinical mastitis incidence by 83 % in the area of Bogor, 82 % for Boyolali region and by 80 % for Malang region. Benda *et al.* [6] suggest that the two bacterial pathogens causing mastitis are often found in the *S. agalactiae* (92 %) and *S. aureus* (67 %). The number of mastitis cases in Indonesia would increase from year to year.

The properties of S. aureus that is, the form of coccid bacteria cells, are gram positive, facultative anaerobic, catalase positive, produces lactic acid, and grows well at 37 ° C. Cultures form dense colonies of golden yellow [7]. One strain of S. aureus have more than one hemolysine namely, alpha, beta, and gamma. S. aureus has the ability to ferment mannitol and inflate the plasma oxalate. The main problem is in overcoming the infection of S. aureus is the problem of antibiotic resistance. Characters of several strains of S. aureus is currently reported to have been resistant to nearly all commercial antibiotics, so there is no hope for a new antibiotic [7]. This study aims to knowing the percentage of S. aureus as a cause of subclinical mastitis, knowing the character of the pigment from S. aureus in the milk collecting line of East Java.

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## MATERIALS AND METHODS

### Material

This study used 40 dairy cow milk samples from 10 dairy cows taken on a dairy farm in Jabung areas to test the level of subclinical mastitis incidence and biochemical properties of S. aureus. For the characteristic color of the colony used 30 isolates of S. aureus from five areas, namely: Karangploso 11 isolates; Jabung 5 isolates; Batu 8 isolates; Pujon 3 isolates, and Dau 3 isolates. Other materials used to support this study were reagent IPB-1, rabbit plasma, hydrogen peroxide ($H_2O_2$) and antibiotic disks. Media used for bacterial identification of S. aureus is an Agar Blood Frame (ABF), heart influsion Broth, Mannitol Salt Agar (MSA).

The tools used for collection and identification of samples used Peddle, sterile test tubes and containers used for storing milk samples, test tubes, glass slides, vortex mixers, incubators, water bath, refrigerator, freezer, usa, Bunsen lamps, microscopes, and Centrifuge.

### Method

#### 1. Subclinical mastitis test with reagents IPB-1

Detection of subclinical mastitis is done by taking milk straight from the udder quarters at milking time. Udder cleans with warm water or soap. Milk first beam was removed; a second beam housed in the paddle, and then inserted reagents IPB-1 by volume ratio of 1: 1. If the amount of each paddle is not the same milk, milk disposed of in a way that is tilted flat on each paddle. Mixed shake horizontally for approximately 15-20 seconds for the IPB-1 reagent can be blended with milk, then observed to occur inflate or not. Dairy cow tested positive for subclinical mastitis in case inflate and if it does not happen then give negative inflate or normal results. Milk stored in sterile tubes after mastitis detection tests for a study sample [3]. Milk sample in a sterile tube is used to test the characteristics of S. Aureus, catalase test, coagulation test, mannitol fermentation test and test the sensitivity of microorganisms to antibiotics.

#### 2. Isolate and Characterization S. aureus

A bacterium can be identified based on biochemical properties of the resulting [8]. Identification of Staphylococcus aureus can be carried out by using tests biochemist include: catalyses test, coagulate test, and planting on the MSA to determine the ability to ferment mannitol. Isolation of S. aureus milk samples carried out by planting media ABF. Usa which has been sterilized dipped in milk and in the culture to the ABF was done by using a line isolation plates and incubated at 37 ° C for 18-24 hours. Colonies that grew on the ABF grouped or separated into several groups according to colony morphology. Bacteria purified by re-planting on media ABF performed with isolation technique of plate lines, incubated 37° C for 18-24 hours. Colonies suspected Staphylococcus coccus shape, color Yellow, Orange or White were separated and purified again by planting the ABF to do further tests. Pure breed storage done by planting cultured on ABF or media Hearth Inflution Broth.

#### Culture in Brain Hearth Infusion (BHI)

Planting in BHI broth media is done by taking a pure colony of media usa ABF with aseptic technique and put into tubes containing BHI broth. Subsequently incubated is at 37 ° C for 18-24 hours. BHI broth which had been overgrown with bacteria used for the tube coagulate test, and planting in the MSA.

#### Coagulate test

Coagulate test principle is to distinguish S. aureus and S. epidermis. S. aureus produces the enzyme coagulate, while S. epidermis not producing it. Positive results are marked by the presence of granular precipitate the fine grains. Slide coagulate test function aware of any production which is the bond coagulate which coagulate enzyme bound to the cell [7].

Isolates suspected Staphylococcus sp coagulate test performed. Tube coagulate test can be performed using cultures in BHI medium with a volume of 0.5. S. aureus showed positive reactions in the coagulate test by exposing the gel-like clot in the tube and the negative reaction is characterized by the absence of clumping in the tube [9]. Slide coagulate test begins with the manufacture of suspension cultures of ABF media with physiological NaCl on glass objects. Rabbit plasma dripped on the suspension and mixed gently using the usa. Changes were observed and compared with the negative control NaCl suspension physiology and rabbit plasma.

Bacteria suspected S. aureus grown on MSA medium, incubated at 37 ° C for 24 hours, observe the color of the colonies or discoloration of the media. Planting is done by bacterial isolation techniques of plate lines and incubated at 37 ° for 18-24 hours and observed the color change of media. The results compared with the control of the media are not planted MSA bacteria. Positive results S. aureus bacterial colonies are when seen yellow color with a yellow zone around it [9].

## RESULTS AND DISCUSSION

### Determination of Subclinical Mastitis

The results of incidence of subclinical mastitis determination are by IPB-1 reagent in Jabung areas and four Malang areas. The results of screening of subclinical mastitis with IPB-1 reagent was obtained positive subclinical mastitis at Jabung areas of 57.5% (22 of 40 samples of milk), and the incidence rate of subclinical mastitis in 5 Malang areas by 54% (107 of 198 samples of milk). When compared with previous studies in Boyolali of 85.5% [10], the incidence rate of subclinical mastitis in Malang is lower. Sleman 78.05%, Bantul 65.75%, Klaten 77.5% and Baturaden 73.2% [10].

The incidence rate of subclinical mastitis in the Territory Dau is 34.2%, Pujon by 20%, Jabung of 57.5%, Batu by 45%, and Karangploso of 67.5%. By taking the same time in some areas of the region of Malang was subclinical mastitis incidence rate of each region is different (Figure l). Judging from the incidence rate of subclinical mastitis in five Malang areas average redemptions of 54% in this study was lower than the results of Wahyuni research [10] amounting to 85.5% in the same.

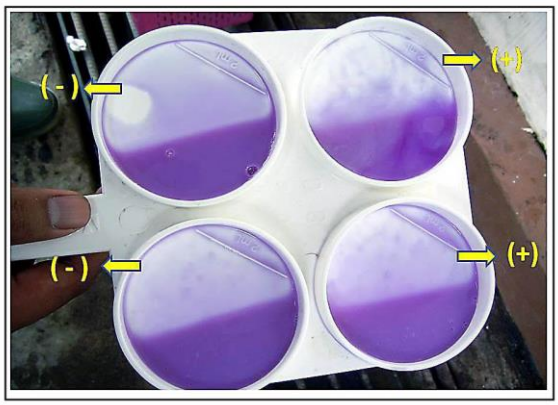


Figure 1. The results of screening subclinical mastitis milk with IPB-1Description:
(+). Positive reaction, there is inflate
(-). Negative reaction, no inflate

All results both positive and negative subclinical mastitis be identified further by planting on a ABF medium, Gram staining, catalyses test, slide and tube coagulate test, and the MSA test for the identification of the character S. aureus.

In the media ABF, S. aureus colony forming circular, cream-colored colonies with a flat edge and corner convex boundary. In the grouping of the 34 colonies of Staphylococcus alleged Jabung area, Yellow colonies (28 colonies) compared with White colonies (6 colonies). White color is possible has not produced the pigment that makes the colony became lipochrome colored golden yellow and orange yellow. The formation of this pigment is one of the criteria in the differentiation of S. aureus.

Percentage of S. aureus in the Jabung reached 42.1%, Pujon 48.15%, Dau 40.3%, Karang Ploso 66.7% and Batu areas 48.6%. The results of S. aureus average percentage in five areas by 49.37%. From these data known to the percentage of S. aureus vary for each area areas in Malang, East Java. Based on the results of research by using 30 isolates of S. aureus milk of dairy cows in Malang 11 isolates (36.67%) produced yellow colored pigment, 17 isolates (56.67%) White pigment, 1 isolates (3.33%) Orange pigment and 1 isolates (3.33%) does not produce pigment.

Observations S. Aureus Yellow colonies in areas Jabung by 73.6%, 17.6% white colonies, and Orange colonies 8.8%; areas Karangploso S. Aureus Yellow colonies 42.3%, 44.2% white colonies , and Orange colonies 13.5%; areas Batu S. aureus colonies Yellow 76.2%, 23.8% white colonies; areas Dau S. aureus yellow colonies 31.4%, white colonies 60%, and orange colonies 31, 4%. In the ABF's media Lipochrom aureus produce pigment that makes the colony appears golden yellow and orange yellow. This yellow pigment which is distinguishes S. aureus S. epidermidis that produce white pigment. The formation of this pigment is one of the criteria in the differentiation of S. aureus [11].

### Cell morphology

The identification of S. aureus cells on the microscopic observation with a magnification of 1,000 times round the studied isolates, clustered, showing purple / violet results from Gram staining (Figure 2). These results are in accordance with Foster [7] that the characteristics of S. aureus have the form of round cells, clustered like grapes.

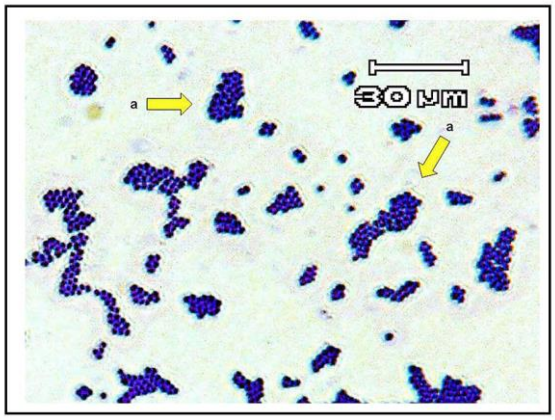


Figure 2. The results of Gram staining of bacteria S aureus showed visible direction of the arrow-shaped coccus cell morphology, clustered like grapes, and purple

The results obtained by Gram staining purple colour. This suggests that bacteria in this study is a Gram-positive bacteria, as Gram-positive bacteria are able to maintain the color of the first painting by using gentian violet is the colour purple. This ability is caused by the content on the cell wall of Gram-positive bacteria is thicker than the Gram-negative [12]. Positive results are indicated by Violet or Purple colour with red-colour background, while negative looks like the color red that looks like a Gram negative. This is consistent opinion Fardiaz [9], that S. Aureus on Gram staining of bacteria retain the gentian violet paint first time faded by alcohol. Gram colour difference is because the content of the cell wall of Gram-positive bacteria content in peptidoglycan thicker than with Gram-negative bacteria. Jawetz, *et al* [13] says that the colouring of S. aureus is a Gram positive bacterium that produces a color Purple on Gram stain, but in old cultures of bacteria can produce a red colour according to Gram negative.

**Biochemical properties**

In the catalase test by using hydrogen peroxide ($H_2O_2$) showed positive results. Positive catalase reaction can be used to differentiate between Streptococcus and Staphylococcus [14]. Catalase test results showed a positive reaction, indicated by the presence of oxygen gas bubbles. Catalase test serves to detect the production of enzymes catalase and $H_2O_2$ parse function which is the result of metabolism in aerobic bacteria into $H_2O$ and O2. S. aureus is catalase positive bacteria [14].

Slide coagulate test using rabbit plasma obtained isolates showed a positive reaction in the form of fibrin precipitates that leads to S. Aureus (Figure 3). Coagulate test may be used to distinguish the bacteria. S. aureus and S.epidermis. The main character of S. aureus is coagulating produce enzymes that are used to distinguish Staphylococcus (and micrococci) other [14]. Enzymes produced coagulate S. aureus activates prothrombine and its derivatives that will transform fibrinogen into fibrin [14]. Coagulate an extracellular protein produced by S. aureus can crumple the plasma with the help of a factor present in serum. Staphylococcus aureus has the ability to agglomerate, especially human blood plasma and rabbit. Coagulate functioning in the face of the process of phagocytosis. After all the observations of colony morphology, cell, and biochemical tests performed with positive results that lead to S. aureus, isolates 30 isolates selected for testing sensitivity to antibiotics.

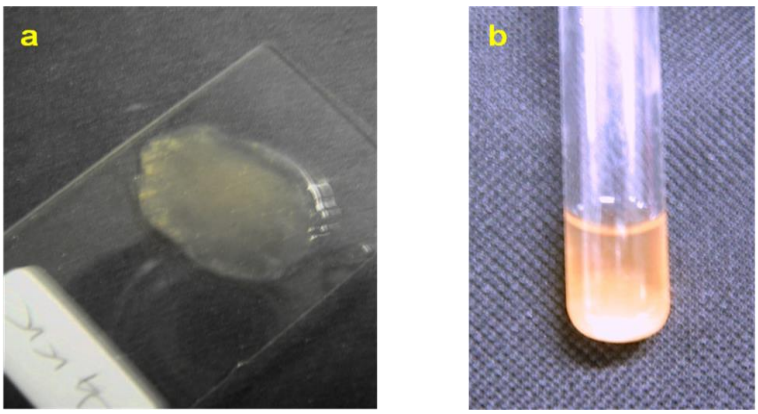


Figure 3: The coagulate test, (b) the resulting clot in the tube coagulate test, (a) fibrin precipitates resulting from the slide coagulate test

## CONCLUSION

From the results of research can be concluded that he incidence rate of subclinical mastitis milk samples are from 198 dairy cattle, 107subclinical mastitis positive samples (54%) and 91 (46%) negative subclinical mastitis and the based on pigment production of the S. aureus isolated, 11 isolates (36.67%) produces a yellow pigment, 17 isolates (56.67%) white pigment, 1 isolates (3.33%) and an orange pigment isolates (3.33%) are not pigmented.

## SUGGESTION

Need to do more research on the properties of S. auerus both yellow-orange colonies and white animal experiments to determine the level of its pathogen.

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