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Cases of *Methicillin-Resistant Staphylococcus aureus* (MRSA) from Raw Milk in East Java, Indonesia

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Abstract: The aim of this study was to isolate and identify the strain of *methicillin-resistant Staphylococcus aureus* (MRSA) from raw milk in East Java, Indonesia. Raw milk samples of 196 samples obtained from four dairy farms. Bacterial identification was based on the growth in Mannitol Salt Agar (MSA) and blood agar media, Gram staining and catalase, & coagulase tests. 87 (44.39%) out of 196 milk samples were for positive *Staphylococcus aureus* isolation. Antibiotic sensitivity testing using *oxacillin* antibiotic showed 36 isolates were resistant to the *oxacillin.* MRSA confirmation test conducted on MRSA isolates showed that 31 isolates were positive by using *Oxacillin Screen Agar Test.* It was concluded that the raw milk can be a potential reservoir for MRSA strains to threat public health.

Key words: *Staphylococcus aureus* · MRSA · Raw Milk · Public Health

INTRODUCTION

The bacteria *Staphylococcus aureus* (*S. aureus*) are the main causes of clinical and subclinical mastitis in dairy cows [1]. Treatment of mastitis is still using antibiotics, while the use of antibiotics cause resistance of *S. aureus* to antibiotics [2]. Methicillin-resistant Staphylococcus aureus (MRSA) is a strain of bacteria that has become a serious problem around the world and is often associated as a cause mastitis in cows [3]. MRSA is a strain of *S. aureus* that are resistant to various antibiotics, including methicillin. The mecA gene is a gene that encodes a modified Penicillin Binding Proteins (PBP2a) so resistant to the antibiotic methicillin. Currently MRSA has emerged worldwide as nosocomial pathogens in animals and humans and are zoonotic [4]. Problems faced to address the cause of this disease is antibiotic resistance.

MRSA is known as one of the main causes of nosocomial infections in hospitals worldwide since the 1980s with an average prevalence of 50% [4]. Only vancomycin is said to be effective for the treatment of MRSA infections [5]. MRSA problems are further complicated by the emergence of vancomycin-resistant MRSA strain and the emergence of a new strain of MRSA that is completely unrelated to nosocomial infections or hospital infection called community associated MRSA (CA-MRSA) [6].

MRSA transmission can be divided into three forms, namely in humans, animals, the environment and the origin of food products. Transmission usually occurs in animals are asymptomatic colonies in nasal or rectal areas. Pets career MRSA may be a reservoir for the disease itself and transmit MRSA to other animals or humans [7]. The incidence of MRSA in humans can be transmitted through direct contact mainly through the hands with an infected person, contaminated food, aerosols and may occur in the mother during childbirth [8]. The aims of this study were to determine the prevalence rate and *methicillin-resistant Staphylococcus aureus* (MRSA) in cow raw milk in East Java, Indonesia.

MATERIALS AND METHODS

Sampling: Samples were taken from four dairy farms of 196 samples. Sampling was carried out through simple random sampling method. Milk samples were stored in the ice box for later observation.

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Isolation and Identification of *Staphylococcus aureus*: The samples were processed immediately upon arrival using aseptic techniques. To detect *S. aureus*, 1mL of each milk sample was inoculated on Baird - Parker agar (Difco, Detroit, Michigan, USA). After 24 - 48 h of incubation at 37°C, suspected colonies were sub-cultured on blood agar plate (Difco, Detroit, Michigan, USA) and incubated for 24 h at 37°C. Suspected Staphylococcus sp colonies with the characteristics of a round, smooth, prominent and shiny and gray to dark golden yellow were subjected to identification.

The identification was done by microscopic examination after staining with Gram stain then continued with catalase test, coagulase test and the haemolysis test on Blood Agar. Microscopic colonies which ferment mannitol using the Gram stain will be visible forms of cocci purplish blue with an array of clustered like grapes [9]. Catalase test was done, then coagulase test was done using rabbit blood plasma [10]. Purified colonies of suspected were streaked on Blood agar medium and, incubated for 24 h at 37°C. *S. aureus* will produce β-haemolysis around colonies [10].

Antibiotic Sensitivity Test: One strain from each *S. aureus* positive sample was selected for susceptibility tests. Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method using Mueller-Hinton agar. The oxacillin antibiotic (5 µg) was placed on the agar surface with minimal pressure using sterile forceps. The incubation was done at 37°C for 18-24 hours [11]. The size of the diameter of the inhibition zone was used to determine the sensitivity of bacteria to antibiotic oxacillin which are grouped into three categories: sensitive (S), intermediate (I) and resistance (R) based on standards issued by the National Committee for Clinical Laboratory Standards (NCCLS) [11].

Confirmation test for MRSA: Confirmation test was done to enforce the presence of MRSA by streaking colonies of the identified bacteria with a zigzag manner in oxacillin screen agar. Positive bacteria resistant to oxacillin media will change its color into blue [12].

RESULTS

Identification of *Staphylococcus aureus*: This study was carried out using 196 samples of cow's milk obtained from four farms in East Java.

Table 1: Number of raw milk samples and positive of *S. aureus*

Dairy Farm	Surabaya	Nongkojajar	Grati	Senduro
Number of Samples	100 (38%)	46 (54%)	35 (46%)	15 (53%)
S. aureus (+)	38	25	16	8

Table 2: Number of Resistant to Oxacillin and MRSA confirm

Dairy Farm	Surabaya	Nongkojajar	Grati	Senduro
Resistant to *oxacillin* 5 µg	14 (79%)	9 (89%)	10 (90%)	3 (100%)
MRSA (+) confirm	11	8	9	3

Fig. 1: MRSA (+) confirm test.

The results of identification of *S. aureus* showed; Staphylococcus sp. shaped cocci and look clustered on electron microscope with a magnification of 1000x. Purple bacteria indicates the Gram-positive bacteria, catalase was positive and *S. aureus* produced β-hemolysis around the examined colonies.

The results of coagulase test showed as many as 87 samples were positive with a lump of plasma. Based on the stages of identification tests that has been carried out on 196 samples of cow's milk, obtained 87 (44.38%) samples were isolates of *S. aureus* (Table 1).

Antibiotic Sensitivity Test Results: The results of the sensitivity test indicated the presence of an antibiotic resistance zone. The antibiotic bottleneck area diameter was measured by using a ruler with millimeter (mm) to the growth of bacteria. If there is bacterial colonies on antibiotic resistance zone, measurements were taken of the distance of the nearest colony with a diameter of inhibition zone. The results showed that antibiotic resistance profiles vary on the 87 isolates of *S. aureus* (Table 2).

The results showed that 36 isolates of *S. aureus* were resistant to oxacillin. The results of a confirmatory test for MRSA on oxacillin screen agar indicated that 31 strains of MRSA isolates were blue in the oxacillin screen agar (Fig. 1).

DISCUSSION

Detection of MRSA resistance can be achieved by using oxacillin disk. Oxacillin and methicillin are chemically in one class. More stable test results occurs with methicillin and oxacillin together but at this time methicillin is no longer commercially produced so on the market is oxacillin [13].

Oxacillin is a β-lactam antibiotic class and is the drug of first choice in the treatment of skin infections caused by *S. aureus* bacteria. Mechanism of action of penicillin inhibits bacterial growth by interfering with transpeptidation reaction in cell wall synthesis [14]. There are four mechanisms of resistance to β-lactams are: inactivation of the antibiotic by β-lactamase; modification of Penicillin-Binding-Protein (PBP) targets; disorder drug penetration to achieve the objectives PBP; and efflux [15].

The production of betalactamase and PBP2a (Penicillin binding protein) could be lead to MRSA strains which are multi-drug resistant [16]. MRSA is a strain of *S. aureus* that are resistant to various antibiotics, including methicillin. The mecA gene is a gene that encodes a modified Penicillin Binding Proteins (PBP2a) so resistant to the methicillin antibiotic [17].

Misused of antibiotics and intramammary preparations used by the owner without the prescription of the veterinarian is attributed to be one of the reasons for increasing incidence of these strains [18]. MRSA is a strain of bacteria that has become a serious problem around the world and is often associated as cause of mastitis in cows [18]. The mecA gene is one part of a mobile genetic element called the staphylococcal cassette chromosome mec (SCCmec or mec DNA) found in all strains of MRSA. Generally SCCmec contains major resistance gene is mecA [19].

CONCLUSIONS

It can be concluded that 87 out of 196 samples of raw milk gave isolation of *S. aureus*. Based on confirmation test on media Oxacillin screen agar thirty-one isolates were tested positive for MRSA.

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