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Submission date: 16-Nov-2020 09:15PM (UTC+0800)
Submission ID: 1447735380
File name: Bukti_C_2.pdf (240.72K)
Word count: 2521
Character count: 13142

Indian Vet. J., November 2019, 96 (11) : 27 - 31

Antibiotic Resistance to Staphylococcus aureus and Methicillin Resistant Staphylococcus aureus (MRSA) Isolated from Dairy Farms in Surabaya, Indonesia

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(Received : May, 2019 **177/19** Accepted : June, 2019)

Abstract

Raw milk samples (80) were obtained from four dairy farms. Bacterial identification was based on the growth in Mannitol Salt Agar, Gram staining, catalase, and coagulase tests. 14 (17.5%) out of 80 milk samples were positive for *Staphylococcus aureus*. Antibiotic sensitivity test using Cefoxitin, Penicillin, Amphicillin, Oxacillin and Tetracyclin showed resistance 14(100%), 14(100%), 12(85,7%), 9(64,3%) and 0(0%) to the antibiotics in that order, 9 isolates were positive for MRSA. It was concluded that the raw milk can be a potential reservoir for MRSA strains which is a threat to public health.

Key words: Staphylococcus aureus, Antibiotic Sensitivity, Milk, Indonesia

Milk is a good medium for the growth and development of *S. aureus*. Contamination can occur, during milking or processing. The main reservoir of *S. aureus* is in infected quarters (Akineden *et al.*, 2001) which causes (Sasia *et al.*, 2004) subclinical or chronic mastitis in dairy cows leading to considerable losses to the dairy industry (Katsuda *et al.*, 2005).

S. aureus infection in humans, especially Methicillin Resistant S. aureus (MRSA) is difficult to treat since they are known to be resistant to various antibiotics (Hata *et al.*, 2010). Staphylococcal infections occurs due to postoperative injuries, infection during hemodialysis, (Fournier *et al.*, 2008).

In Surabaya, Methiclin Resistant *S. aureus* (MRSA) was isolated from raw milk from four dairy farms. Based on its antibiotic sensitivity test it was decided to assess the distribution of MRSA strains.

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Materials and Methods

Twenty milk samples were taken, each from Kaliwaron, Wonocolo, Pogot and Bendul Merisi Farms during the morning milking time.

10 ml of milk samples in test tubes collected from different farms were used for isolation of *S. aureus* by streaking on the MSA isolation media were incubated 37°C for 24 hrs. The appearances of Yellow coloured colonies were subjected to grams staining (Effendi *et al.* 2018).

The presence of MSA was assessed by Catalase test by addition of 3% hydrogen peroxide on the yellow coloured colonies. The appearance of gas bubbles revealed the presence of *S. aureus* (Fig 1). The catalase test was performed by inoculating the yellowish colonies in nutrient brooth, incubated at 37°C for 24 hrs followed by addition of 1 ml rabbit plasma and evenly mixed and incubated for 24 hrs. The clotting of plasma confirms the presence of S. aureus (Effendi *et al.* 2019).

The antibiotic sensitivity test for *S.aureus* was carried out as per Kirby-Baurer method using Cefoxitin, Penicillin, Amphicillin, Oxacilin and Tetracyclin (Fig 2) (CLSI, 2017).

Results and Discussion

The results of isolation and identification on 80 samples of raw milk from 4 dairy farms in Surabaya revealed at 14 (17.5%) positive samples of Staphylococcus aureus (Tabel I).

Fourteen positive samples for Staphylococcus aureus were subjected to antibiotic sensitivity test using five antibiotics and the results are presented in Table II.

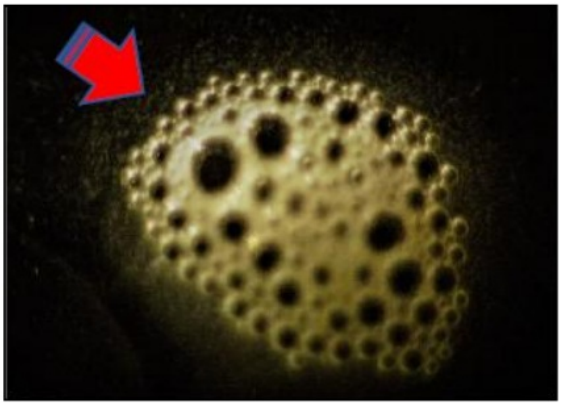

Fig 1. Catalase test on Staphylococcus aureus shows positive bubbles

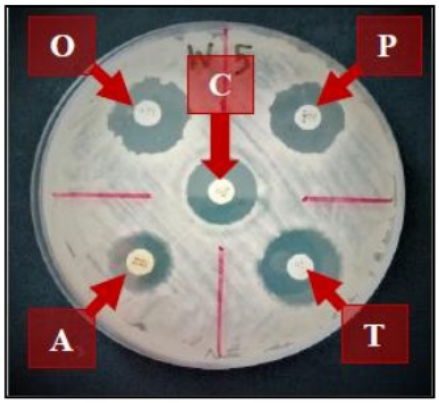

Fig 2. Inhition zone on antibiotic sensitivity test of isolate code W2. Information : C = Cefoxitin 30 µg; P = Penicillin 10 µg; A = Amphicillin 10 µg; O = Oxacillin 10 µg; T = Tetracyclin 30 µg

Table I. Presence of Staphylococcus aureus from milk samples in Surabaya, Indonesia.

Location of farm	Sample size	(+) MSA media	(+) Gram staining	(+) Catalase	(+) Coagulase
Kaliwaron (K)	20	5	5	5	2
Pogot (P)	20	5	5	5	4
Wonocolo (W)	20	6	6	4	2
Bendul Merisi (B)	20	7	7	6	6
Total	**80**	**23**	**23**	**20**	**14 (17.5%)**

The prevalence of *S. aureus* in 14 samples (17.5%) indicate necessity for clean milk production pratices to reduce the contamination of S. aureus. The prevalence of S. aureus in this study is comparable with that of Ayano *et al.*, (2013) who reported a prevalence of 13.8%. The lower prevalence of 6.6% and 10.8% was reported in India (Kumar and Prasad, 2010) and Brazil (Fagundes *et al.*, 2010), respectively.

While a higher prevalence of 40%, and 100% respectively has been reported in Morocco (Bendahou *et al.*, 2008), and South Africa (Ateba *et al.*, 2010). The prevalence observed may be due to the presence of subclinical infection and negligence of hygienic practices such as improper milking procedures, milk handling techniques, and improper storage which increases *S. aureus* in milk.

The level of bacterial resistance to antibiotics according to the assessment standards of antibiotic inhibitory zone based on Clinical Laboratory Standards Institute (CLSI) are grouped into three categories, namely sensitive, intermediate, and resistant (CLSI, *loc. cit*).

Penicillin, Amphicillin, Cefoxitin and Oxacillin are antibiotics belonging to the class ß-lactam antibiotics. B-lactam antibiotics are antibiotics that are often used in the treatment of mastitis cases in dairy cows. The beta-lactam group has the ability to inhibit bacterial growth by influencing bacterial cell wall synthesis. This antibiotic has activity in S. aureus through the interaction of three heavy molecules and one mild molecule in penicillin binding proteins. The mechanism of action of antibiotics ß-lactam begins with penicillin binding protein (PBPs) in bacteria (Quinn *et al.*, 2002). The function of penicillin binding protein is to have an effect on the synthesis of peptidoglycan cell walls and cell growth. B-lactam antibiotics bind and inhibit penicillin binding protein (PBPs), which is an enzyme for the synthesis of Peptidoglycan

Table II. Measurement of inhibition zone on antibiotic sensitivity test of Staphylococcus aureus.

No	Isolates Code	Diameter of inhibition zone of antibiotics in mm				
		Cefoxitin	Penicillin	Amphicillin	Tetracyclin	Oxacillin
		30 µg	10 µg	10 µg	30 µg	10 µg
1.	B1	15 (R)	26 (R)	27 (R)	22 (S)	21 (R)
2.	B2	16 (R)	26 (R)	20 (R)	22 (S)	15 (R)
3.	B3	20 (R)	24 (R)	19 (R)	23 (S)	16 (R)
4.	B4	20 (R)	22 (R)	19 (R)	15 (I)	22 (S)
5.	B5	18 (R)	20 (R)	19 (R)	20 (S)	27 (S)
6.	B6	20 (R)	25 (R)	29 (S)	17 (I)	15 (R)
7.	K1	18 (R)	19 (R)	15 (R)	18 (I)	27 (S)
8.	K2	20 (R)	26 (R)	22 (R)	23 (S)	25 (S)
9.	P1	21 (R)	20 (R)	18 (R)	17 (I)	24 (S)
10.	P2	20 (R)	17 (R)	14 (R)	23 (S)	19 (R)
11.	P3	20 (R)	24 (R)	21 (R)	23 (S)	21 (R)
12.	P3	17 (R)	25 (R)	29 (S)	17 (I)	14 (R)
13.	W1	18 (R)	22 (R)	18 (R)	23 (S)	11 (R)
14.	W2	19 (R)	20 (R)	18 (R)	16 (I)	21 (R)

Information: R: Resistant I: Intermediate S: Sensitive

(Effendi, 2009). The resistance to ß-lactam can be caused by S. aureus being able to produce ß-lactamase which can break up the ß-lactam ring or the expression of PBP 2a which has a low affinity for oxacillin and other ß-lactams.

Sensitivity on Cefoxitin antibiotics was observed in fourteen isolates which were 100% resistant to Cefoxitin. Cefoxitin is used as a test antibiotic to detect the resistance properties of antibiotic substances Cefoxitin and methicillin to *S. aureus* (Datta *et al*., 2011). Isolates which showed resistance to Cefoxitin and Penicillin was also used in this study as a marker of Staphylococcus aureus in milk. The isolates from several dairy farms in the Surabaya were resistant to beta-lactam antibiotics.

The resistance of *Staphylococcus aureus* to ß-lactam antibiotics is a problem that is quite common in several places, which is becoming more prevalent if treatment with ß-lactam antibiotics is not based on dosage and appropriate use (Effendi, *loc. cit*). The development of bacterial resistance to antibiotics is influenced by the intensity of antibiotic exposure in an area, and uncontrolled use of antibiotics tends to increase the resistance of germs that were originally sensitive (Shryock and Richwine, 2010).

Based on the study that tetracyclin antibiotics can still be used in cases of infection by Staphylococcus aureus because they still have sensitivity to several *S. aureus* isolates. Tetracyclin has a broad spectrum activity against gram-positive and gram-negative bacteria by inhibiting protein synthesis (Velhner and Milanov, 2015).

Resistance to antibiotics caused by bacteria can be divided into three, among others, innate resistance (primary), acquired resistance (secondary), and episomal resistance. Innate (primary) resistance due to the presence of antibiotic decomposing enzymes in bacteria so that naturally these bacteria can break down antibiotics, resistance can be obtained (secondary) due to mutations in bacteria that occur quickly and can also occur for a long time and episomal resistance where bacteria have a factor R on plasmids that can be transmitted to other bacteria that have species links through conjugate or transduction cell contact (Jagielski

et al., 2014). The study showed that Cefoxitin and penicillin were ineffective for the treatment of *S. aureus* infection. While for Oxacillin also showed that 5 samples that were still sensitive in samples code B4, B5, K1, K2 and P1. Samples code B6 and P4 were samples still sensitive against amphicillin. Samples that are still sensitive to ß-lactam show that ß-lactamase is still capable of hydrolyzing the ß-lactam ring (Elsayed *et al*., 2015). Tetracyclin can effectively be used in the treatment of *S. aureus* infections. In this study, there were 6 intermediate isolates against tetracyclin which were thought to be resistant. However, there were still 8 sensitive isolates.

We found that cefoxitin discs, as recommended by Jain *et al*., 2008, is a good method for detecting MRSA by combining oxacillin discs so as to confirm MRSA presence. It is always recommended to combine the two methods, one with high sensitivity and the other with high specificity. According to the present results, 9 (64.28%) included MRSA. It is concluded that the disc diffusion (DD) test on oxacillin is more specific but less sensitive than the cefoxitin DD test. This finding is important to confirm the existence of MRSA and advice the government to take steps to control MRSA sourced from raw milk.

Summary

MRSA is a bacterium that is resistant to antibiotic treatment. The presence of MRSA in raw milk in Surabaya requires proper combination of antibiotics based on antibiotic sensitivity test and the government intervention on the treatment schedule with required antibiotics.

Acknowledgement

This study was supported in part with the PENELITIAN UNGGULAN FAKULTAS Funds from Airlangga University, Indonesia.

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