Patterns of Antimicrobial Resistance on *Staphylococcus Aureus* Isolated From Raw Milk from Dairy Farms in Surabaya

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Abstract

The aim of this research was to show the pattern of *S. aureus* bacteria isolated from raw milk from two dairy farms in Surabaya against antibiotics oxacillin, penicillin, amphicillin, chloramphenicol, and gentamicin. The study used purposive sampling method. About 72 samples isolated on *Mannitol Salt Agar* media (MSA) from the result which identified as *Staphylococcus sp* was 14 (19.44%) showed the positive samples on coagulase test. Antibiotic disk used i.e oxacillin, penicillin, amphicillin, chloramphenicol, gentamicin by using Kirby Bauer method. Inhibiton diameter zone measured at millimeter to determined a sensitivity level of antibiotic. The result showed about 14 (100%) of samples was resistant to Antibiotic oxacillin type, 14 (100%) of samples was resistant to penicillin, 9 (64,2%) samples was resistant, and 5 (35,7%) of samples was sensitive to amphicillin, 14 (100%) of samples was sensitive to chloramphenicol, and 14 (100%) of samples was sensitive to gentamicin. These results will open up our view that cooking milk is important to eliminate the danger of S. aureus isolates which present in milk and have potential hazards to public health.

***Key words** : Dairy farms, S. aureus, Antimicrobial Resistance, Raw milk.*

Introduction

Staphylococcus aureus is a pathogenic bacteria that usually causes mastitis in dairy cows, can cause economic losses for dairy farmers [(1)]. The incidence of mastitis caused by S. aureus bacteria is the highest incidence rate[(2, 3)]. For a long time S. aureus has been a causative agent of mastitis, besides that the bacteria can also cause food borne disease (FBD), because these bacteria can contaminate processed dairy products during processing, it is caused by hygiene factors from workers, equipment that used less sterile, the environment also affects the sanitation of the cage that is not good [(1)].

The use of antibiotics is a step of choice in treatment in cases of infections caused by bacteria known as the drug of choice, because one mechanism of action of antibiotics is to damage the bacterial cell wall, so that the bacteria can die. Recorded from some documentation about S. aureus for antibiotics has two relevant effects, the first is the reduction in cure rates after treatment, and the second is the potential impact of transmission of resistant bacteria to humans through the food chain [(3, 4)].

Antibiotics that are usually recommended in animal husbandry include β-lactam, tetracycline, and macrolide groups. However, the β-lactam group is most often used in the treatment of mastitis [(5)], due to S. aureus bacteria can produce β-lactamase. Therefore, almost all isolates of S. aureus bacteria are resistant to penicillin class antibiotics, it is caused by β-lactamase which can break the β-lactam ring found in penicillin group [(6, 7)].

In Surabaya, the number of cases of subclinical mastitis required treatment of antibiotic treatment, so we need data on antimicrobial patterns, especially from beta lactam antibiotics as the main antibiotics for the treatment of mastitis. Therefore, this study was to identify S. aureus antimicrobial resistance isolated from raw cow

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milk samples from two dairy farms in Surabaya, East Java, Indonesia based on antibiotic sensitivity testing and to understand patterns of antimicrobial resistance.

Materials and Methods

Sample collection

Thirty eight milk samples were taken from Kaliwaron Farm, and thirty four milk samples were taken Bendul Merisi Farm 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^{o}C for 24 hrs. The appearances of yellow coloured colonies were subjected to grams staining [8].

Identification of Staphylococcus aureus

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^{o}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, as shown Fig.2 [9].

Antibiotic sensitivity test

The antibiotic sensitivity test for *S.aureus* was carried out as per Kirby-Baurer method using Oxacillin, Penicillin, Amphicillin, Chloramphenicol and Gentamicin (Fig. 3) [10].

Results and Discussion

The results of isolation and identification on 72 samples of raw milk from 2 dairy farms in Surabaya revealed at 14 (19.45%) positive samples of Staphylococcus aureus (Tabel 1.). Fourteen positive samples for Staphylococcus aureus were subjected to antibiotic sensitivity test using five antibiotics and the results are presented in Table 2.

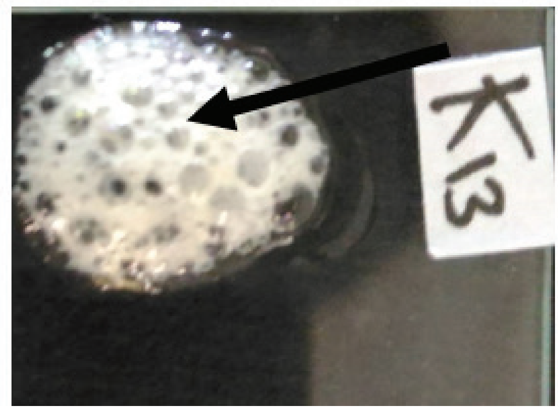

Figure 1. S. aureus catalase test shows positive presence of bubbles

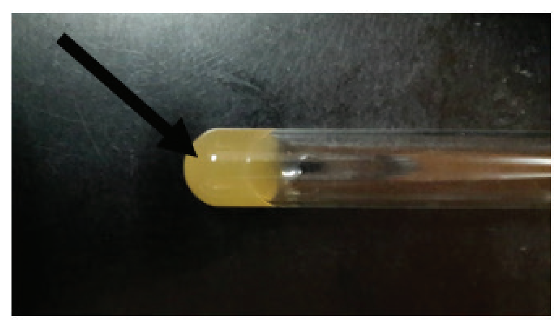

Figure 2. The positive S. aureus coagulase test has a lump in plasma

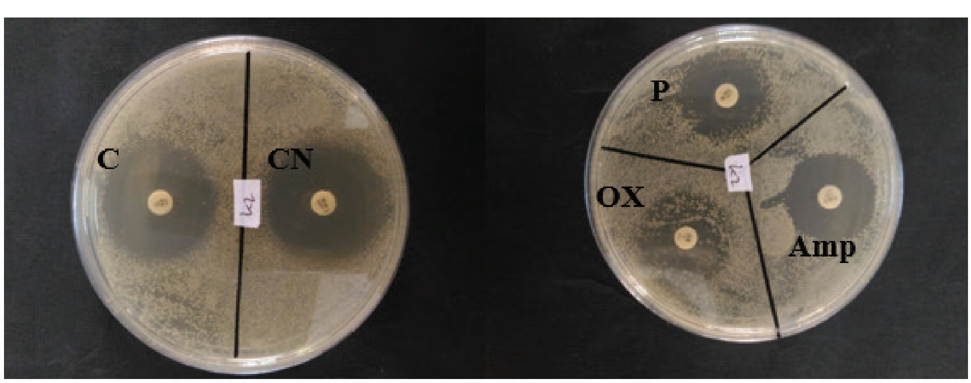

Figure 3. Zones of inhibition of antibiotic sensitivity test on the K2 sample

Note : OX= Oxacillin; P= Penicillin; AMP= Amphicillin; C= Chloramphenicol;

CN= Gentamicin

Table 1. Presence of Staphylococcus aureus from milk samples in Surabaya, Indonesia.

Location of farm	Sample size	(+) S. aureus
Kaliwaron (K)	38	7
Bendul Merisi (B)	34	7
Total	72	14

Table 2. Results of measurement of antibiotic sensitivity test on S. aureus

Sample code	Antibiotic disk inhibition zone diameter in mm				
	Oxacillin	Penicillin	Amphicillin	Chloramphenicol	Gentamicin
K2	28(R)	18(R)	29(S)	31(S)	30(S)
K3	27(R)	16(R)	29(S)	27(S)	26(S)
K13	19(R)	19(R)	18(R)	24(S)	22(S)
K16	0(R)	18(R)	20(R)	28(S)	30(S)
K28	0(R)	0(R)	19(R)	26(S)	18(S)
K29	0(R)	0(R)	19(R)	28(S)	27(S)
K33	27(R)	21(R)	16(R)	31(S)	19(S)
B1	26(R)	0(R)	21(R)	30(S)	27(S)
B12	22(R)	18(R)	18(R)	28(S)	31(S)
B13	27(R)	26(R)	19(R)	30(S)	26(S)
B17	26(R)	17(R)	42(S)	26(S)	30(S)
B19	26(R)	19(R)	40(S)	27(S)	29(S)
B25	22(R)	18(R)	12(R)	27(S)	30(S)
B27	28(R)	18(R)	40(S)	29(S)	28(S)

Note : R = resistant; S = sensitive

Based on the results of the S. aureus resistance test against several antibiotics, there were 14 (100%) samples resistant to oxacillin antibiotics, 14 (100%) samples were resistant to penicillin, 9 (64.2%) samples were resistant and 5 (35.7%) samples sensitive to amphicillin, 14 (100%) samples were sensitive to chloramphenicol, and 14 (100%) samples were sensitive to gentamicin.

Oxacillin, penicillin, and amphicillin belong to the β-lactam antibiotic class. B-lactam antibiotics are antibiotics that are often used in the treatment of mastitis in dairy cows. This antibiotic has activity in S. aureus through the interaction of three heavy molecules and one light molecule in penicillin binding proteins. The function of penicillin binding protein has an effect on the synthesis of peptidoglycan cell walls and cell growth. B-lactam antibiotics bind to and inhibit penicillin binding protein (PBPs) which is an enzyme for Peptidoglycan synthesis [6, 11].

According to Quinn et al., (2002) stated that beta-lactam antibiotics have the ability to inhibit bacterial growth by influencing the process of bacterial cell wall synthesis[12]. The mechanism of action of β-lactam antibiotics starts with penicillin binding protein (PBPs) in bacteria. Obstruction of bacterial cell wall synthesis occurs because the process of transpeptidation between the peptidoglycan chains is disrupted, then the activation of proteolytic enzymes in cell walls [13].

Resistance that occurs with oxacillin, penicillin and amphicillin antibiotics in S. aureus from fresh cow's milk in Surabaya is probably due to the presence of PBP 4 gene. PBP 4 gene is a PBP group that has a low BM and is associated with β-lactam antibiotic resistance, and possibly PBP 4 causes the ability to tolerate β-lactam antibiotics. The nature of PBP 4 causes penicillin deacylates about 10-20 faster than PBPs with high BM. This is possible because PBP 4 binds oxacilin, penicillin and amphicillin at higher concentrations. Some PBP 4 molecules that are not bound to the concentration level that has been binding to PBP 2a molecules will tolerate β-lactam antibiotics given [6, 14]. Resistance to β-lactams that occur can be caused by S. aureus being able to produce β-lactamases which can break down β-lactam rings or the expression of PBP 2a which has a low affinity for oxacillin or other β-lactams. The resistance of S. aureus bacteria to lactam β group antibiotics is caused by this broad-spectrum antibiotic, both in vivo and in vitro and against a large number of Gram positive and Gram negative bacteria [15, 16].

B-lactam antibiotics in this study showed that Oxacillin and penicillin were no longer effective for the treatment of S. aureus infections because based on the results of the study 14 samples were resistant to these antibiotics. Whereas amphicillin also showed the same thing even though there were 5 samples that were still sensitive in samples B17, B19, B27, K2, and K3. Samples that are sensitive to β-lactam show that β-lactamase is still able to hydrolyze the β-lactam ring which can cause sensitivity [17].

Gentamicin antibiotics are very well used because 14 samples are sensitive with 100% effectiveness for S. aureus. Gentamicin is an aminoglycoside class of antibiotics. Gentamicin has a sugar group (glycoside) that is bound to the NH2 (amino) group by a mechanism of action that binds irreversibly to the ribosome 30s sub unit of bacteria, namely by inhibiting or preventing cells from making proteins properly and causing errors in the translocation of the genetic code [18, 19] .

The results also showed that 14 samples were 100% sensitive to the antibiotic Chloramphenicol, making it very effective for S. aureus. Chloramphenicol is an antibiotic group that can inhibit the growth of bacteria with a broad spectrum of work against S. aureus bacteria. Chloramphenicol works by breaking the binding of new amino acids in the peptide chains that begin to arise, largely because Chloramphenicol inhibits peptidyl transferase in the process of protein production. Chloramphenicol is primarily bacteriostatic and the growth of microorganisms begins again when drug administration is stopped [20, 21].

The overall discussion of this study is that β-lactam antibiotics can still be the first choice for the treatment of S. aureus infections, but it is necessary to be aware of the resistance and transfer of antibiotic resistance that occurs in bacteria. The use of alternatives for treatment using Chloramphenicol and Gentamicin is highly recommended because it is very sensitive and effective for treatment, also provides information that in animal products namely raw cow's milk in Surabaya has the potential for food borne disease (FBD) through S. aureus bacteria.

Conclusion

Based on the research results it can be concluded that 14 (100%) samples are resistant to oxacillin, 14 (100%) penicillin resistant samples, 9 (64.2%) resistant samples and 5 (35.7%) amphicillin sensitive samples, 14 (100%) chloramphenicol sensitive samples, and 14 (100%) gentamicin sensitive samples. These results will open up our view that cooking milk is important to eliminate the danger of S. aureus isolates present in milk and have potential hazards to public health.

Designation :

Wiwiek Tyasningsih as an associate professor on Universitas Airlangga

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Ethical Clearance: Raw milk were used in this study, hence ethical clearance was not necessary. Raw milk samples were collected from two dairy farms in Surabaya, East Java province, Indonesia.

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Conflict of Interest: Nil

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