Eco. Env. & Cons. 28 (January Suppl. Issue) : 2022; pp. (S68-S72)
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ISSN 0971–765X

http://doi.org/10.53550/EEC.2022.v28i01s.009

Prevalence and antibiotic resistance of *Staphylococcus aureus* isolated from Ettawa Crossbreed Goat Raw Milk on Wonosari sub-distric, Malang, Indonesia

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(Received 9 August, 2021; Accepted 7 September, 2021)

ABSTRACT

This research was aimed to determine the prevalence and profile of antibiotic resistant of *Staphyloccocus aureus* bacteria collected from Ettawa crossbreed goat raw milk at Bagelanfarm in Wonosari, Malang, East Java. A total 100 raw milk samples were collected from Bagelan farm. Isolation and identification of *Staphyloccocus aureus* (*S. aureus*) were used Mannitol Salt Agar(MSA) media, Gram staining, catalase test, and coagulase test. The antibiotic sensitivity test was done by using diffusion disc Kirby-Bauer method. The results showed that 26 isolates were confirmed as *S. aureus*. The antibiotic sensitivity test result showed about 4 samples (15%) resistance to cloramfenicol, 5 samples (19%) resistance to tetracyclin, and 14 samples (54%) resistance to erythromycin. *S. Aureus* were sensitive to Gentamicin and cefoxitin antibiotics (100%). About 1 sample was *Multiple Drug Resistance* (MDR) on antibiotic tetracyclin, erythromycin, and cloramfenicol. In conclusion, the presence of antibiotic resistant and MDR of *S. aureus* in Ettawa crossbreed goat raw milk should raise public awareness about the dangers posed by these isolates to spread and threaten human health.

Keywords : *Antibiotic resistant, Ettawa crossbreed goat raw milk, Staphylococcus aureus, Human health*

Introduction

Foodstuffs originating from milk are expected to have good nutritional content and are safe for consumption, however milk is a good growth medium for bacteria, both pathogenic bacteria and spoilage bacteria because it contains a number of nutrients (Altalhi and Hassan, 2009). Milk quality is influenced by cage management, milking method, and milk storage. Poor management of the cage, unhygienic milking methods and improper storage can cause milk to become more contaminated with bacteria. Bacteria that are often isolated from food products of animal origin include *Salmonella, Escherichia coli, Camphylobacter, Listeria monocytogenes, Brucella, Mycobacterium, Yersinia, Bacillus cereus* and *Staphylococcus aureus* (*S. aureus)* (Chey *et al.*, 2004). All of these bacteria can cause a source of disease trans-

mission that is harmful to the health of consumers.

S. aureus in raw milk can cause Milk Borne Disease (MBD) for consumers because *S. aureus* bacteria can produce a toxin in the form of Enterotoxin which can cause food poisoning syndrome in humans and animals (Sasidharan *et al*., 2011). The need for good handling of milk before, during, after milking, and storage of milk. So that the quality and nutritional content of the milk is maintained until it is consumed by consumers (Kupradit *et al*., 2020).

S. aureus bacteria is a bacterium that causes infection in goats which is commonly called mastitis. According to Khairullah *et al*., (2020) mastitis is an event that can harm farmers in Indonesia because it can reduce milk production and quality. Mastitis is generally divided into clinical and subclinical mastitis. Clinical bacteria are characterized by inflammation of the udder, whereas subclinical mastitis is characterized by a healthy, well-defined udder that is often not seen by farmers (Alian *et al.,* 2012). Milk that is mastitis can be dangerous for consumers if it is drunk.

Antibiotics are chemicals for treatment in dealing with infectious diseases that are good for human life, which are known as the drug of choice. The use of antibiotics is not only for humans, but also used in the field of animal health. Antibiotic therapy in the livestock sector is generally used to control subclinical mastitis (Jamali *et al.,* 2015). Antibiotics are used for the treatment, control and prevention of infectious diseases. Continuous use of antibiotics can cause bacteria to become resistant to antibiotic treatment. *S. Aureus* is known to be resistant to several antibiotics (Economou *et al.,* 2015). The definition of resistance is not inhibited the growth of bacteria given antibiotics. So that bacteria survive and multiply which can cause therapy using antibiotics to fail (Yunita *et al.,* 2020).

For this reason, it is necessary to conduct research to obtain an overview of the antibiotic resistant profile of *S. aureus* isolated from raw milk of Ettawacrossbreed goat. The hope to be obtained is to increase public awareness about the dangers of antibiotic resistance from *S. aureus* which is a threat to public health.

Materials and Methods

Sample Collection

This research will be conducted from September 2020 to November 2020 at the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Airlangga University. The sample was Ettawa crossbreed goat raw milk from Bangelan farm, Wonosarisub District, Malang, East Java. The samples taken were 100 raw milk samples with each sample of 10 ml.

Bacterial isolates

A total of 26 *S. Aureus* isolates from Ettawa crossbreed goat raw milk were obtained from Bagelan farm, Wonosari sub district, Malang in East Java, Indonesia, were used in this study that shown in Table-1. The isolation and identification were performed for counting bacteria using conventional phenotyping method involved mannitol salt phenol red agar growth (E. Merck, Darmstadt, Germany), Gram staining (Effendi *et al.,* 2018), microscopic observation, catalase test, and tube coagulase test (Effendi *et al.,* 2019).

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 tetracycline, gentamicin, cefoxitin, erythromycin, and chloramphenicol antibiotics were placed on the agar surface with minimal pressure using sterile forceps. The incubation was done at 37 °C for 18-24 hours (Decline *et al*., 2020). The size of the diameter of the inhibition zone was used to determine the sensitivity of bacteria to antibiotics based on standards issued by the Clinical Laboratory Standards International (CLSI, 2020).

Results and Discussion

Prevalence and antibiotic resistance of *Staphylococcus aureus*

The test results found that there were 26 (26%) *S. aureus* isolates from 100 Ettawa crossbreed goat raw milk samples taken from on Bagelan farm, Wonosari, Malang, in East Java, Indonesia based on morphological characteristics, catalase and coagulase tests, as shown in Table 1.

Based on the isolation results from 100 samples, 64 (64%) samples were able to ferment mannitol on MSA media. Changes can be seen in the MSA media which was initially red to yellow and colonies were

Table 1. Prevalence and Antimicrobial Resistance Profile of *Staphylococcus aureus* collected from Ettawa crossbreed goat raw milk on Bagelan farm, Wonosasi, Malang, Indonesia

Location	Sample Size	Confirmed *S. aureus*	Resistant to					MDR
			TE	C	FOX	E	CN	
Bagelan Farm	100	26	5	4	0	14	0	1
TOTAL	100	26	5	4	0	14	0	1
Percentage (%)	100	26	19.2	15.4	0	53.8	0	3.8

Note : TE = Tetracycline (30 µg); FOX= Cefoxitin (30 µg); C= Chloramphenicol (30 µg); E= Erythromycin (15µg); CN= Gentamicin (10µg); MDR = Multidrug Resistant.

formed. The color change occurs because *S. aureus* bacteria produce acid which causes changes in phenol red in agar which changes from red to yellow, S. aureus bacteria are said to be able to ferment mannitol on MSA media (Tyasningsih *et al*., 2019). MSA media is a selective and differential media. MSA media contains 7.5% NaCl so that many bacteria cannot stand the NaCl concentration except Staphylococcus and micrococcus.

Staphylococcus aureus is a gram-positive and cocci-shaped bacteria seen from a purple microscope. The genus *Stapylococcus* itself has a round shape with a clustered arrangement like grapes and is purple or violet in color (Dhanashekar *et al.,* 2012). The results of microscopic examination of 64 samples showed that all samples formed colonies of *S. aureus* bacteria with violet colored characteristics and had a coccus shape. *S. aureus*is a Gram-positive bacterium, in the form of cocci seen from a purple microscope because the bacteria retains the first color, namely gentian violet. Samples that have seen the morphology are then continued with the catalase test. The function of the catalase test is to distinguish between *Staphylococcus* bacteria and Streptococcus bacteria, where Staphylococcus bacteria are positive in the catalase test. *S. Aureus* is a bacterium that can produce the enzyme coagulase. Coagulase test is performed to see and distinguish *S. Aureus* bacteria from other *Staphylococcus* bacteria, because *S. aureus* can coagulate plasma with the help of factors in serum. In the coagulase test of 64 samples, 26 isolates produced positive coagulase with characteristics on BPW media that had been inoculated with *S. aureus* isolates turned into a jelly-like or clot-like shape, which means that the isolates were *S. aureus* bacteria and can be used for confirmation (Figure 1) (Ramandinianto *et al.,* 2020).

The results of the diameter of the inhibition zone from the test of *S. aureus* resistance to 5 antibiotics were then compared with the CLSI standard. The

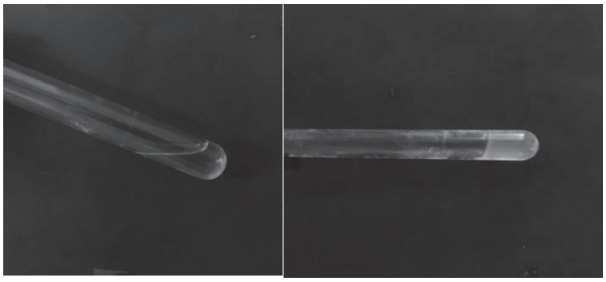

Fig. 1. Negative and positive results of the coagulase test. Positive isolates looked lumpy and had a jelly-like consistency.

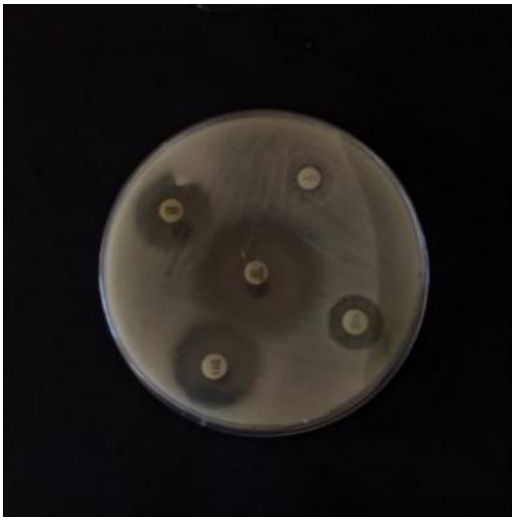

Fig. 2. Results of antibiotic sensitivity test on *S. aureus* with 5 antibiotics

results of the Tetracycline antibiotic resistance test were 5 resistant isolates (19%), 5 intermediate isolates (19%), and 16 sensitive isolates (62%). A total of 26 isolates showed sensitivity (100%) to the antibiotics Gentamicin and Cefoxitin. There were 14 isolates resistant (54%), 18 isolates intermediate (31%), and 4 isolates sensitive (15%) to the antibiotic Erythromycin. In chloramphenicol antibiotics, 4 isolates

were resistant (15%), 6 isolates were intermediate (23%), and 16 isolates were sensitive (62%). Based on the average diameter of the zone of inhibition of bacteria against antibiotics, the average of the antibiotics Tetracycline, Gentamicin, Cefoxitin and Chloramphenicol showed sensitive results with values of 19 mm, 23 mm, 25 mm, and 19 mm, respectively. The average yield of Erythromycin antibiotic is 14 mm, when compared with the standard diameter, it shows intermediate results (Figure 2).

Based on the results of resistance to five antibiotics in the form of Tetracycline, Gentamicin, Cefoxitin, Erythromycin, and Chloramphenicol, it was found that *S. aureus* bacteria in Bangelan farm were still sensitive to Gentamicin and Cefoxitin antibiotics. This is in accordance with the results obtained in 26 samples which showed zones that were still sensitive to the two antibiotics. This result is because the two antibiotics may have never been used on goats in Bangelan farm (Landers *et al*., 2012).

Cefoxitin is a second-generation cefamycin betalactam antibiotic with broad spectrum activity. Cefoxitin has a mechanism of inhibiting bacterial cell wall synthesis. While gentamicin, is an aminoglycoside that has a mechanism to inhibit protein synthesis (Khairullah *et al.,* 2020) Gentamicin has two or more amino groups attached to the benzene group and is bactericidal. In the mechanism of inhibition of protein synthesis, gentamicin binds to the 30S ribosomal subunit of bacteria or some is bound to the 50S ribosomal subunit and inhibits peptidyl-tRNA translocation, resulting in bacteria unable to synthesize vital proteins for growth (Ramandinianto *et al.,* 2020).

The diameter of the inhibition zone on tetracycline antibiotics, chloramphenicol, and erythromycin showed various resistance results. The results of tetracycline diameter showed that 19% isolates were resistant, 19% intermediate, and 62% sensitive. Tetracycline is a tetracycline class antibiotic that has a broad spectrum of action and its usefulness has decreased due to increased bacterial resistance. Some of the sensitive isolates showed that these bacteria still have target recognition sites for tetracyclines. This occurs due to changes in the permeability of the microbial cell envelope. For sensitive bacteria, the drug will be in the environment and will not leave the cell, while in bacteria that are drug resistant it cannot be actively transported into the cell or will disappear quickly so that the minimum inhibitory concentration cannot be maintained (Neu and Gootz, 2001).

Chloramphenicol showed the smallest resistance in the form of 15% isolates, 6% isolates experienced an intermediate state, and 62% were sensitive. Chloramphenicol is a class of antibiotics from chloramphenicol which has a broad spectrum of action. Chloramphenicol prevents protein synthesis by binding to the 50s ribosomal subunit and inhibiting the peptidyl transferase enzyme so that peptide bonds are not formed in the bacterial synthesis process (Rahmaniar *et al.,* 2020). Meanwhile, 54% of isolates were resistant to Erythromycin, 31% intermediate, 15% sensitive. Erythromycin is a macrolide antibiotic that is bacteriostatic, which works to inhibit protein synthesis in bacteria by blocking new proteins from leaving the ribosome (Novak, 2015). *S. Aureus* forms the formation of ribosomal subunits and one of them is the 50S ribosome, the 50S subunit erythromycin binds and prevents the formation of these subunits, as a result of this inhibition, bacteria cannot develop properly (Effendi, 2019). According to Todar (2008), erythromycin resistance occurs due to the efflux pump mechanism by removing antibiotics from bacterial cells.

Antibiotics have a minimum dose to achieve a therapeutic effect; high doses can cause bacteria to mutate due to stronger selection pressure (Raymond, 2019). Giving antibiotics under doses can cause bacteria not to be completely destroyed, so that the remaining bacteria adapt and become resistant. This situation can harm consumers. Errors in diagnosing a disease, administration of inappropriate drug therapy doses, and incomplete drug administration result in not inhibiting bacteria, but can accelerate bacteria to experience mutations and the occurrence of resistance that can harm humans. Bacteria that are resistant to some antibiotics will continue to replicate and pass down resistance genes in other bacteria. Humans who are exposed to resistant bacteria through consumed dairy products (milk borne disease), will be difficult to treat and cause death (Harijani *et al*., 2020).

Conclusion

In conclusion, the presence *S. aureus* and the presence of MDR of *S. aureus* should raise public awareness about the dangers posed by these isolates. Although the results showed that MDR of *S. aureus* from Ettawa crossbreed goat raw milk had a relatively low prevalence at the antibiotic sensitivity test

showed the potential to spread and threaten public health from these isolates.

Acknowledgements

This article was supported in part with the Penelitian Hibah Mandat funding from Airlangga University, Indonesia in the fiscal year 2020, with grant number: 368/UN3.14/PT/2020.

References

Alian, F., Rahimi, E., Shakerian, A., Momtaz, H., Riahi, M. and Momeni, M. 2012. Antimicrobial Resistance of *Staphylococcus aureus* Isolated from Bovine, Sheep and Goat Raw Milk. *Global Veterinaria*. 8(2): 111-114.

Altalhi, A. D. and Hassan, S. A. 2009. Bacterial quality of raw milk investigated by *Escherichia coli* and isolates analysis for specific virulence-gene markers. *Food Control*. 20 : 913–917.

Clinical and Laboratory Standard Institue., 2020. *Performance Standard for Antimicrobial Susceptibility Testing*, 30th Edition. CLSI Document M100-ED30. USA.

Chey, F.Y., Abdullah, A. and Ayobb, M.K. 2004. Bacteriological Quality and Safety of Raw Milk in Malaysia. *Food Microbiol.* 21 : 535–541.

Decline, V., Effendi, M. H., Rahmaniar, R. P., Yanestria, S. M. and Harijani, N. 2020. Profile of antibiotic-resistant and presence of methicillin-resistant *Staphylococcus aureus* from nasal swab of dogs from several animal clinics in Surabaya, Indonesia. *Intl J One Health*. 6(1) : 90-94.

Dhanashekar, R., Akkinepalli, S. and Nellutla, A. 2012. Milk-borne infections. An analysis of their potential effect on the milk industry. *GERMS.* 2(3): 101-109.

Economou, V. and Gousia, P. 2015. Agriculture and Food Animals as A Source of Antimicrobial-Resistant Bacteria. *Infect Drug Resist.* 8 : 49-61.

Effendi, M.H., Oktavianto, A. and Hastutiek, P. 2018. Tetracycline Resistance Gene in Streptococcus Agalactiae Isolated from Bovine Subclinical Mastitis in Surabaya, Indonesia. *Philipp. Journal of Veterinary Medicine*. 55 (SI) : 115-120.

Effendi, M. H., Hisyam, M. A. M., Hastutiek, P. and Tyasningsih, W. 2019. Detection of coagulase gene in *Staphylococcus aureus* from several dairy farms in East Java, Indonesia, by polymerase chain reaction, *Vet. World*. 12(1): 68-71.

Harijani, N., Wandari, A., Effendi, M.H. and Tyasningsih. W. 2020. Molecular Detection of Encoding Enterotoxin C Gene and Profile of Antibiotic Resistant on *Staphylococcus aureus* Isolated from Several Dairy Farms in East Java, Indonesia. *Biochem. Cell. Arch.* 20 (1) : 3081-3085.

Jamali, H., Paydar, M., Radmehr, B., Ismail, S. and Dadrasnia, A. 2015. Prevalence and antimicrobial resistance of *Staphylococcus aureus* isolated from raw milk and dairy products. *Food Control*. 54 : 383-388.

Khairullah, A.R., Sudjarwo, S.A., Effendi, M.H., Harijani, N., Tyasningsih, W., Rahmahani, J., Permatasari, D.A., Ramandinianto, S.C., Widodo, A. and Riwu, K.H.P. 2020. A Review of Methicillin-Resistant *Staphylococcus aureus* (MRSA) on Milk and Milk Products: Public Health Importance. *Sys Rev Pharm.* 11(8): 59-69.

Khairullah, A. R., Ramandinianto, S.C. and Effendi, M.H. 2020. A Review of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* (LA-MRSA) onBovine Mastitis. *Sys Rev Pharm.* 11(7) : 172-183.

Kupradit, C., Innok, S., Woraratphoka, J., Ketudat-Cairns, M. 2020. Prevalence and Characterization of Pathogenic Bacteria in Bulk Tank Raw Milk, Thailand. *Walailak J Sci & Tech.* 17(6): 588-599.

Landers, T.F., Cohen, B., Wittum, T.E. and Larson, E.L. 2012. A review of antibiotic use in food animals: Perspective, policy, and potential. *Public Health Rep.* 127 (1): 4-22. DOI: 10.1177/003335491212700103.

Neu, H. C and Gootz, T. D. 2001. Antimicrobial Chemoterapy. Medmicro. The University of Texas Medical Branch.

Novak, I. and Branka, K. 2015. Electronic Structure of Antibiotic Erythromicin. Spectrochimica Acta Part A: Molecula and Biomolecular Spectroscopy. Vol. 138.

Rahmaniar, R. P., Yunita, M. N., Effendi, M. H. and Yanestria, S. M. 2020. Encoding Gene for Methicillin Resistant *Staphylococcus aureus* (MRSA) Isolated from Nasal Swab of Dogs. *Indian Vet. J.* 97(02), 37-40.

Ramandinianto, S.C., Khairullah, A.R. and Effendi, M.H. 2020. *Mec*A gene and methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from dairy farms in East Java, Indonesia. *Biodiversitas.* 21(8) : 3562-3568.

Ramandinianto, S.C., Khairullah, A.R., Effendi, M.H. and Hestiana, E. P. 2020. Profile of Multidrug Resistance (MDR) and Methicillin Resistant *Staphylococcus aureus* (MRSA) on Dairy Farms in East Java Province, Indonesia. *Indian Journal of Forensic Medicine & Toxicology.* 14(4):3439-3445.

Raymond, B. 2019. Five Rules For Resistance Management In The Antibiotic Apocalypse, a Road Map For Integrated Microbial Management. *Evolutionary Applications.* 12 : 1079-1091.

Sasidharan, S., Prema, B. and Yoga Latha, L.L. 2011. Antimicrobial drug resistance of *Staphylococcus aureus* in dairy products. *Asian Pac J Trop Biomed*. 1 (2) : 130-132. DOI: 10.1016/S2221-1691(11)60010-5

Tyasningsih, W., Effendi, M. H., Budiarto, B. and Syahputra, I. R. 2019. Antibiotic Resistance to *Staphylococcus aureus* and Methicillin Resistant Staphylococcus aureus (MRSA) Isolated from Dairy Farms in Surabaya, Indonesia. *Indian Vet. J.* 96(11) : 27-31.

Yunita, M. N., Effendi, M. H., Rahmaniar, R. P., Arifah, S. and Yanestria, S. M. 2020. Identification of Spa Gene For Strain Typing of Methicillin Resistant *Staphylococcus aureus* (MRSA) Isolated from Nasal Swab of Dogs. *Biochem. Cell. Arch.* 20 (1) : 2999-3004.