BIODIVERSITAS
Volume 24, Number 1, January 2023
Pages: 423-430

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240149

Detection of multidrug-resistant *Staphylococcus aureus* isolated from dairies milk in Medowo Village of Kediri District, Indonesia

GIOVANNI DWI SYAHNI PUTRA[1], ASWIN RAFIF KHAIRULLAH[2], MUSTOFA HELMI EFFENDI[3,♥], MOCHAMAD LAZUARDI[4], SHENDY CANADYA KURNIAWAN[5], DANIAH ASHRI AFNANI[6], OTTO SAHAT MARTUA SILAEN[7], YUSAC KRISTANTO KHODA WARUWU[1], SAUMI KIREY MILLANNIA[1], AGUS WIDODO[8], SAFIRA RAMADHANI[1], MUHAMMAD THORIQ IHZA FARIZQI[1], KATTY HENDRIANA PRISCILIA RIWU[2]

[1]Profession Program in Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia
[2]Doctoral Program in Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia
[3]Division of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia.♥email: mhelmieffendi@gmail.com
[4]Division of Basic Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia
[5]Master program of Animal Sciences, Department of Animal Sciences, Specialisation in Molecule, Cell and Organ Functioning, Wageningen University and Research. Wageningen 6708 PB, Netherlands
[6]Master Program in Veterinary Disease and Public Health, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia
[7]Doctoral Program in Biomedical Science, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No. 6 Senen, Jakarta 10430, Indonesia
[8]Program of Veterinary Paramedic, Faculty of Vocational Studies, Universitas Airlangga. Jl. Airlangga No.4-6, Kampus B Airlangga, Surabaya 60115, East Java, Indonesia

Manuscript received: 27 November 2022. Revision accepted: 16 January 2023.

Abstract. *Putra GDS, Khairullah AR, Effendi MH, Lazuardi M, Kurniawan SC, Afnani DA, Silaen OSM, Waruwu YKK, Millannia SK, Widodo A, Ramadhani S, Farizqi MTI, Riwu KHP. 2023. Detection of multidrug-resistant (MDR)* Staphylococcus aureus *isolated from dairies milk in Medowo Village of Kediri District, Indonesia. Biodiversitas 24: 423-430.* Bacterial resistance has emerged as a major concern in dairy farms in Indonesia due to the pervasive usage of antibiotics. Furthermore, no specific research has been done to explain the prevalence of *Staphylococcus aureus* in isolated milk from dairy cows in Kediri, particularly in Medowo Village, and their antibiotic resistance. Moreover, to control the emergence of diseases in humans brought on by dairy cow's milk or infections transmitted through milk, additional research on the prevalence and resistance of bacteria in dairy farms in the Kediri district is urgently required. In Medowo, the Village Kandangan of District, Kediri Regency, Indonesia, 100 dairy cow's milk samples were taken from farms in numerous hamlets. The Kirby-Bauer method performed an antibiotic sensitivity test using disk diffusion. The sensitivity test was attached with antibiotic discs on tetracycline, penicillin, gentamicin, erythromycin, and cefoxitin. According to sample evaluation results, *S. aureus* was detected in 94 (94%) of the 100 isolated milk samples based on morphological culture features, Gram staining, and biochemical assays. According to the profile of antibiotic resistance derived from the findings of the *S. aureus* antibiotic resistance test, 23 isolates (24.47%) were proven to be multidrug-resistant (MDR) because they were resistant to three to four classes of antibiotics. The use of antibiotics typically rises in response to an increase in the prevalence of disease in cattle, which could lead to higher levels of antibiotic residue in milk and possibly higher levels of bacterial resistance to antibiotics. Therefore, dairy farming requires methods for prudently and correctly employing antibiotics.

Keywords: Milk, multidrug resistance, public health, *Staphylococcus aureus*

INTRODUCTION

The amount of milk consumed annually by Indonesians has increased due to growing awareness of milk consumption for its health benefits (Sjarif et al. 2019). Milk has health advantages because it is a source of calcium for developing teeth and bones and helps people build immunity (Ratajczak et al. 2021). The yield of nutritious milk can be impacted by the health management of dairy cows (Erickson and Kalscheur 2020). Keeping livestock healthy is crucial when raising dairy cattle because they produce at their best when they are healthy (Dallago et al. 2021). Numerous factors need to be considered when it comes to cattle health, beginning with the issues of disease, treatment, and prevention (Kimman et al. 2013).

As a result of milk's chemical constituents, harmful bacteria can flourish and spread in an environment rich in nutrients (Berhe et al. 2020). Milk primarily consists of water (87.2%), fat (3.7%), protein (3.5%), lactose (4.9%), and minerals (0.7%) (Bekuma and Galmessa 2018). The milk pH generally lies between 6.5 and 6.6, which is ideal for microorganisms since the pH level is near neutral. Unfortunately, this pH is usually suitable for bacterial development and causes milk to deteriorate quickly (Marouf and Elmhal 2017).

One harmful bacteria that can contaminate milk and potentially causes sickness is *Staphylococcus aureus* (Gebremedhin et al. 2022; Yunita et al. 2020). As long as it is handled inappropriately, milk contaminated with dangerous bacteria (milk-borne pathogens) can readily become infected anytime and anywhere (Berhe et al. 2020). Some of the risk factors for bacterial contamination include the farmer's dirty hands while milking (Naing et al. 2019), less hygienic dairy equipment (Du et al. 2020), unclean cowshed surroundings (Aliyo and Teklemariam 2022), nearby manure disposal sites (Alegbeleye et al. 2018), and the distance between the cowshed to the well (Pfister et al. 2018; Knight-Jones et al. 2016). During milking and processing, *S. aureus* contamination in milk is possible (Regasa et al. 2019). In dairy cows, *S. aureus* bacterial contamination can result in udder irritation or mastitis, lowering milk output and quality (Khairullah et al. 2020). Even though *S. aureus* contamination does not result in physical changes to the milk, it is obviously hazardous because consumers cannot detect its presence (Zeaki et al. 2019; Virdis et al. 2010).

One of the communities in Kediri Regency in Medowo Village consists of the majority of people as dairy farmers (Solikin et al. 2018). Medowo Village produces 11,500 liters of milk each month (Abadi 2019). The high mastitis cases on dairy farms and the high milk production in Medowo Village are related (Fawaid 2020). Antibiotics are recommended for treating bacterial contamination or are sometimes referred to as preferred treatments (Lee et al. 2013). Tetracycline, gentamicin, and penicillin, for example, are broad-spectrum antibiotics that are frequently used (Decline et al. 2020). Because it is simple to obtain and reasonably priced, these antibiotics are commonly used (Khairullah et al. 2019). Injecting antibiotics directly or combining them with straw, bran, tofu dregs, and grass is generally adopted by farmers (Mdegela et al. 2021).

Bacterial resistance has developed due to Indonesia's dairy farm's extensive use of antibiotics (Ramandinianto et al. 2020a). Uncontrolled antibiotic use tends to make previously susceptible microorganisms more resistant (Mancuso et al. 2021). Dairy farmers in Kediri frequently practice antibiotics based on self-medication (Nepal and Bhatta 2018). The breeder's lack of understanding increases the probability of new issues and the formation of multidrug-resistant (MDR) S. aureus germs (Liu et al. 2022). Additionally, no specific research has been done to explain the prevalence of *S. aureus* resistance found in dairy cows in Kediri, particularly in Medowo Village.

Fresh milk contaminated with *S. aureus* and antibiotic resistance can result in toxic shock syndrome (Tamendjari et al. 2021). Furthermore, *S. aureus* infections brought on by resistant strains are more challenging to treat than infections brought on by non-resistant strains, and the bacteria do not react well to treatments therein (Chambers and Deleo 2009). As a result, antibiotic treatment becomes challenging and expensive, and it may not always heal effectively (Ventola 2015). Therefore, the present study investigated the diversity and prevalence of *Staphylococcus aureus* isolated milk from dairy cows in Kediri, particularly in Medowo Village, and their antibiotic resistance.

MATERIALS AND METHODS

Study area and sample collection

The research was carried out between March and July 2022. A sampling of 100 dairy cow milk was carried out from dairy farms in several hamlets (Medowo hamlet, Sidorejo hamlet, Mulyorejo hamlet, and Ringinagung hamlet) in Medowo Village of Kandangan District, Kediri Regency. In addition, laboratory experimentation on isolation and identification and tests for antibiotic sensitivity of bacteria was carried out at the Faculty of Veterinary Medicine, Airlangga University, Surabaya's Veterinary Public Health Laboratory.

Isolation and identification of *Staphylococcus aureus*

A test tube containing 9 mL of Buffered Peptone Water (BPW) medium and up to 1 mL of the milk sample were used for the incubation process, which took place for 24 hours at 37°C. First, bacterial isolation was carried out on Mannitol Salt Agar (MSA) media by adding one ose inoculant from BPW with structure-containing beads. After that, MSA media were streaked to obtain the initial bacterial growth carried out in a zigzag pattern. MSA was then incubated for 24 hours at 37°C (Rahmaniar et al. 2020).

The next stage is the Gram stain test to determine the species of Gram bacteria in the colony. Next, the catalase and coagulase assays were also conducted to identify the S. aureus bacterium. Next, 3% H2O2 hydrogen peroxide was dripped on a clean glass object to perform the catalase test. Then, mix one dose of inoculum from MSA in the object glass (Ismail et al. 2018). The coagulase test involved aseptically inserting 200 µl of plasma into a sterile microtube. Then add 3-4 culture colonies and incubate the mixture at 37°C for 24 hours to detect the presence of free coagulase (Kumari et al. 2020).

Antibiotic sensitivity test

Kirby-Bauer method of antibiotic sensitivity test was used in this study using Mueller Hinton Agar (MHA) media and Disk Diffusion Agar. In this test, *S. aureus* isolates were partially removed from sterile loops and combined with 8 mL of physiological NaCl in a test tube. The solution was then homogenized with a vortex until it reached the McFarland turbidity standard of 0.5. Next, a sterile cotton swab dispensed up to 0.2 mL of bacterial suspension in physiological NaCl, then evenly inoculated onto MHA media (Afnani et al. 2022). Then place the antibiotic discs with tetracycline 30 µg, penicillin 10 µg, gentamicin 10 µg, erythromycin 15 µg, and cefoxitin 30 µg using a tweezer. The media was incubated at 37°C for 24 hours after receiving an antibiotic disc.

By measuring the diameter of the inhibition zone formed around the antibiotic disc with a vernier caliper, the disc diffusion method was used to read the antibiotic sensitivity test results (Fanissa et al. 2022). The Clinical and Laboratory Standards Institute (CLSI) 2020 is used to analyze measurements of the bacterial inhibition zone.

RESULTS AND DISCUSSION

Bacterial isolates

Based on the sample evaluation and the results of the morphological culture, Gram staining, and biochemical testing, 94 (94%) of the 100 isolated milk samples were confirmed to be positive for *S. aureus* (Table 1). Yellow bacterial colonies on MSA media indicated a positive morphological culture of *S. aureus* (Figure 1). Purple colonies and grouped cocci that indicate positive Gram stain results can be seen (Figure 2). a biochemical test for *S. aureus* has produced positive results (Figure 4) when bubbles emerge on the object glass during the catalase test (Figure 3), and clout appears at the bottom of the microtube during the coagulase test.

Antibiotic resistance of *Staphylococcus aureus*

Out of a total of 94 *S. aureus* isolates, 24 (25.53%) were resistant to the one class of antibiotics tested, while, according to the profile of antibiotic resistance derived from the findings of the S. aureus resistance test to antibiotics. In comparison, 39 isolates (41.49%) were antimicrobial resistant to two classes, and 23 isolates (24.47%) were confirmed to be multidrug resistant because they were resistant to 3 to 4 antibiotic classes (Figure 5). Furthermore, the multidrug resistance was dominated by the pattern of TE – GM – P (Tetracycline, gentamicin, penicillin) antibiotic resistance. The pattern of TE followed as many as 11 (11.70%) isolates – P – E antibiotic resistance (Tetracycline, penicillin, erythromycin) by seven isolates (7.45%) (Table 2).

Milk samples from the Sidorejo sub-village contained 11 MDR isolates of *S. aureus* were found most frequently, followed by six isolates from the Medowo sub-village (Table 3). That may account for the high number of MDR *S. aureus* cases, 23 isolates from 100 milk samples examined found in dairy farms in Medowo Village, Kediri District. In addition, the study found that 86 isolates of *S. aureus* had the highest level of penicillin resistance.

Table 1. Isolation of *Staphylococcus aureus* from milk samples in Medowo village

Location	Sample code	Sample size	*S. aureus* (%)
Medowo hamlet	PSI, PBT, PED, PJ	25	25 (100%)
Sidorejo hamlet	PW, PS, PNG, PDIK	25	22 (88%)
Mulyorejo hamlet	PL, PMI, PDI, PD, PPIT	25	23 (92%)
Ringinagung hamlet	PH, PM, PG, PP	25	24 (96%)
Total		100	94 (94%)

Note: % (Percentage of positive).

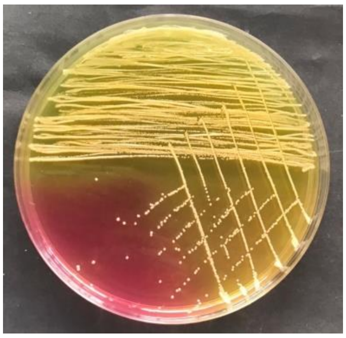

Figure 1. *Staphylococcus aureus* colonies in MSA

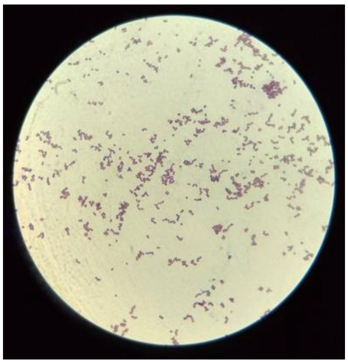

Figure 2. Gram-stained *Staphylococcus aureus* colonies under a microscope

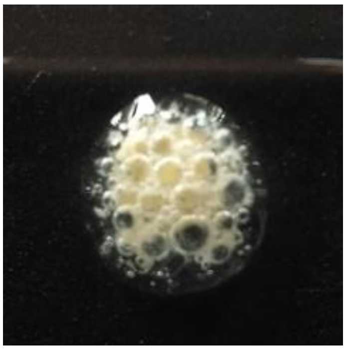

Figure 3. Catalase test results indicate *Staphylococcus aureus* positivity

Figure 4. Coagulation test results indicate *Staphylococcus aureus* positivity

Table 2. Isolated *Staphylococcus aureus* resistance profile by antibiotic group

Group of antibiotics	Resistance profile	Number of isolates (n=94)	Total number of isolates (%)
		Resistant isolates (%)	
0	No one is resistant	8 (8.51%)	8 (8.51%)
1	P	24 (25.53%)	24 (25.53%)
	TE – P	34 (36.17%)	39 (41.49%)
2	GM – P	3 (3.19%)	
	P – E	2 (2.13%)	
	FOX – TE – P	1 (1.06%)	23 (24.47%)
	FOX – GM – P	3 (3.19%)	
≥3	TE – GM – P	11 (11.70%)	
	TE – P – E	7 (7.45%)	
	TE – GM – P – E	1 (1.06%)	

Note: FOX: Cefoxitin, E: Erythromycin, GM: Gentamicin, TE: Tetracycline, P: Penicillin.

Table 4. *Staphylococcus aureus* isolates with a profile MDR

Hamlet	Sample code	Resistance profile	Resistance profile				
			FOX	TE	GM	P	E
Medowo	PBT 1	TE – P – E	–	✓	–	✓	✓
	PED 5	TE – P – E	–	✓	–	✓	✓
	PJ 3	TE – GM – P	–	✓	✓	✓	–
	PJ 4	TE – P – E	–	✓	–	✓	✓
	PJ 5	TE – P – E	–	✓	–	✓	✓
	PJ 6	TE – GM – P – E	–	✓	✓	✓	✓
Sidorejo	PW 6	FOX – GM – P	✓	–	✓	✓	–
	PW 7	TE – GM – P	–	✓	✓	✓	–
	PW 9	FOX – GM – P	✓	–	✓	✓	–
	PW 10	FOX – GM – P	✓	–	✓	✓	–
	PW 12	TE – GM – P	–	✓	✓	✓	–
	PW 16	TE – GM – P	–	✓	✓	✓	–
	PW 17	TE – GM – P	–	✓	✓	✓	–
	PW 18	TE – GM – P	–	✓	✓	✓	–
	PW 19	TE – GM – P	–	✓	✓	✓	–
	PDIK 1	TE – P – E	–	✓	–	✓	✓
	PDIK 2	TE – P – E	–	✓	–	✓	✓
Mulyorejo	PDI 3	TE – P – E	–	✓	–	✓	✓
	PD 2	TE – GM – P	–	✓	✓	✓	–
Ringinagung	PM 1	TE – GM – P	–	✓	✓	✓	–
	PM 2	TE – GM – P	–	✓	✓	✓	–
	PM 5	TE – GM – P	–	✓	✓	✓	–
	PP 1	FOX – TE – P	✓	✓	–	✓	–

Note: ✓: Resistant, FOX : Cefoxitin, E : Erythromycin, GM : Gentamicin, TE : Tetracycline, P : Penicillin.

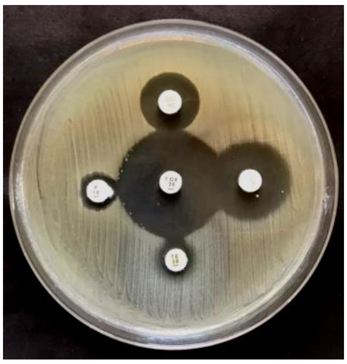

Figure 5. Susceptibility to antibiotics of *Staphylococcus aureus* isolates cultured on MHA

Discussion

Milk frequently contains the bacterium *S. aureus*, which can cause infectious mastitis (Khairullah et al. 2020). A contagious bacterium that dwells on a cow's udder and teat surface cause contagious mastitis, an inflammation of the udder (Kerro et al. 2020). During the milking process, this bacterium spreads from sick cows or quarters to healthy cows or quarters (Cheng and Han 2020). The skin is quickly colonized by *Staphylococcus*, which then enters the nipple duct, where infection occurs (Tong et al. 2015). Mastitis can be split into two categories based on bacterial contamination: mastitis brought on by agents from within the quarter and mastitis brought on by environmental contamination agents (Cobirka et al. 2020). Compared to subclinical mastitis produced by environmental disease agents, the incidence rate of disease caused by pathogens from inside the quarter is higher (Widodo et al. 2022).

Mastitis is frequently brought on by *S. aureus* bacterial infection in dairy cows, which also lowers milk quality and production (Côté-Gravel and Malouin 2019). According to an epidemiological investigation by Birhanu et al. (2017), *S. aureus* bacteria were shown to be the 65% causal agent of subclinical mastitis isolated from milk samples. During the milking process, dairy cows' nipples can exchange *S. aureus* infection, resulting in transmission (Antanaitis et al. 2021). In this investigation, *S. aureus* was identified in 94 (94%) of 100 milk samples. This proportion is greater than that found in the study by Gebremedhin et al. (2022), which examined 486 milk samples, 52 of which (10.70%) had *S. aureus* verified. In addition, Khairullah et al. (2022) did a study in which they evaluated 150 milk samples, only 80 of which (53.33%) had *S. aureus* verified.

The emergence of bacterial resistance to antibiotics coincides with the rising prevalence of antimicrobial medications and the introduction of new drugs (Cook and Wright 2022). The failure of an antibiotic to work against a bacterium is known as antibiotic resistance (Kapoor et al. 2017). Plasmid alterations or exchanges between members of the same bacterial species give germs their survival ability (Zwanzig 2020). The disc diffusion test method is most frequently used to determine the sensitivity status of bacteria to antibiotics (Balouiri et al. 2016). Disk diffusion involves the absorption of the antibacterial portion to be tested on disc paper, attaching it to agar media that has been homogenized with bacteria, and incubating the mixture until an inhibition zone is apparent in the vicinity of the disc (Mawardi et al. 2020). The antibiotic's capacity to diffuse, the media's thickness, the antibiotic's concentration in the disc, and the organism's sensitivity to the antibiotic all impact how big the inhibition zone forms (Bonev et al. 2008).

The antibiotic sensitivity test results are used to create an antibiotic resistance profile that serves as scientific data for infection control (Badulla et al. 2020). Data on antibiotic resistance profiles can also be used to track the prevalence of antibiotic resistance across various organisms and geographical locations (Cave et al. 2021). Poor infection control and the extensive use of antibiotics contribute to the spread of antibiotic resistance (Ayukekbong et al. 2017). Antibiotic sensitivity monitoring enables the detection of infections with multi-resistant traits, the potential to spread to the population, and the amount of resistance rising yearly (Gajic et al. 2022). According to Duran et al. (2012) incidences of antibiotic resistance are frequently observed in *S. aureus*.

According to the study findings of Lobanovska and Pilla (2017), 86 isolates of *S. aureus* had the highest resistance to penicillin medicines. Because of their frequent use, penicillin antibiotic resistance is possible (Lobanovska and Pilla 2017). Mastitis in dairy cows is commonly treated with penicillin, a form of β-lactamase antibiotic (Fejzic et al. 2014). Penicillin is frequently used to treat and manage bacterial infections on dairy farms, which causes the level of *S. aureus* resistance to be extremely high in some regions (Virto et al. 2022). In this investigation, the disc diffusion method identified 23 isolates (24.47%) of *S. aureus* that were multidrug resistant to more than three same classes of antibiotics. The proportion in this study is still higher than that found in Ramandinianto et al. (2020b), which recovered 76 *S. aureus* isolates from 170 milk samples, 13 of which (17.10%) were multidrug-resistant *S. aureus*. Even so, this study's percentage is still lower than that of other research by Mohammed et al. (2018), who identified 46 isolates of *S. aureus* from 117 milk samples, of which 12 isolates (26.09%) were multidrug-resistant *S. aureus*.

Many factors contribute to the development of antibiotic resistance in microorganisms, including their inappropriate (irrational) use and the prevalence of community self-medication. Also contributed was the rise in unnecessary health care costs, the government's lax oversight of the distribution of antibiotics, and the absence of expert research will find new antibiotics (Prestinaci et al. 2015). Although *S. aureus* is naturally susceptible to antibiotics, it also possesses a remarkable capacity to become resistant to nearly every antimicrobial to which it is exposed (Keman and Soyer 2019). According to Selim et al. (2022), the possibility of transmission makes the presence of *S. aureus* resistant to antibiotics in milk hazardous to the public health sector.

A latent resistance gene's expression, a gene with a resistance determinant or genetic mutation, is the primary way bacterial colonies survive in a threatened state (Sharma et al. 2019). Moreover, chromosomes encode most bacterial information, multidrug resistance in bacteria develops due to multistep mutations that gradually increase resistance (Gogry et al. 2021). Extrachromosomal genes, which can be found in plasmids or bacteriophages, are present in some bacteria (Deutsch et al. 2018). Resistance genes can be passed from chromosomes to plasmids or the other way around on transposons and integrons, where the factor R plasmids, also known as transmitting plasmids, can be transferred (Partridge et al. 2018).

Plasmids that experience multiple resistance and the existence of genes in chromosomes that convey resistance qualities can also create multidrug-resistant bacteria (McMillan et al. 2019). For example, an integron comprises two DNA segments with an antibiotic resistance gene on one side, as opposed to a transposon, which is one gene or a small number of resistance genes that are directly repeated or reverse-bound to a DNA sequence (Sultan et al. 2018).

The use of antibiotics typically rises in response to an increase in the prevalence of disease in cattle, which could lead to higher levels of antibiotic residue in milk and possibly higher levels of bacterial resistance to antibiotics (Ghimpețeanu et al. 2022). *S. aureus* resistant to medicines can spread through several risk factors, including cow udders, cow bodies, airborne dust, unsanitary milking equipment, and humans who are less aseptic when milking (Kalayu et al. 2020). This study revealed that *S. aureus* is a multidrug-resistant, and rational and prudent use of antibiotics is required (Karaman et al. 2020). In addition, there is a risk of acquiring antibiotic resistance when using broad-spectrum antibiotics and applying inappropriate treatment techniques (Rhee et al. 2020).

In conclusion, according to the findings of this investigation, 23 multidrug-resistant (MDR) *S. aureus* isolates were discovered in several dairy farms in Medowo Village, Kediri Regency. The high multidrug resistance levels used in the Kediri district led to this figure. Additional research on bacterial resistance in dairy farms in the Kediri district is urgently required to stop the occurrence of human diseases brought on by dairy cow's milk or milk-borne illnesses. Furthermore, methods for using antibiotics sensibly and effectively are required to manage infections caused by multidrug-resistant *S. aureus.*

ACKNOWLEDGMENTS

This study was partly supported by the *Penelitian Hibah Mandat* funding from Universitas Airlangga, Indonesia, in the fiscal year 2022, with grant number: 220/UN3.15/PT/2022.

REFERENCES

Abadi I. 2019. Agribusiness development strategy farms dairy cattle in the District Kediri. Manag Agribus: J Agribus 19 (2): 9-25. DOI: 10.32503/agribisnis.v19i2.648.

Afnani DA, Fatih N, Effendi MH, Tyasningsih W, Kairullah AR, Kurniawan SC, Silaen OSM, Ramandianto SC, Widodo A, Hendriana K, Riwu KHP. 2022. Profile of Multidrug Resistance and Methicillin-Resistant *Staphylococcus aureus* (MRSA) isolated from cats in Surabaya, Indonesia. Biodiversitas 23 (11): 5703-5709. DOI: 10.13057/biodiv/d231121.

Alegbeleye OO, Singleton I, Sant'Ana AS. 2018. Sources and contamination routes of microbial pathogens to fresh produce during field cultivation: A review. Food Microbiol 73: 177-208. DOI: 10.1016/j.fm.2018.01.003.

Aliyo A, Teklemariam Z. 2022. Assessment of milk contamination, associated risk factors, and drug sensitivity patterns among isolated bacteria from raw milk of borena zone, Ethiopia. J Trop Med 2022: 3577715. DOI: 10.1155/2022/3577715.

Antanaitis R, Juozaitienė V, Jonike V, Baumgartner W, Paulauskas A. 2021. Milk lactose as a biomarker of subclinical mastitis in dairy cows. Animals 11 (6): 1736. DOI: 10.3390/ani11061736.

Ayukekbong JA, Ntemgwa M, Atabe AN. 2017. The threat of antimicrobial resistance in developing countries: causes and control strategies. Antimicrob Resist Infect Control 6: 47. DOI: 10.1186/s13756-017-0208-x.

Badulla WFS, Alshakka M, Ibrahim MIM. 2020. Antimicrobial resistance profiles for different isolates in Aden, Yemen: A cross-sectional study in a resource-poor setting. Biomed Res Intl 2020: 1810290. DOI: 10.1155/2020/1810290.

Balouiri M, Sadiki M, Ibnsouda SK. 2016. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal 6 (2): 71-79. DOI: 10.1016/j.jpha.2015.11.005.

Bekuma A, Galmessa U. 2018. Review on hygienic milk products practice and occurrence of mastitis of mastitis in cow's milk. Agric Res Technol: Open Access J 18 (2): 556053. DOI: 10.19080/ARTOAJ.2018.18.556053.

Berhe G, Wasihun AG, Kassaye E, Gebreselasie K. 2020. Milk-borne bacterial health hazards in milk produced for commercial purpose in Tigray, northern Ethiopia. BMC Public Health 20: 894. DOI: 10.1186/s12889-020-09016-6.

Birhanu M, Leta S, Mamo G, Tesfaye S. 2017. Prevalence of bovine subclinical mastitis and isolation of its major causes in Bishoftu Town, Ethiopia. BMC Res Notes 10 (1): 767. DOI: 10.1186/s13104-017-3100-0.

Bonev B, Hooper J, Parisot J. 2008. Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. J Antimicrob Chemother 61 (6): 1295-1301. DOI: 10.1093/jac/dkn090.

Cave R, Cole J, Mkrtchyan HV. 2021. Surveillance and prevalence of antimicrobial resistant bacteria from public settings within urban built environments: Challenges and opportunities for hygiene and infection control. Environ Intl 157: 106836. DOI: 10.1016/j.envint.2021.106836.

Chambers HF, Deleo FR. 2009. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. Nat Rev Microbiol 7 (9): 629-641. DOI: 10.1038/nrmicro2200.

Cheng WN, Han SG. 2020. Bovine mastitis: risk factors, therapeutic strategies, and alternative treatments - A review. Asian-Australas J Anim Sci 33 (11): 1699-1713. DOI: 10.5713/ajas.20.0156.

Cobirka M, Tancin V, Slama P. 2020. Epidemiology and Classification of Mastitis. Animals (Basel) 10 (12): 2212. DOI: 10.3390/ani10122212.

Cook MA, Wright GD. 2022. The past, present, and future of antibiotics. Sci Transl Med 14 (657): eabo7793. DOI: 10.1126/scitranslmed.abo7793.

Côté-Gravel J, Malouin F. 2019. Symposium review: Features of *Staphylococcus aureus* mastitis pathogenesis that guide vaccine development strategies. J Dairy Sci 102 (5): 4727-4740. DOI: 10.3168/jds.2018-15272.

Dallago GM, Wade KM, Cue RI, McClure JT, Lacroix R, Pellerin D, Vasseur E. 2021. Keeping dairy cows for longer: A critical literature review on dairy cow longevity in high milk-producing countries. Animals 11 (3): 808. DOI: 10.3390/ani11030808.

Decline V, Effendi MH, Rahmaniar RP, Yanestria SM, 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. DOI: 10.14202/ijoh.2020.90-94.

Deutsch DR, Utter B, Verratti KJ, Sichtig H, Tallon LJ, Fischetti VA. 2018. Extra-chromosomal DNA sequencing reveals episomal prophages capable of impacting virulence factor expression in *Staphylococcus aureus*. Front Microbiol 9: 1406. DOI: 10.3389/fmicb.2018.01406.

Du B, Meng L, Liu H, Zheng N, Zhang Y, Guo X, Zhao S, Li F, Wang J. 2020. Impacts of milking and housing environment on milk microbiota. Animals 10 (12): 2339. DOI: 10.3390/ani10122339.

Duran N, Ozer B, Duran GG, Onlen Y, Demir C. 2012. Antibiotic resistance genes & susceptibility patterns in staphylococci. Indian J Med Res 135 (3): 389-396.

Erickson PS, Kalscheur KF. 2020. Nutrition and feeding of dairy cattle. Anim Agric 157-180. DOI: 10.1016/B978-0-12-817052-6.00009-4.

Fanissa F, Effendi MH, Tyasningsih W, Ugbo EN. 2022. Multidrug-resistant *Salmonella* species from chicken meat sold at Surabaya Traditional Markets, Indonesia. Biodiversitas 23 (6): 2823-2829. DOI: 10.13057/biodiv/d230606.

Fawaid, B. 2020. Cage sanitation, hygiene of dairy farmer, physical quality and microorganism of dairy cattle milk in Medowo, Kediri, East Java. J Environ Health 12 (1): 69-77. DOI: 10.20473/jkl.v12i1.2020.69-77.

Fejzic N, Begagic M, Šerić-Haračić S, Smajlovic M. 2014. Beta lactam antibiotics residues in cow's milk: comparison of efficacy of three screening tests used in Bosnia and Herzegovina. Bosn J Basic Med Sci 14 (3): 155-159. DOI: 10.17305/bjbms.2014.3.109.

Gajic I, Kabic J, Kekic D, Jovicevic M, Milenkovic M, Mitic Culafic D, Trudic A, Ranin L, Opavski N. 2022. Antimicrobial susceptibility testing: A comprehensive review of currently used methods. Antibiotics 11 (4): 427. DOI: 10.3390/antibiotics11040427.

Gebremedhin EZ, Ararso AB, Borana BM, Kelbesa KA, Tadese ND, Marami LM, Sarba EJ. 2022. Isolation and identification of *Staphylococcus aureus* from milk and milk products, associated factors for contamination, and their antibiogram in Holeta, Central Ethiopia. Vet Med Intl 2022: 6544705. DOI: 10.1155/2022/6544705.

Ghimpețeanu OM, Pogurschi EN, Popa DC, Dragomir N, Drăgotoiu T, Mihai OD, Petcu CD. 2022. Antibiotic use in livestock and residues in food-a public health threat: A Review. Foods 11 (10): 1430. DOI: 10.3390/foods11101430.

Gogry FA, Siddiqui MT, Sultan I, Haq QMR. 2021. Current update on intrinsic and acquired colistin resistance mechanisms in bacteria. Front Med 8: 677720. DOI: 10.3389/fmed.2021.677720.

Ismail YS, Yulvizar C, Mazhitov B. 2018. Characterization of lactic acid bacteria from local cow's milk kefir. IOP Conf Ser: Earth Environ Sci 130: 012019. DOI: 10.1088/1755-1315/130/1/012019.

Kalayu AA, Woldetsadik DA, Woldeamanuel Y, Wang SH, Gebreyes WA, Teferi T. 2020. Burden and antimicrobial resistance of *S. aureus* in dairy farms in Mekelle, Northern Ethiopia. BMC Vet Res 16 (1): 20. DOI: 10.1186/s12917-020-2235-8.

Kapoor G, Saigal S, Elongavan A. 2017. Action and resistance mechanisms of antibiotics: A guide for clinicians. J Anaesthesiol Clin Pharmacol 33 (3): 300-305. DOI: 10.4103/joacp.JOACP_349_15.

Karaman R, Jubeh B, Breijyeh Z. 2020. Resistance of Gram-Positive Bacteria to Current Antibacterial Agents and overcoming approaches. Molecules 25 (12): 2888. DOI: 10.3390/molecules25122888.

Keman D, Soyer F. 2019. Antibiotic-resistant *Staphylococcus aureus* does not develop resistance to vanillic acid and 2-hydroxycinnamic acid after continuous exposure in vitro. ACS Omega 4 (13): 15393-15400. DOI: 10.1021/acsomega.9b01336.

Kerro Dego O, Pacha PA, Gillespie BE, Pighetti GM. 2020. Experimental *Staphylococcus aureus* mastitis infection model by teat dipping in bacterial culture suspension in dairy cows. Animals (Basel) 10 (5): 751. DOI: 10.3390/ani10050751.

Khairullah AR, Raharjo D, Rahmahani J, Suwarno, Tyasningsih W, Harijani N. 2019. Antibiotics resistant at *Staphylococcus aureus* and *Streptococcus* sp isolated from bovine mastitis in Karangploso, East Java, Indonesia. Indian J Forensic Med Toxicol 13 (4): 439-444. DOI: 10.5958/0973-9130.2019.00329.3.

Khairullah AR, Ramandinianto SC, Effendi MH. 2020. A Review of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* (LA-MRSA) on Bovine Mastitis. Syst Rev Pharm 11 (7): 172-183. DOI: 10.31838/srp.2020.7.28.

Khairullah AR, Sudjarwo SA, Effendi MH, Ramandininto SC, Gelolodo MA, Widodo A, Riwu KHP, Kurniawati DA, Rehman S. 2022. Profile of Multidrug Resistance and Methicillin-Resistant *Staphylococcus aureus* (MRSA) on dairy cows and risk factors from farmer. Biodiversitas 23 (6): 2853-2858. DOI: 10.13057/biodiv/d230610.

Kimman T, Hoek M, de Jong MCM. 2013. Assessing and controlling health risks from animal husbandry. NJAS - Wagening J Life Sci 66: 7-14. DOI: 10.1016/j.njas.2013.05.003.

Knight-Jones TJ, Hang'ombe MB, Songe MM, Sinkala Y, Grace D. 2016. Microbial contamination and hygiene of fresh cow's milk produced by smallholders in Western Zambia. Intl J Environ Res Public Health 13 (5): 737. DOI: 10.3390/ijerph13070737.

Kumari H, Chakraborti T, Singh M, Chakrawarti MK, Mukhopadhyay K. 2020. Prevalence and antibiogram of coagulase negative *Staphylococci* in bioaerosols from different indoors of a university in India. BMC Microbiol 20: 211. DOI: 10.1186/s12866-020-01875-8.

Lee CR, Cho IH, Jeong BC, Lee SH. 2013. Strategies to minimize antibiotic resistance. Intl J Environ Res Public Health 10 (9): 4274-4305. DOI: 10.3390/ijerph10094274.

Liu J, Wang X, Bi C, Mehmood K, Ali F, Qin J, Han Z. 2022. Molecular characterization of multi-drug-resistant *Staphylococcus aureus* in mastitis bovine milk from a dairy farm in Anhui, China. Front Vet Sci 9: 966533. DOI: 10.3389/fvets.2022.966533.

Lobanovska M, Pilla G. 2017. Penicillin's Discovery and Antibiotic Resistance: Lessons for the Future? Yale J Biol Med 90 (1): 135-145.

Mancuso G, Midiri A, Gerace E, Biondo C. 2021. Bacterial Antibiotic Resistance: The Most Critical Pathogens. Pathogens 10 (10): 1310. DOI: 10.3390/pathogens10101310.

Marouf AAS, Elmhal SI. 2017. Monitoring pH During Pasteurization of Raw Cow's Milk using Nd: YAG Laser. Intl J Adv Res Phys Sci 4 (12): 1-4. DOI: 10.20431/IJARPS.2017.4.12.2.

Mawardi RH, Sulistyani N, Nurkhasanah, Desyratnaputri R. 2020. Antibacterial activity and tlc-bioautography analysis of the active fractions of *Muntingia calabura* L. leaves against *Staphylococcus aureus*. J Pharm Sci Community 17 (2): 69-75. DOI: 10.24071/jpsc.002362.

McMillan EA, Gupta SK, Williams LE, Jové T, Hiott LM, Woodley TA, Barrett JB, Jackson CR, Wasilenko JL, Simmons M, Tillman GE, McClelland M, Frye JG. 2019. Antimicrobial resistance genes, cassettes, and plasmids present in *Salmonella enterica* associated with United States food animals. Front Microbiol 10: 832. DOI: 10.3389/fmicb.2019.00832.

Mdegela RH, Mwakapeje ER, Rubegwa B, Gebeyehu DT, Niyigena S, Msambichaka V, Nonga HE, Antoine-Moussiaux N, Fasina FO. 2021. Antimicrobial use, residues, resistance and governance in the food and agriculture sectors, Tanzania. Antibiotics (Basel) 10 (4): 454. DOI: 10.3390/antibiotics10040454.

Mohammed J, Ziwa MH, Hounmanou YMG, Kisanga A, Tuntufye HN. 2018. Molecular typing and antimicrobial susceptibility of Methicillin-Resistant *Staphylococcus aureus* isolated from bovine milk in Tanzania. Intl J Microbiol 2018: 4287431. DOI: 10.1155/2018/4287431.

Naing YW, Wai SS, Lin TN, Thu WP, Htun LL, Bawm S, Myaing TT. 2019. Bacterial content and associated risk factors influencing the quality of bulk tank milk collected from dairy cattle farms in Mandalay Region. Food Sci Nutr 7 (3): 1063-1071. DOI: 10.1002/fsn3.945.

Nepal G, Bhatta S. 2018. Self-medication with Antibiotics in WHO Southeast Asian Region: A Systematic Review. Cureus 10 (4): e2428. DOI: 10.7759/cureus.2428.

Partridge SR, Kwong SM, Firth N, Jensen SO. 2018. Mobile Genetic Elements Associated with Antimicrobial Resistance. Clin Microbiol Rev 31 (4): e00088-17. DOI: 10.1128/CMR.00088-17.

Pfister H, Madec L, Cann PL, Costet N, Chouvet M, Jouneau S, Vernhet L. 2018. Factors determining the exposure of dairy farmers to thoracic organic dust. Environ Res 165: 286-293. DOI: 10.1016/j.envres.2018.04.031.

Prestinaci F, Pezzotti P, Pantosti A. 2015. Antimicrobial resistance: a global multifaceted phenomenon. Pathog Glob Health 109 (7): 309-318. DOI: 10.1179/2047773215Y.0000000030.

Rahmaniar RP, Yunita MN, Effendi MH, Yanestria SM. 2020. Encoding gene for methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from nasal swab of dogs. Indian Vet J 97 (2): 37-40.

Ramandinianto SC, Khairullah AR, Effendi MH. 2020a. *Mec*A gene and methicillin resistant *Staphylococcus aureus* (MRSA) isolated from dairy farms in East Java, Indonesia. Biodiversitas 21 (8): 3562-3568. DOI: 10.13057/biodiv/d210819.

Ramandinianto SC, Khairullah AR, Effendi MH, Hestiana EP. 2020b. Profile of Multidrug Resistance (MDR) and Methicillin Resistant *Staphylococcus aureus* (MRSA) on Dairy Farms in East Java Province, Indonesia. Indian J Forensic Med Toxicol 14 (4): 3439-3445. DOI: 10.37506/ijfmt.v14i4.12157.

Ratajczak AE, Zawada A, Rychter AM, Dobrowolska A, Krela-Kaźmierczak I. 2021. Milk and Dairy Products: Good or bad for human bone? practical dietary recommendations for the prevention and management of Osteoporosis. Nutrients 13 (4): 1329. DOI: 10.3390/nu13041329.

Regasa S, Mengistu S, Abraha A. 2019. Milk safety assessment, isolation, and antimicrobial susceptibility profile of *Staphylococcus aureus* in selected dairy farms of Mukaturi and Sululta Town, Oromia Region, Ethiopia. Vet Med Intl 2019: 3063185. DOI: 10.1155/2019/3063185.

Rhee C, Kadri SS, Dekker JP, Danner RL, Chen HC, Fram D, Zhang F, Wang R, Klompas M. 2020. Prevalence of antibiotic-resistant pathogens in culture-proven sepsis and outcomes associated with inadequate and broad-spectrum empiric antibiotic use. JAMA Netw Open 3 (4): e202899. DOI: 10.1001/jamanetworkopen.2020.2899.

Selim A, Kelis K, AlKahtani MDF, Albohairy FM, Attia KA. 2022. Prevalence, antimicrobial susceptibilities and risk factors of Methicillin resistant *Staphylococcus aureus* (MRSA) in dairy bovines. BMC Vet Res 18: 293. DOI: 10.1186/s12917-022-03389-z.

Sharma D, Misba L, Khan AU. 2019. Antibiotics versus biofilm: an emerging battleground in microbial communities. Antimicrob Resist Infect Control 8: 76. DOI: 10.1186/s13756-019-0533-3.

Sjarif DR, Yuliarti K, Iskandar WJ. 2019. Daily consumption of growing-up milk is associated with less stunting among Indonesian toddlers. Med J Indones 28 (1): 70-76. DOI: 10.13181/mji.v28i1.2607.

Solikin N, Hartono B, Fanani Z, Ichsan MN. 2018. The Potential of Economicbase of the Livestock Sector in Kediri, East Java. J Dev Res 2 (1): 9-14. DOI: 10.28926/jdr.v2i1.48.

Sultan I, Rahman S, Jan AT, Siddiqui MT, Mondal AH, Haq QMR. 2018. Antibiotics, Resistome and Resistance Mechanisms: A Bacterial Perspective. Front Microbiol 9: 2066. DOI: 10.3389/fmicb.2018.02066.

Tamendjari S, Bouzebda FA, Chaib L, Aggad H, Ramdani M, Bouzebda Z. 2021. Antibiotic resistance of *Staphylococcus aureus* isolated from raw cow and goat milk produced in the Tiaret and Souk Ahras areas of Algeria. Vet World 14 (7): 1929-1934. DOI: 10.14202/vetworld.2021.1929-1934.

Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG. 2015. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev 28 (3): 603-661. DOI: 10.1128/CMR.00134-14.

Ventola CL. 2015. The antibiotic resistance crisis: part 1: causes and threats. Pharm Ther 40 (4): 277-283.
Virdis S, Scarano C, Cossu F, Spanu V, Spanu C, De Santis EP. 2010. Antibiotic resistance in *Staphylococcus aureus* and coagulase negative Staphylococci isolated from goats with subclinical mastitis. Vet Med Intl 2010: 517060. DOI: 10.4061/2010/517060.
Virto M, Santamarina-García G, Amores G, Hernández I. 2022. Antibiotics in dairy production: Where is the problem? Dairy 3: 541-564. DOI: 10.3390/dairy3030039.
Widodo A, Lamid M, Effendi MH, Khairullah AR, Riwu KHP, Yustinasari LR, Kurniawan SC, Ansori ANM, Silaen OSM, Dameanti FNAEP. 2022. Antibiotic sensitivity profile of multidrug-resistant (MDR) *Escherichia coli* isolated from dairy cow's milk in Probolinggo, Indonesia. Biodiversitas 23 (10): 4971-4976. DOI: 10.13057/biodiv/d231002.
Yunita MN, Effendi MH, Rahmaniar RP, Arifah S, Yanestria SM. 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. DOI: 10.35124/bca.2020.20.S1.2999.
Zeaki N, Johler S, Skandamis PN, Schelin J. 2019. The role of regulatory mechanisms and environmental parameters in Staphylococcal food poisoning and resulting challenges to risk assessment. Front Microbiol 10: 1307. DOI: 10.3389/fmicb.2019.01307.
Zwanzig M. 2020. The ecology of plasmid-coded antibiotic resistance: a basic framework for experimental research and modeling. Comput Struct Biotechnol J 19: 586-599. DOI: 10.1016/j.csbj.2020.12.027.