






RESEARCH ARTICLE

REVISED Detection of *mecA* gene and methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from milk and risk factors from farms in Probolinggo, Indonesia [version 3; peer review: 2 approved]

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Abstract

Background: *Staphylococcus aureus* is commonly found in dairy cows and is a source of contamination in milk. *S. aureus* that are resistant to beta-lactam antibiotics (especially cefoxitin) are referred to as methicillin-resistant *Staphylococcus aureus* (MRSA). The spread of MRSA cannot be separated from sanitation management during milking; it can originate from milk collected from the udder or from the hands of farmers during the milking process. The purpose of this study was to examine the level of MRSA contamination in dairy cow's milk and farmer's hand.

Methods: A total of 109 samples of dairy cow's milk and 41 samples of farmer's hand swabs were collected at a dairy farm in Probolinggo, East Java, Indonesia. Samples were cultured and purified using mannitol salt agar (MSA). The profile of *S. aureus* resistance was

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established by disk diffusion test using a disk of beta-lactam antibiotics, namely oxacillin and ceftiofur.

Results: The *S. aureus* isolates that were resistant to oxacillin and ceftiofur antibiotics were then tested for oxacillin resistance screening agar base (ORSAB) as a confirmation test for MRSA identity. *S. aureus* isolates suspected to be MRSA were then tested genotypically by polymerase chain reaction (PCR) method to detect the presence of the *mecA* gene. The results of the isolation and identification found 80 isolates (53.33%) of *S. aureus*. The results of the resistance test found that 42 isolates (15%) of *S. aureus* were resistant to oxacillin and 10 isolates (12.5%) were resistant to ceftiofur. The ORSAB test found as many as 20 isolates (47.62%) were positive for MRSA. In PCR testing to detect the presence of the *mecA* gene, three isolates (30%) were positive for the *mecA* gene.

Conclusions: This study shows that several *S. aureus* isolates were MRSA and had the gene encoding *mecA* in dairy farms.

Keywords

Staphylococcus aureus, MRSA, Milk, Swab's hand, Public health




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REVISED Amendments from Version 2

Based on reviewer comments, we have removed some unnecessary information, we have added some information to the isolation and identification results, we have added the research sample calculation formula to the method, and we have added some research pictures. We have improved the discussion section accordingly. We have corrected the minor typos, and grammar and we want to thanks the reviewers for their valuable comments which improved the quality of manuscript.

Any further responses from the reviewers can be found at the end of the article

Introduction

Staphylococcus aureus is a pathogenic bacteria that can cause public health problems, because these bacteria often contaminate products of animal origin, including milk or commonly known as milk-borne disease (MBD).¹ This opportunistic bacterial pathogen that can be found in animals and humans. This bacterium can cause various diseases ranging from mild to systemic skin infections such as pneumonia, arthritis, and meningitis.²⁻⁴ In previous studies, *S. aureus* was mostly transmitted to humans through contaminated milk.⁵ *S. aureus* is commonly found on the skin and mucosa of livestock, especially dairy cows with subclinical or clinical mastitis, which is a source of contamination in milk.⁶ If these bacteria are resistant to beta-lactam antibiotics is referred to as methicillin-resistant *S. aureus* (MRSA).⁷

It has been noted in earlier investigations that MRSA can result in new health issues for both people and animals.⁸ The high rate of MRSA contamination in dairy farms due to excessive administration of antibiotics in the treatment of dairy cows and the spread of these bacteria cannot be separated from sanitation management during milking.³ Contamination can happen from milk that is collected from the udder as well as from the hands of farmers during the milking process.⁹ The Probolinggo Regency, specifically in Krucil District, is one of the largest milk-producing centers in Indonesia.¹⁰ Antibiotics have been widely used as treatment in cases of infection in dairy cattle in Probolinggo, especially in cases of mastitis, so contamination by MRSA in dairy farms in Probolinggo¹¹ is possible.

S. aureus evolved into strain MRSA because it received the insertion of a large DNA element between 20-100 kb called staphylococcal cassette chromosome *mec* (SCC *mec*), that underlies the change in normal penicillin-binding protein (PBP), namely PBP2 to PBP2a.¹² PBP2a is expressed by the gene encoding *mecA* contained in SCC *mec* which has a very low affinity for beta-lactams, so that event cultured on media containing high concentrations of beta-lactams, MRSA survives.¹³ Molecular detection of the *mecA* gene using polymerase chain reaction (PCR) is often carried out to confirm the presence of MRSA isolates, but cannot be done in all laboratories because of the ability and cost constraints.¹⁴ Constraints in the use of PCR can be replaced by examining MRSA using the disk diffusion method with the antibiotics oxacillin and cefoxitin, which is then continued with an examination using oxacillin resistance screening agar base (ORSAB).¹⁵

The purpose of this study was to examine the level of MRSA contamination in dairy cow's milk and farmer's hand in Probolinggo, Indonesia, as well as to compare phenotypic detection methods using screening with oxacillin and cefoxitine diffusion disks, ORSAB, and confirming genotypes using PCR to detect *mecA*-coding genes. The sensitivity and specificity of the test show the effectiveness and ease of application of the MRSA detection method.

Methods**Sampling**

Milk samples were taken from the udders of female cows who were in lactation period, while the samples of farmer's hand swabs were taken from farmers who were milking. The sample size in this study refers to the formula used by Regasa *et al.*¹⁶ in the study of the milk safety assessment of *Staphylococcus aureus* as follows:

$$n = \frac{Z^2 \times P(1 - P)}{d^2}$$

$$n = \frac{1.96^2 \times 0.048(1 - 0.048)}{0.04^2} = \frac{3.8416 \times 0.045696}{0.0016} = \frac{0.1755457536}{0.0016}$$

$$n = 109.72 \sim 109 \text{ dairy cow's}$$

Note:

n = Sample size

Z = Z value at 95% confidence level (1.96)¹⁶

P = Expected prevalence is 4.8%¹⁷

d = Desired absolute precision (4%)

Based on these calculations, 109 milk samples was obtained with the selection of dairy cooperatives purposively based on the amount of milk production in an area and the willingness of dairy cooperatives to participate in the study. Meanwhile, the number of farmer hand swab samples was adjusted to the number of dairy cows owned by each farmer in the dairy cooperative area, of which 41 cattle were obtained from 109 cows.

A total of 109 samples of dairy cow's milk and 41 samples of farmer's hand swabs were collected at a dairy farm in the Probolinggo region, East Java, Indonesia from July to September 2021. Dairy cow's milk samples were taken from each cow in the third press as much as 30 ml which was then stored in a 60 ml sample bottle; the farmer's hand swab samples were taken from each farmer after the milking process using a sterile cotton swab which was then stored on Amies medium.

Bacteria isolation and identification

As much as 1 ml of each milk sample was put into a 20 ml test tube filled with 9 ml of Mannitol Salt Broth (MSB) medium while for hand swab samples, the Amies medium was vortexed until it became liquid and then 1 ml was added into a 20 ml test tube which has been filled with 9 ml of MSB media. The test tube containing MSB which had been mixed with the sample was incubated in an incubator (Isuzu Model 2-2195, Jica) at 37°C for 24 hours. The samples were cultured and purified using Mannitol Salt Agar (MSA) (Oxoid CM0085) and then incubated at 37°C for 24 hours.

Microscopic examination of bacteria was done through Gram staining to visualise Gram-positive bacteria in the form of cocci and clusters.¹⁸ The biochemical examination was carried out using a catalase test and a coagulase test. The catalase test was carried out by dripping 3% hydrogen peroxide (H₂O₂) on bacterial colonies that had been placed on the surface of the glass.¹⁹ The coagulase test was carried out by dripping 200 µl of rabbit plasma into a coagulase test tube containing bacterial colonies, which was then incubated at 37°C for 24 hours.²⁰

Oxacillin and cefoxitin disk diffusion methods

The test was carried out following the Clinical and Laboratory Standards Institute (CLSI) 2020 guidelines: *S. aureus* was tested for susceptibility to the antibiotics oxacillin 1 µg and cefoxitin 30 µg (Oxoid) on Muller Hinton Agar (MHA) plates (Oxoid, CM0337). The identified isolates were purified on mannitol salt agar (HiMedia Pvt. Ltd., M118) and incubated at 37°C for 24 hours. Using a sterile cotton swab (AKD 10903610549), standardized isolates (0.5 McFarland standard) were evenly streaked on the surface of the MHA medium (Oxoid, CM0337). The oxacillin (1 µg) and cefoxitin (30 µg) antibiotic disks were placed side by side with a distance of 50 mm on MHA that had been inoculated with isolates, and then incubated at 37°C for 24 hours to measure the inhibition zone.

Oxacillin resistance screen agar test

S. aureus isolates resistant to oxacillin 1 µg and cefoxitin 30 µg (Oxoid) were confirmed by ORSAB (HiMedia M1415) using *S. aureus* isolates from the MHA media; plus Oxacillin Resistance Selective Supplement (Supplement, HiMedia Pvt. Ltd., FD191).²¹

Detection of the *mecA* gene

All *S. aureus* isolates that were resistant to cefoxitin 30 µg and positive on ORSAB examination were then subjected to a PCR test to detect the presence of the *mecA* gene.²² The DNA extraction process was carried out according to the QIAamp DNA Mini Kit protocol (51304 & 51306), where previously the isolates were purified on MSA (HiMedia Pvt. Ltd, M118) and inoculated on MHA (Oxoid, CM0337). The primer used was *mecA* F: 5'-AAA ATC GAT GGT AAA GGT TGG C-3' and *mecA* R: 5'-AGT TCT GCA GTA CCG GAT TTG C-3'.²³ The PCR master mix used GoTaq Green Master Mix (Promega, 9PIM712) which is a ready-to-use solution mixture containing Taq DNA polymerase, dNTPs, MgCl₂, and a reaction buffer. DNA was amplified using a Thermal Cycler T100 machine (Bio-Rad, 186-1096) for 40 cycles in 25 µl of the reaction mixture with the following steps: denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 min with a final extension at 72°C for 5 min. A total of 10 µl of PCR product were analyzed by 2% agarose gel electrophoresis, and the gel was visualized under ultraviolet light.²⁴ A positive test indicated a PCR product in the 533-base pair (bp) band.

Result

The results of the isolation and identification tests yielded 80 (53.33%) *S. aureus* isolates from 150 samples taken at a dairy farm in Probolinggo, East Java, Indonesia. The 80 isolates that were positive for *S. aureus* consisted of 54 isolates from dairy cow's milk samples and 26 isolates from farmer's hand swab samples as shown in Table 1. *S. aureus* had phenotypic colony characteristics on MSA medium, namely a change in color in the medium from red to golden-yellow indicating mannitol fermentation, while the colonies had various pigments including white, golden, and yellow as shown in Figure 1. The Gram staining test showed the Gram-positive colonies in the form of cocci and clusters as shown in Figure 2, which were then confirmed by the catalase test and coagulase test as shown in Figures 3 and 4.¹⁹

The disk diffusion method on MHA medium showed that 42 isolates exhibited resistance to oxacillin preparations, with a percentage of 52.5% (28 isolates came from dairy cow's milk samples and 14 isolates came from farmer's hand swab sample); on the other hand, 10 isolates showed resistance to cefoxitin, with a percentage of 12.5% (five isolates came from dairy cow's milk samples and five isolates came from farmer's hand swab samples) as shown in Table 2 and Figure 5.

Table 1. Isolation of *Staphylococcus aureus* by type of sample.

Sample type	Sample code	Sample size	Gram positive	Catalase positive	Coagulase positive	Positive <i>S. aureus</i> (%)
Milk	AS	109	109 (100%)	109 (100%)	54 (49.54%)	54 (49.54%)
Swab hand	AT	41	41 (100%)	41 (100%)	26 (63.41%)	26 (63.41%)
Total		150				80 (53.33%)



Figure 1. The results of positive yellow mannitol fermentation and *Staphylococcus aureus* colonies appear mucoid white on mannitol salt agar (MSA) medium (HiMedia Pvt. Ltd, M118).

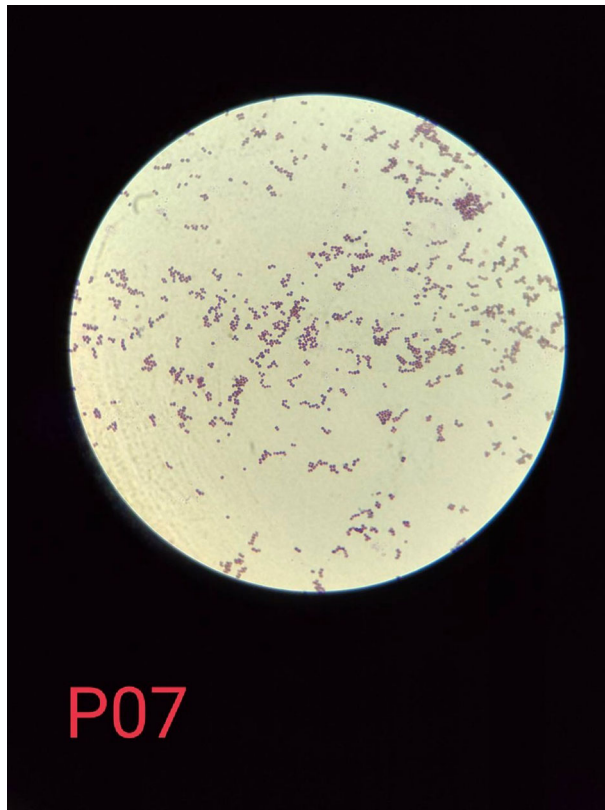


Figure 2. Microscopic picture of Gram stain test on presumptive *Staphylococcus aureus* isolates using a 1000× magnification microscope.

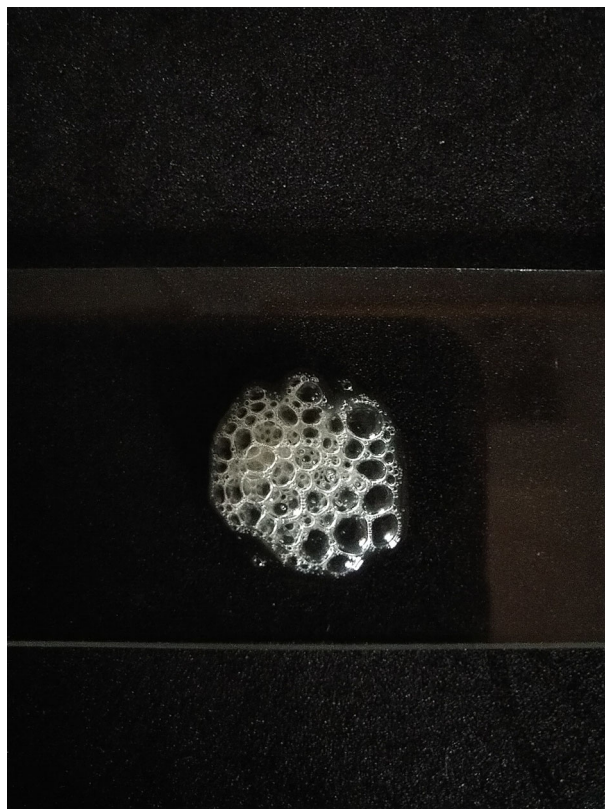


Figure 3. Catalase test results positive for *Staphylococcus aureus* isolates.

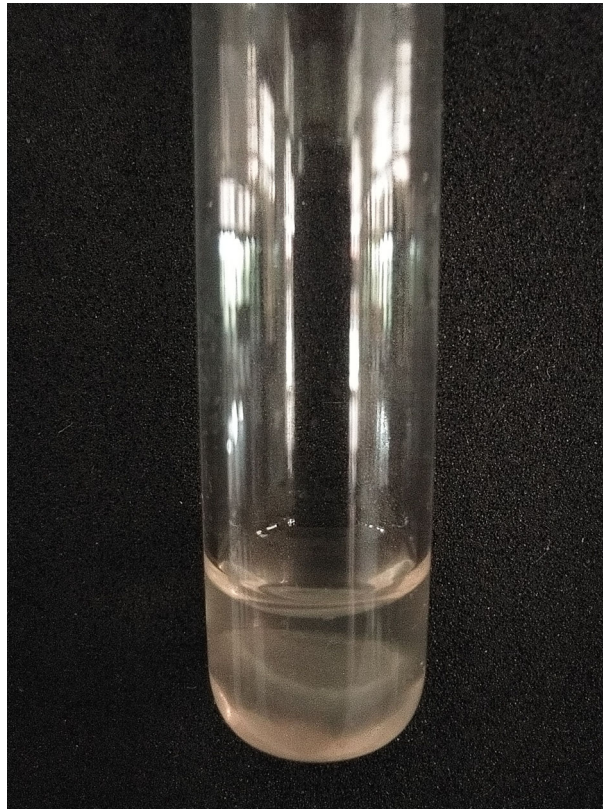


Figure 4. Coagulase test results positive for *Staphylococcus aureus* isolates.

Table 2. Oxacillin and cefoxitin disk diffusion test of *Staphylococcus aureus*.

Sample type	<i>Staphylococcus aureus</i> isolate (n=80)			
	OX disk diffusion		FOX disk diffusion	
	Resistant (%)	Sensitive (%)	Resistant (%)	Sensitive (%)
Milk	28 (35%)	26 (32.5%)	5 (6.25%)	49 (61.25)
Swab hand	14 (17.5%)	12 (15%)	5 (6.25%)	21 (26.25%)
Total	42 (52.5%)	38 (47.5%)	10 (12.5%)	70 (87.5%)

No *S. aureus* isolate was found to simply be resistant to cefoxitin, according to the disc diffusion test results, and all isolates that were found to be resistant to cefoxitin were also found to be resistant to oxacillin, as shown in [Table 3](#).

Confirmation of the phenotype test that for resistance to oxacillin and cefoxitin was followed by ORSAB test, with a blue culture coloration indicating positive results while a white coloration indicated negative results. The ORSAB test showed that of the 42 isolates of *S. aureus* that were resistant to oxacillin, 20 isolates (47.62%) were confirmed MRSA by the disk diffusion method, as shown in [Table 4](#).

S. aureus isolates suspected to be MRSA (Phenotypically resistant to cefoxitin and positive for ORSAB) were then tested genotypically using PCR to detect the presence of the gene encoding *mecA*. A total of 10 isolates suspected to be MRSA were tested, from which three isolates (30% of the total isolates tested by PCR) were detected positive for the *mecA* gene, as shown in [Figure 6](#). The results of the PCR test showed that isolates suspected to be MRSA were found to have the *mecA* gene, which is resistant to the antibiotics cefoxitin and oxacillin, as shown in [Table 3](#).

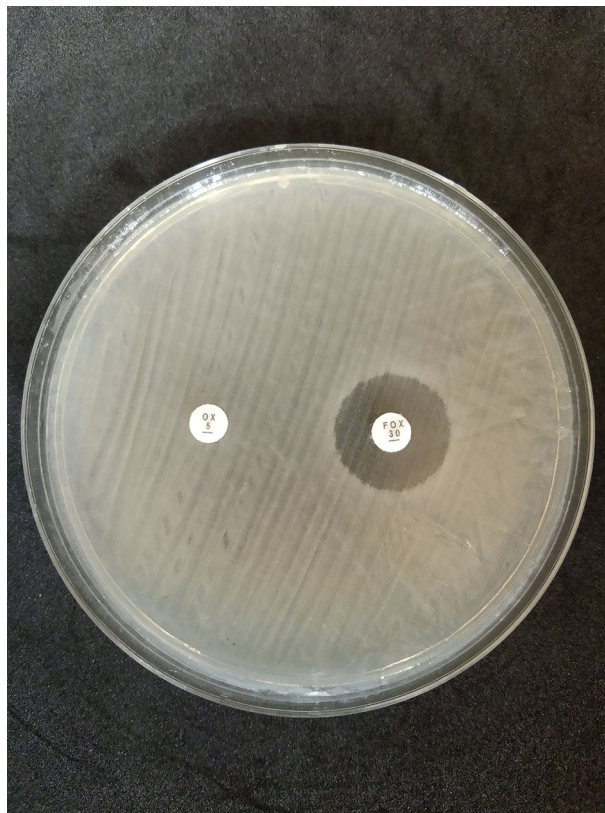


Figure 5. Oxacillin (OX) and ceftiofur (FOX) resistant to disk diffusion test in Mueller Hinton Agar (MHA) (Oxoid, CM0337); OX = oxacillin, FOX = ceftiofur.

Table 3. Positive MRSA confirmed by oxacillin and ceftiofur disk diffusion, ORSAB and *mecA* gene detection.

Sample type	Sample code	Resistance to disk diffusion test		ORSAB Test	<i>mecA</i> detection using PCR	Number positive of MRSA isolates by <i>mecA</i> detection (%)
		OX	FOX			
Milk	AT 21	+	+	+	-	2 (20%)
	AT 28	+	+	+	+	
	AT 29	+	+	+	-	
	AT 33	+	+	+	-	
	AT 41	+	+	+	+	
Swab hand	AS 67	+	+	+	-	1 (10%)
	AS 77	+	+	+	-	
	AS 80	+	+	+	-	
	AS 102	+	+	+	-	
	AS 109	+	+	+	+	
Total						3 (30%)

Table 4. Total number confirmed MRSA by oxacillin resistance screening agar base (ORSAB).

Sample type	Sample code	Number of isolates tested ORSAB (n=42)	Positive ORSAB test
Milk	AS	28 (66.67%)	15 (35.71%)
Hand swab	AT	14 (33.33%)	5 (11.9%)
Total		42 (100%)	20 (47.62%)

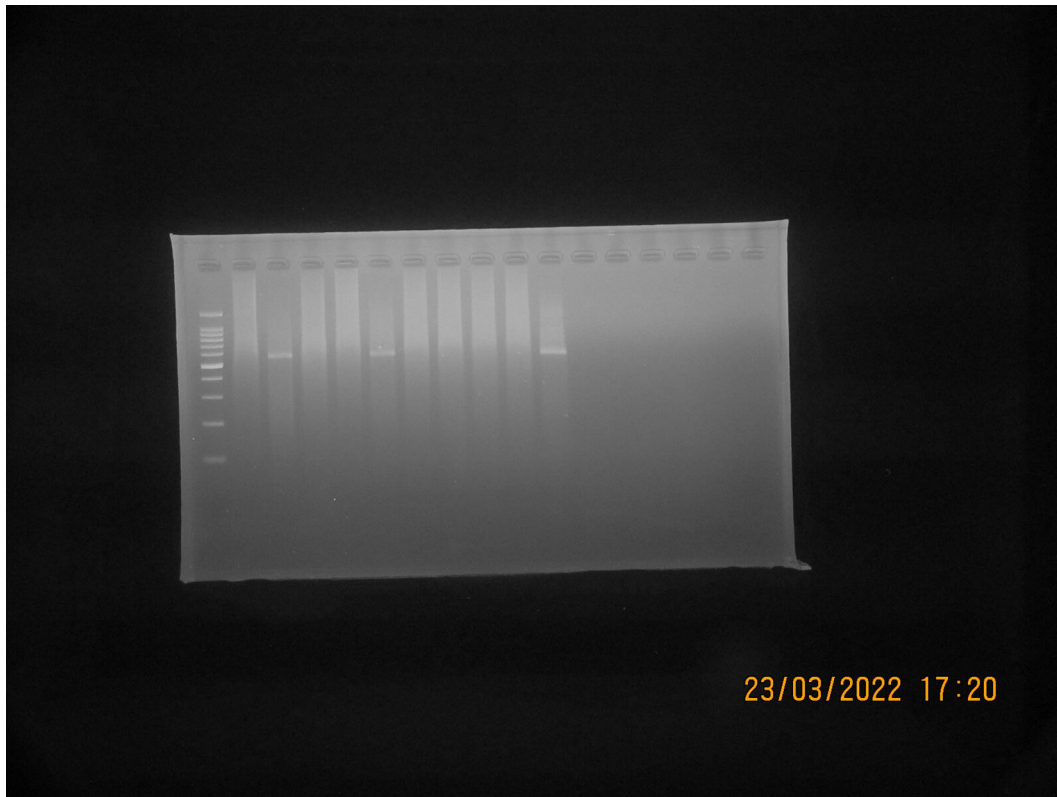


Figure 6. Detection *mecA* PCR results with positive bands at 533 bp; Marker line: 100-bp molecular-weight markers; Line K-: *Staphylococcus aureus* ATCC 25923 (Negative Control); Line AT28, AT41, and AS109: Positive result for *mecA* gene; Line AT21, AT29, AT33, AS67, AS77, AS80, and AS102: Negative result for *mecA* gene.

Discussion

MBD is quite a common public health problem, because it not only has an impact on human health, also has an impact on the health of dairy cows, especially in the milk production and quality sector.²⁵ Several previous studies have reported that the incidence of contaminated milk by *S. aureus* resistant to antibiotics is found in both developed and developing countries.²⁶ Improper and unhygienic handling of milk, especially during the milking process, plays an important role in the occurrence of milk contamination.²⁷ Unhygienic farmer hands when milking can also potentially transmit pathogenic bacteria in milk, including *S. aureus*.²⁸

S. aureus is a pathogenic bacterium that can cause various infectious diseases ranging from skin infections to systemic infections that can lead to death.²⁹ In this study, of 150 milk samples, 80 samples (53.33%) were found to have *S. aureus* contamination; this percentage is higher than the research conducted by Wang *et al.*³⁰ who isolated 90 (46.15%) *S. aureus* from 195 milk samples, and from another study conducted by Jahan *et al.*³¹ who isolated 12 (25.53%) *S. aureus* from 47 milk samples. This study employed a purposive sampling design that was carried out to detect the presence of *S. aureus* strains in dairy farms that have low milking hygiene, which can increase bacterial contamination in cow's milk.³² In line with this, the research conducted by Khiabani *et al.*³³ showed that the difference in the number of isolates found could be influenced by differences in study design such as population and geographic distribution of the sample, infection control practices, and the type of antibiotic used, as seen in Figure 6.

The problem of the incidence of *S. aureus* infection continues to grow with the emergence of MRSA, which is resistant to all beta-lactam antibiotics, including monobactams and cephalosporins, which are a group of antibiotics often used to treat Staphylococcus infections.³⁴ MRSA infection causes treatment problems and facilitates its spread, so prompt and early diagnosis is needed to identify MRSA accurately.³⁵ In this study, 42 samples (52.5%) of *S. aureus* were found to be resistant to oxacillin disks, and 10 samples (12.5%) to cefoxitin disks. Miragaia³⁶ stated that the phenotypic detection of MRSA using disk diffusion still has not shown accurate results, and *mecA* genotyping using PCR is still the main recommendation even though it cannot be done routinely. However, even so, identification of MRSA with disk diffusion is still widely used because it can be done quickly and at a lower cost.³⁷ Diffusion disks using oxacillin and cefoxitin have

the same sensitivity level of 100%, and specificities of 74.07% for oxacillin and 92.59% for ceftazidime.³⁸ However, several previous studies reported that the use of the ceftazidime disk diffusion method had a better sensitivity level than that of oxacillin in detecting MRSA, because the oxacillin disk diffusion method still has a high false positive rate.³⁹ Vyas *et al.*³⁸ stated that false positives could be influenced by beta-lactamase hyperproduction, resulting in the phenotypic expression of oxacillin resistance but without a genotypic resistance mechanism.

In this study, all isolates detected were resistant to the ceftazidime and oxacillin disks. All isolates detected to be resistant to oxacillin and ceftazidime were confirmed by ORSAB assay, in line with a report by Pourmand *et al.*⁴⁰ which stated that the ORSAB test has a specificity of 100%. In this study, 20 of the 42 isolates (47.62%) were found to be positive for MRSA. The sensitivity level confirmed the resistance strain being tested while the specificity was to the minimum inhibitory concentration (MIC).⁴¹ Ceftazidime-resistant and ORSAB-positive *S. aureus* isolates were tested genotypically using PCR to detect the presence of the gene encoding *mecA*; these isolates also had positive results in all phenotypic methods (resistance to ceftazidime and oxacillin in the disk diffusion method and positive results in the ORSAB test). These results are similar to those from research conducted by Ramandinianto *et al.*⁴² The antibiotic ceftazidime is a good inducer for the expression of the *mecA* gene because it can increase the expression of PBP2a, which is encoded by the *mecA* gene.⁴³ This also agrees with Reichmann and Pinho⁴⁴ and Anand *et al.*⁴⁵

From this study, it can be concluded that the occurrence of MRSA contamination in milk can be caused by various factors including the unhygienic hands of farmers when milking.⁴⁶ MRSA contamination poses a serious public health risk, which increases the potential for the spread of difficult-to-treat staphylococci.⁴⁷ Therefore, microbiology laboratory examinations are very important to isolate and identify MRSA isolates quickly, accurately, and cost-effectively from food samples of animal origin.⁴⁸ Genotypic detection using PCR to detect the presence of the gene encoding *mecA* is a molecularly accurate MRSA test; however, in laboratories that cannot perform molecular testing, the ceftazidime disk diffusion method can be used to detect MRSA.⁴⁹ This is based on the ability of the ceftazidime disk diffusion test in detecting the expression of the *mecA* gene which can be a more effective and efficient MRSA screening method.⁵⁰

Conclusions

This study shows that several *S. aureus* isolates are Methicillin-Resistant *S. aureus* (MRSA) and have the gene encoding *mecA* in dairy farms. The spread of *S. aureus* that is MRSA can be a threat to public health. Thus, prevention and control measures are needed to suppress the spread of *S. aureus* infection on a dairy farm in Probolinggo, East Java, Indonesia.

Data availability

Underlying data

Figshare: Detection of *mecA* gene and methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from milk and risk factors from the farmer in Probolinggo, Indonesia, <https://doi.org/10.6084/m9.figshare.19784005>.

This project contains the following underlying data:

- CMT data and code sample (Argopuro).xlsx
- Results of Isolation and Identification (Argopuro).xlsx
- Bacterial resistance test results (Argopuro).xlsx
- MRSA confirmatory test results (Argopuro).xlsx

Extended data

Figshare: Detection of *mecA* gene and methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from milk and risk factors from the farmer in Probolinggo, Indonesia, <https://doi.org/10.6084/m9.figshare.19784005>.

This project contains the following extended data:

- Table and Figure.docx

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/) (CC-BY 4.0).

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References

- Ayele Y, Gutema FD, Edao BM, *et al.*: **Assessment of *Staphylococcus aureus* along milk value chain and its public health importance in Sebeta, central Oromia, Ethiopia.** *BMC Microbiol.* 2017; **17**(1): 141.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Jin T, Mohammad M, Pullerits R, *et al.*: **Bacteria and Host Interplay in *Staphylococcus aureus* Septic Arthritis and Sepsis.** *Pathogens.* 2021; **10**(2): 158.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Dai J, Wu S, Huang J, *et al.*: **Prevalence and Characterization of *Staphylococcus aureus* Isolated From Pasteurized Milk in China.** *Front. Microbiol.* 2019; **10**: 641.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Nhatsave N, Garrine M, Messa A, *et al.*: **Molecular Characterization of *Staphylococcus aureus* Isolated from Raw Milk Samples of Dairy Cows in Manhiça District, Southern Mozambique.** *Microorganisms.* 2021; **9**(8): 1684.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Mekonnen SA, Lam TJGM, Hoekstra J, *et al.*: **Characterization of *Staphylococcus aureus* isolated from milk samples of dairy cows in small holder farms of North-Western Ethiopia.** *BMC Vet. Res.* 2018; **14**: 246.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Pumipuntu N, Tunyong W, Chantratita N, *et al.*: ***Staphylococcus* spp. associated with subclinical bovine mastitis in central and northeast provinces of Thailand.** *PeerJ.* 2019; **7**: e6587.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Panchal VV, Griffiths C, Mosaei H, *et al.*: **Evolving MRSA: High-level β -lactam resistance in *Staphylococcus aureus* is associated with RNA Polymerase alterations and fine tuning of gene expression.** *PLoS Pathog.* 2020; **16**(7): e1008672.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Aires-de-Sousa M: **Methicillin-resistant *Staphylococcus aureus* among animals: currentThis study shows overview.** *Clin. Microbiol. Infect.* 2017; **23**(6): 373–380.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Schnitt A, Tenhagen BA: **Risk Factors for the Occurrence of Methicillin-Resistant *Staphylococcus aureus* in Dairy Herds: An Update.** *Foodborne Pathog. Dis.* 2020; **17**(10): 585–596.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Retnowati N, Iskandar R, Perambang R: **Implementation of Kaizen Culture to Increase the Efficiency and Quality of Pasteurized Milk Products in KUD Argopuro Krucil Probolinggo.** *Proceeding of the 1st International Conference on Food and Agriculture.* 2020; 111–116.
- Ramandinianto SC, Khairullah AR, Effendi MH, *et al.*: **Profile of Multidrug Resistance (MDR) and Methicillin Resistant *Staphylococcus aureus* (MRSA) on Dairy Farms in East Java Province, Indonesia.** *Indian J. Forensic Med. Toxicol.* 2020; **14**(4): 3439–3445.
- Santiago C, Pang EL, Lim KH, *et al.*: **Inhibition of penicillin-binding protein 2a (PBP2a) in methicillin resistant *Staphylococcus aureus* (MRSA) by combination of ampicillin and a bioactive fraction from *Duabanga grandiflora*.** *BMC Complement. Altern. Med.* 2015; **15**: 178.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Rolo J, Worning P, Boye Nielsen J, *et al.*: **Evidence for the evolutionary steps leading to *mecA*-mediated β -lactam resistance in *Staphylococci*.** *PLoS Genet.* 2017; **13**(4): e1006674.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Rocchetti TT, Martins KB, Martins PYF, *et al.*: **Detection of the *mecA* gene and identification of *Staphylococcus* directly from blood culture bottles by multiplex polymerase chain reaction.** *Braz. J. Infect. Dis.* 2018; **22**(2): 99–105.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Apfalter P, Assadian O, Kalczyk A, *et al.*: **Performance of a new chromogenic oxacillin resistance screen medium (Oxoid) in the detection and presumptive identification of methicillin-resistant *Staphylococcus aureus*.** *Diagn. Microbiol. Infect. Dis.* 2002; **44**(2): 209–211.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Regasa S, Mengistu S, Abraha A: **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. Int.* 2019; **2019**(2): 1–11.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Kou X, Cai H, Huang S, *et al.*: **Prevalence and Characteristics of *Staphylococcus aureus* Isolated From Retail Raw Milk in Northern Xinjiang, China.** *Front. Microbiol.* 2021; **12**:705947.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Effendi MH, Oktavianto A, Hastutiek P: **Tetracycline resistance gene in *Streptococcus agalactiae* isolated from bovine subclinical mastitis in Surabaya, Indonesia.** *Philippine J. Vet. Med.* 2018; **55**: 115–120.
- Effendi MH, Hisyam MAM, Hastutiek P, *et al.*: **Detection of coagulase gene in *Staphylococcus aureus* from several dairy farms in East Java, Indonesia, by polymerase chain reaction.** *Vet World.* 2019; **12**(1): 68–71.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Tyasningsih W, Effendi MH, Budiarto B, *et al.*: **Antibiotic resistance to *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from dairy farms in Surabaya, Indonesia.** *Indian Vet. J.* 2019; **96**(11): 27–31.
- Decline V, Effendi MH, Rahmaniar RP, *et al.*: **Profile of antibiotic-resistant and presence of methicillin-resistant *Staphylococcus aureus* from nasal swab of dogs from several animal clinics in Surabaya, Indonesia.** *Int. J. One Health.* 2020; **6**(1): 90–94.
[Publisher Full Text](#)
- Ho PL, Liu MC, Tong MK, *et al.*: **Evaluation of disk diffusion tests and agar screening for predicting *mecA*-mediated oxacillin resistance in *Staphylococcus lugdunensis* revealed a cefoxitin-susceptible, *mecA*-positive *S. lugdunensis* clonal complex 27 clone.** *J. Glob. Antimicrob. Resist.* 2020; **20**: 260–265.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Nam LV, Quyet D, Hung PN, *et al.*: **Antibiotic Resistance Profile and Methicillin-Resistant Encoding Genes of *Staphylococcus aureus* Strains Isolated from Bloodstream Infection Patients in Northern Vietnam.** *Open Access Maced J. Med. Sci.* 2019; **7**(24): 4406–4410.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Rahmaniar RP, Yunita MN, Effendi MH, *et al.*: **Encoding gene for methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from nasal swab of dogs.** *Indian Vet. J.* 2020; **97**(2): 37–40.
- Berhe G, Wasihun AG, Kassaye E, *et al.*: **Milk-borne bacterial health hazards in milk produced for commercial purpose in Tigray, northern Ethiopia.** *BMC Public Health.* 2020; **20**(1): 894.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Abdi RD, Gillespie BE, Vaughn J, *et al.*: **Antimicrobial Resistance of *Staphylococcus aureus* Isolates from Dairy Cows and Genetic Diversity of Resistant Isolates.** *Foodborne Pathog. Dis.* 2018; **15**(7): 449–458.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Amenu K, Wieland B, Szonyi B, *et al.*: **Milk handling practices and consumption behavior among Borana pastoralists in southern Ethiopia.** *J. Health Popul. Nutr.* 2019; **38**: 6.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Nyokabi S, Luning PA, de Boer IJM, *et al.*: **Milk quality and hygiene: Knowledge, attitudes and practices of smallholder dairy farmers in central Kenya.** *Food Control.* 2021; **130**: 108303.
[Publisher Full Text](#)
- Cheung GYC, Bae JS, Otto M: **Pathogenicity and virulence of *Staphylococcus aureus*.** *Virulence.* 2021; **12**(1): 547–569.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Wang W, Lin X, Jiang T, *et al.*: **Prevalence and Characterization of *Staphylococcus aureus* Cultured From Raw Milk Taken From**

- Dairy Cows With Mastitis in Beijing, China.** *Front. Microbiol.* 2018; **9**: 1123.
[PubMed Abstract](#) | [Publisher Full Text](#)
31. Jahan M, Rahman M, Parvej MS, *et al.*: **Isolation and characterization of *Staphylococcus aureus* from raw cow milk in Bangladesh.** *J. Adv. Vet. Anim. Res.* 2014; **2**(1): 49–55.
[Publisher Full Text](#)
32. Tohoyessou MG, Mousse W, Sina H, *et al.*: **Toxin Production and Resistance of *Staphylococcus* Species Isolated from Fermented Artisanal Dairy Products in Benin.** *J. Pathog.* 2020; **2020**: 1–12.
[Publisher Full Text](#)
33. Khiabani NO, Motamedzadegan A, Raisi SN, *et al.*: **Chemical, textural, rheological, and sensorial properties of wheyless feta cheese as influenced by replacement of milk protein concentrate with pea protein isolate.** *J. Texture Stud.* 2020; **51**(3): 488–500.
[PubMed Abstract](#) | [Publisher Full Text](#)
34. Bush K, Bradford PA: **β -Lactams and β -Lactamase Inhibitors: An Overview.** *Cold Spring Harb. Perspect. Med.* 2016; **6**(8): a025247.
[PubMed Abstract](#) | [Publisher Full Text](#)
35. Mehta Y, Hegde A, Pande R, *et al.*: **Methicillin-resistant *Staphylococcus aureus* in Intensive Care Unit Setting of India: A Review of Clinical Burden, Patterns of Prevalence, Preventive Measures, and Future Strategies.** *Indian J. Crit. Care Med.* 2020; **24**(1): 55–62.
[PubMed Abstract](#) | [Publisher Full Text](#)
36. Miragaia M: **Factors Contributing to the Evolution of *mecA*-Mediated β -lactam Resistance in *Staphylococci*: Update and New Insights From Whole Genome Sequencing (WGS).** *Front. Microbiol.* 2018; **9**: 2723.
[PubMed Abstract](#) | [Publisher Full Text](#)
37. Bennett K, Sharp SE: **Rapid differentiation of methicillin-resistant *Staphylococcus aureus* and methicillin-susceptible *Staphylococcus aureus* from blood cultures by use of a direct cefoxitin disk diffusion test.** *J. Clin. Microbiol.* 2008; **46**(11): 3836–3838.
[PubMed Abstract](#) | [Publisher Full Text](#)
38. Vyas A, Sharma M, Kumar S, *et al.*: **A comparative study of Oxacillin screen agar, Oxacillin disk diffusion and Cefoxitin disk diffusion, Oxacillin E-test method for routine screening of methicillin-resistant *Staphylococcus aureus*.** *Int. J. Curr. Res. Rev.* 2015; **7**(10): 55–60.
39. Broekema NM, Van TT, Monson TA, *et al.*: **Comparison of Cefoxitin and Oxacillin Disk Diffusion Methods for Detection of *mecA*-Mediated Resistance in *Staphylococcus aureus* in a Large-Scale Study.** *J. Clin. Microbiol.* 2009; **47**(1): 217–219.
[PubMed Abstract](#) | [Publisher Full Text](#)
40. Pourmand MR, Hassanzadeh S, Mashhadi R, *et al.*: **Comparison of four diagnostic methods for detection of methicillin resistant *Staphylococcus aureus*.** *Iran J. Microbiol.* 2014; **6**(5): 341–344.
[PubMed Abstract](#)
41. Kowalska-Krochmal B, Dudek-Wicher R: **The Minimum Inhibitory Concentration of Antibiotics: Methods, Interpretation, Clinical Relevance.** *Pathogens.* 2021; **10**(2): 165.
[PubMed Abstract](#) | [Publisher Full Text](#)
42. Ramandinianto SC, Khairullah AR, Effendi MH: ***MecA* gene and methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from dairy farms in East Java, Indonesia.** *Biodiversitas.* 2020; **21**(8): 3562–3568.
[Publisher Full Text](#)
43. Koupahi H, Jahromy SH, Rahbar M: **Evaluation of Different Phenotypic and Genotypic Methods for Detection of Methicillin Resistant *Staphylococcus aureus* (MRSA).** *Iran. J. Pathol.* 2016; **11**(4): 370–376.
[PubMed Abstract](#)
44. Reichmann NT, Pinho MG: **Role of SCC mec type in resistance to the synergistic activity of oxacillin and cefoxitin in MRSA.** *Sci. Rep.* 2017; **7**: 6154.
[PubMed Abstract](#) | [Publisher Full Text](#)
45. Anand KB, Agrawal P, Kumar S, *et al.*: **Comparison of cefoxitin disk diffusion test, oxacillin screen agar, and PCR for *mecA* gene for detection of MRSA.** *Indian J. Med. Microbiol.* 2009; **27**(1): 27–29.
[PubMed Abstract](#) | [Publisher Full Text](#)
46. Khairullah AR, Ramandinianto SC, Effendi MH: **A Review of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* (LA-MRSA) on Bovine Mastitis.** *Sys. Rev. Pharm.* 2020; **11**(7): 172–183.
47. Chambers HF, Deleo FR: **Waves of resistance: *Staphylococcus aureus* in the antibiotic era.** *Nat. Rev. Microbiol.* 2009; **7**(9): 629–641.
[PubMed Abstract](#) | [Publisher Full Text](#)
48. Khairullah AR, Sudjarwo SA, Effendi MH, *et al.*: **A Review of Methicillin-Resistant *Staphylococcus aureus* (MRSA) on Milk and Milk Products: Public Health Importance.** *Sys. Rev. Pharm.* 2020; **11**(8): 59–69.
49. Ramandinianto SC, Khairullah AR, Effendi MH, *et al.*: **Detection of Enterotoxin type B gene on Methicillin Resistant *Staphylococcus aureus* (MRSA) isolated from raw milk in East Java, Indonesia.** *Sys. Rev. Pharm.* 2020; **11**(7): 290–298.
50. Boubaker IBB, Abbes RB, Abdallah HB, *et al.*: **Evaluation of a cefoxitin disk diffusion test for the routine detection of methicillin-resistant *Staphylococcus aureus*.** *Clin. Microbiol. Infect.* 2004; **10**(8): 762–765.
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Version 3

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F M Yasir Hasib 

Department of Infectious Diseases and Public Health, Jockey Club College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Hong Kong, China

Thank you for the revised version of the manuscript. I think the authors did a great job in addressing all the comments related to the initial review.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Antibiotic resistance, Public health, Bioinformatics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 2

Reviewer Report 13 September 2022

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Ikechukwu Benjamin Moses 

¹ Department of Applied Microbiology, Faculty of Science, Ebonyi State University, Abakaliki, Nigeria

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Paulo, Brazil

My concerns have been addressed by the authors.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Antimicrobial resistance surveillance in veterinary and human medicine, and the development of alternative therapeutic approaches.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 05 September 2022

<https://doi.org/10.5256/f1000research.134188.r149189>

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Ikechukwu Benjamin Moses 

¹ Department of Applied Microbiology, Faculty of Science, Ebonyi State University, Abakaliki, Nigeria

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General comments:

The article entitled "*Detection of mecA gene and methicillin-resistant Staphylococcus aureus (MRSA) isolated from milk and risk factors from farms in Probolinggo, Indonesia*" was well-written. The study design and objectives of the manuscript were good and address a critical public health issue, especially with regards to foodborne illnesses and the spread of clinically-relevant bacterial pathogens (MRSA) in livestock and their by-products. However, the authors need to make a few grammatical and technical corrections which I have already suggested in my attached comments. The research findings are in very good tandem with the research aim and objectives. If the authors are able to make the few corrections I suggested, it can be indexed.

Abstract:

The abstract is good and well-structured but needs a few corrections:

- *Sentence 1:* "*S. aureus* that are resistant to beta-lactam antibiotics are referred to as methicillin-resistant *Staphylococcus aureus* (MRSA)."

Comment 1: Authors should rephrase/restructure the above sentence in the abstract section because beta-lactam antibiotics include penicillin, ampicillin, etc. So, if for example,

an isolate is resistant to penicillin or even ampicillin, it can't be referred to as methicillin-resistant. The disc diffusion method using cefoxitin disc (30 µg) is more reliable in identifying methicillin-resistant *S. aureus* (MRSA). So, I suggest that authors should re-write this sentence as "*S. aureus* that are resistant to beta-lactam antibiotics (**especially cefoxitin**) are referred to as methicillin-resistant *Staphylococcus aureus* (MRSA).

- *Sentence 2*: "The purpose of this study was to examine the level of MRSA contamination in dairy cow's milk and farmer's hand swabs."

Comment 2: Authors should delete the word "swabs" in the sentence because what is being actually assessed are the hands of the farmers. The swab is just a tool used to collect the sample.

Comment 3: The keyword "**Swab's hand**" should be changed to "**hand swabs**" in the list of keywords.

Introduction:

The introduction was generally very good. I will suggest that the authors make a change in the last paragraph of this section:

- **Last paragraph of introduction**: The purpose of this study was to examine the level of MRSA contamination in dairy cow's milk and farmer's hand **swab** in Probolinggo, Indonesia, as well as to compare phenotypic detection methods using screening with oxacillin and cefoxitine diffusion disks, ORSAB, and confirming genotypes using PCR to detect *mecA*-coding genes.

Comment: I think the authors should remove the word "swab" as what is being actually assessed are the **farmers' hands**, just like I mentioned in my earlier suggestion in the abstract section.

Methods:

The methodology was well-detailed except for some important technical corrections which I have suggested:

- **Oxacillin and cefoxitin disk diffusion methods**
The test was carried out following the Clinical and Laboratory Standards Institute (CLSI) 2020 guidelines: *S. aureus* was tested for susceptibility to the antibiotics **oxacillin 30 µg** and cefoxitin 30 µg (Oxoid) on Muller Hinton Agar (MHA) plates (Oxoid, CM0337). The identified isolates were purified on mannitol salt agar (HiMedia Pvt. Ltd., M118), incubated at 37°C for 24 hours **as a 0.5 McFarland suspension, and then taken using a sterile cotton swab of size S (AKD 10903610549). They were then wiped** evenly on the surface of the MHA medium (Oxoid, CM0337). **Disk**. The oxacillin **30 µg** and cefoxitin 30 µg antibiotic disks were placed side by side with a distance of **5 cm** on MHA that had been inoculated with isolates, and then incubated at 37°C for 24 hours to measure the inhibition zone.

Comment 1: Authors should correct the concentration of oxacillin antibiotic disc to **1 µg** because oxacillin disc concentration from Oxoid, UK is **1 µg** while that of cefoxitin is correct at the **30 µg** indicated. I think this might have been an oversight during the writing of the manuscript.

Comment 2: Authors should take note of the **bolded sections** in the sentence and make corrections as I indicated below for the sentence to be more comprehensive and understandable. Also, 5cm is the same as 50mm, so it is preferable to indicate that the distance between the oxacillin and cefoxitin antibiotics was 50 mm instead of 5 cm since distance units in the CLSI charts are in mm. As I mentioned earlier, the sentence in the last section should be written as: "The identified isolates were purified on mannitol salt agar (HiMedia Pvt. Ltd., M118) **and** incubated at 37°C for 24 hours. **Using a sterile cotton swab** (AKD 10903610549), **standardized isolates (0.5 McFarland standard) were evenly streaked** on the surface of the MHA medium (Oxoid, CM0337). The oxacillin (**1 µg**) and cefoxitin (**30 µg**) antibiotic disks were placed side by side with a distance of 50 mm on MHA that had been inoculated with isolates, and then incubated at 37°C for 24 hours to measure the inhibition zone."

Comment 3: The concentration of all the oxacillin discs in the manuscript should be changed to **1 µg**.

Results:

The results are very clear and understandable. Data were properly interpreted and comprehensive. However, I suggested some important changes and corrections:

- **Comment 1:** The colour of *S. aureus* on mannitol salt agar (MSA) is golden-yellow. I will suggest authors use this all through the manuscript.
- *Sentence:* "Based on the results of the disk diffusion test, no *S. aureus* isolate was to only be resistant to cefoxitin: all *S. aureus* isolates that were detected to be resistant to cefoxitin were also identified as resistant to oxacillin as shown in Table 3."

Comment 2: I suggest that authors should rephrase this sentence to be more understandable.

Comment 3: I suggest that the authors delete the column "**mecA detection using PCR**" in **Table 3** as it is empty and serves no function since the last column is already indicating the total isolates that harboured the *mecA* gene.

Discussion:

The discussion is good but needs some critical changes in some confusing sentences which I have suggested below:

- *Sentence:* ..contamination; this percentage is higher than the research conducted by Wang et al.²⁷ which isolated 195 milk samples, of which 90 samples (46.15%) were contaminated with *S. aureus*, and from another study conducted by Jahan et al.²⁸ who isolated 47 milk samples, of which 12 (25.53%) were contaminated with *S. aureus*.

Comment 1: There is a mix-up in the sentence above. The sentence is stating that milk samples were isolated while what was actually isolated was the *S. aureus* from the milk samples. I will suggest that authors should re-write this section as "*..contamination; this percentage is higher than the research conducted by Wang et al.²⁷ who isolated 90 (46.15%) S. aureus from 195 milk samples, and from another study conducted by Jahan et al.²⁸ who isolated 12 (25.53%) S. aureus from 47 milk samples*".

- *Sentence:* ...mecA PCR results with a positive band at 533 bp. Marker line: 100-bp molecular-weight markers; Line K-: Staphylococcus aureus ATCC 25923 (Negative Control); Line AT28, AT41, and AS109: positive result for mecA gene detection; Line AT21, AT29, AT33, AS67, AS77, AS80, and AS102: negative result for mecA gene.

Comment 2: I think the authors should delete this sentence since Figure 3 already has text under the gel picture depicting what was positive for mecA, including the targeted fragment size and what was negative.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Antimicrobial resistance surveillance in veterinary and human medicine, and the development of alternative therapeutic approaches.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 06 Sep 2022

Aswin Rafif Khairullah, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia

Respected reviewer Moses, thanks a lot for your able input. We have followed all your kind suggestions accordingly. Kindly find our response:

Thanks for your valuable comments and suggestions on the manuscript entitled:
"Detection of *mecA* gene and methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from milk and risk factors from farms in Probolinggo, Indonesia"

We welcome feedback. We have made modifications to the study on the following points:

Abstract section:

Authors should rephrase/restructure the above sentence in the abstract section because beta-lactam antibiotics include penicillin, ampicillin, etc. So, if for example, an isolate is resistant to penicillin or even ampicillin, it can't be referred to as methicillin-resistant. The disc diffusion method using cefoxitin disc (30 µg) is more reliable in identifying methicillin-resistant *S. aureus* (MRSA). So, I suggest that authors should re-write this sentence as "S. aureus that are resistant to beta-lactam antibiotics (especially cefoxitin) are referred to as methicillin-resistant *Staphylococcus aureus* (MRSA).

Response: We have revised and rephrased/restructured this sentence: "*S. aureus* that are resistant to beta-lactam antibiotics are referred to as methicillin-resistant *Staphylococcus aureus* (MRSA)."

Authors should delete the word "swabs" in the sentence because what is being actually assessed are the hands of the farmers. The swab is just a tool used to collect the sample.

Response: We have deleted the word "swabs" in the sentence.

Introduction section

I think authors should remove the word "swab" as what is being actually assessed are the **farmers' hands** just like I mentioned in my earlier suggestion in the abstract section.

Response: We have removed the word swabs accordingly as you suggested to us.

Methodology section

Authors should correct the concentration of oxacillin antibiotic disc to **1 µg** because oxacillin disc concentration from Oxoid, UK is **1 µg** while that of cefoxitin is correct at the **30 µg** indicated. I think this might have been an oversight during the writing of the manuscript.

Response: According to your suggestion we have corrected the concentration of oxacillin antibiotic disc to **1 µg**.

Authors should take note of the **bolded sections** in the sentence and make corrections as I indicated below for the sentence to be more comprehensive and understandable. Also, 5cm is the same as 50mm. So, it is preferable to indicate that the distance between the oxacillin and cefoxitin antibiotics were 50 mm instead of 5 cm since distance units in the CLSI charts are in mm. Like I mentioned earlier, the sentence in the last section should be written as: "The identified isolates were purified on mannitol salt agar (HiMedia Pvt. Ltd., M118) **and** incubated at 37°C for 24 hours. **Using a sterile cotton swab** (AKD 10903610549), **standardized isolates (0.5 McFarland standard) were evenly streaked** on the surface of the MHA medium (Oxoid, CM0337). The oxacillin (**1 µg**) and cefoxitin (**30 µg**) antibiotic disks were placed side by side with a distance of 50 mm on MHA that had been inoculated with isolates, and then incubated at 37°C for 24 hours to measure the inhibition zone."

Response: We have corrected the whole section accordingly as you suggested. The concentration of all the oxacillin discs in the manuscript should be changed to **1µg**.

Results section

The colour of *S. aureus* on mannitol salt agar (MSA) is golden-yellow. I will suggest authors use this all through the manuscript.

Response: We have corrected this sentence throughout the manuscript.

I suggest that authors should rephrase this sentence to be more understandable.

Response: We have rephrased the whole sentence of table three so that it is understandable.

I suggest that authors delete the column of "**mecA detection using PCR**" in **Table 3** as it is empty and serves no function since the last column is already indicating the total isolates that harboured mecA gene

Response: We have followed your suggestions and made changes in Table 3.

Discussion section

There is a mix up in the sentence above. The sentence is stating that milk samples were isolated while what was actually isolated were the *S. aureus* from the milk samples. I will suggest that authors should re-write this section as "**..contamination; this percentage is higher than the research conducted by Wang *et al.*²⁷ who isolated 90 (46.15 %) *S. aureus* from 195 milk samples, and from another study conducted by Jahan *et al.*²⁸ who isolated 12 (25.53%) *S. aureus* from 47 milk samples**".

Response: We have corrected your recommended sentence accordingly in the whole section of the manuscript.

I think Authors should delete this sentence since Figure 3 already has a writing under the gel picture depicting what was positive for mecA, including the targeted fragment size and what as negative.

Response: We have deleted this sentence.

Competing Interests: no

Reviewer Report 26 July 2022

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F M Yasir Hasib 

Department of Infectious Diseases and Public Health, Jockey Club College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Hong Kong, China

Keywords:

1. No need for 'Swab's hand' as a keyword.

Introduction:

1. "*Staphylococcus aureus* is a pathogenic bacteria...", "*S. aureus* is an opportunistic bacterial pathogen..." - The two lines are almost similar and seem redundant. However, using pathogenic bacteria on the first line is unnecessary.
2. "In previous studies, *S. aureus* mainly was transmitted to humans through contaminated milk..." - In this line, you should add at least three references.
3. "These bacteria can quickly evolve into antibiotic-resistant strains due to inappropriate antibiotic administration" - Please delete the line or re-write it. It's challenging to make such a bold statement regarding drug administration.
4. "*S. aureus* that is resistant to beta-lactam antibiotics is referred to as methicillin-resistant *S. aureus* (MRSA)" - Why this line again? Please check the coherence of the lines.
5. "In previous studies, it was reported that the presence of MRSA can cause new health problems..." - Please re-write the introduction section to improve the flow of the introduction - sorry.

Methods:

1. How was the sample size calculated?
2. Please describe the sampling strategy.
3. What was the condition of the cows? i.e., lactation, age, antibiotic administration, housing type, mastitis condition, etc.
4. How do you select the dairy animals from the herd?
5. Why were hand swabs taken after milking? Before milking seems more accurate. Before milk collection, what process did you follow for the aseptic milk collection?
6. I don't find any importance in microscopic examination here.
7. What was the positive isolate used as a standard?
8. For MRSA, you can do only Cefoxitin disk diffusion for the confirmation. Oxacillin MIC is recommended according to CLSI (13th edition, CLSI, M02, Page 30¹).

Results:

1. "The Gram staining test showed the Gram-positive colonies in the form of cocci and clusters, which were then confirmed by the catalase test and coagulase test" - Change the line and add both biochemical test results (+/-).
2. You did not include the correlation between the isolates recovered from milk or hand swab.
3. "...milk can be caused by various factors including the unhygienic hands of farmers when milking..." - You did not take the swab before milking, how do you argue about it?

Discussion and Conclusions:

1. I think the authors did a great job describing the findings.

References

1. Clinical and Laboratory Standards Institute: M02 Performance Standards for Antimicrobial Disk Susceptibility Tests, 13th Edition. 2018. 30 [Reference Source](#)

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Antibiotic resistance, Public health, Bioinformatics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 13 Sep 2022

Aswin Rafif Khairullah, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya,

Indonesia

Thanks for your valuable comments and suggestions on the manuscript entitled:
“**Detection of *mecA* gene and methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from milk and risk factors from farms in Probolinggo, Indonesia**”

Abstract section:

No need for 'Swab's hand' as a keyword.

Response: We have removed the "swab hand's" description in the keywords.

Introduction section

"*Staphylococcus aureus* is a pathogenic bacteria...", "*S. aureus* is an opportunistic bacterial pathogen..." - The two lines are almost similar and seem redundant. However, using pathogenic bacteria on the first line is unnecessary.

Response: We have removed the description of *S. aureus* in the second sentence so that there is no repetition of the word.

"*Staphylococcus aureus* is a pathogenic bacteria...", "*S. aureus* is an opportunistic bacterial pathogen..." - The two lines are almost similar and seem redundant. However, using pathogenic bacteria on the first line is unnecessary.

Response: We have removed the description of *S. aureus* in the second sentence so that there is no repetition of the word.

"In previous studies, *S. aureus* mainly was transmitted to humans through contaminated milk..." - In this line, you should add at least three references.

Response: We have added two more references to the sentence so that there are three references in the sentence.

"These bacteria can quickly evolve into antibiotic-resistant strains due to inappropriate antibiotic administration" - Please delete the line or re-write it. It's challenging to make such a bold statement regarding drug administration.

Response: We have deleted the sentence.

"*S. aureus* that is resistant to beta-lactam antibiotics is referred to as methicillin-resistant *S. aureus* (MRSA)" - Why this line again? Please check the coherence of the lines.

Response: We have corrected the sentence to make it clearer.

"In previous studies, it was reported that the presence of MRSA can cause new health problems..." - Please re-write the introduction section to improve the flow of the introduction

Response: We have corrected the sentence to make it clearer.

Methodology section

How was the sample size calculated?

Response: We have added the sample calculation formula to the methodology

Please describe the sampling strategy.

Response: Based on these calculations, a sample was obtained with the selection of dairy cooperatives purposively based on the amount of milk production in an area and the willingness of dairy cooperatives to participate in the study.

What was the condition of the cows? i.e., lactation, age, antibiotic administration, housing type, mastitis condition, etc.

Response: Milk samples were taken from the udders of female cows who were in the lactation period.

How do you select the dairy animals from the herd?

Response: The condition of most dairy farms in Indonesia is not in a herd, but a few dairy cows are kept in cages.

Why were hand swabs taken after milking? Before milking seems more accurate. Before milk collection, what process did you follow for the aseptic milk collection?

Response: Because after the milking process there is cross-contamination of MRSA transmission from cows to humans and humans to cows. We have sterilized the sample bottles that we will use to take samples and milk samples taken directly from the cow's nipples.

I don't find any importance in microscopic examination here.

Response: We have added a description of the microscopic examination in Table 1 along with a picture of the results of Gram staining observed through a microscope.

What was the positive isolate used as a standard?

Response: Isolates were tested positive for *Staphylococcus aureus* when they had yellow colonies on Mannitol Salt Agar (MSA) media accompanied by positive results on Gram-positive staining and biochemical tests (catalase test and coagulase test).

For MRSA, you can do only Cefoxitin disk diffusion for the confirmation. Oxacillin MIC is recommended according to CLSI (13th edition, CLSI, M02, Page 301).

Response: Because cefoxitin has a higher sensitivity and specificity than oxacillin in

detecting MRSA.

Results section

"The Gram staining test showed the Gram-positive colonies in the form of cocci and clusters, which were then confirmed by the catalase test and coagulase test" - Change the line and add both biochemical test results (+/-).

Response: We have added the description of the Gram stain examination, catalase test, and coagulase test in Table 1 along with pictures.

You did not include the correlation between the isolates recovered from milk or hand swab.

Response: Because in this study we focused on observing risk factors for MRSA transmission originating from dairy cows.

"...milk can be caused by various factors including the unhygienic hands of farmers when milking..." - You did not take the swab before milking, how do you argue about it?

Response: Because we had difficulty in sampling on the farms, especially when sampling farmers' hand swabs due to the large number of milk samples we took. Besides that, it was very difficult to get samples of farmers' hand swabs before milking on several farms at the same time.

Competing Interests: No competing interests were disclosed.

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