

# Distribution of antimicrobial resistance genes of methicillin-resistant *Staphylococcus aureus* isolated from animals and humans in Yogyakarta Indonesia

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## Abstract

**Background and Aim:** Methicillin-resistant *Staphylococcus aureus* (MRSA) has been known as a highly pathogenic bacteria in animals and humans, which is still becoming a global health issue. The prevalence of MRSA infection continues to increase worldwide and has become a global concern as a dangerous zoonotic disease. The World Health Organization estimates that by 2050 MRSA will be the leading cause of death. This study aimed to estimate the prevalence of MRSA in *S. aureus* isolates of veterinary and human origin in Yogyakarta, Indonesia.

**Materials and Methods:** A total of 42 cases of *S. aureus* infection were examined in this study, consisting of nine isolates from cattle, five from goat, and 28 from human. All isolates were confirmed as *S. aureus* based on bacterial culture and detection of 23S rRNA and thermonuclease *nuc* gene by polymerase chain reaction (PCR).

**Results:** Among 42 isolates, 35 isolates (83.3%) were identified as MRSA by PCR positive of *mecA* gene encoding methicillin resistance. Most MRSA strains were found in human isolates (100%), followed by cattle isolates (55.5%) and goats (40%). All MRSA strains were also multi-resistant to penicillin (*blaZ* gene) and tetracycline (*tetK*, and *tetM* genes) with a prevalence of about 98%.

**Conclusion:** MRSA prevalence in humans and animals has increased significantly in Yogyakarta, Indonesia, compared to the previous study. The antimicrobial resistance pattern of MRSA animal isolates tends to be similar to humans and, thus, raises public health concerns about MRSA zoonotic spread.

**Keywords:** animal, antimicrobial resistance, human, methicillin-resistant *Staphylococcus aureus*, *Staphylococcus aureus*.

## Introduction

Multidrug-resistant strains of *Staphylococcus aureus* have emerged as a result of the discovery and widespread usage of antibiotics. Methicillin-resistant *Staphylococcus aureus* (MRSA) has been a global health challenge because it can cause severe infection in animals and humans. MRSA, one of *S. aureus* strains that has evolved, was first found in hospitals in the 1960s. The MRSA infection expanded to other parts of the world and developed a reservoir known as healthcare-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) in both

settings [1, 2]. Data reveal that from 94,000 cases in America, the frequency of MRSA infection is on the rise globally, with a morbidity rate of 18,650 cases. Conversely, Asia has the highest frequency of HA-MRSA and CA-MRSA infections worldwide, with an MRSA infection prevalence of 70% [2, 3]. Indonesia has a prevalence incidence of 3.1%, particularly in Java and Bali [3]. Antibiotic resistance, also known as antimicrobial resistance (AMR), has emerged as a worldwide health problem that has to be taken seriously. According to the World Health Organization (WHO) survey, antibiotic resistance is a significant issue that threatens the entire world. The death rate from antibiotic resistance, which was estimated by the WHO to be around 700,000/year in 2014, is expected to rise to 10 million per year by 2050, exceeding the death rate from cancer [4].

*S. aureus* can cause a wide range of illnesses, from mild skin infections to more serious conditions such as urogenital tract infections, toxic shock, septicemia,

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pneumonia, and endocarditis [5]. It is difficult to treat and eradicate resistant bacterial strains due to their capacity to respond to different types of antibiotics. The staphylococcal cassette chromosome, which contains the gene (*mecA*) encoding a penicillin-binding protein 2a (PBP2a), is acquired by bacteria. Bacteria are unable to bind to lactam antimicrobials because of the changed PBP2a [5, 6]. The WHO [4] reports that antibiotics that have also been identified as resistant include sulfonamides, ciprofloxacin, fluoroquinolones, the early and third generation of cephalosporins, and even colistin resistance has also been reported in several countries. This complicates infection control and increases the risk of death and the cost of treating AMR problems.

MRSA strains exhibit the traits of expressing a variety of virulence factors, including leukocidin, fibronectin, and super antigenic toxins [7, 8]. As a result, MRSA is one of the pathogens that contribute significantly to the development of hospital-acquired nosocomial infections (HA-MRSA), which also result in infections that are acquired in the community (CA-MRSA). According to estimates, the organisms asymptotically infect 20%–30% of the human population [9]. *S. aureus* is located in the anterior nares of humans. Therefore, patients admitted to the hospital may contract an infection from hospital staff who are infected and a source of infection in the nares. Human AMR is a global public health concern, particularly in developing nations with subpar healthcare infrastructure [9–11]. MRSA infection in animals was originally discovered in cattle, horses, and dogs in the 1970s and 1990s, respectively. Pyoderma, surgical wound infections, otitis, urinary tract infections, and arthropathy are the most common conditions reported to be linked to MRSA infections in small animals [11, 12]. The main problem with *S. aureus* infection in cattle is the formation of mastitis (udder infection), which has led to significant financial losses [13, 14]. *S. aureus* infection in livestock can also produce the same disease as in humans. With incidence rates ranging from 1.1% in Japan to 52.2% in Egypt, MRSA infection in animals poses a concern to the public's health [15–17]. With a prevalence rate of 42.9%, many MRSA infections in dairy cattle and goats have been documented in Indonesia [18–20]. Antimicrobials used as growth promoters and for preventive and therapeutic purposes are to blame for the rise of MRSA in livestock [5, 21]. As resistant genes can be transferred from farm animals to humans through direct contact or the food chain, MRSA infection in livestock is progressively becoming a risk to the public's health [5, 22]. In addition, the kinds of antibiotics used in agriculture are the same ones that are professionally prescribed in hospitals for humans. Therefore, excessive use can undoubtedly be linked to a rise in MRSA cases in both humans and animals [23]. The results of prior study of sensitivity tests to different antibiotics revealed that numerous *S. aureus* isolates from humans and animals

have developed multi-drug resistance [24]. Using molecular analysis to detect resistance genes in this study will yield more accurate outcomes.

This study aimed to evaluate the prevalence of MRSA in veterinary and human cases. By evaluating antibiotic sensitivity and identifying MRSA resistance genes, the result of this study could be valuable information to determine effective AMR control strategies.

## Materials and Methods

### Ethical approval

Ethical permission for human isolates complied with the ethical clearance issued by the Medical and Health Research Ethics Committee (MHREC) Universitas Gadjah Mada with Ref. No. KE/FK/0131/EC/2023. Milk samples were obtained from the Veterinary Clinical Pathology laboratory's top management.

### Study period and location

This study was conducted from June 2022 to September 2023 in the Clinical Pathology Laboratory, Faculty of Veterinary Medicine, Universitas Gadjah Mada.

### Bacterial isolates

A total of 42 *S. aureus* isolates used in this study were retrieved from Integrated Laboratory Installation Section of Dr. Sardjito Hospital, Yogyakarta, Indonesia (28 human isolates) with various clinical cases, and from Veterinary Clinical Pathology Laboratory, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia (14 animal isolates; nine cattle; and five goats) with udder infection (mastitis).

### Isolation and identification of *S. aureus* strains

On blood agar plates containing 5% of freshly drawn, defibrinated sheep blood, *S. aureus* isolates from both humans and animals were cultivated. The plates were then incubated at 37°C for 18 to 24 h. The form of the colony and biochemical and microbiological assays were used to identify *S. aureus*-specific colonies that had been streaked into single colonies. The samples were identified using Gram staining, fermentation on mannitol salt agar (MSA), catalase, and coagulase assays. The catalase test was carried out by applying a drop of hydrogen peroxide to a microscope slide. Hydrogen peroxide was mixed with a small amount of the bacterial isolate; the bacteria were catalase-positive and showed oxygen bubbles. The coagulase test was carried out using rabbit plasma to cultivate the bacteria in the test tube. At 6 and 24 h, coagulation was seen to be present. As a quality control organism, *S. aureus* strain ATCC 25923 was employed.

### DNA isolation and purification

The DNA from *S. aureus* was purified using a QIAmp DNA small kit (Qiagen, Germany) in accordance with the manufacturer's instructions. The bacterial strains were grown for 24 h at 37°C on a blood agar base (Oxoid, Germany) with 5% defibrinated

sheep blood. A total of 5~10 *S. aureus* colonies were suspended with 180 µL TE buffer (10 mM Tris-HCl and 1 mM EDTA [pH 8]) containing 5 µL lysostaphin (1.8 U µL<sup>-1</sup>; Sigma, USA) in 2 mL microfuge tubes. The suspension was incubated for 1 h at 37°C and 25 µL of proteinase K (14.8 mg mL<sup>-1</sup>; Sigma) and 200 µL of AL buffer (containing reagents AL1 and AL2; Qiagen) were then added. The suspensions were incubated for 30 min at 56°C, followed by 10 min at 95°C, and then briefly spun at 6000× g for a few seconds. Each sample received an additional 420 µL of ethanol before being put into a spin QIAamp column. The sample was washed twice with 500 µL of AW buffer (Qiagen) and the spin columns were then placed in a clean collecting tube after centrifugation at 6000× g for 1 min. After the second wash and centrifugation at 6000× g for 3 min, the QIAamp spin columns were placed in a clean 2 mL microfuge tube, and the DNA was eluted twice with 200 and 100 µL of AE buffer (Qiagen). DNA was stored at -20°C [25].

#### Molecular identification and detection of gene encoding antibiotic resistance

Molecular identification of *S. aureus* was done according to the amplification of 23S rRNA gene and *nuc* gene with the polymerase chain reaction (PCR) technique. The *mecA* gene encoding for methicillin resistance was detected by PCR as previously described [26]. The other antibiotic resistance gene, such as *blaZ* encoding for penicillin resistance, *tetK* and *tetM* encoding for tetracycline resistance, was detected by PCR technique with the program and primer design as described in Table-1 [27–29]. The reaction mixture (25 µL) contained 1 µL primer 1 (20 pmol), 1 µL primer 2 (20 pmol; IDT, USA), 12.5 µL PCR mix containing Taq DNA polymerase, MgCl<sub>2</sub>, and dNTPs (MyTaq Red mix, Bioline, UK), 2 µL of DNA template, and 8.5 µL distilled water. According to the manufacturer's instructions, the QIAamp DNA mini kit (Qiagen) was used to prepare the isolates' DNA. An American thermal cycler (Benchmark) was used to amplify the genes. The PCR products were separated by gel electrophoresis in Tris Borate EDTA buffer containing a tris base, boric acid, and EDTA solution on a 1.5% (w/v) agarose gel (Invitrogen, USA). A 100

bp DNA ladder (Invitrogen) was employed as a size marker. A SU 2 of the *S. aureus* strain was used as a positive control of MRSA [19], and ATCC 25923 of the methicillin-susceptible *S. aureus* strain was used as a negative control [30]. Redsafe (Intron, Korea) staining and ultra-violet transillumination were used to see the resulting bands.

#### Results

Based on the cultural and biochemical test, all 42 human and animal isolates in this study were identified as *S. aureus* with features of Gram-positive, catalase-positive, coagulase, and fermented mannitol in MSA. The identification of isolates was then confirmed genetically by PCR amplification of species-specific 23S rRNA and thermonuclease *nuc* genes. All isolates were confirmed as *S. aureus* with the amplicons size 1250 bp and 279 bp, respectively (Figures-1 and 2).

All 28 (100%) *S. aureus* strains isolated from humans used in this study were identified as methicillin-resistant by detection of the *mecA* gene by PCR with an amplicon size of 532 bp (Figure-3). In veterinary cases, 7 (50%) *S. aureus* strains were identified as methicillin-resistant, with 5 isolates (55.5%) from cattle, and 2 isolates (40%) from goats (Figure-4). According to this research, more MRSA strains have been identified from humans and animals in Yogyakarta than in an earlier study conducted in Indonesia [24].

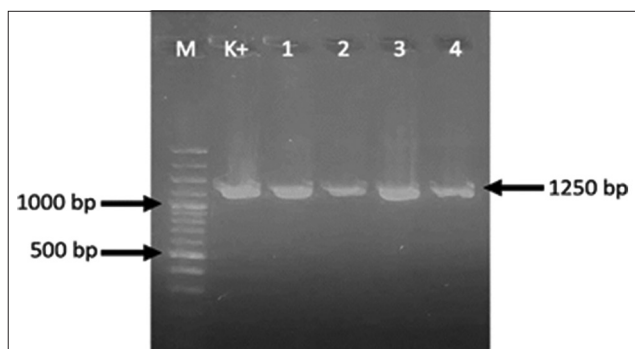
This present study also shows that MRSA strains were multi-resistant to other antibiotics, such as penicillin and tetracycline, with a high average resistance of 98% in both cases. The multi-resistant prevalence rate in human medical cases was 100%, while cattle 80%, and goat 100% (Figure-4). Most of the *S. aureus* strains were detected to be positive for *blaZ* gene encoding penicillin-resistant (95.2%) and *tetK* and *tetM* genes encoding tetracycline-resistant (97.6%) (Figure-5).

#### Discussion

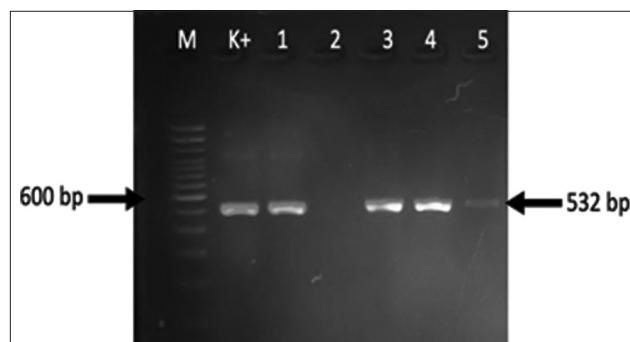
The presence of *S. aureus* in dairy cattle has been one of the key concerns in the dairy industry. The *S. aureus* colonization in the udder and skin leads

**Table-1:** Oligonucleotide primers and PCR programs used for amplifying the genes of 23S rRNA, *nuc*, *mecA*, *blaZ*, *tetK*, and *tetM*.

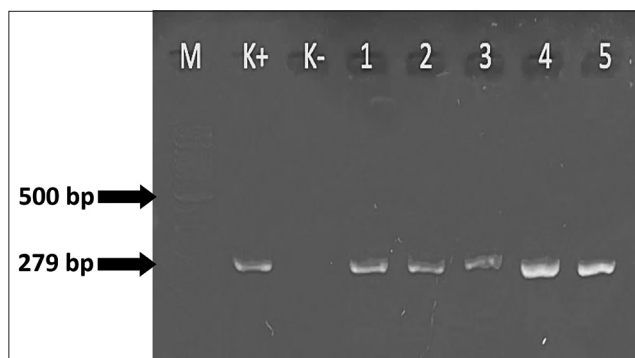
Gene	Sequence (5'-3')	Amplicon size (bp)	Reference
<i>mecA</i>	AAA ATC GAT GGT AAA GGT TGG AGT TCT GCA GTA CCG GAT TTG	532	[26]
23S rRNA	ACG GAG TTA CAA AGG ACG AC AGC TCA GCC TTA ACG AGT AC	1250	[27]
<i>nuc</i>	GCG ATT GAT GGT GAT ACG GTT ACG CAA GCC TTG ACG AAC TAA AGC	279	[28]
<i>blaZ</i>	ACT TCA ACA CCT GCT GCT TTC TGA CCA CTT TTA TCA GCA ACC	173	[29]
<i>tetK</i>	GTA GCG ACA ATA GGT AAT AGT GTA GTG ACA ATA AAC CTC CTA	360	[29]
<i>tetM</i>	AGT GGA GCG ATT ACA GAA CAT ATG TCC TGG CGT TGT CTA	158	[29]



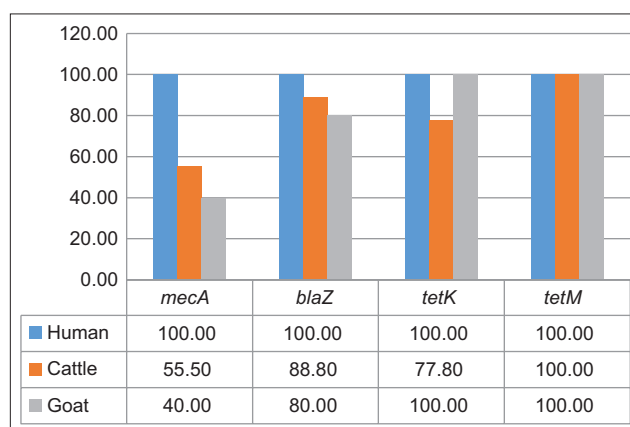
**Figure-1:** Amplicons of the gene encoding 23S rRNA of selected *S. aureus* strains with the molecular size of 1250 bp. Lane K+: *S. aureus* control strain, Lane 1-4: selected *S. aureus*, Lane M: 100 bp molecular-size DNA ladder. *S. aureus*=*Staphylococcus aureus*.



**Figure-3:** Amplicons of *mecA* gene with 532 bp molecular size. A positive control of MRSA (Lane K+), negative strain (Lane 2, no band), Lane 1, 3, 4, 5: *S. aureus* selected strain which showed clear bands confirmed that, all the isolates were Methicillin-resistant *Staphylococcus aureus*, Lane M: 100 bp molecular-size DNA ladder. *S. aureus*=*Staphylococcus aureus*.



**Figure-2:** Amplicons of thermonuclease (*nuc*) gene of selected *S. aureus* strains, with the molecular size of 279 bp. Lane K+: *S. aureus* control positive strain, Lane K-: A negative isolate, Lane 1-5: *S. aureus* selected strains. Lane M: 100 bp molecular-size DNA ladder. *S. aureus*=*Staphylococcus aureus*.

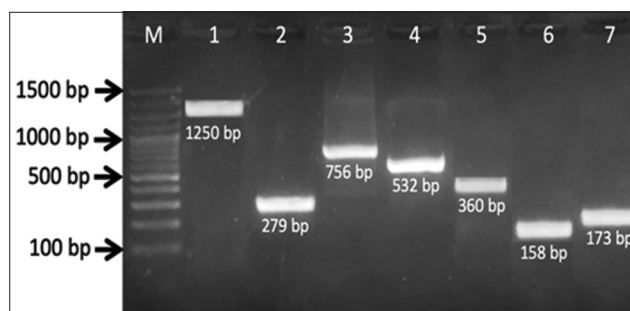


**Figure-4:** Antibiotic resistance gene of *S. aureus* isolated from human and veterinary medical cases. *mecA*: Encoding methicillin-resistant, *blaZ*: Encoding penicillin-resistant, *tetK* and *tetM*: encoding tetracycline-resistant.

to mastitis, which possibly contaminates either dairy products or livestock [31, 32]. Mastitis in cows significantly reduces production, which causes economic loss. Meanwhile, its therapy with antibiotic intervention results in antibiotic residue contamination and animal culling, often performed in the unsuccessful therapy. In particular, therapy for MRSA infection has a low cure rate [32] and causes more burden.

This study identified *S. aureus* isolated from humans (100%) and animals (50%) as MRSA. These findings agree with the previous report that indicated a relative increase of MRSA strains isolated from human and animal origin in Yogyakarta [24]. In addition, the highest prevalence of MRSA was identified from the highest medical cases (90%), cattle (12.82%), and goats (4.17%) [19, 20]. This relatively increased prevalence seriously threatens humans and animals, particularly dairy livestock. This is because resistance genes can be transferred directly from food animals to humans or through the food chain. Moreover, *S. aureus* possesses genes encoding enterotoxins that potentially lead to food poisoning. Concern over the threat that livestock MRSA presents to public health is rising [1].

The multi-resistant prevalence rate reported in this study, both in human and animal isolates,



**Figure-5:** Gene amplicon encoded species-specific and several antibiotics resistance of selected confirmed *S. aureus* isolates. Lane M: 100 bp DNA ladder, Lane 1: 23S rRNA gene (1250 bp), Lane 2: thermonuclease *nuc* (279 bp), Lane 3: *coa* gene (756 bp), Lane 4: *mecA* gene (532 bp), Lane 5: *tetK* gene (360 bp), Lane 6: *tetM* gene (158 bp), Lane 7: *blaZ* gene (173 bp).

indicates a high prevalence of multidrug-resistant bacteria in Yogyakarta, Indonesia. The AMR pattern of *S. aureus* animal isolates appears to be comparable to the human isolates, according to the resistance percentage (Figure-4). Penicillin and tetracycline were common antibiotics used in humans and animals. These antibiotics were the drug of choice for mastitis

treatment in the veterinary field. *S. aureus* is the most pathogenic agent that causes mastitis in cattle and small ruminants [33–35]. Besides that, penicillin and tetracycline are also commonly used as growth promoters in livestock animals [22]. The widespread use of commonly used antibiotics in humans has contributed to the current phenomenon of similar resistance patterns in animals [1].

The result of this study supported the previous findings that the most frequently detected gene was *blaZ* [36]. The previous report also discovered that MRSA is resistant to penicillin G 100% and 99% [24]. Penicillin resistance is mediated by penicillinase, an enzyme that hydrolyzes the  $\beta$ -lactam ring of penicillin. The *blaZ* gene encoding for penicillinase is known to be carried through plasmid that facilitates rapid spread between Staphylococci. The *blaZ* gene has also been found in *S. aureus*, and other coagulase-positive and coagulase-negative staphylococci, suggesting that the *blaZ* gene plays an important role in penicillin resistance in Staphylococcus [37]. The presence of *tetK* and *tetM* genes in dairy cattle mastitis should be known since they not only encode for tetracycline resistance but also potential resistance to other antibiotics such as doxycycline and minocycline. This might limit the drug of choice for treating the infection [17].

Inadequate sanitation and poor farm management techniques contributed to the presence of *S. aureus* in the milk of dairy animals on the farm, despite the fact that it is a normal resident in humans [38, 39]. Hand-milked dairy animals may also be a source of *S. aureus* infection due to a high incidence of transfer [40]. Infection of MRSA was believed to be only a human pathogen for many years until the first MRSA isolated from mastitis in dairy cattle was reported in 1972 [41]. It has now become an increasingly important issue in veterinary medicine, not only reported in livestock but also in companion and wild animals [1, 42, 43]. Since MRSA might be transmitted as a zoonotic disease among humans and animals, the proper use of antibiotics in humans and livestock animals should be applied. Antibiotics used as growth promoters in livestock animals should be avoided since they could increase the risk of antibiotic resistance to humans through the food chain [40]. The outcomes of our surveillance of MRSA from human and animal isolates in Yogyakarta, Indonesia, provided information about the evolution of these bacteria's resistance pattern, which may be used to choose appropriate AMR control measures.

## Conclusion

This study confirmed a significant increase in the prevalence of MRSA strain isolated from human and veterinary medical cases in Yogyakarta, Indonesia compared to a previous similar studies [19, 20, 24]. MRSA was most commonly found in human isolates (100%), followed by isolates from cattle (55.5%) and goats (40%). The MRSA strains observed in this study were also multi-resistant to penicillin and tetracycline,

with a prevalence of about 98%, indicating the current high prevalence of multidrug resistance. It was also a particularly interesting finding that the AMR pattern of MRSA animal isolates tends to be similar to humans. The presence of *mecA* gene in cattle and goat isolates indicates the important transmission of MRSA among humans and animals.

## Authors' Contribution

MF: Performed the experiment, data analysis, and wrote the manuscript. SIOS: Conceptualization, supervising, and wrote the manuscript. OS, US, REK, and AD: Sample collection and preparation. FA and FBL: Data analysis and reviewed the manuscript. MW: Data analysis and drafted the manuscript. All authors have read, reviewed, and approved the final manuscript.

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## Competing Interests

The authors declare that they have no competing interests.

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