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Molecular evidence of *mec*A gene encoding methicillin-resistant *Staphylococcus aureus* isolated from cats in Surabaya, Indonesia

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a novel strain of this bacterium that is resistant to β-lactam antibiotics with multidrug-resistant (MDR) features. Probable MRSA reservoirs have been identified in pet animals. This investigation sought to determine the *mec*A gene, which confers methicillin resistance in MRSA in cats. A total of 150 cats were collected from animal clinics and veterinary hospitals in five regions of Surabaya, Indonesia. *S. aureus* isolates were tested for antibiotic resistance using the Kirby-Bauer diffusion method, which consisted of streaking bacterial suspensions according to the 0.5 McFarland standard and then placing five different antibiotic disks on Mueller–Hinton Agar (MHA). Oxacillin resistance screening agar base (ORSAB) was used to continue cultivating cefoxitin-resistant *S. aureus* isolates as an MRSA confirmation test. Eighteen (12%) *S. aureus* isolates were found as a result of the identification and isolation. The antibiotic resistance test results revealed 7 (38.88%) multidrug-resistant (MDR) isolates: 3 (16.66%) MDR *S. aureus* isolates and 4 (22.22%) MDR *S. aureus* isolates testing positive for ORSAB, which were identified as MDR and MRSA isolates, respectively. Four MRSA isolates were then subjected to molecular detection using polymerase chain reaction (PCR), with positive results revealed by a band that appeared at 310 bp. This study unearthed molecular evidence for the *mec*A gene that confers methicillin resistance in MRSA. It can be concluded that strict monitoring for MRSA in cats is required due to the significance of these bacteria and their potential for zoonotic transmission.

Introduction

Ownership of a cat as a pet is becoming increasingly popular, especially in urban areas, which has increased the possibility for bacteria to be transmitted between people and animals (Aires-de-Sousa, 2017). Mammals and birds' skin and mucous membranes naturally contain *Staphylococcus aureus*, but it has also developed into a common opportunistic pathogen in veterinary and human medicine (Ma *et al*., 2020). Once inside the body, *S. aureus* can cause a wide range of ailments, from minor skin infections to serious invasive infections that can be lethal (Ramandinianto *et al*., 2020; Bertelloni *et al*., 2021). It is Gram-positive, 0.8 mm in diameter, aerobic or anaerobic, and grows best at 37 °C and pH 7.4 (Gardete and Tomasz, 2014). Resistance by mutation is a process wherein genetic alterations in *S. aureus* that modify the target DNA gyrase or reduce outer membrane proteins might result in drug resistance (Yang *et al*., 2020). One kind of plasmid-mediated resistance is acquired resistance. For instance, plasmid-mediated transduction, transformation, and induction of drug-resistance genes can result in the production of excessive lactamase, which makes bacteria resistant (Haaber *et al*., 2017; Permatasari *et al*., 2025).

MRSA resistance is mostly brought on by the transmission of drug resistance genes mediated by plasmids, which can expand the genome and enable the subsequent transfer of resistance genes between *S. aureus* and other bacteria (Vestergaard *et al*., 2019). Complex interactions between bacterial species from multiple environments amplify antimicrobial resistance between humans, animals, and the environment (Kraemer *et al*., 2019). Methicillin-resistant *Staphylococcus aureus* (MRSA) is a strain of *S. aureus* that is resistant to the antibiotic methicillin and has become a global threat. It has appeared in the population among individuals who do not have risk factors for acquiring MRSA, posing a novel danger. In addition to MRSA's known presence in healthcare settings, the following discovery of MRSA colonizing or infecting animals and in animal-derived products was especially concerning since it identified additional MRSA reservoirs. The MRSA era began in 1961 when *S. aureus* first developed resistance to methicillin and other lactam drugs (Harkins *et al*., 2017). The high rates of disease and mortality linked to MRSA infection have drawn the attention of medical establishments worldwide due to the serious hazards to human health. In fact, data indicate that MRSA infection is among the most prevalent infectious infections in the world (Hassoun *et al*., 2017).

Methicillin resistance is brought on by the *mec*A gene, which generates the new protein Penicillin Protein Binding 2a (PBP2a), a member of an enzyme family necessary for the formation of bacterial cell walls (Khairullah *et al*., 2020; Khairullah *et al*., 2023). The clonal types of MRSA infecting humans who cohabit with dogs and cats are similar to those that are prevalent in these pets (Vincze *et al*., 2014; Silva *et al*., 2022). In 1988, an infected domestic cat caused the first reported MRSA human outbreak of feline origin, which affected patients and staff in an elderly nursing facility in the UK (Shoaib *et al*., 2022). MRSA transmission is facilitated by close interaction between pets and their owners, which is reflected

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in how pet owners are more likely to acquire MRSA than the general population. This shows that animals can act as reservoirs for infection (Haenni *et al*., 2012). Meanwhile, several studies have revealed that intimate contact with humans is associated with an increased risk of *S. aureus* colonization in pets, and MRSA in cats is predominantly of human origin (Bierowiec *et al*., 2016). MRSA has been found in cats, dogs, horses, cattle, rabbits, cockroaches, and chinchillas, among other animals (Islam *et al*., 2016; Yunita *et al*., 2020; Khairullah *et al*., 2024). Numerous animals' unknown carrier status, inadequate cleaning and disinfection procedures, high population density, the possibility of nosocomial transmission from humans, and some possible reasons of this exposure (Dalton *et al*., 2020).

Beta-lactam antibiotics have a relatively low affinity for PBP2a (Islam *et al*., 2016); in fact, this protein confers resistance to methicillin and several other beta-lactam antibiotics (Pantosti *et al*., 2007). The staphylococcal cassette chromosome (*mec*), a moveable segment of genetic material that is introduced into the *S. aureus* chromosome proximal to orf X, contains the *mec*A gene (Katayama *et al*., 2000). The frequent stroking, petting, and licking that companion animals engage in with their owners exposes them to dangerous MRSA infections. Given that MRSA is a serious public health issue, this work is extremely important.

Materials and methods

Ethical approval

Nasal swabs from cats were used in this study, so ethical approval was not necessary. These swab samples were collected from five regions in Surabaya, East Java, Indonesia.

Study planning and sample collection

A total of 150 samples were obtained from five regions (North, East, West, Central, and South) of Surabaya. The swabs were collected from veterinary clinics and hospitals between May 2022 and July 2022.

Bacterial isolates

Amies medium transport was used to collect cat nasal swab samples, which were then kept in an icebox at 4°C. *S. aureus* was isolated using a sterilized Amies cotton swab and streaking on mannitol salt agar (MSA). The bacterial inoculum was cultured for 24 hours at 37°C on MSA medium (Effendi *et al*., 2018). *S. aureus* was identified using Gram staining, positive results from the Voges-Proskauer, coagulase, and catalase tests, as well as yellow colonies with yellow zones on MSA media (Effendi *et al*., 2019).

Tests for antibiotic susceptibility and MRSA confirmation

The Kirby–Bauer diffusion method was used to examine the antibiotic sensitivity for five different antibiotics: cefoxitin (5 µg), erythromycin (15 µg), tetracycline (30 µg), ciprofloxacin (5 µg), and chloramphenicol (30 µg). Purified cultures were made into bacterial solutions using the equivalent of 0.5 McFarland units. Five separate antibiotic disks were placed after the Mueller–Hinton agar (MHA) (Oxoid-CM00337) surface had been streaked with a sterile cotton swab to create the inoculum. The disks were then incubated at 35°C for 24 h. The results of antibiotic sensitivity tests were calculated using the width of the inhibition zone of the clear region surrounding the antibiotic disk, which is measured in millimeters according to the specification provided by the Clinical and Laboratory Standards Institute (CLSI, 2021). Several *S. aureus* isolates that were cefoxitin-resistant on MHA (Oxoid-CM00337) were kept for an MRSA confirmation test.

The MRSA confirmation test were streaked on oxacillin resistance screening agar base (ORSAB) (HiMedia M1415) and then oxacillin resistance selective supplement (Supplement, HiMedia FD191).

PCR analyses of mecA gene

DNA was extracted from MRSA isolates and analyzed using a QIAamp DNA Mini Kit 50 (Qiagen, Germany), in accordance with the manufacturer's guidelines. MRSA isolates were supplemented with 180 µl of lysozyme (20 mg/ml) and incubated for 45 min at 37°C. Then, the extracted DNA was diluted with 20 µl of Proteinase K and 200 µl of Buffer kit. Three microliters of DNA solution was used for the PCR amplification. Pure *S. aureus* genomic DNA was used as a template for amplification using a Promega GoTag Master Mix, following the manufacturer's guidelines. PCR amplification was carried out using the primers *mec*A (Forward) 5′- GTA GAA ATG ACT GAA CGT CCG ATA A - 3′ and *mec*A (Reverse) 5′- CCA ATT CCA CAT TGT TTC GGT CTA A - 3′ with a length of 310 base pairs (bp) (Rahmaniar *et al*., 2020). The amplification process included initial denaturation at 94°C for 4 min; 40 cycles of denaturation at 94°C for 45 s, annealing at 58°C for 45 s, and extension at 72°C for 1 min; and then final extension at 72°C for 3 min. Following the amplification reaction, the PCR products were electrophoretically separated on a 2% agarose gel using Ultrapore Agarose Gel (Invitrogen, USA) and stained with Safe DNA gel stain (Invitrogen, USA) at 100 V for 45 min in 10X UltrapureTM TBE buffer (Invitrogen) before being illuminated by a UV transilluminator and visualized.

Results

Identification of S. aureus

A total of 150 feline nasal swab samples were examined, 18 (12%) of which were found to be positive for *S. aureus* based on observations of colony morphology shown in Figure 1. Gram staining was used for identification, and catalase, coagulase, and Vogues–Proskauer biochemical tests were also performed. All samples that passed all biochemical tests with a positive outcome were categorized as *S. aureus* isolates.

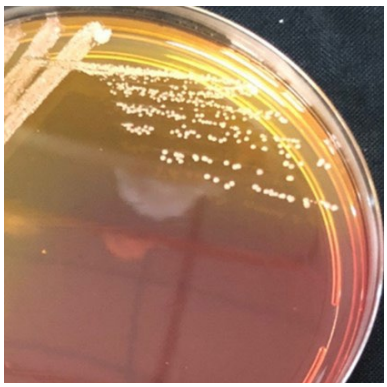

Fig. 1. The appearance of *S. aureus* in MSA

MRSA confirmation test

Among the 18 *S. aureus* isolates that were discovered (12%), one was tetracycline-resistant, two were tetracycline- and erythromycin-resistant, and seven were labeled as multidrug-resistant (MDR) because they had three or more classes of resistance. In terms of the patterns of antibiotic resistance, a total of 4 *S. aureus* isolates were resistant to cefoxitin, ciprofloxacin, tetracycline, erythromycin, and chloramphenicol, as shown in Figure 2, followed by 3 *S. aureus* isolates with resistance to erythromycin, tetracycline, and chloramphenicol, and 7 with no antibiotic resistance found (Table 1).

As a confirmation test for MRSA, four *S. aureus* isolates that were resistant to cefoxitin were classed as presumptive MRSA isolates and continued to streak on ORSAB. Figure 3 illustrates the ORSAB test results, in which blue indicated positive findings and white indicated negative results. Since all four presumptive MRSA isolates (22.22%) tested with ORS-

AB produced positive results, they were all considered to have been phenotypically identified as MRSA isolates. Polymerase chain reaction (PCR) assays were performed by targeting the presence of the *mec*A gene; the molecular test revealed that four (22.22%) isolates were MRSA-positive, based on a band representing 310 bp as shown in Figure 4.

Table 1. Isolates of *S. aureus* with different antibiotic resistance profiles.

Resistance profile	Resistant isolates (%)
	Number of *S. aureus* isolates (n = 18)
No antibiotic resistance	38.8% (7/18)
TE	16.6% (3/18)
TE–E	5.55% (1/18)
TE–E–C	16.6% (3/18)
E–C–FOX–CIP	5.55% (1/18)
E–C–FOX–CIP–TE	16.6% (3/18)

Note: TE: Tetracycline, E: Erythromycin, C: Chloramphenicol, FOX: Cefoxitin, CIP: Ciprofloxacin

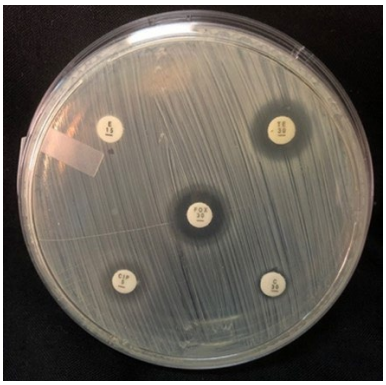

Fig. 2. Antibiotic sensitivity test on a presumptive MRSA isolate in MHA

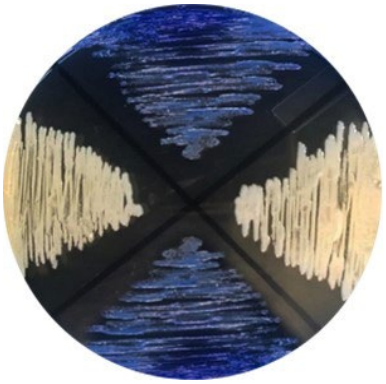

Fig. 3. ORSAB as a confirmation test for MRSA; aniline blue color represents positive results and white or pale color represents negative results

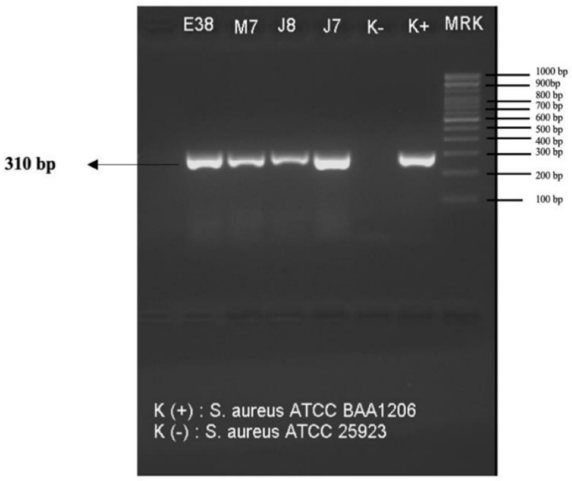

Fig. 4. Gel electrophoresis demonstrates the presence of *mec*A gene at a nucleotide length of 310 bp. E38, M7, J8, and J7 are sample codes indicating positive results. MRK stands for DNA ladder marker (100–1000 bp), K- stands for *S. aureus* ATCC 25923 as a negative control, K+ stands for *S. aureus* ATCC BAA1206 as a positive control.

Discussion

The term "superbugs" refers to several bacterial and viral strains that are resilient to a number of currently used antimicrobials, including a semi-synthetic antibiotic called methicillin that was originally employed to stop the spread of staphylococcal infections (Tyasningsih *et al*., 2019). MRSA is a variant of *S. aureus* and is also called a superbug because it consistently evades antibiotics and other medications that are often used to treat both severe and minor infections (Nandhini *et al*., 2022). MRSA isolates exhibit a high rate of antibiotic resistance because of excessive or frequent methicillin use, which has rendered the drug ineffective for treating bacterial infections (Mustapha *et al*., 2016). In total in this study, *S. aureus* was detected in 18/150 (12%) samples. This corroborates an earlier study's findings of a high prevalence of infection (Ruiz-Ripa *et al*., 2021). It was also stated that *S. aureus* isolates were mostly found on the skin and mucosa of cats. MRSA was found to colonize 2.1% of cats in veterinary clinics. MRSA in companion animals can have spread globally, but the prevalence varies in many European countries, North America, and Singapore (Lee *et al*., 2018). The most common staphylococcal species in cats is *S. aureus* because these germs can occasionally thrive as opportunistic infections on domestic animals (Tyasningsih *et al*., 2019).

The number of MRSA isolates confirmed in this study was 4/150 (2.6%), which constituted 4/18 (22.2%) of the *S. aureus* isolates. Nosocomial infections are a common source of *S. aureus* strains that are resistant to a number of antimicrobial drugs. MRSA strains found in companion animals including cats, dogs, and horses are often distinct from those found in animals used for food. The first case of strains reported resembled human hospital-acquired methicillin-resistant *S. aureus* (HA-MRSA), but the second case appeared to be a unique clone that was modified for use in animals and is unconnected to the majority of human HA-MRSA (Mustapha, 2014).

Companion animals are now common in homes in developed nations. In the USA, more than 50% of homes have pet animals (Bhat, 2021). Recently, reports of *Staphylococcus* in dogs and cats revealed various coagulase-positive pets, with dogs, and cats in particular having been found to carry *S. aureus* (Weese, 2010). MRSA transmission is more likely to occur when there is close contact between animals and people, as is the case with most cats, which have frequent direct human contact (Crespo-Piazuelo and Lawlor, 2021). Furthermore, studies have shown that MRSA can spread through inanimate items and aerosols, raising concerns that pets could act as MRSA reservoirs and infect humans (O'Rourke, 2003). The transfer of bacterial strains between cats and their owners is suggested by the numerous instances of identical MRSA strains found in cohabiting pets and people (Rahmaniar *et al*., 2020). A healthy cat was the source of one of the four MRSA isolates that tested positive in this study, which proves that healthy cats can carry MRSA even in the absence of any clinical symptoms (Bierowiec *et al*., 2016). It is commonly assumed that companion animals acquire MRSA from humans because nosocomial MRSA isolates closely resemble those from cats and dogs. This is because both humans and animals have a higher colonization rate than infection rate, and both can act as MRSA reservoirs to spread strains within a household (Ferreira *et al*., 2011). Pet owners had a significantly higher risk of MRSA colonization (18%) than the general population (1%–2%), according to a study done in the US and Canada (Petinaki and Spiliopoulou, 2015). Furthermore, in a UK healthcare retirement home, the same strain of MRSA colonized residents, staff, and a household cat (Horner *et al*., 2013). Furthermore, one study reported that MRSA has been found in subclinical carriers, including cats and other animals (Harrison *et al*., 2014). The MRSA strain was present on the paws and fur of cats, which may be important in terms of the possibility of transmission (Ruiz-Ripa *et*

al., 2021). Direct contact with companion animals is still thought to be an efficient route for MRSA to spread to people, even though research has indicated that indirect contact with these animals is a significant pathway to infection (Goerge *et al*., 2017). Another earlier study found that veterinarians who treat dogs and cats were more likely to be colonized and to have the same strain as the 12.8% of household contacts who tested positive for MRSA (Walther *et al*., 2012). This was confirmed by a study conducted in the UK, which found that MRSA was present in 12.3% and 7.5% of veterinarians and pet owners, respectively, who had come into contact with animals (Jordan *et al*., 2011).

Culture can be used to identify *Staphylococcus aureus* infections, including colonization. MRSA can colonize multiple anatomical sites, so both nasal and rectal swabs should be performed whenever possible (Kottler *et al*., 2010). *S. aureus* is the dominant commensal bacterium found in the nasal mucosa, and is physiologically located in the nose. This is supported by a previous study by Hardy *et al*. (2020) showing that nasal swabs are better than pharyngeal, axillary, and perineal swabs. In another study it was mentioned that MRSA was more common in nasal swabs than in swabs of pus or wounds in mammals (Habibullah, 2017).

Colony morphology, gram staining, and biochemical tests were employed to detect the bacterium in clinical specimens using standard microbiological procedures (Bierowiec *et al*., 2016). Yellow colonies with yellow zones were considered to be presumptive *S. aureus* colonies on mannitol salt agar (MSA) as the selective medium (Javid *et al*., 2018). A coagulase test as a biochemical test was used to differentiate *S. aureus* from other staphylococci. Methicillin-resistant strains could be found if *S. aureus* was isolated using antibiotic sensitivity testing (CDC, 2009). Since methicillin is no longer sold commercially in the US, most antibiotic susceptibility testing uses oxacillin or cefoxitin (Khairullah *et al*., 2022). Testing for antibiotic sensitivity has some limitations when compared with finding *mec*A or PBP2a (Naccache *et al*., 2019). The "gold standard" for identifying *mec*A in MRSA was reported to be genetic testing, such as PCR (Koupahi *et al*., 2016). Finding the *mec*A gene is the main evidence for detecting an MRSA isolate, which is in agreement with previously reported findings in dogs (Rahmaniar *et al*., 2020) and in milk (Ramandinianto *et al*., 2020). In addition, our findings in this study suggest that the isolates that were resistant to cefoxitin all contained the *mec*A gene (Ramandinianto *et al*., 2020).

The lack of an effective treatment for MRSA infection necessitates controlling and preventing its spread in both humans and animals (Khairullah *et al*., 2020). Early detection by microbiological surveillance and careful antibiotic administration can help prevent MRSA (Johnson, 2015). Animals with known MRSA infections should be segregated and barrier precautions should be taken when treating them (Jaradat *et al*., 2020). When there is a possibility of infection via contact with bodily fluids, protective gear such as gloves and specific clothing that may be disinfected at the clinic should be worn (Verbeek *et al*., 2020). Particularly when using invasive devices such as intravenous catheters and urine catheters, good infection control procedures should be employed (Haque *et al*., 2018). Guidelines should also be established by reputable veterinary facilities to reduce cross-contamination by MRSA and other methicillin-resistant staphylococci (Waruwu *et al*., 2023). Maintaining proper hygiene, which includes hand washing and environmental disinfection, is essential for prevention (Hillier, 2020). Every opportunity should also be taken to cover MRSA-infected wounds (Millannia *et al*., 2023). According to one study, if infection control protocols are followed, the possibility of transfer from contaminated surgical employees should be miniscule, despite the fact that colonized individuals can transfer MRSA to animals (Turner *et al*., 2019). Rapid isolation of MRSA carriers and the implementation of barrier precautions to avoid contact with animals are made possible by admission screening. Diagnostics should also be performed on animals that have or may have been exposed to MRSA infection or colonized staff. MRSA carriers must be immediately isolated and barrier procedures must be implemented to avoid contact with animals, which are made possible by admission screening (Currie *et al*., 2019). All hospitalized animals may require costly routine examinations that are only helpful for referral procedures (Bert *et al*., 2016). For this reason, some authors advise screening specific groups, such as animals with diseases that are resistant to antibiotics, are not recovering, or have nosocomial infection, as well as animals owned by medical professionals or families with a confirmed MRSA infection (Lagler *et al*., 2022). MRSA colonization was naturally eliminated in a number of animals, including dogs, cats, and horses, when the environment was regularly cleansed, disinfected, and re-infection was reduced (Horner *et al*., 2013). Antimicrobial decolonization is currently not advised for pets on a regular basis, but it may be taken into consideration in specific situations to prevent transmission to people or other animals (Shane *et al*., 2017). It has been considered that topical treatment for nasal carriage in animals, such as mupirocin, is indeed not practicable (Agarwal *et al*., 2015). The risk groups such as pet owners, veterinarians, paramedics, and veterinary clinic staff, as well as the general public, should be advised to avoid unnecessary interaction with pets, take proper hygiene precautions, and prevent inappropriate use of antibiotics. Given that pets have the potential to act as reservoirs and endanger public health, veterinary medical staff should be aware of MRSA colonization and infection (Decline *et al*., 2020).

Conclusion

The molecular finding of the *mec*A gene encoding mrsa from cats is important for proving the potential for transmission to humans. MRSA transmission in companion animals, particularly cats, is considered a global concern. Numerous reports on the prevalence of MRSA in pets have recently been published. The majority of MRSA infections in pets are linked to exposure to animal clinics and human contact. To control and prevent mrsa infections, in addition to increasing public awareness, the importance of rational use of antibiotics in the treatment of sick animals. To reduce transmission in veterinary clinics, control mechanisms must be improved, and additional studies must be conducted to determine the true prevalence of mrsa in healthy and sick cats, which serve as a reservoir for human infection.

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Conflict of interest

The authors have no conflict of interest to declare.

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