Detection mecA gene and Staphylococcus aureus resistance to several antibiotics isolated from cat ear swabs at a veterinary hospital located at Surabaya – Indonesia

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ABSTRACT
Cats are susceptible to S. aureus, which mainly colonizes the nose and ears of these feline species. Otitis externa in cat ears is one of the illnesses produced by S. aureus in animals. Antibiotic therapy for affected animals is the conventional treatment for infections by S. aureus. Antibiotic use during prolonged treatment and given at the wrong doses can cause germs to become resistant. Given this context, research on S. aureus isolated from cat ears and tests for antibiotic resistance and the mecA gene is required. Samples of cat ears were obtained from the Amies media using a sterile cotton swab. Bacterial isolation was done on MSA media, and then the catalase and coagulase assays were used to identify the bacteria. S. aureus isolates were evaluated for sensitivity using disks of the antibiotics cefoxitin, tetracycline, erythromycin, gentamicin, and chloramphenicol connected to MHA media. All positive isolates of S. aureus underwent MRSA testing, and then the mecA gene was detected. The sample investigation revealed that 91% (91/100) were positive for S. aureus, and 3.30% (3/91) were confirmed to be multidrug-resistant (MDR) because they are resistant to 3–4 antibiotic classes. Out of the 12 MRSA isolates analyzed, the mecA gene was detected in one isolate. Inappropriate antibiotic use causes bacterial resistance in pets. Additionally, excessive antibiotic use in a population might develop acquired bacterial resistance to an antibiotic. Antibiotic use in animals must be assessed to administer medication and prevent the development of antibiotic resistance appropriately.

Keywords: Staphylococcus aureus. Multi-drug resistance. Cat. Public health.

RESUMO
Gatos são suscetíveis a adquirir S. aureus que colonizam principalmente as narinas e os ouvidos de espécies de felinos. A otite externa no ouvido dos gatos é uma das doenças produzidas pelo S. aureus nos animais. A terapia com antibióticos é o tratamento convencional para as infecções produzidas pelo S. aureus. Os antibióticos utilizados durante o prolongado tratamento e o emprego de sub doses podem selecionar microorganismos resistentes. Com base em tais argumentos torna-se necessária a pesquisa de S. aureus isolados do ouvido dos gatos, bem como, a realização de testes para a resistência a antibióticos e do gene mecA. Empregando swabs estéreis de algodão foram obtidas amostras dos ouvidos dos gatos em meio de Amies. O isolamento bacteriano foi efetuado em meio MAS e os testes catalase e coagulase foram realizados para a identificação das bactérias. A sensibilidade dos isolados de S. aureus foi avaliada com o emprego de discos dos antibióticos cefoxitin, tetraciclinia, eritromicina, gentamicina e cloranfenicol, incorporados no meio MHA. Todos os
isolados positivos de *S. aureus* foram submetidos ao test MRSA para a detecção do gene *mecA*. A amostra investigada revelou 91% (91/100) de positivos para *S. aureus*, dos quais, 3,30% (3/91) foram resistentes a múltiplas drogas (MDR) pois foram resistentes a 3-4 classes de antibióticos. De 12 MRSA isolados analisados o gene *mecA* foi detectado em um isolado. O uso inapropriado de antibióticos é a causa da resistência bacteriana em pets. Adicionalmente o emprego excessivo de antibióticos em uma população pode resultar no desenvolvimento de resistência bacteriana adquirida a antibióticos. O uso de antibióticos em animais deve ser ordenado por uma administração de medicamentos apropriada para prevenir o desenvolvimento da resistência.


**Introduction**

*Staphylococcus aureus* is a Gram-positive bacteria that typically lives in the skin tissue of both humans and animals (Rahmaniar et al., 2020). *S. aureus* can cause various skin disorders in animals, including dogs and cats. It can be isolated from the skin mucosa of those animals (Khairullah et al., 2022). Cats are susceptible to acquiring *S. aureus*, which mainly colonizes the nose and ears of these feline species (Yunita et al., 2020). One of the infections that can be caused by *S. aureus* in animals is otitis externa in cat ears (Avberšek et al., 2021), which is an inflammatory condition of the external ear canal outside of the tympanic membrane (Awosile et al., 2018; Rosenfeld et al., 2014).

This bacterial infection will not typically result in significant issues (Ramandinianto et al., 2020), which is present in clinically healthy animals. However, it can cause infections when the immune system is weakened (Decline et al., 2020). The two bacterial species most frequently infect cats are *S. aureus* and *S. pseudointermedius* (Bierowiec et al., 2021). Phenotypically, these microorganisms are non-motile, have a spherical shape, measure 0.8-1 µm in diameter, cluster together like a bunch of grapes, and do not produce spores (Tahi et al., 2021). This bacterium can thrive at a temperature of 37 °C and can procreate by binary fission (Medvedová et al., 2019).

It is recognized that *S. aureus* bacteria can spread from pets to people or vice-versa (Afnani et al., 2022). Given that pets are frequently treated as family members, it is impractical to enable physical interaction between people and animals, including skin-to-skin contact, aerosols from coughing, sneezing, and saliva (Damborg et al., 2016). Compared to street cats, domestic cats related to their owners run the highest risk of spreading *S. aureus* (Overgaauw et al., 2020). The spread of these bacteria in veterinary hospitals will be a problem for public health (Haag et al., 2019).

The conventional treatment for *S. aureus* infections in animals is antibiotic therapy (Mala et al., 2021). Prolonged and incorrect antibiotic use can lead to the development of resistant bacteria (Paterson et al., 2016), reducing the effectiveness of antibiotics (Friedman et al., 2016). Antibiotic resistance poses a significant threat, making infections more difficult to cure (Melander & Melander, 2017). Cats, as home pets, may receive antibiotics similar to those prescribed for humans and can transmit zoonotic bacteria to their owners through close contact, contaminated food, or the environment (Foreman-Worsley et al., 2021; Zanen et al., 2022). As a result, it might enable cats to act as a possible reservoir for zoonotic germs that are resistant to antibiotics, such as *S. aureus* (Dafale et al., 2020). The close interaction between pets and their owners increases the risk of spreading harmful microorganisms, posing a threat to public health (Joosten et al., 2020).

Antibiotic-resistant bacteria are a challenge in the health sector that must be addressed (Dhingra et al., 2020). There is fear that the level of sensitivity of these bacteria to antibiotics will continue to decrease and will not be limited to one type of antibiotic (Reygaert, 2018). Bacteria that can resist three or more antibiotics are said to have multidrug resistance (Yang et al., 2021). *S. aureus* resistance is critical since it makes it harder to treat infections caused by this bacterium because it does not respond well to routinely used antibiotics (Kaur & Chate, 2015).

*S. aureus* is well-known for its characteristics of multidrug resistance and resistance to β-lactam-class antibiotics.
sometimes known as methicillin-resistant Staphylococcus aureus (MRSA) (Green et al., 2012). MRSA and other antibiotic-resistant bacteria have led to nosocomial infections (Nimer, 2022). All β-lactam medications, such as cephalosporins and carbapenems, are ineffective against MRSA strains resistant to oxacillin and cefoxitin (Gajdács, 2019). Animal MRSA isolates were shown to have considerably higher levels of gentamicin, ciprofloxacin, and clindamycin resistance than human MRSA isolates (Fitrandia et al., 2023).

MRSA strains mediate through the mecA gene (Rajabiani et al., 2014). A cellular genetic component called the staphylococcal cassette chromosome mec (SCCmec) contains this gene (Saber et al., 2017). Penicillin-binding protein 2a (PBP 2a), produced by this gene, has a lower affinity for β-lactam antibiotics than ordinary PBP (Fergestad et al., 2020). Thus, methicillin-resistant Staphylococcus aureus containing the mecA gene will resist β-lactam-class antibiotics (Ramandinianto et al., 2020).

Surabaya is the largest city in Indonesia, which has animal hospitals, including the Airlangga University Educational Animal Hospital and the Animal Husbandry Service Animal Hospital of East Java Province. The Surabaya City Veterinary Hospital still conducts very little research on the mecA gene and S. aureus resistance in cats. Given that S. aureus is one of the most common causes of infection in cats, this will hurt the decision of the type of medication for disease prevention (Dalton et al., 2020).

In recent years, severe epidemics have been brought on by antibiotic resistance. As a result, it is essential to conduct resistance tests and culture experiments to track bacterial antibiotic resistance. Given this context, research on S. aureus isolated from cat ears and tests for antibiotic resistance are required.

**Materials and Methods**

**Study area and sample collection**

This research was conducted from March to April 2022. Bacterial isolation and sensitivity tests were conducted at the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Airlangga University. At the same time, cat ear swab samples were taken in Surabaya, Indonesia, namely the Airlangga University Teaching Animal Hospital and the East Java Provincial Animal Husbandry Hospital. As many as 100 cat ear swab samples were collected in this study. Sampling was done by swabbing the cat’s ear cavity using a sterile cotton swab from Amies transport medium. A cotton swab was placed into an Amies transport container, labeled, and then placed inside a cooler that had already been filled with ice packs.

**Isolation and identification of S. aureus**

Making a Manitol Salt Agar (MSA) medium is the first step in bacterial isolation. The sample was extracted from the Amies transport medium tube using a loop that had been pre-burned to render it sterile after being removed from the coolbox. Using a streaking technique, a Petri dish with sterile MSA media is inoculated with bacteria. It took 24 h for the MSA media to incubate at 37 °C. S. aureus is thought to cause bacterial colonies on MSA media that turn yellow. The Gram stain test is the next step, identifying the Gram bacteria species present in the colony. The catalase and coagulase assays are the next step, which identify the S. aureus bacterium.

The first step in the Gram staining procedure was to make the thinnest preparation of bacterial colonies on MSA media that are thought to be S. aureus by combining a small amount of the bacterial culture with one drop of physiological NaCl on an object glass and fixing it on a Bunsen burner. The crystal violet dye was then drip-applied to the fixed preparation and allowed to stand for 2 min. After removing the leftover color, the preparations were washed under running water. Lugol was applied to the preparation and left for 1 min in the following step. After that, the lugol was discarded, and the preparations were rinsed under running water. The mixtures were diluted with 96% alcohol, allowed to sit for 10–20 sec, and then rinsed under running water. After 2 min, the preparation was then dyed with safranin. After removing the color, it was cleaned under running water and allowed to dry. Finally, immersion oil was sprayed into the preparation, and bacterial morphology was viewed under a 1000x magnification microscope.

A single loop of a bacterial culture thought to be S. aureus on MSA media was used for the catalase test. Then, 3% hydrogen peroxide (H₂O₂) was drip-applied to a glass object before one layer of the bacterial culture was spread over it and mixed. The existence of gas bubbles (O₂) produced by the genus Staphylococcus is a sign that the catalase test was successful. In the coagulase test, plasma was extracted from rabbit blood using EDTA anticoagulant, followed by 15 min centrifugation at 10,000 rpm. Using a loop, the bacterium culture on MSA media that was thought to be S. aureus was placed in 1 ml of nutrient broth for further incubation for 24 h at 37 °C. Using a syringe, the next step was to add 1 ml of rabbit plasma to the nutrient broth previously contaminated with bacteria—in incubation for 4–24 h. On the coagulase test, clots in the nutrient broth medium indicated a positive result for S. aureus.
Antibiotic sensitivity test
The Kirby Bauer disc diffusion method was used for an antibiotic sensitivity test. After being isolated and identified, the S. aureus bacteria were suspended by taking the bacterial culture, placing it in a test tube with 9 ml of physiological NaCl, and homogenizing it with a vortex. The comparison of suspension turbidity was to the McFarland criterion of 0.5. Afterward, the suspension was gently inoculated using a cotton swab to wipe the entire surface of the MHA media. After 15 min, the cotton swab was removed to allow the bacterial colonies to adhere to the MHA medium. On the surface of the media, tweezers were used to place paper discs containing the antibiotics cefoxitin (30 µg), tetracycline (30 µg), erythromycin (15 µg), gentamicin (10 µg), and chloramphenicol (30 µg). The bacterial culture was kept at 37 °C for 24 h.

A transparent region surrounding the paper disk, known as the zone of inhibition of bacterial growth, indicates the success of this method’s tests. Vernier calipers measure the diameter in millimeters of the area around the antibiotic disc that inhibits bacterial growth. The inhibitory zone’s diameter will then be classified as sensitive (S), intermediate (I), and resistance (R). Following that, the measurement findings are contrasted with the guidelines provided by the Clinical & Laboratory Standards Institute (2020).

Confirmatory MRSA test
All isolates that were positive for S. aureus underwent an MRSA test. Oxacillin Resistance Screening Agar Base (ORSAB) media combined with Oxacillin Resistance Selective Supplement was used to inoculate bacterial colonies from MHA media to confirm the presence of MRSA. The ORSAB medium was then incubated for 24 h at 37 °C.

mecA detection
The DNA used as a template is 5 µl. The primers were specific for the meca gene (Table 1). The mixture for the PCR reaction with a volume of 25 µl consisting of 12.5 µl PCR Mastermix (0.2 mM dNTP, 0.5 U Taq Polymerase, Buffer, and 1.5 mM MgCl₂), 1.25 µl for each primer and 5 µl DNA template. The PCR was conducted under the following conditions: initial denaturation at 94 °C for 7 min, 35 cycles of 96 °C for 50 sec of denaturation, 50 °C for 40 sec of annealing, 72 °C for 1 min of extension, and finally, 10 min of extension at 72 °C.

The PCR product was visualized in 1.5% agarose. A total of 5 µl of the PCR product was combined with 1 µl of the marker, placed on parafilm, and submerged in an ethidium bromide solution for 10 min in a dark place. We observed the PCR product formed with a UV transilluminator with a wavelength of 360 nm.

Results

Bacterial isolates
Based on morphological culture characteristics, Gram staining, and biochemical testing, the sample investigation revealed that, of the 100 isolated cat nose swab samples, 91 isolates (91%) were determined to be positive for S. aureus (Table 2). Yellow bacterial colonies on MSA media indicated a positive morphological culture of S. aureus (Figure 1). Purple colonies and grouped cocci that indicate a positive Gram stain

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequences (5’ to 3’)</th>
<th>Target gene</th>
<th>Amplicons size</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>meca forward</td>
<td>5’-AAA ATC GAT GGT AAA GGT TGG C-3’</td>
<td>meca</td>
<td>533 bp</td>
<td>Khairullah et al. (2022)</td>
</tr>
<tr>
<td>meca reverse</td>
<td>5’-AGT TCT GCA GTA CCG GAT TGG C-3’</td>
<td>meca</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2 – Isolation of S. aureus, MRSA, and meca gene from cat ear swabs

<table>
<thead>
<tr>
<th>Veterinary hospital</th>
<th>Sample code</th>
<th>Sample size</th>
<th>S. aureus positive</th>
<th>MRSA positive</th>
<th>meca positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Airlangga University Educational Animal Hospital</td>
<td>A</td>
<td>50</td>
<td>46 (92%)</td>
<td>5 (10%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>East Java Provincial Animal Husbandry Hospital</td>
<td>B</td>
<td>50</td>
<td>45 (90%)</td>
<td>7 (14%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>91 (91%)</td>
<td>12 (12%)</td>
<td>1 (1%)</td>
<td></td>
</tr>
</tbody>
</table>

Note: % (Percentage of positive).
result can be seen (Figure 2). The emergence of bubbles on the object glass in the catalase test (Figure 3) and the appearance of clout at the bottom of the microtube in the coagulase test signify a positive biochemical test for *S. aureus* (Figure 4).

**Antibiotic resistance of *S. aureus***

In this investigation, 38.46% (35/91) of *S. aureus* had the highest resistance to the antibiotic erythromycin. The level of *S. aureus* resistance to numerous additional antibiotics, including 13.19% (12/91) of tetracycline, 8.79% (8/91) of chloramphenicol, 4.39% (4/91) of gentamicin, and none of the isolates (0%) were resistant to cefoxitin (Table 3).

Antibiotic resistance profile from the results of the *S. aureus* resistance test to antibiotics showed that 32.97% (30/91) were detected as resistant to 1 class of antibiotics tested. In comparison, 10.99% (10/91) were resistant to two classes of antibiotics, and 3.30% (3/91) confirmed to be multidrug resistance (MDR) because they are resistant to 3 to 4 antibiotic classes (Figure 5) with GM–C–E antibiotic resistance pattern (Gentamicin, chloramphenicol, erythromycin) of 2.20% (2/91) and antibiotic resistance pattern GM–TE–E (Gentamicin, tetracycline, erythromycin) as much as 1.10% (1/91) (Table 3). The investigation found that 35 isolates of *S. aureus* had the highest level of erythromycin resistance.

MDR *S. aureus* isolates were found in cat ear swab samples from the Airlangga University Animal Teaching Hospital and two from the Animal Husbandry Service Animal Hospital (Table 4).

Table 3 – Isolated *S. aureus* resistance profile by antibiotic group

<table>
<thead>
<tr>
<th>Group of antibiotics</th>
<th>Resistance profile</th>
<th>Number of isolates (n=91)</th>
<th>Total number of isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Resistant isolates (%)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>No one is resistant</td>
<td>48 (52.75%)</td>
<td>48 (52.75%)</td>
</tr>
<tr>
<td>1</td>
<td>TE</td>
<td>8 (8.79%)</td>
<td>30 (32.97%)</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>22 (24.17%)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>GM – E</td>
<td>1 (1.10%)</td>
<td>10 (10.99%)</td>
</tr>
<tr>
<td></td>
<td>TE – E</td>
<td>3 (3.30%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C – E</td>
<td>6 (6.59%)</td>
<td></td>
</tr>
<tr>
<td>≥3</td>
<td>GM – C – E</td>
<td>2 (2.20%)</td>
<td>3 (3.30%)</td>
</tr>
<tr>
<td></td>
<td>GM – TE – E</td>
<td>1 (1.10%)</td>
<td></td>
</tr>
</tbody>
</table>

Note: C = Chloramphenicol; E = Erythromycin; GM = Gentamicin; TE = Tetracycline.

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

Figure 3 – Catalase test results indicate *S. aureus* positivity.

Figure 4 – Coagulation test results indicate *S. aureus* positivity.

Figure 5 – Analyze the susceptibility to antibiotics of an *S. aureus* isolate cultured on MHA.
The ORSAB test revealed 12 out of 91 isolates of *S. aureus* (Table 2). This shows that the MRSA infection level in the Surabaya Veterinary Hospital is still low, namely, 12 MRSA isolates from 100 samples analyzed.

**mecA detection**

The presence of the *mecA* gene was subsequently determined by genotyping the 12 isolates that the ORSAB test had identified as MRSA. One isolate was found to carry the *mecA* gene based on the electrophoresis results of the 12 isolates investigated; three of these isolates got their *mecA* gene from a cat ear swab (Table 2). The emergence of a single band on the electrophoresis findings indicates a positive *mecA* gene (Figure 6).

**Discussion**

**Bacterial isolates**

After performing a lengthy sequence of isolation and identification, 91 out of 100 samples obtained from two veterinary facilities in Surabaya were positive for *S. aureus*. The finding of *S. aureus* in cat ears is connected to *S. aureus*, a typical animal flora typically present in animals (Bruce et al., 2022). The nose, ears, throat, and skin of humans and numerous animal species have the common commensal bacterium *S. aureus* (Kumpitsch et al., 2019).

This study discovered samples that were thought to be *S. aureus* bacteria, which were identifiable by a switch in the color of the MSA media from red to golden yellow. *S. aureus* colonies in MSA media culture exhibit the phenotypic trait of altering the color of the medium from red to yellow, a sign that the colony is fermenting mannitol (Thakur et al., 2017). Staphyloxanthin pigments in *S. aureus* colonies on MSA media express colors like orange and golden yellow (Gu et al., 2022). As a result of the high salt concentration in MSA medium and bacterial mannitol fermentation, growth and color changes of colonies on MSA media are thought to be predictive criteria in the detection of *S. aureus* using MSA media (Yambise et al., 2020).

Gram-positive *S. aureus* is a purple coccus-shaped bacteria with a coccus form (Khairullah et al., 2019). According to Thairu et al. (2014), Gram-specific differences are determined by elements in the cell wall. For example, Gram-positive bacteria have a thicker peptidoglycan layer than Gram-negative bacteria, which makes the Gram staining process seem purple. Peptidoglycan, which is made from a three-dimensional bond between the amino sugar N-acetylglucoaminase and N-acetyl muramic acid, will keep the crystal violet's purple color because it has a more robust cell wall (DeMeester et al., 2018). The peptide cross-links between the peptidoglycan chains give Gram-positive bacteria cell walls their strength (Kim et al., 2015).

The presence of gas bubbles (O₂) produced by the genus *Staphylococcus* sp indicates a positive catalase test. These gas bubbles demonstrate the presence of the catalase enzyme, which can convert hydrogen peroxide into gaseous water and oxygen in *Staphylococcus* sp bacteria (Effendi et al., 2019).

The coagulase test revealed clots in samples verified to be positive for the bacterium *S. aureus* in this investigation. *S. aureus* produces a protein called coagulase that clots plasma with substances found in serum (McAdow et al., 2012). A positive coagulase reaction is necessary to identify *S. aureus* from other *Staphylococcus* species. Coagulase-negative *Staphylococci* (CoNS), as shown by the coagulase test, were absent from 9 out of 100 samples.

**Antibiotic resistance of *S. aureus***

The Kirby-Bauer method was used to conduct sensitivity tests on isolates with verified *S. aureus* to ascertain their antibiotic resistance profile to chloramphenicol, tetracycline, gentamicin, erythromycin, and cefoxitin. This test uses an
antibiotic disk mounted to a disk, and bacteria are cultivated on MHA media all around the antibiotic disk. The width of the clear zone on the media is then measured to determine the results. An indirect indicator of an antibiotic’s capacity to prevent harmful germs from growing around the disc is its presence or absence (Beceiro et al., 2013). Based on the sensitive, moderate, and resistant categories, this measurement yields qualitative categories evaluated in CLSI 2020.

The *S. aureus* isolate sensitivity test revealed resistance to nearly all tested antibiotics, varying degrees of resistance for each type except cefoxitin. The results showed that *S. aureus* isolates from cat ear swab samples at the Surabaya City Veterinary Hospital had resistance to antibiotics of 47.25% (43/91), precisely 23 isolates from the East Java Animal Husbandry Service Animal Hospital and 20 isolates from the Airlangga University Teaching Animal Hospital. *S. aureus* isolates in this study were resistant to tetracycline 13.19% (12/91), erythromycin 38.46% (35/91), gentamicin 4.39% (4/91), and chloramphenicol 8.79% (8/91). *S. aureus* isolates in cat ear swabs at the Airlangga University Teaching Animal Hospital and East Java Animal Husbandry Service Veterinary Hospital experienced resistance to the antibiotics tetracycline, erythromycin, gentamicin, and chloramphenicol. These findings support the claims made by Maslikov et al. (2019) that *S. aureus* isolated from cats at the Teaching Animal Hospital in Ukraine has antibiotic resistance.

*S. aureus* resistance mechanisms can be brought on by several mechanisms, including the enzymatic inactivation of antibiotics, alteration of antibiotic targets, augmentation of efflux pumps, and restriction of drug absorption (Zhang & Cheng, 2022). The type of antibiotic being used has an impact on the resistance mechanism since bacteria can use one or many defense mechanisms (Uddin et al., 2021). The highest to lowest resistance patterns of *S. aureus* occurred in the antibiotics erythromycin 38.46% (35/91), tetracycline 13.19% (12/91), chloramphenicol 8.79% (8/91), and gentamicin 4.39% (4/91). In addition, no *S. aureus* isolates were found against 0% cefoxitin (0/91) in this study. Antibiotics in earlier treatments strongly correlate with the prevalence of *S. aureus* resistance in animals, particularly cats (Elnageh et al., 2021). Cats might additionally develop antibiotic resistance from their environment (Gargano et al., 2022).

Regular antibiotic use can stress bacteria, triggering resistance in the bacterial DNA and leading to mutations and genetic alterations in bacterial cells (Corona & Martinez, 2013). The inappropriate use of antibiotics causes bacterial resistance in pets (Dache et al., 2021). The efficacy of treatment and the prevention of antibiotic resistance depends significantly on prudent antibiotic use (Wallinga et al., 2022). Additionally, excessive antibiotic usage in a population might develop acquired bacterial resistance to an antibiotic (Wales & Davies, 2015). The overuse of new antibiotics and prolonged antibiotic use are additional variables that encourage the development of bacterial resistance in animal hospitals (prolonged administration of antibiotics provides an opportunity for the growth of more resistant bacteria) (Van et al., 2020).

The two most frequently used antibiotics to treat cats and dogs are tetracycline and chloramphenicol (Stefańska et al., 2022). Resistance develops as a result of this condition. Penicillin, tetracycline, and erythromycin resistance were prevalent in isolates obtained from cats and dogs (Garcés et al., 2022). The findings of this study are likewise consistent with those of Awosile et al. (2018), who reported that 13 isolates of *S. aureus* isolated from cats exhibited excellent resistance to antibiotics, including 100% chloramphenicol, 82% erythromycin, and 94% tetracycline. *S. aureus* isolates from pet ears, including cats, were shown to be resistant to the medicines erythromycin, chloramphenicol, and tetracycline, according to Bierowiec et al. (2016).

The findings of this investigation demonstrated that *S. aureus* had a lower level of resistance to gentamicin antibiotics than the others, at 4.39% (4/91), and that no *S. aureus* isolates had been discovered to be resistant to cefoxitin antibiotics. The findings of this study are consistent with those of Feßler et al. (2022), who found that the *S. aureus* isolates from inpatient cats at the veterinary clinic in Germany have a low degree of gentamicin resistance. The use of gentamicin must still be watched even though the pattern of gentamicin resistance in this study was only 4.39% because of the findings of this investigation, which revealed that four isolates of *S. aureus* were already gentamicin-resistant. The antibiotic gentamicin belongs to the aminoglycoside class and is frequently used with other antibiotics in clinical settings to treat Gram-positive and Gram-negative bacteria. The findings of this study are also in line with those of Jung et al. (2020), which found that resistance to cefoxitin in cats was less common than resistance to methicillin and oxacillin. Gram-negative bacteria, including *E. coli*, Klebsiella, Proteus, and *Staphylococcus* sp, are susceptible to the second-generation cephalosporin drug cefoxitin (Shaikh et al., 2015).

This study also found *S. aureus* bacteria with an MDR of 3.30% (3/91), namely isolate A03 from the Teaching Animal Hospital of Airlangga University and isolates B03 and B41 from the East Java Provincial Animal Husbandry Hospital. These findings align with those of Waruwu et al. (2023), who discovered higher MDR *S. aureus* infections in cats at a...
rate of 19.51% (16/82). Afmami et al. (2022) found a 38.89% (7/18) MDR of *S. aureus* in cats. This could account for the three isolates from 100 cat nose swab samples representing Surabaya's remaining low-MDR *S. aureus* infections.

A multidrug resistance (MDR) bacterium resists three or more drugs (Widodo et al., 2023). MDR events can arise for various reasons, including the incorrect use of antibiotics, incorrect diagnosis, and incorrect bacteria that cause them (Widodo et al., 2022). Multidrug resistance incidents become challenging to treat and cause resistance to spread (Nwobodo et al., 2022). Plasmid mediation methods can cross multidrug-resistant bacteria (Elshamy et al., 2020). Chromosomes encode most information in bacteria. However, some bacteria also carry extrachromosomal genes in plasmids or bacteriophages (Deutsch et al., 2018). Plasmids typically carry resistance genes, a common source of resistance in clinical isolates due to their plasmid-based resistance mechanism (Darphorn et al., 2021). Because plasmid-borne genes are more mobile than those on chromosomes, they can spread to other cells, causing them to acquire resistance genes (Lehtinen et al., 2021).

The findings of the resistance test on 91 isolates of *S. aureus* from cat ear swabs from the Surabaya City Veterinary Hospital were the main topic of discussion in this study. Based on the results of the resistance test in this investigation, no isolates were discovered to be resistant to the β-lactam antibiotic cefoxitin, demonstrating that it is still helpful in treating *S. aureus* infections. Samples still sensitive to β-lactams demonstrate that β-lactamase can still hydrolyze the β-lactam ring, which can result in sensitivity (Bonomo, 2017). There were no methicillin-resistant *Staphylococcus aureus* (MRSA) isolates, as evidenced by the lack of cefoxitin antibiotic resistance. Cefoxitin was used in this investigation as a test antibiotic to determine *S. aureus* sensitivity to β-lactam-class antibiotics (Akanbi et al., 2017). *S. aureus* infections can still be treated with other antibiotics, including gentamicin, tetracycline, and chloramphenicol. A high level of erythromycin resistance was discovered in 35 out of 91 *S. aureus* isolates. Hence, extra care must be taken while using erythromycin as a therapy for *S. aureus* infection.

**Confirmatory MRSA**

This study confirmed 12 isolates from 91 isolates tested by ORSAB as MRSA isolates. *Staphylococcal* cassette chromosome mec (SCCmec), a significant DNA insertion between 20 and 100 kb, led to the evolution of *S. aureus* into a methicillin-resistant strain of the bacteria (Rolo et al., 2017). SCCmec integrates into the *S. aureus* chromosome in a region near the origin of chromosome replication (Noto et al., 2008). Due to alterations made to the natural penicillin-binding protein (PBP), specifically PBP 2 to PBP 2a, MRSA isolates are now invulnerable to all medications in the β-lactam class (Otto, 2013). Because PBP 2a has a relatively low affinity for β-lactams, the MRSA strain may persist and build the bacterial cell wall even when this bacterium is grown in conditions with high concentrations of β-lactams (Fergestad et al., 2020).

**mecA detection**

Out of the 12 MRSA isolates analyzed, the *mecA* gene was detected in one isolate. The pathogenic bacteria *S. aureus* with MRSA features are encoded by numerous resistance genes, including the *mecA* gene (Hiramatsu et al., 2013). Antibiotic resistance is caused by bacteria with the *mecA* gene found in MRSA (Wielders et al., 2002). Due to the presence of the PBP 2a protein, this antibiotic resistance affects the β-lactam antibiotic class (Kakoullis et al., 2021).

The *mecA* gene, which encodes a specific transpeptidase that renders bacteria resistant to methicillin and β-lactam medications, is located on the SCCmec chromosome of MRSA (Fisher & Mobashery, 2016). This gene produces penicillin-binding protein 2a (PBP 2a) (Hiramatsu et al., 2013). The β-lactam class of antibiotics has a modest affinity for this protein (Ramandinianto et al., 2020). The bacteria that produce this protein will resist all β-lactam antibiotic types (Fisher & Mobashery, 2016). As a result, the *mecA* gene, which causes a resistance reaction and mainly inhibits the β-lactamase antibiotic group, is thought to be responsible for the MRSA bacteria's resistance (Miragaia, 2018).

The *mecA* gene in MRSA isolates spread directly by touch, aerosols, and inanimate objects (Ramandinianto et al., 2020). The transfer of bacterial strains between pets and owners has been linked to transmission of the same MRSA strains in cats and people residing in the same household, according to molecular identification of the *mecA* gene (Afnani et al., 2022). Humans and pets act as household reservoirs of the *mecA* gene for MRSA because they are more likely to be colonized than infected (Khairullah et al., 2022).

Due to the lack of effective treatments for the illness, *mecA* gene transmission from animals to humans and humans to animals must be managed (Laurent et al., 2012). To reduce MRSA cross-contamination, veterinarians, hospitals, and animal clinics must strictly adhere to established health protocols (Elnageh et al., 2021). Prevention of transmission can be done by maintaining excellent hygiene, which includes washing hands and cleaning the environment (Avberšek et al., 2021). Barrier precautions should be used when caring for animals with MRSA infections carrying...
the mecA gene, including wearing gloves and masks and isolating infected animals (Algammal et al., 2020). Early molecular detection is essential to identify animals infected with MRSA that carry the mecA gene (Dey et al., 2023).

Based on the findings of this study, a thorough assessment of the impact of using antibiotics to prevent the spread of MRSA in veterinary hospitals on the general public’s health is required in light of the detection of MRSA isolates bearing the mecA gene in the Surabaya veterinary hospital.

Conclusion

This study collected 100 ear swab samples from cats at the Airlangga University Teaching Animal Hospital and the East Java Provincial Animal Husbandry Hospital. Of these samples, 91 were confirmed positive for S. aureus, three isolates were identified as multidrug-resistant (MDR), and one carried the mecA gene. Although the finding of multi-drug resistant isolates and the mecA gene in this study is limited, caution regarding the use of antibiotics in pets, especially cats, must increase.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Ethics Statement

Ear swabs were used in this study. Hence, ethical approval was unnecessary. Ear swab samples were collected from the veterinary hospital in Surabaya, Indonesia, per standard collection procedure.

References


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