FULL ARTICLE

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

Detecção do gene mecA e resistência a diversos antibióticos em Staphylococcus aureus isolados do ouvido de gatos em um hospital localizado em 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 *mec*A 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 *mec*A 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 *mec*A. 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, tetraxiclina, 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 *mec*A. 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 *mec*A 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.

Palavras-chave: *Staphylococcus aureus*. Resistência múltipla a drogas. Gatos. Saúde pública.

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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 (Medveďová 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 (Fitranda et al., 2023).

MRSA strains mediate through the *mec*A gene (Rajabiani et al., 2014). A cellular genetic component called the staphylococcal cassette chromosome *mec* (SCC*mec*) 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 *mec*A 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 *mec*A 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 (H_2O_2) 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 (O_2) 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—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 *mec*A 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 $MgCl_2$), 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

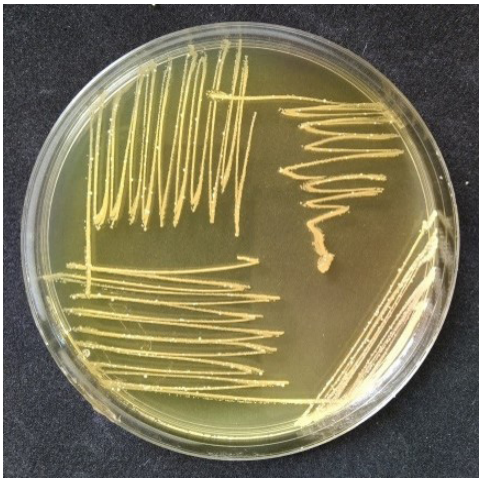

Figure 1 – *S. aureus* colonies in MAS.

Table 1 – Details of primers used in this study

Primers	Sequences (5′ to 3′)	Target gene	Amplicons size	References
*mec*A *forward*	5′-AAA ATC GAT GGT AAA GGT TGG C-3′	*mec*A	533 bp	Khairullah et al. (2022)
*mec*A *reverse*	5′-AGT TCT GCA GTA CCG GAT TTG C-3′	*mec*A		

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

Veterinary hospital	Sample code	Sample size	*S. aureus* positive	MRSA positive	*mec*A positive
Airlangga University Educational Animal Hospital	A	50	46 (92%)	5 (10%)	0 (0%)
East Java Provincial Animal Husbandry Hospital	B	50	45 (90%)	7 (14%)	1 (2%)
Total		**100**	**91 (91%)**	**12 (12%)**	**1 (1%)**

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).

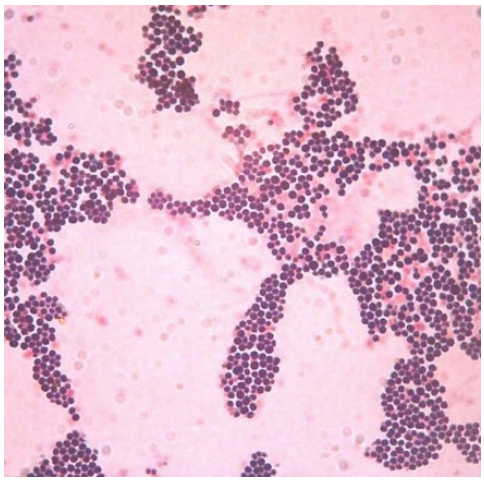

Figure 2 – Gram-stained *S. aureus* colonies under a microscope.

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

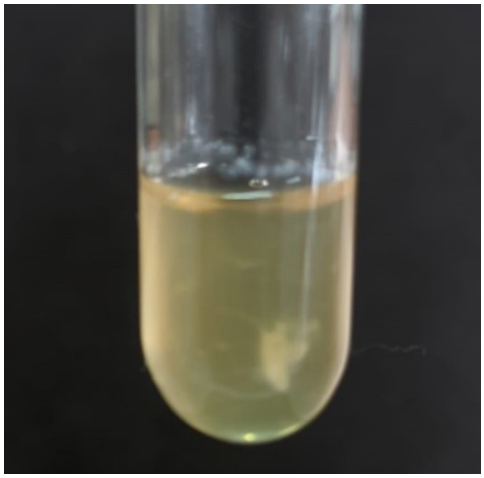

Figure 4 – Coagulation test results indicate *S. aureus* positivity.

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

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

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

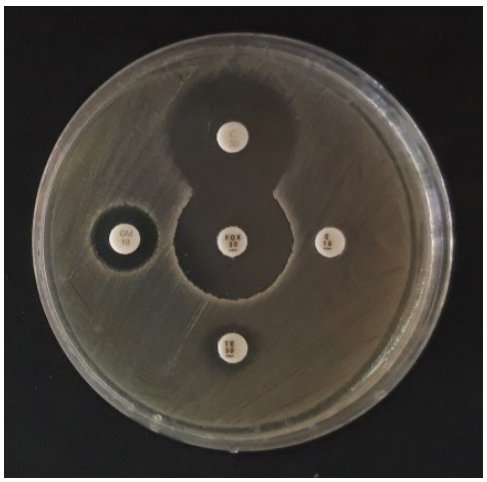

Figure 5 – Analyze the susceptibility to antibiotics of an *S. aureus* isolate cultured on MHA.

Table 4 – *S. aureus* isolates with a profile MDR

Veterinary hospital	Sample code	Resistance profile	Antibiotic				
			GM	C	TE	E	FOX
Airlangga University Educational Animal Hospital	A 03	GM – C – E	✓	✓	–	✓	–
East Java Provincial Animal Husbandry Hospital	B 03	GM – C – E	✓	✓	–	✓	–
	B 41	GM – TE – E	✓	–	✓	✓	–

Note: ✓= Resistant; C = Chloramphenicol; E = Erythromycin; FOX = Cefoxitin; GM = Gentamicin; TE = Tetracycline.

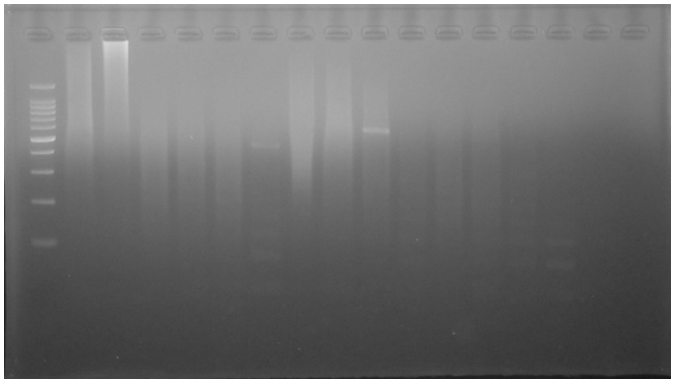

Figure 6 – *mec*A gene detection results.

Confirmatory MRSA

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 *mec*A gene was subsequently determined by genotyping the 12 isolates that the ORSAB test had identified as MRSA. One isolate was found to carry the *mec*A gene based on the electrophoresis results of the 12 isolates investigated; three of these isolates got their *mec*A gene from a cat ear swab (Table 2). The emergence of a single band on the electrophoresis findings indicates a positive *mec*A 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 (O_2) 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 (Garcês 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). Afnani 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* (SCC*mec*), 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 *mec*A gene was detected in one isolate. The pathogenic bacteria *S. aureus* with MRSA features are encoded by numerous resistance genes, including the *mec*A gene (Hiramatsu et al., 2013). Antibiotic resistance is caused by bacteria with the *mec*A 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 *mec*A gene, which encodes a specific transpeptidase that renders bacteria resistant to methicillin and β-lactam medications, is located on the SCC*mec* chromosome of MRSA (Fishovitz et al., 2014). The 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 *mec*A 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 *mec*A 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 *mec*A gene (Afnani et al., 2022). Humans and pets act as household reservoirs of the *mec*A 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, *mec*A 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 *mec*A 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 *mec*A 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 *mec*A 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 *mec*A gene. Although the finding of multi-drug resistant isolates and the *mec*A 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

Afnani DA, Fatih N, Effendi MH, Tyasningsih W, Khairullah AR, Kurniawan SC, Silaen OSM, Ramandianto SC, Widodo A, Hendriana K, Riwu KHP. Profile of multidrug resistance and methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from cats in Surabaya, Indonesia. Biodiversitas. 2022;23(11):5703-9. http://dx.doi.org/10.13057/biodiv/d231121.

Akanbi OE, Njom HA, Fri J, Otigbu AC, Clarke AM. Antimicrobial susceptibility of *Staphylococcus aureus* isolated from recreational waters and beach sand in Eastern Cape Province of South Africa. Int J Environ Res Public Health. 2017;14(9):1001. http://dx.doi.org/10.3390/ijerph14091001. PMid:28862669.

Algammal AM, Hetta HF, Elkelish A, Alkhalifah DHH, Hozzein WN, Batiha GE, El Nahhas N, Mabrok MA. Methicillin-resistant *Staphylococcus aureus* (MRSA): one health perspective approach to the bacterium epidemiology, virulence factors, antibiotic-resistance, and zoonotic impact. Infect Drug Resist. 2020;13:3255-65. http://dx.doi.org/10.2147/IDR.S272733. PMid:33061472.

Avberšek J, Papić B, Kušar D, Erjavec V, Seme K, Golob M, Zdovc I. Feline otitis externa caused by methicillin-resistant *Staphylococcus aureus* with mixed hemolytic phenotype and overview of possible genetic backgrounds. Antibiotics. 2021;10(5):599. http://dx.doi.org/10.3390/antibiotics10050599. PMid:34070191.

Awosile BB, McClure JT, Saab ME, Heider LC. Antimicrobial resistance in bacteria isolated from cats and dogs from the Atlantic Provinces, Canada from 1994-2013. Can Vet J. 2018;59(8):885-93. PMid:30104781.

Beceiro A, Tomás M, Bou G. Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world? Clin Microbiol Rev. 2013;26(2):185-230. http://dx.doi.org/10.1128/CMR.00059-12. PMid:23554414.

Bierowiec K, Miszczak M, Korzeniowska-Kowal A, Wzorek A, Płókarz D, Gamian A. Epidemiology of *Staphylococcus pseudintermedius* in cats in Poland. Sci Rep. 2021;11(1):18898. http://dx.doi.org/10.1038/s41598-021-97976-z. PMid:34556720.

Bierowiec K, Płoneczka-Janeczko K, Rypuła K. Is the colonisation of *Staphylococcus aureus* in pets associated with their close contact with owners? PLoS One. 2016;11(5):e0156052. http://dx.doi.org/10.1371/journal.pone.0156052. PMid:27227897.

Bonomo RA. β-lactamases: a focus on current challenges. Cold Spring Harb Perspect Med. 2017;7(1):a025239. http://dx.doi.org/10.1101/cshperspect.a025239. PMid:27742735.

Bruce SA, Smith JT, Mydosh JL, Ball J, Needle DB, Gibson R, Andam CP. Shared antibiotic resistance and virulence genes in *Staphylococcus aureus* from diverse animal hosts. Sci Rep. 2022;12(1):4413. http://dx.doi.org/10.1038/s41598-022-08230-z. PMid:35292708.

Clinical & Laboratory Standards Institute – CLSI. Performance standards for antimicrobial susceptibility testing in CLSI supplement M100. Wayne: CLSI; 2020.

Corona F, Martinez JL. Phenotypic resistance to antibiotics. Antibiotics. 2013;2(2):237-55. http://dx.doi.org/10.3390/antibiotics2020237. PMid:27029301.

Dache A, Dona A, Ejeso A. Inappropriate use of antibiotics, its reasons and contributing factors among communities of Yirgalem town, Sidama regional state, Ethiopia: a cross-sectional study. SAGE Open Med. 2021;9:20503121211042461. http://dx.doi.org/10.1177/20503121211042461. PMid:34504704.

Dafale NA, Srivastava S, Purohit HJ. Zoonosis: an emerging link to antibiotic resistance under "one health approach". Indian J Microbiol. 2020;60(2):139-52. http://dx.doi.org/10.1007/s12088-020-00860-z. PMid:32255846.

Dalton KR, Rock C, Carroll KC, Davis MF. One health in hospitals: how understanding the dynamics of people, animals, and the hospital built-environment can be used to better inform interventions for antimicrobial-resistant gram-positive infections. Antimicrob Resist Infect Control. 2020;9(1):78. http://dx.doi.org/10.1186/s13756-020-00737-2. PMid:32487220.

Damborg P, Broens EM, Chomel BB, Guenther S, Pasmans F, Wagenaar JA, Weese JS, Wieler LH, Windahl U, Vanrompay D, Guardabassi L. Bacterial zoonoses transmitted by household pets: state-of-the-art and future perspectives for targeted research and policy actions. J Comp Pathol. 2016;155(1, Suppl 1):S27-40. http://dx.doi.org/10.1016/j.jcpa.2015.03.004. PMid:25958184.

Darphorn TS, Bel K, Koenders-van Sint Anneland BB, Brul S, Ter Kuile BH. Antibiotic resistance plasmid composition and architecture in *Escherichia coli* isolates from meat. Sci Rep. 2021;11(1):2136. http://dx.doi.org/10.1038/s41598-021-81683-w. PMid:33483623.

Decline V, Effendi MH, Rahmaniar RP, Yanestria SM, Harijani N. 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):90-4. http://dx.doi.org/10.14202/IJOH.2020.90-94.

DeMeester KE, Liang H, Jensen MR, Jones ZS, D'Ambrosio EA, Scinto SL, Zhou J, Grimes CL. Synthesis of functionalized N-acetyl muramic acids to probe bacterial cell wall recycling and biosynthesis. J Am Chem Soc. 2018;140(30):9458-65. http://dx.doi.org/10.1021/jacs.8b03304. PMid:29986130.

Deutsch DR, Utter B, Verratti KJ, Sichtig H, Tallon LJ, Fischetti VA. Extra-chromosomal DNA sequencing reveals episomal prophages capable of impacting virulence factor expression in *Staphylococcus aureus*. Front Microbiol. 2018;9:1406. http://dx.doi.org/10.3389/fmicb.2018.01406. PMid:30013526.

Dey TK, Shome BR, Bandyopadhyay S, Goyal NK, Lundkvist Å, Deka RP, Shome R, Venugopal N, Grace D, Sharma G, Rahman H, Lindahl JF. Molecular characterization of methicillin-resistant Staphylococci from the dairy value chain in two Indian states. Pathogens. 2023;12(2):344. http://dx.doi.org/10.3390/pathogens12020344. PMid:36839616.

Dhingra S, Rahman NAA, Peile E, Rahman M, Sartelli M, Hassali MA, Islam T, Islam S, Haque M. Microbial resistance movements: an overview of global public health threats posed by antimicrobial resistance, and how best to counter. Front Public Health. 2020;8:535668. http://dx.doi.org/10.3389/fpubh.2020.535668. PMid:33251170.

Effendi MH, Hisyam MAM, Hastutiek P, Tyasningsih W. 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. http://dx.doi.org/10.14202/vetworld.2019.68-71. PMid:30936656.

Elnageh HR, Hiblu MA, Abbassi MS, Abouzeed YM, Ahmed MO. Prevalence and antimicrobial resistance of Staphylococcus species isolated from cats and dogs. Open Vet J. 2021;10(4):452-6. http://dx.doi.org/10.4314/ovj.v10i4.13. PMid:33614441.

Elshamy AA, Aboshanab KM, Yassien MA, Hassouna NA. Prevalence of plasmid-mediated resistance genes among multidrug-resistant uropathogens in Egypt. Afr Health Sci. 2020;20(1):190-8. http://dx.doi.org/10.4314/ahs.v20i1.24. PMid:33402907.

Fergestad ME, Stamsås GA, Morales Angeles D, Salehian Z, Wasteson Y, Kjos M. Penicillin-binding protein PBP2a provides variable levels of protection toward different β-lactams in *Staphylococcus aureus* RN4220. MicrobiologyOpen. 2020;9(8):e1057. http://dx.doi.org/10.1002/mbo3.1057. PMid:32419377.

Feßler AT, Scholtzek AD, Schug AR, Kohn B, Weingart C, Schink A-K, Bethe A, Lübke-Becker A, Schwarz S. Antimicrobial and biocide resistance among feline and canine *Staphylococcus aureus* and *Staphylococcus pseudintermedius* isolates from diagnostic submissions. Antibiotics. 2022;11(2):127. http://dx.doi.org/10.3390/antibiotics11020127. PMid:35203730.

Fisher JF, Mobashery S. β-lactam resistance mechanisms: gram-positive bacteria and *Mycobacterium tuberculosis*. Cold Spring Harb Perspect Med. 2016;6(5):a025221. http://dx.doi.org/10.1101/cshperspect.a025221. PMid:27091943.

Fishovitz J, Hermoso JA, Chang M, Mobashery S. Penicillin-binding protein 2a of methicillin-resistant *Staphylococcus aureus*. IUBMB Life. 2014;66(8):572-7. http://dx.doi.org/10.1002/iub.1289. PMid:25044998.

Fitranda M, Salasia SIO, Sianipar O, Dewananda DA, Arjana AZ, Aziz F, Wasissa M, Lestari FB, Santosa CM. Methicillin-resistant *Staphylococcus aureus* isolates derived from humans and animals in Yogyakarta, Indonesia. Vet World. 2023;16(1):239-45. http://dx.doi.org/10.14202/vetworld.2023.239-245. PMid:36855365.

Foreman-Worsley R, Finka LR, Ward SJ, Farnworth MJ. Indoors or outdoors? An international exploration of owner demographics and decision making associated with lifestyle of pet cats. Animals. 2021;11(2):253. http://dx.doi.org/10.3390/ani11020253. PMid:33498511.

Friedman ND, Temkin E, Carmeli Y. The negative impact of antibiotic resistance. Clin Microbiol Infect. 2016;22(5):416-22. http://dx.doi.org/10.1016/j.cmi.2015.12.002. PMid:26706614.

Gajdács M. The continuing threat of methicillin-resistant *Staphylococcus aureus*. Antibiotics. 2019;8(2):52. http://dx.doi.org/10.3390/antibiotics8020052. PMid:31052511.

Garcês A, Lopes R, Silva A, Sampaio F, Duque D, Brilhante-Simões P. Bacterial isolates from urinary tract infection in dogs and cats in portugal, and their antibiotic susceptibility pattern: a retrospective study of 5 years (2017-2021). Antibiotics. 2022;11(11):1520. http://dx.doi.org/10.3390/antibiotics11111520. PMid:36358175.

Gargano V, Gambino D, Orefice T, Cirincione R, Castelli G, Bruno F, Interrante P, Pizzo M, Spada E, Proverbio D, Vicari D, Salgado-Caxito M, Benavides JA, Cassata G. Can stray cats be reservoirs of antimicrobial resistance? Vet Sci. 2022;9(11):631. http://dx.doi.org/10.3390/vetsci9110631. PMid:36423080.

Green BN, Johnson CD, Egan JT, Rosenthal M, Griffith EA, Evans MW. Methicillin-resistant *Staphylococcus aureus*: an overview for manual therapists. J Chiropr Med. 2012;11(1):64-76. http://dx.doi.org/10.1016/j.jcm.2011.12.001. PMid:22942844.

Gu K, Ouyang P, Hong Y, Dai Y, Tang T, He C, Shu G, Liang X, Tang H, Zhu L, Xu Z, Yin L. Geraniol inhibits biofilm formation of methicillin-resistant *Staphylococcus aureus* and increase the therapeutic effect of vancomycin in vivo. Front Microbiol. 2022;13:960728. http://dx.doi.org/10.3389/fmicb.2022.960728. PMid:36147840.

Haag AF, Fitzgerald JR, Penadés JR. *Staphylococcus aureus* in animals. Microbiol Spectr. 2019;7(3):GPP3-0060-2019. http://dx.doi.org/10.1128/microbiolspec.GPP3-0060-2019.

Hiramatsu K, Ito T, Tsubakishita S, Sasaki T, Takeuchi F, Morimoto Y, Katayama Y, Matsuo M, Kuwahara-Arai K, Hishinuma T, Baba T. Genomic basis for methicillin resistance in *Staphylococcus aureus*. Infect Chemother. 2013;45(2):117-36. http://dx.doi.org/10.3947/ic.2013.45.2.117. PMid:24265961.

Joosten P, Van Cleven A, Sarrazin S, Paepe D, De Sutter A, Dewulf J. Dogs and their owners have frequent and intensive contact. Int J Environ Res Public Health. 2020;17(12):4300. http://dx.doi.org/10.3390/ijerph17124300. PMid:32560155.

Jung WK, Shin S, Park YK, Lim SK, Moon DC, Park KT, Park YH. Distribution and antimicrobial resistance profiles of bacterial species in stray cats, hospital-admitted cats, and veterinary staff in South Korea. BMC Vet Res. 2020;16(1):109. http://dx.doi.org/10.1186/s12917-020-02326-2. PMid:32272916.

Kakoullis L, Papachristodoulou E, Chra P, Panos G. Mechanisms of antibiotic resistance in important gram-positive and gram-negative pathogens and novel antibiotic solutions. Antibiotics. 2021;10(4):415. http://dx.doi.org/10.3390/antibiotics10040415. PMid:33920199.

Kaur DC, Chate SS. Study of antibiotic resistance pattern in methicillin resistant *Staphylococcus aureus* with Special reference to newer antibiotic. J Glob Infect Dis. 2015;7(2):78-84. http://dx.doi.org/10.4103/0974-777X.157245. PMid:26069428.

Khairullah AR, Raharjo D, Rahmahani J, Suwarno, Tyasningsih W, Harijani N. Suwarno, Tyasningsih W, Harijani N. Antibiotics resistant at *Staphylococcus aureus* and *Streptococcus* sp isolated from bovine mastitis in Karangploso, East Java, Indonesia. Indian J Forensic Med Toxicol. 2019;13(4):439-44. http://dx.doi.org/10.5958/0973-9130.2019.00329.3.

Khairullah AR, Rehman S, Sudjarwo SA, Effendi MH, Ramandininto SC, Gelolodo MA, Widodo A, Riwu KHP, Kurniawati DA. Detection of *mec*A gene andmethicillin-resistant *Staphylococcus aureus* (MRSA) isolated from milk

and risk factors from farms in Probolinggo, Indonesia. F1000 Res. 2022;11:722. http://dx.doi.org/10.12688/f1000research.122225.1.

Kim SJ, Chang J, Singh M. Peptidoglycan architecture of Gram-positive bacteria by solid-state NMR. Biochim Biophys Acta. 2015;1848(1, Suppl 1 Pt B):350-62. http://dx.doi.org/10.1016/j.bbamem.2014.05.031. PMid:24915020.

Kumpitsch C, Koskinen K, Schöpf V, Moissl-Eichinger C. The microbiome of the upper respiratory tract in health and disease. BMC Biol. 2019;17(1):87. http://dx.doi.org/10.1186/s12915-019-0703-z. PMid:31699101.

Laurent F, Chardon H, Haenni M, Bes M, Reverdy ME, Madec JY, Lagier E, Vandenesch F, Tristan A. MRSA harboring *mec*A variant gene *mec*C, France. Emerg Infect Dis. 2012;18(9):1465-7. http://dx.doi.org/10.3201/eid1809.111920. PMid:22932400.

Lehtinen S, Huisman JS, Bonhoeffer S. Evolutionary mechanisms that determine which bacterial genes are carried on plasmids. Evol Lett. 2021;5(3):290-301. http://dx.doi.org/10.1002/evl3.226. PMid:34136276.

Mala L, Lalouckova K, Skrivanova E. Bacterial skin infections in livestock and plant-based alternatives to their antibiotic treatment. Animals. 2021;11(8):2473. http://dx.doi.org/10.3390/ani11082473. PMid:34438930.

Maslikov SN, Bely DD, Samoiliuk VV, Vakulik VV, Spitsyna TL. Pathogenetic role of *Staphylococcus aureus* in purulent keratoconjunctivitis in cats. Regul Mech Biosyst. 2019;10(4):507-12. http://dx.doi.org/10.15421/021974.

McAdow M, Missiakas DM, Schneewind O. *Staphylococcus aureus* secretes coagulase and von Willebrand factor binding protein to modify the coagulation cascade and establish host infections. J Innate Immun. 2012;4(2):141-8. http://dx.doi.org/10.1159/000333447. PMid:22222316.

Medveďová A, Havlíková A, Lehotová V, Valík Ľ. *Staphylococcus aureus* 2064 growth as affected by temperature and reduced water activity. Ital J Food Saf. 2019;8(4):8287. http://dx.doi.org/10.4081/ijfs.2019.8287. PMid:31897398.

Melander RJ, Melander C. The challenge of overcoming antibiotic resistance: an adjuvant approach? ACS Infect Dis. 2017;3(8):559-63. http://dx.doi.org/10.1021/acsinfecdis.7b00071. PMid:28548487.

Miragaia M. Factors contributing to the evolution of *mec*A-Mediated β-lactam resistance in Staphylococci: update and new insights from Whole Genome Sequencing (WGS). Front Microbiol. 2018;9:2723. http://dx.doi.org/10.3389/fmicb.2018.02723. PMid:30483235.

Nimer NA. Nosocomial infection and antibiotic-resistant threat in the Middle East. Infect Drug Resist. 2022;15:631-9. http://dx.doi.org/10.2147/IDR.S351755. PMid:35241915.

Noto MJ, Kreiswirth BN, Monk AB, Archer GL. Gene acquisition at the insertion site for SCCmec, the genomic island conferring methicillin resistance in *Staphylococcus aureus*. J Bacteriol. 2008;190(4):1276-83. http://dx.doi.org/10.1128/JB.01128-07. PMid:18083809.

Nwobodo DC, Ugwu MC, Anie CO, Al-Ouqaili MTS, Ikem JC, Chigozie UV, Saki M. Antibiotic resistance: the challenges and some emerging strategies for tackling a global menace. J Clin Lab Anal. 2022;36(9):e24655. http://dx.doi.org/10.1002/jcla.24655. PMid:35949048.

Otto M. Coagulase-negative Staphylococci as reservoirs of genes facilitating MRSA infection: staphylococcal commensal species such as *Staphylococcus epidermidis* are being recognized as important sources of genes promoting MRSA colonization and virulence. BioEssays. 2013;35(1):4-11. http://dx.doi.org/10.1002/bies.201200112. PMid:23165978.

Overgaauw PAM, Vinke CM, van Hagen MAE, Lipman LJA. A one health perspective on the human-companion animal relationship with emphasis on zoonotic aspects. Int J Environ Res Public Health. 2020;17(11):3789. http://dx.doi.org/10.3390/ijerph17113789. PMid:32471058.

Paterson IK, Hoyle A, Ochoa G, Baker-Austin C, Taylor NG. Optimising antibiotic usage to treat bacterial infections. Sci Rep. 2016;6(1):37853. http://dx.doi.org/10.1038/srep37853. PMid:27892497.

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

Rajabiani A, Kamrani F, Boroumand MA, Saffar H. *mec*-A-mediated resistance in *Staphylococcus aureus* in a referral Hospital, Tehran, Iran. Jundishapur J Microbiol. 2014;7(4):e9181. http://dx.doi.org/10.5812/jjm.9181. PMid:25147695.

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

Reygaert WC. An overview of the antimicrobial resistance mechanisms of bacteria. AIMS Microbiol. 2018;4(3):482-501. http://dx.doi.org/10.3934/microbiol.2018.3.482. PMid:31294229.

Rolo J, Worning P, Nielsen JB, Bowden R, Bouchami O, Damborg P, Guardabassi L, Perreten V, Tomasz A, Westh H, de Lencastre H, Miragaia M. Evolutionary origin of the Staphylococcal Cassette Chromosome *mec* (SCC*mec*). Antimicrob Agents Chemother. 2017;61(6):e02302-16. http://dx.doi.org/10.1128/AAC.02302-16. PMid:28373201.

Rosenfeld RM, Schwartz SR, Cannon CR, Roland PS, Simon GR, Kumar KA, Huang WW, Haskell HW, Robertson PJ. Clinical practice guideline: acute otitis externa. Otolaryngol Head Neck Surg. 2014;150(1, Suppl):S1-24. http://dx.doi.org/10.1177/0194599813514365. PMid:24491310.

Saber H, Jasni AS, Jamaluddin TZMT, Ibrahim R. A review of Staphylococcal Cassette Chromosome *mec* (SCC*mec*) types in Coagulase-Negative Staphylococci (CoNS) species. Malays J Med Sci. 2017;24(5):7-18. http://dx.doi.org/10.21315/mjms2017.24.5.2. PMid:29386968.

Shaikh S, Fatima J, Shakil S, Rizvi SM, Kamal MA. Antibiotic resistance and extended spectrum beta-lactamases: types, epidemiology and treatment. Saudi J Biol Sci. 2015;22(1):90-101. http://dx.doi.org/10.1016/j.sjbs.2014.08.002. PMid:25561890.

Stefańska I, Kwiecień E, Kizerwetter-Świda M, Chrobak-Chmiel D, Rzewuska M. Tetracycline, macrolide and lincosamide resistance in *Streptococcus canis* strains from companion animals and its genetic determinants. Antibiotics. 2022;11(8):1034. http://dx.doi.org/10.3390/antibiotics11081034. PMid:36009903.

Tahi AA, Sousa S, Madani K, Silva CLM, Miller FA. Ultrasound and heat treatment effects on *Staphylococcus aureus* cell viability in orange juice. Ultrason Sonochem. 2021;78:105743. http://dx.doi.org/10.1016/j.ultsonch.2021.105743. PMid:34525437.

Thairu Y, Nasir IA, Usman Y. Laboratory perspective of gram staining and its significance in investigations of infectious diseases. Sub-Saharan Afr J Med. 2014;1(4):168-74. http://dx.doi.org/10.4103/2384-5147.144725.

Thakur P, Nayyar C, Tak V, Saigal K. Mannitol-fermenting and tube coagulase-negative Staphylococcal isolates: unraveling the diagnostic dilemma. J Lab Physicians. 2017;9(1):65-6. http://dx.doi.org/10.4103/0974-2727.187926. PMid:28042222.

Uddin TM, Chakraborty AJ, Khusro A, Zidan BRM, Mitra S, Emran TB, Dhama K, Ripon MKH, Gajdács M, Sahibzada MUK, Hossain MJ, Koirala N. Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects. J Infect Public Health. 2021;14(12):1750-66. http://dx.doi.org/10.1016/j.jiph.2021.10.020. PMid:34756812.

Van TTH, Yidana Z, Smooker PM, Coloe PJ. Antibiotic use in food animals worldwide, with a focus on Africa: pluses and minuses. J Glob Antimicrob Resist. 2020;20:170-7. http://dx.doi.org/10.1016/j.jgar.2019.07.031. PMid:31401170.

Wales AD, Davies RH. Co-selection of resistance to antibiotics, biocides and heavy metals, and its relevance to foodborne pathogens. Antibiotics. 2015;4(4):567-604. http://dx.doi.org/10.3390/antibiotics4040567. PMid:27025641.

Wallinga D, Smit LAM, Davis MF, Casey JA, Nachman KE. A review of the effectiveness of Current US policies on antimicrobial use in meat and poultry production. Curr Environ Health Rep. 2022;9(2):339-54. http://dx.doi.org/10.1007/s40572-022-00351-x. PMid:35477845.

Waruwu YKK, Khairullah AR, Effendi MH, Lukiswanto BS, Afnani DA, Kurniawan SC, Silaen OSM, Riwu KHP, Widodo A, Ramandinianto SC. Detection of methicillin-resistant *Staphylococcus aureus* and multidrug resistance isolated from cats in animal clinic at Sidoarjo District, East Java, Indonesia. Biodiversitas. 2023;24(1):106-11. http://dx.doi.org/10.13057/biodiv/d240114.

Widodo A, Lamid M, Effendi MH, Khailrullah AR, Kurniawan SC, Silaen OSM, Riwu KHP, Yustinasari LR, Afnani DA, Dameanti FNAEP, Ramandinianto SC. Antimicrobial resistance characteristics of multidrug resistance and extended spectrum beta-lactamase producing *Escherichia coli* from several dairy farms in Probolinggo, Indonesia. Biodiversitas. 2023;24(1):215-21. http://dx.doi.org/10.13057/biodiv/d240126.

Widodo A, Lamid M, Effendi MH, Khairullah AR, Riwu KHP, Yustinasari LR, Kurniawan SC, Ansori ANM, Silaen OSM, Dameanti FNAEP. Antibiotic sensitivity profile of multidrug-resistant (MDR) *Escherichia coli* isolated from dairy cow's milk in Probolinggo, Indonesia. Biodiversitas. 2022;23(10):4971-6. http://dx.doi.org/10.13057/biodiv/d231002.

Wielders CL, Fluit AC, Brisse S, Verhoef J, Schmitz FJ. *mec*A gene is widely disseminated in *Staphylococcus aureus* population. J Clin Microbiol. 2002;40(11):3970-5. http://dx.doi.org/10.1128/JCM.40.11.3970-3975.2002. PMid:12409360.

Yambise D, Ariestanti CA, Budiarso TY. Isolation and Identification of Biofilm-Forming *Staphylococcus aureus* in Commercial Cow Milk Products. Sciscitatio. 2020;1(2):87-93. http://dx.doi.org/10.21460/sciscitatio.2020.12.33.

Yang X, Ye W, Qi Y, Ying Y, Xia Z. Overcoming multidrug resistance in bacteria through antibiotics delivery in surface-engineered nano-cargos: recent developments for future nano-antibiotics. Front Bioeng Biotechnol. 2021;9:696514. http://dx.doi.org/10.3389/fbioe.2021.696514. PMid:34307323.

Yunita MN, Effendi MH, Rahmaniar RP, Arifah S, Yanestria SM. Identification of spa gene for strain typing of methicillin resistant *Staphylococcus aureus* (MRSA) isolated from nasal swab of dogs. Biochem Cell Arch. 2020;20(1):2999-3004. http://dx.doi.org/10.35124/bca.2020.20.S1.2999.

Zanen LA, Kusters JG, Overgaauw PAM. Zoonotic Risks of Sleeping with Pets. Pathogens. 2022;11(10):1149. http://dx.doi.org/10.3390/pathogens11101149. PMid:36297206.

Zhang F, Cheng W. The mechanism of bacterial resistance and potential bacteriostatic strategies. Antibiotics. 2022;11(9):1215. http://dx.doi.org/10.3390/antibiotics11091215. PMid:36139994.

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