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Profile of Multidrug Resistance and Methicillin-Resistant *Staphylococcus aureus* (MRSA) isolated from cats in Surabaya, Indonesia

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Abstract. *Afnani DA, Fatih N, Effendi MH, Tyasningsih W, Kairullah AR, Kurniawan SC, Silaen OSM, Ramandianto SC, Widodo A, Hendriana K, Riwu KHP. 2022. Profile of Multidrug Resistance and Methicillin-Resistant* Staphylococcus aureus *(MRSA) isolated from cats in Surabaya, Indonesia. Biodiversitas 23: 5703-5709.* Methicillin-Resistant *Staphylococcus aureus* (MRSA) is the name given to *Staphylococcus aureus* that has multidrug-resistance (MDR) characteristics and is resistant to β-lactam drugs. Compared to other livestock, companion animals have been mentioned as potential MRSA reservoirs more frequently. This study aimed to identify MDR and detects MRSA strains from cats in Surabaya. A total of 150 nasal swab of cats were taken from several clinics and veterinary hospital. Samples were swabbed using Amies Medium Transport then identified using microbiological standard method. Kirby-Bauer diffusion method was performed for *S. aureus* antibiotic resistance profile on five different antibiotic discs. As a confirmatory test for MRSA, *S. aureus* isolates that were resistant to cefoxitin continued to grow on Oxacillin Resistance Screening Agar Base (ORSAB). The findings of the isolation and identification and process revealed 18 (12%) *S. aureus* isolates. The antibiotic resistance test revealed 3 (2%) MDR *S. aureus* isolates and 4 (2.6%) MDR *S. aureus* isolates that were ORSAB positive. It has been discovered that there are MDR and MRSA *S. aureus* isolates. It could be used as an indicator of irrational usage of antibiotics and it explained that cats could be source of infection to public health.

Keywords: Cats, MDR, MRSA, public health

INTRODUCTION

Pets such as dogs and cats have increased substantially in modern society (Habibullah et al. 2017). Companion animals are frequently regarded as family members, and daily close proximity or direct contact between humans and their pets puts them at risk of contracting a variety of pathogenic germs, including multidrug-resistant (MDR) bacteria (Kaspar et al. 2018; Riwu et al. 2020). In addition to being a natural component of the flora of the skin and mucous membranes of mammals and birds, *Staphylococcus aureus* has developed into significant opportunistic pathogens in human and veterinary medicine (Gemma et al. 2020). The colonies of the Gram-positive, non-spore-forming coccus *S. aureus* are spherical, smooth, and shiny (Mustapha et al. 2014; Khairullah et al. 2022a; Khairullah et al. 2022b). Staphylococci have experienced evolutionary processes in reaction to the presence of antimicrobial medications in biological systems, coinciding with the beginning of antimicrobial drug use in the practice of modern human and veterinary medicine (Cook and Wright 2022). This evolution suggested the amplification and spread of clinically significant strains of pathogenic staphylococci affecting human and animal populations, as well as the acquisition of mechanisms for antimicrobial treatment resistance (János et al. 2021). The ubiquity of *Staphylococcus* sp. which inhabit all ecological niches may be linked to Antimicrobial Resistant (AMR) characteristics. AMR between humans, animals, and the environment is amplified by complex interactions between bacterial species from various 'environments' creating a general public health issue (Palma et al. 2020). *Staphylococcus aureus* has an anti-antibiotic defense mechanism that involves changing in the binding and active sites, producing trans membrane proteins known as efflux proteins, and producing plasmids that include genes for antibiotic resistance (Munita and Arias 2016).

The first report of *S. aureus* developing resistance to methicillin and other β-lactam drugs occurred in 1961, heralding the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) (Harkins et al. 2017). *S. aureus* is referred to as MDR if it is resistant to at least three drugs (Hiramatsu et al. 2014; Khairullah et al. 2019). When bacteria become resistant to β-lactam antibiotics, they are referred to as MDR *S. aureus* or MRSA (Guo et al. 2020). MRSA has been identified in multiple surfaces and pieces of equipment indicating that the environment may be a significant source of MRSA infection (Hoet et al. 2011). Methicillin resistance results from the emergence of the *mec*A gene (Ramandinianto et al. 2020a; Khairullah et al. 2022c), which produces a novel protein called PBP2a (Penicillin Protein Binding 2a), a member of the family of enzymes required for bacterial cell wall synthesis (Fishovitz et al. 2014). Methicillin and other β-lactam antibiotics are resistant to the protein (PBP2a), which has a very low affinity for them (Miragaia 2018). The *S. aureus* chromosome upstream of X contains a movable genetic element called staphylococcal cassette chromosome *mec* (SCC*mec*) *mec*A, which contains the *mec*A gene (McClure et al. 2020).

Dogs, cats, lambs, cattle, horses, rabbits, seals, cockroaches, guinea pigs, and chinchillas have all been reported to have MRSA (Chandrasekaran et al. 2014; Gulani et al. 2016; Khairullah et al. 2020a; Islam et al. 2016). This exposure may be caused by a number of factors, including a high population density, the potential for nosocomial transmission from humans, inadequate cleaning and disinfection procedures, the unidentified carrier status of numerous animals, or a stressful environment (Dalton et al. 2020). Although, domestic animals are often colonized by different *Staphylococci* species, these germs do occasionally dwell on them (Rahmaniar et al. 2020). When *S. aureus* enters the body, it can cause a range of diseases, from minor skin infections to serious invasive infections that can be fatal (Pantosti 2012; Ramandinianto et al. 2020b). Due to companion animal's frequent intimate physical contact with their owners through caressing, petting, and licking, which exposes them to harmful MRSA germs (Pomba et al. 2017). Recent findings of MRSA isolation from small animals in the UK imply that MRSA is significantly more common in veterinary clinic because animals and people both have a higher colonization rate than infection rates, also both of them can serve as MRSA reservoirs for the spread of strains within the same environment (Walther et al. 2017). A study conducted in Canada and the US found that pet owners have a considerably higher colonization risk of MRSA (18%) than the general population (1-2%) (Cuny et al. 2022). This supports earlier hypotheses that bacterial "spillover" from owners to their dogs would establish a reservoir for infection and re-colonization, MRSA is becoming a public health concern (Overgaauw et al. 2020). This study is significant since there may be MRSA transmission between cats and people.

MATERIALS AND METHODS

Study area and sample collection

A total of 150 nasal swab samples of cats came from several clinics and veterinary hospitals in each five regions of Surabaya, East Java, Indonesia, between May 2022 until July 2022. Sample testing carried out in Department of Veterinary Microbiology and Mycology, Faculty of Veterinary, Airlangga University, Indonesia.

Isolation and identification of *Staphylococcus aureus*

Nasal swab samples from cats were obtained using Amies as a medium transport and kept in the icebox at 4°C. *S. aureus* was isolated using sterile cotton swab samples from the Amies medium transport and streaked on Mannitol Salt Agar (MSA) and then 24 hours were spent incubating bacterial inoculum on MSA media at 37°C (Effendi et al. 2019). Gram staining, positive catalase, coagulase, and Voges-Proskauer test results, as well as yellow colonies with yellow zones on MSA media, were used to isolate and identify *S. aureus* (Effendi et al. 2018).

Antibiotic sensitivity test and MRSA confirmation test

The isolates that have been isolated and identified will be purified on Mannitol Salt Agar (MSA) (HiMedia Pvt. Ltd., M118) and incubated at 37°C for 24 hours as a 0.5 Mc Farland suspension and then taken using a sterile cotton swab of size S (AKD 10903610549). Then wipe evenly on the surface of the Muller Hinton Agar (MHA) media (Oxoid, CM0337). Place the antibiotic disks were cefoxitin 5 μg, erythromycin 15 μg, tetracycline 30 μg, ciprofloxacin 5 μg, and chloramphenicol 30 μg (Oxoid) side by side with a distance of 5 cm on Muller Hinton Agar (MHA) media that has been inoculated with isolates and then incubated at 37°C for 24 hours to measure the inhibition zone.

MRSA confirmation test done by using several *S. aureus* isolates from MHA (Oxoid-CM00337) that are resistant to cefoxitin then streaking on Oxacillin Resistance Screening Agar Base (ORSAB) (HiMedia M1415) plus Oxacillin Resistance Selective Supplement (Supplement, HiMedia FD191) (Decline et al. 2020).

RESULTS AND DISCUSSION

Bacterial isolates

Of the total of 150 nasal swab samples collected from cat analyzed, 18 (12%) samples were found positive for *S. aureus* Based on morphological culture and gram staining appearance (Figures 1 and 2). The positive number of *S. aureus* can be caused by several factors, Staphylococcal infection in humans can be transmitted from animals (Haag et al. 2019) and *S. aureus* reported as the most common staphylococcal strain in cats because these organisms occasionally live on domestic animals as an opportunistic pathogen (Pantosti 2012).

Antibiotic resistance *Staphylococcus aureus*

Two *S. aureus* isolates (1.3%) were found to be resistant to one class of antibiotics during antibiotic susceptibility testing, then seven *S. aureus* isolates (4.6%) (Figure 3) and were categorized as MDR because they were resistant to three classes of antibiotics and dominated by the pattern of antibiotic resistance FOX-CIP-E-TE-C (Cefoxitin, Ciprofloxacin, Erythromycin, Tetracycline, Chloramphenicol) with a total of 4 S. aureus isolates followed by 3 *S. aureus* isolates with antibiotic resistance pattern E-TE-C (Erythromycin, Tetracycline, Chloramphenicol) (Table 1). MRSA testing was carried out on *S. aureus* isolates that had developed a resistance to the antibiotic cefoxitin. According to the study's findings, four of the *S. aureus* isolates mentioned in Table 2 that were thought to be MRSA later tested positive for ORSAB (Figure 4). Four of the *S. aureus* isolates in this investigation were MRSA, whereas the other three were MDR.

Discussion

Based on the proportion of antibiotic resistance test results displayed in Table 2, it can be determined whether the center, north, and eastern regions lack antibiotic resistance because no *S. aureus* isolate was discovered. West region has a resistance of one type of antibiotic and South region has the highest percentage of antibiotic resistance (Asbell et al. 2018). A high resistance rate of antibiotics can indicate irrational and frequent use so that the antibiotic becomes no longer effective as a therapy for bacterial infections (Mustapha et al. 2014). The prevalence of *S. aureus* isolates carried on the skin and mucosa of cats was reported in several earlier research (Ruiz-Ripa et al. 2021). In other research, 40% of healthy cats and 50% of cats with pyodermas were found to have *S. aureus* (Bierowiec et al. 2016)

The percentage of confirmed MRSA isolates was 2.6% (4/150) of the total number of 22.2% (4/18) *S. aureus* isolates. The presence of MRSA in pet animals especially cats similar to other studies, Pantosti (2012) mentioned 2.1% of cats in Veterinary clinics were colonized by MRSA. According to Algammal et al. (2020), MRSA has been identified in a number of other domesticated species, including horses, dogs, chickens, and cats. Geographic distribution of the presence of MRSA can be found worldwide, MRSA detected in many European countries, North America, and Singapore (Lee et al. 2018).

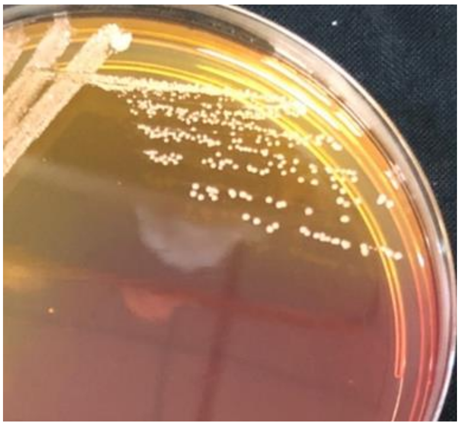

Figure 1. Colonies of *Staphylococcus aureus* in MSA

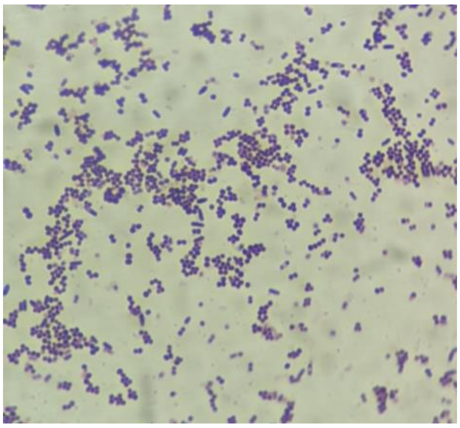

Figure 2. *Staphylococcus aureus* colonies stained with Gram under a microscope

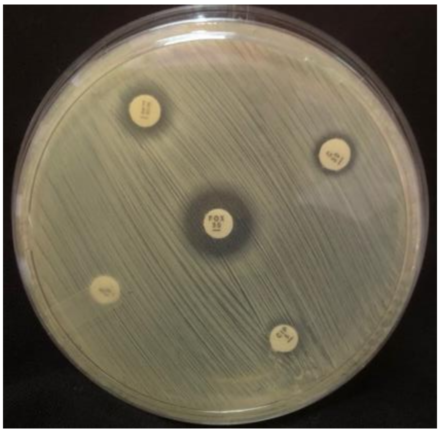

Figure 3. Test for antibiotic sensitivity on an MRSA isolate grown on MHA

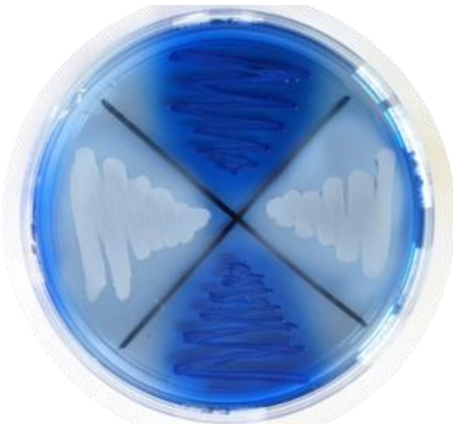

Figure 4. The color blue represents positive confirmation results from the ORSAB test for MRSA, while the color white indicates negative confirmation result

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

Group of antibiotics	Resistance profile	Resistant isolates (%) Number of *Staphylococcus aureus* isolates (n=18)	Total number of isolates (%)
0	No antibiotic resistance	38.8% (7/18)	7 (38.8%)
1	TE	16.6% (3/18)	3 (16.6%)
2	TE-E	5.55% (1/18)	1 (5.55%)
>3	TE-E-C	16.6% (3/18)	3 (16.6%)
	E-C-FOX-CIP	5.55% (1/18)	1 (5.55%)
	E-C-FOX-CIP-TE	16.6% (3/18)	3 (16.6%)

Note: TE: Tetracycline, E: Erythromycin, C: Chloramphenicol, FOX: Cefoxitin, CIP: Ciprofloxacin. Note: %: percentage. Info: Total 8 isolates Resistance with >3 Group of Antibiotics are classified as MDR, Total of 4 isolates that Resistance with Cefoxitin are MRSA continued to ORSAB.

Table 2. Results of the region-based MRSA screening from cat nasal swabs and the proportion of antibiotic resistance test (%)

List of antibiotics resistant / MRSA	Region of pet clinic and veterinary hospital location in Surabaya				
	Central	North	South	East	West
TE (Tetracycline)	0 %	0%	44.4% (8/18)	0%	11.1% (2/18)
E (Erythromycin)	0%	0%	38.8% (7/18)	0%	5.55% (1/18)
C (Chloramphenicol)	0%	0%	22.2% (4/18)	0%	0%
FOX (Cefoxitin)	0%	0%	22.2% (4/18)	0%	0%
CIP (Ciprofloxacin)	0%	0%	22.2% (4/18)	0%	0%
MRSA	0	0	4	0	0

Note: Percentage (%) of *Staphylococcus aureus* isolates, MRSA: Methicillin-Resistant *Staphylococcus aureus*.

Close interaction between animals and humans increasing the possibility transmission of MRSA because most cats frequently direct contact with humans (Crespo-Piazuelo and Lawlor 2021). There are 4 positive MRSA isolates, one of them classified as a healthy cat. It indicates that MRSA can carried by healthy cats without showing any clinical manifestations (Bierowiec et al. 2016). MRSA has been discovered in asymptomatic carriers, including cats and other animals, according to Harrison et al. (2014). Cats harbored the MRSA strain on the fur and paws, which also plays an important rule as probable vehicle of the transmission (Pantosti 2012). It is connected to a prior study by Petinaki et al. (2015) in the USA, the most prevalent MRSA type in pets was identified as USA100 (ST5), which is a close relative of hospital-acquired methicillin-resistant *Staphylococcus aureus* (HA-MRSA) in humans. MRSA recovered from cats is identical to those affecting humans with a similar regional distribution (Aires-de-Sousa 2017). MRSA can also spread through direct contact, aerosols, and inanimate items, according to studies (Domon et al. 2016).

The prevalence of identical MRSA strains in cats and people residing in the same home has been linked to the transmission of bacterial strains between companion animals and their owners, according to molecular detection analyses (Oh et al. 2020). Given that cat nasal swabs resemble nosocomial MRSA and that both humans and companion animals are more likely to become colonized than infected, both can serve as reservoirs for the recirculation of MRSA strains within the same household (Morris et al. 2012). Companion animals are thought to contract MRSA from humans (Vincze et al. 2014). Direct companion animals exposure is considered effective way of MRSA transmission to human, some other studies show that indirect exposure is a relevant pathway to acquire colonization, based on research results found by Hermes et al. (2012) 12.8 % of household contacts of MRSA positive, veterinarians treating cats and dogs were also more frequently colonized and carried the same strain (Jordan et al. 2011). This was confirmed in a study from the UK where veterinarians and pet owners who had contact with pets were colonized with MRSA in 12.3% and 7.5%, respectively (Loeffler et al. 2010). According to a different study conducted in Canada and the US, although pet owners don't seem to be at an increased risk for MRSA infections, their MRSA colonization rate (18%) is much higher than that of the general population (1-2%) (Faires et al. 2010).

By using culture, MRSA infections, including colonization, are detected (Lee et al. 2015). Nasal swab sampling should be done using media transport then cultured on selective media for *S. aureus* (Yagüe et al. 2021). The approach employed for identifying and isolating the bacterium can affect how easily it is detected in clinical specimens, biochemical tests such coagulase test are used to differentiate *Staphylococcus aureus* from other *Staphylococci* also Vogues Proskauer to differentiate *S. aureus* and *S. pseudintermedius* (Klaschik et al. 2015). MRSA can be identified via antibiotic susceptibility tests like the disk diffusion test, the majority of these tests used cefoxitin because methicillin is no longer commercially accessible (Bonjean et al. 2016). Compared to the identification of *mec*A or PBP2a, antibiotic susceptibility testing has some limitations, *S. aureus* isolates that resistant to cefoxitin continued to ORSAB as phenotypic test, depending on factors like temperature, it may also change during growth (Meng et al. 2020). Genotypic test to detect gen *mec*A that encodes MRSA is Polymerase Chain Reaction (PCR) as the golden standard for identification (Pournajaf et al. 2014).

Since there is now no effective treatment for MRSA, it is necessary to regulate and prevent MRSA transmission from animals to animals and from animals to humans by adopting healthy lifestyle practices (Khairullah et al. 2020b; Lee et al. 2018). MRSA in animals and humans can also be prevented by early detection with microbiological surveillance and rational usage of antibiotics (Okwu et al. 2019). Some animals have spontaneously eradicated MRSA when the environment was regularly cleansed and disinfected and re-infection was avoided, and colonization in cats and other animals frequently appears to be temporary (Petinaki and Spiliopoulou 2015). To reduce MRSA cross-contamination, veterinary hospitals and animal clinics must closely enforce their established procedures (Traverse and Aceto 2015). Prevention requires practicing good hygiene, including hand washing and disinfecting the surroundings (Bloomfield et al. 2007). When treating animals that are diagnosed with MRSA infections, barrier precautions should be practiced such as wearing gloves and masks properly also those animals should be isolated (López-Alcalde et al. 2015). Screening upon entry enables quick isolation of MRSA carriers (Leung et al. 2013). Regular inspection of all accepted animals may be expensive and only useful for referral practices (Hernandez et al. 2018).

In conclusion, from this study, it can be concluded that MRSA is an important global issue, cases of MRSA are not only limited to human health, there are multi transmission routes between humans and animals. Antibiotic sensitivity tests from cat nasal swabs gave a different antibiotic resistance profile and as a screening test for MRSA proved that cats can act as a reservoir for the spreading and colonization of MRSA to humans and endanger public health. The importance of MDR and MRSA isolates could be used as a reference to control and prevent MRSA infection and also to increase public awareness, especially for those risk groups of humans such as veterinarians, paramedics, and pet owners. Further studies are needed to find out how long the duration of MRSA colonization and infection in companion animals, also protocols for the prevention of MRSA transmission among species.

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