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Encoding Gene for Methicillin Resistant Staphylococcus aureus (MRSA) Isolated from Nasal Swab of Dogs

Reina Puspita Rahmaniar, Maya Nurwartanti Yunita, Mustofa Helmi Effendi[1], and Sheila Marty Yanestria

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Abstract

The purpose of this study was to isolate and identify encoding gene for *methicillin-resistant Staphylococcus aureus* (MRSA) from nasal swab of dogs in Surabaya, Indonesia. Nasal swab of dogs of 85 samples obtained from five areas in Surabaya. Bacterial identification was based on the growth in Mannitol Salt Agar, Gram staining, catalase, coagulase and VP tests. 43 (50.59%) out of 85 samples were for positive *Staphylococcus aureus* isolation. MRSA confirmation by Oxacillin Resistant Screen Agar Base (ORSAB) were 25(29.41%). The molecular identification on *mec*A gene by PCR showed that 5(5.88%) isolates were positive contain *mec*A gene. It was concluded that the dogs as companion animals can be a potential reservoir for MRSA strains to threat public health.

Key words: *Staphylococcus aureus*, MRSA, Dogs, *mec*A gene

The problem of *Staphylococcus aureus* resistance to the methicillin antibiotic poses a serious threat to people throughout the world, which is also reported in nosocomial infection in humans (Batabyal *et al.,* 2012), and also in dogs and cats (Loeffler *et al.,* 2010). Transmission from humans to animals or vice versa can occur can lead to bacterial transmission (Faires *et al.,* 2009), and pets can act as a reservoir in spreading infection to humans, when in contact with the animal. MRSA infection in various forms like from minor skin infections, blood vessel infections, pneumonia, pericarditis, infections of the central nervous system, wound infections, surgical site infections, pyoderma, otitis, and urinary tract infections (Jarvis *et al.,* 2012).

Rachel *et al.,* (2009) reported that methicillin-resistant Staphylococcus from healthy pets, even though methicillin is not used for animal therapy, the transfer of resistance will increase the spread of MRSA infections between animals and humans or vice versa (Duquette and Nuttall, 2004). Based on this background, it is necessary to conduct research on MRSA in animals and identification of the encoding gene so that the spread of MRSA can be prevented.

Materials and Methods

Nasal swab samples taken from sick dogs with symptoms of pain, diarrhea, vomiting, tremors; healthy dogs showing no symptoms of illness and samples from animal hospitals, animal clinics, dog shops in Surabaya were collected.

Isolation and identification *Staphylococcus aureus* was done using Manitol Salt Agar (MSA) media and identified isolates. The presence of *Staphylococcus aureus* is characterized by plasma clotting and *Voges Proskauer* (VP) test positive, and shown on Fig 1 and Fig 2. (Effendi *et al*., 2019).

Confirmation tests were carried out for the presence of Methicillin Resistant Staphylococcus aureus (MRSA) by planting colonies from MSA media in streaks on Oxacillin Resistant Screen Agar Base (ORSAB) media. Positive results are shown by changing the color of the media to bluish (Fig 3) (Anand *et al.,* 2009).

All MRSA isolates were subcultured on MSA and incubated at 37°C for 24 h before DNA extraction. The DNA of all S. aureus isolates in this study was extracted using QIAamp® DNA

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Table I. Data of MRSA isolates in this study

Location	Number of samples	Positive *S. aureus*	MRSA Confimation by ORSAB	mecA gene
Center of Surabaya	20	13	8	2
Western of Surabaya	15	5	3	1
Eastern of Surabaya	20	6	4	1
Southern of Surabaya	20	11	6	0
Northern of Surabaya	10	8	4	1
Total	85	43(50.59%)	25(29.41%)	5(5.88%)

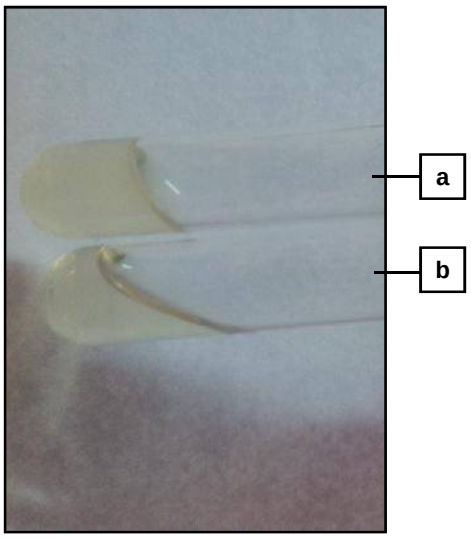

Fig 1. Coagulase test on *Staphylococcus aureus* shows positive clotting of plasma (a) and negative *Staphylococcus aureus* shows no cloting of plasma (b)

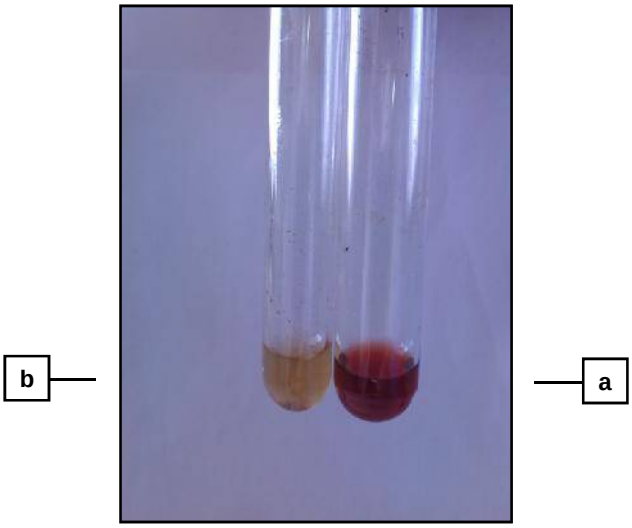

Fig 2. VP test results (a) positive VP positive *Staphylococcus aureus* ; (b) negative VP negative *S. aureus*.

Mini Kit (QIAGEN, Singapore) and done using the manufacturer method (Effendi et al., *loc cit*).

For PCR amplification, a total of 50 µl reaction mixture contained 28 µl Go taq green master mix (Promega, Germany), 20 µl RNase free water, and 1 µl of each forward and reverse primer was prepared. The primer used for mecA gene amplification as described by Sangeetha *et al.,* (2012) was 5'-GTA GAA ATG ACT GAA CGT CCG ATA A - 3'and 5'- CCA ATT CCA CAT TGT TTC GGT CTA A -3').

A total of 2.5µl of DNA template were added to the mixture. The mixture then amplified using PCR cycler according to the protocol of Sangeetha et al., (2012). with modification as following: Pre denaturation 94°C for one minute, Denaturation 94°C for 45 seconds, Annealing 58°C for 45 seconds, Extension 72°C for one minute and Final extension 72°C for 3 minutes with 35 cycles (Sangeetha et al., loc cit). The presence of PCR products was determined by electrophoresis of 10 µl of products in 2% agarose gel with TBE buffer as described by Elhassan *et al.,* (2015)and 100 bp DNA ladder as a marker (Promega, Germany).

Results and Discussion

Based on the results of isolation and identification carried out on 85 samples of nasal swab of dogs from 5 areas in Surabaya there were 43(50.59%) positive samples of *Staphylococcus aureus* (Table I).

Fourty three positive samples of *Staphylococcus aureus* identified for and confirmation of MRSA test using ORSAB media, shown on figure 3. The results shown on Table I.

Basically *Staphylococcus aureus* is a commensal organism that is found as part of

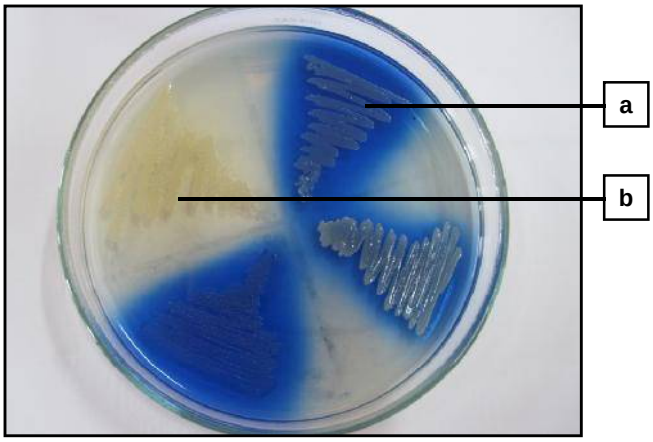

Fig 3. MRSA confirmation test on Oxacillin Resistant Screen Agar Base (ORSAB) media with the results of (a) Methicillin Resistant Staphylococcus aureus (MRSA) and (b) Methicillin Sensitive Staphylococcus aureus (MSSA).

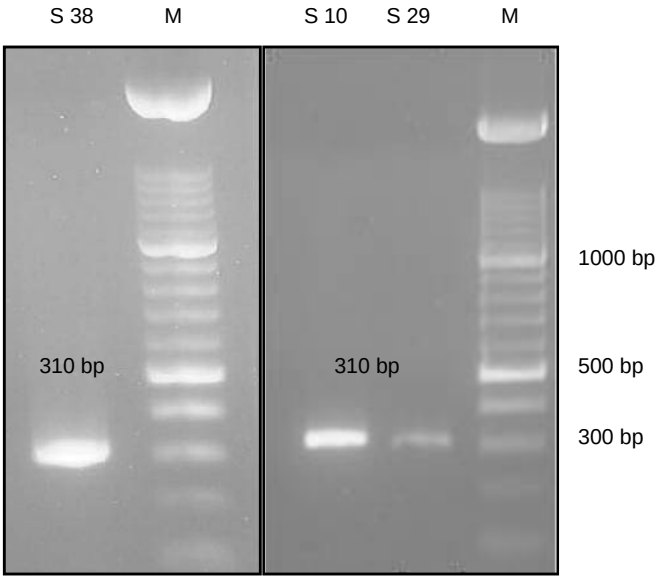

Fig 4. Electrophoretic product PCR results, the *mec*A gene is shown in the presence of band bands at 310 bp
Information : M = Marker ; S38 = Sample 38 ; S10 = Sample 10 ; S29 = Sample 29

the normal flora of humans and animals, and 30 percentage of the population is a source of human pathogens cause the disease (Ibrahem, 2010).

Out of the 43 isolates 25 (29.41%) were Methicillin-resistant (MRSA). Bhanderi and Jhala (2011) stated that resistance to methicillin which is caused by changes in the nature of penicilin binding protein 2a (PBP2a) (Malachowa and Frank, 2010). This is in accordance with previous studies that succeeded in isolating MRSA in dogs. MRSA strains in dogs were identical from owners and infected pets. The dominance of human MRSA strains in household pets can re-propagate MRSA to humans or other species (Loeffler *et al., loc. cit*; Miller and Diep, 2008).

The results of this study, MRSA are found in samples from affected and healthy subjects. This is consistent with the results of Faires *et al.,* (*loc. cit*) who has reported that MRSA is increasingly being identified in dogs and cats with infections as well as healthy dogs and cats.

To control and prevent the MRSA transmission from animals to animals, and from animals to humans must be carried clean living habits of pet animals and human (Hafez *et al.,* 2009). The present findings of MRSA from nasal swabs (29.41 %) in Surabaya concurrs the findings of Elhassan *et al., loc. cit* (45.5 %) in Japan; Kunishima *et al*., 2010 (45.5%) and Jarvis *et al., loc. cit* (61.8 %) in U.S

The finding of the *mec*A gene is the main evidence for detection of MRSA isolate, which is agreement with that of findings in Sudan (Elhassan *et al., loc. cit*), and in India (Mehndiratta *et al*., 2009). However, our findings are in this study suggests the low *mec*A gene 5/25 (20%) may open the door to look for other intrinsic factors which can compete with the *mec*A gene in producing resistance with high MRSA prevalence. In on the other hand, the absence of the *mec*A gene in resistant Staphylococcal isolates are registered throughout the world (Hawraa *et al.,* 2014). These discoveries suggests that there are other mechanisms for the presence of the *mec*A gene responsible for beta-lactam resistance and molecular methods for *mec*A gene alone are not sufficient for the confirmation and characterization of MRSA isolates. Another novel encoding gene *mec*C also have a role for detection MRSA isolates (Rania *et al.,* 2017). Although the data obtained are few *mec*A genes, that dogs can be a source of MRSA transmission to humans and their surroundings.

Summary

Molecular identification of the *mec*A gene can be used to prove the presence of MRSA in dogs. Thefore, the presence of MRSA on the dogs in Surabaya requires the government to respond to encourage antibiotic use in pet animals to be appropriate and rational. Which is an important step to reduce the incidence of MRSA sourced from pet animal origin, especially dogs.

Acknowledgement

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