Phenotypic and genotypic characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from dogs and cats at University Veterinary Hospital, Universiti Putra Malaysia

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**Abstract.** Methicillin-resistant *Staphylococcus aureus* (MRSA) is known to cause nosocomial infections and is now becoming an emerging problem in veterinary medicine. The objective of the study was to determine the presence of MRSA in 100 cats and dogs sampled between November 2007 and April 2008 at the University Veterinary Hospital, Universiti Putra Malaysia. MRSA was detected in 8% of pets sampled. Ten percent (5/50) and 6% (3/50) of the isolates were from dogs and cats, respectively. All MRSA isolates possessed the *mecA* gene and were found to be resistant to at least three antimicrobials with a minimum of Oxacillin MIC of 8 µg/mL. One isolate (CT04) had an extremely high MIC of >256 µg/mL. The MLST type ST59 found in this study have been reported earlier from Singapore and other countries as a strain from animal and community-associated MRSA respectively. Pulsed-field gel electrophoresis revealed five pulsotypes. Two isolates from cats (CT27 and CT33) and three isolates from dogs (DG16, DG20, and DG49) were respectively assigned to pulsotypes B and D. The study suggests that cats and dogs in Malaysia are potential reservoirs for MRSA.

**INTRODUCTION**

Methicillin-resistant *Staphylococcus aureus* (MRSA) emerged as an important nosocomial pathogen in the early 1960s. Since then, MRSA has been causing hospital-associated infections throughout the world; however, recent epidemiological shift has been observed as MRSA started to prevail in the community and causing associated diseases (Ochoa et al., 2005; Zetola et al., 2005). Moreover several recent reports showed that MRSA is becoming an emerging problem in veterinary medicine, particularly in small animal and equine practices (Leonard & Markey, 2008). The first case of MRSA in veterinary species was identified in the milk of a cow with mastitis in 1972 (Duquette & Nuttall, 2004; O’Mahony et al., 2005). Methicillin resistance in *S. aureus* is mediated by the penicillin-binding protein PBP2a, which has a low binding affinity to β-lactam antibiotics. It is encoded by the *mecA* gene located on one of the six types of staphylococcal cassette chromosome mec (SCCmec) (Hiramatsu et al., 2001; Morgan, 2008).

Initially, reports of MRSA colonization in small animals were infrequent, however, marked increment of case reports have been appearing in recent years (Boag et al., 2004; van Duijkeren et al., 2004; Rich & Roberts, 2004). Inter-transmission of MRSA between animals and humans have been reported (Manian, 2003; Weese et al., 2005) and companion animals have been indicated as a potential reservoirs of MRSA.
This is becoming a public health concern because companion animals often are in close contact with their owners, risking them to the transmission of pathogenic bacteria (Gurdabassi et al., 2004). Studies conducted on MRSA in Malaysia so far have been conducted on humans, mainly focusing on major hospitals in the country (Neela et al., 2008; Norazah et al., 2009). However, data related to MRSA isolated from cats and dogs are scarce. Therefore, the objectives of this study were to detect MRSA in cats and dogs referred to the University Veterinary Hospital, Universiti Putra Malaysia and to determine the molecular typing of the isolates using Pulsed-field gel electrophoresis (PFGE).

**MATERIALS AND METHODS**

**Isolation and Identification**

Fifty cats and 50 dogs were included in this study. The animals were residents, inpatients, and visiting cases at the University Veterinary Hospital (UVH), Universiti Putra Malaysia. Samples were collected between November 2007 and April 2008. Two separate sterile swabs with Stuart Transport Medium (Oxoid, UK) were used to hygienically collect samples from each nostrils and the perianal region of the animals. The swabs were placed into 10 mL Tryptone Soya Broth (Oxoid, UK) for 48 h for enrichment and later streaked onto 7% blood agar and incubated at 37°C overnight. Catalase and oxidase positive colonies were subcultured onto mannitol salt agar and further confirmed by Staphytect Plus® (Oxoid, UK). The selection of MRSA were made on Oxacillin-resistant agar base media (ORSAB; Oxoid, UK) supplemented with ORSAB selective supplement (Oxoid, UK), consisting of oxacillin and polymyxin B. Inoculated plates were aerobically incubated at 37°C for 24-48 h.

**Antimicrobial Susceptibility Test**

Inocula were prepared from overnight MRSA cultures on blood agar supplemented with 2% sodium chloride (NaCl). Pure colonies were emulsified into normal saline (0.85% NaCl) and turbidity of the suspension was equilibrated to 0.5 MacFarland and spread onto Mueller-Hinton agar (MHA; Oxoid, UK). Fifteen antimicrobial discs were used in this study. The interpretative criteria for each antimicrobial tested were made according to the recommendations published by the Clinical and Laboratory Standards Institute (CLSI, 2006). Oxacillin MIC was determined by using E test (AB Biodisk, Solana, Sweden) on MHA with 2% NaCl.

**Detection of mecA gene by Polymerase Chain Reaction (PCR)**

Extraction of genomic DNA from MRSA culture was performed using DNeasy Blood and Tissue DNA purification kit (Qiagen) per procedures recommended by the manufacturer. The PCR amplification was done as described earlier by Murakami et al. (1991), with the following sequences: mecA1, 5'-AAA ATC GAT GGT AAA GGT TGG C-3' and mecA2, 5'-AGT TCT GCA GTA CCG GAT TTG C-3'. The PCR amplification mixture consisted of 200 µM of dNTPs, 1 IU of Taq polymerase, 1.5 mM MgCl₂ (PROMEGA Corporations, USA), 2 µM of each primer, and 50 to 100 ng of template. The amplification was carried using the following parameters: 94°C for 45 s, 30 cycles of (94°C for 45 s, 55°C for 1 min, 72°C for 2 min) and 72°C for 2 min. PCR products were resolved by electrophoresis with 1% agarose gel, stained with ethidium bromide and visualized using UV-Gel Doc system (Bio-Rad). *Staphylococcus aureus* ATCC 29253 (negative mecA) and *S. aureus* ATCC 43300 (positive mecA) were used as controls.

**spa Typing**

Staphylococcal protein A (spa) and MLST typing were conducted on three selected isolates, two dog isolates (DG16, DG36), and one cat isolate (CT04). Accordingly
amplification of the spa repeat region was performed using primers spa-1113f (5'‐AAAGACGATCTTCCGGTGAGC‐3') and spa-1514r (5'‐AGCAGTAGTGCCGTTTGTCTT‐3') as described previously by Harmsen et al. (2003). A PCR mixture of 50 µl final volume was constituted by 1.25 IU Taq polymerase, 1 mM MgCl₂, 200 µM of dNTP mix, 0.2 µM of each primer (spa-1113f, and spa-1514r), 5 µl of 10x PCR buffer and 3 µl of genomic DNA. The cycling condition was done for 5 min at 80ºC, followed by 35 cycles of 45s denaturation at 94ºC, 45 s of annealing at 60ºC, 90s of extension at 72ºC, and a final extension step of 10 min at 72ºC. Purification of the PCR product was done by using QIAquick PCR Purification Kit (QIAGEN) and sent for sequencing. The sequences were aligned and the analysis and subsequent typing were done by feeding sequence repeats into online spa serve (http://spaserver.ridom.de/)

MLST Typing
Multilocus sequence typing of the same selected isolates has been conducted by amplifications of internal fragments of the seven housekeeping genes of S. aureus according to Enright et al. (2000) with minor modifications. The master mix for each pair of primer consisting 3 µl of DNA template, 200 µM dNTP mix, 1.25U Taq polymerase, 0.25 µM of each primer, 5 µl of 10x PCR buffer, sterile deionised distilled water was added to make 50 µl of reaction volume. The PCR was conducted with an initial 5-min denaturation at 95ºC, followed by 30 cycles of annealing at 55ºC for 1 min, extension at 72ºC for 1 min, and denaturation at 95ºC for 1 min, followed by a final extension step of 72ºC for 5 min. The annealing temperature was raised to 60ºC for amplification of gmk and pta genes. The PCR products were purified by QIAquick PCR Purification Kit (QIAGEN) and sent for sequencing. The sequenced products were aligned and assignment of the allele and sequence type was made using online database (http://saureus.mlst.net).

PFGE Typing
Pulsed-field gel electrophoresis of the isolates was conducted according to Harmony protocol (Murchan et al., 2003). S. aureus colonies from overnight cultures were embedded into agarose plugs. After bacterial lysis with lysozyme (100 mg/L), lysostaphin (10 000 U/mL) and RNase (50 mg/L) for 2 h at 37ºC, genomic DNA was digested using SmaI (40 U/mL) at room temperature, overnight. Separation of the digested fragments (PFGE) was done using a CHEF DRII (Bio‐Rad). The parameters of electrophoresis were 6v/cm with an angle of 120º and initial switch time of 5s to 15s for 10 hrs and final switch time of 15s to 60s at 14ºC. Gel pictures were obtained by using Alpha Imager (Bio‐Rad).

RESULTS
Isolation of MRSA
MRSA were detected in 5 dogs (10%) and 3 cats (6%) of the 100 pets sampled. Among the MRSA positive dogs, 8% (4/50) and 2% (1/50) were inpatient and outpatient dogs respectively. In cats, MRSA was isolated from inpatients only.

Antimicrobial Susceptibility
All isolates (100%) were resistant to at least 3 antimicrobials including oxacillin, methicillin and amoxicillin. Whereas 75% (6/8) of the isolates have shown resistance to amikacin and cefoxitin. All isolates were susceptible to impenem (10 µg/mL), vancomycin (30 µg/mL), and rifampicin (15 µg/mL) (Figure 1). All isolates had MICs of at least 8 µg/mL towards oxacillin, confirming that that the isolates are oxacillin-resistant. It was found that isolate CT04 had a high level of resistance with an MIC of 256 µg/mL (Table 1).

PCR Amplification of the mecA
All the isolates were positive for mecA (533 bp), the gene mostly responsible for methicillin (oxacillin) resistance (Figure 2).
Table 1. MIC, meca, spa typing and MLST results of MRSA isolates from dogs and cats.

<table>
<thead>
<tr>
<th>Isolate ID</th>
<th>Source</th>
<th>MIC in µg/mL</th>
<th>meca</th>
<th>spa Type</th>
<th>MLST(ST)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DG13</td>
<td>Dog</td>
<td>12 µg/ml</td>
<td>Positive</td>
<td>ND*</td>
<td>ND</td>
</tr>
<tr>
<td>DG16</td>
<td>Dog</td>
<td>16 µg/ml</td>
<td>Positive</td>
<td>t3590</td>
<td>ST59</td>
</tr>
<tr>
<td>DG20</td>
<td>Dog</td>
<td>8 µg/ml</td>
<td>Positive</td>
<td>t267</td>
<td>ST59</td>
</tr>
<tr>
<td>DG36</td>
<td>Dog</td>
<td>16 µg/ml</td>
<td>Positive</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>DG49</td>
<td>Dog</td>
<td>12 µg/ml</td>
<td>Positive</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CT04</td>
<td>Cat</td>
<td>&gt;256 µg/ml</td>
<td>Positive</td>
<td>t346</td>
<td>ST55</td>
</tr>
<tr>
<td>CT27</td>
<td>Cat</td>
<td>32 µg/ml</td>
<td>Positive</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CT33</td>
<td>Cat</td>
<td>32 µg/ml</td>
<td>Positive</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*ND: Not determined

MLST and spa Typing

The results from MLST assigned the two isolates from dogs (DG 16 and DG 20) to ST59 whereas the isolate from a cat (CT04) was typed as ST55. On the other hand, all the three isolates tested were typed to be different by spa typing (Table 1).

PFGE Results

Using the criteria proposed by Tenover et al. (1995), visual analysis of the PFGE patterns revealed five pulsotypes. Two cat isolates (CT27 and CT33) and three dog isolates (DG16, DG20, and DG49) shared indistinguishable PFGE patterns. Isolate
Figure 2. Agarose gel electrophoresis of amplified 533bp segment of the mecA gene. (Lanes: M, 100bp size markers; 1, ATCC29253; 2, DG13; 3, DG16; 4, DG20; 5, DG36; 6, DG49; 7, CT04; 8, CT27; 9, CT33; 10, ATCC43300)

CT04 and DG13 had band difference of ≥ 6 with respect to all other isolates, hence they were assigned to distinct pulsotypes (Figure 3).

DISCUSSION

MRSA, emerging soon after methicillin was used in treating infections had caused nosocomial infections worldwide. The organism has since been isolated from livestock and companion animals. Cats and dogs represent potential sources of the spread of MRSA due to the extensive use of the antimicrobial agents and their close contact with humans (Gurdabasi et al., 2004).

The current study revealed that 8% (8/100) of pets sampled at the UVH, UPM was positive for MRSA. Of this, 10% (5/50) and 6% (3/50) were from dogs and cats, respectively. Other studies have also reported more MRSA in dogs (Rich & Roberts, 2004; O’Mahony et al., 2005; Morgan, 2008); compared to cats. However, the prevalence rates of MRSA found in several studies were much lower compared to that of the current study. Kown et al. (2006) reported MRSA in 1.9% (3/157) of hospitalized dogs in Korea, Malik et al. (2006) reported MRSA in 0.8% (2/252) of dogs and cats in Australia and Lilienbaum et al. (1998) found MRSA in 2% of healthy cats in Brazil. In Malaysia, a previous study by Jacklyn (2006) reported MRSA in 1.93% (7/362) of cats and dogs, a rate that is much lower that reported in the current study. The current finding is consistent with the recent surveillance of MRSA in small animals in the United States which indicated that MRSA has been detected at increasingly higher rates (Morris et al., 2006; Jones et al., 2007).

All MRSA isolates in the current study were resistant to three or more of the antimicrobials tested including Oxacillin and Methicillin. Although vancomycin resistance (Tiwari & Sen, 2006) and imipenem resistances (Goff & Dozicky, 2007) were reported in previous studies, all the isolates in the current study were susceptible to both antibiotics. In our study, the MIC for all isolates were more than 8 µg/mL and one isolate from a cat had an extremely high MIC of more than 256 µg/mL. This suggests that a highly oxacillin and multidrug resistant S. aureus is also a problem in companion animals. It is most probably that these infections were
acquired by the animals from MRSA of human origin. Several epidemiological studies and case reports have suggested that MRSA transmission from human to companion animals and vice versa is possible (van Duijkeren et al., 2004; Baptiste et al., 2005; Weese et al., 2005; Hanselman et al., 2008).

Referring to the MLST database, the MLST type ST59 found in this study was also reported from neighboring countries Singapore, Australia, Taiwan, Japan, UK, and US. Most of the data referring to ST59 revealed that these MRSA isolates were community-associated and possessed PVL genes (Vandenesch et al., 2003; Takano et al., 2008). While ST55 was reported from UK only, in which it was grouped as an intermediate between major MRSA clones ST36 and ST45, and CC8 clones, it was the first MRSA lineage to be described (Cooper & Feil, 2006). This finding may imply that an epidemic community-associated MRSA is also colonizing pets in Malaysia and this entails that the MRSA strains could have been acquired by the pets from human sources. It has been reported that dogs and other pets living in close contact with human MRSA carriers can become colonized with MRSA of human origin (Duquette & Nuttal, 2004; van Duijkeren et al., 2004). On the other hand the increasing reports of MRSA isolation from companion animals has also raised public health concern as these animals can potentially pose risks to humans by serving as reservoirs (Cefai et al., 1994; Moore & Lindsay, 2002; Manian, 2003; van Duijkeren et al., 2004). In addition to MLST typing, a single spa typing has proven to be the method of choice for its rapidity, low cost and reproducibility in such a way that it can predict clonal lineages of S. aureus (Koreen et al., 2004). The spa type t3590 isolated from a dog in the current study have previously been reported from China. While t267 and t346 were reported from at least five European countries (http://spaserver2.ridom.de/spatypes.shtml). The fact that these spa types are found in pets in this study might indicate the possible dissemination of the strains into Malaysia most likely through human carriers with subsequent transmission of the strains to pet animals. The PFGE patterns of the isolates suggest that there are two main
clonal types of MRSA, characterized by pulsotype B and D that are colonizing cats and dogs presented to the university small animal hospital. This finding may indicate that the two MRSA types here (B and D) have been prevailing and circulating in the small animal hospital and colonizing animals admitted. Furthermore, these MRSA clones can serve as potential contaminants to the environment and source of infection for humans through direct or indirect contact to the pets.

Although the sample size of our study was small, we believe that it gives a picture of the overall occurrence of MRSA in cats and dogs in Malaysia at present. It is a common practice for antimicrobial prescription for the clinical cases to be instituted before confirmatory diagnosis. Such practice invites potential microbes like *S. aureus* to become resistant to a wide range of antimicrobials. Irrespective of the mode of transmission, whether from human-to-animals or vice versa, the high number of positive cases suggests high number of individuals serving as reservoir hosts for the MRSA, who may then become the source of infections or colonization. With the increase in pet-ownership, the inter-transmission of antimicrobial resistant bacteria will persist unless the transmission is interrupted. Therefore, it is important that studies on MRSA in Malaysia be conducted on a larger scale to monitor the MRSA occurrence in the country. This ought to be done by employing more discriminatory identification approaches such as the molecular tools to reveal the epidemiological patterns of the organism. Implementation of appropriate control and prevention methods could help to contain the colonization and/or infection. However, other approaches such as public education for a more stringent personal and environmental hygiene, test and decolonization of carrier individuals, prudent use of antimicrobial agents are among strategies that may also be employed.

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**REFERENCES**


