DISTRIBUTION AND CHARACTERIZATION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) AT THE SMALL ANIMAL HOSPITAL, FACULTY OF VETERINARY MEDICINE, CHIANG MAI UNIVERSITY, THAILAND

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Abstract. Of 416 samples taken from veterinary staff (n = 30), dogs (n = 356) and various environmental sites (n = 30) at the Small Animal Hospital, Faculty of Veterinary Medicine, Chiang Mai University, Thailand, 13 samples contained methicillin-resistant *Staphylococcus aureus* (MRSA), of which 1 (SCC*mec* type II) came from veterinarian, 9 (SCC*mec* types I, III, IVa, V and untypeable) from dogs, and 3 (SCC*mec* types I, III, and IVb) from environmental samples. The MRSA isolates were 100% susceptible to vancomycin (100%), 69% to cephazolin and 62% to gentamicin, but were up to 92% resistant to tetracycline group, 69% to trimethoprim-sulfamethoxazoles and 62% to ceftriaxone. In addition, all MRSA isolates showed multidrug resistance. As the MRSA isolates from the veterinary staff and dogs were of different SCC*mec* types, this suggests there were no cross-infections. However, environmental contamination appears to have come from dogs, and appropriate hygienic practices should be introduced to solve this problem.

Keywords: *Staphylococcus aureus*, MRSA, SCC*mec* type, small animal hospital, Thailand

INTRODUCTION

Staphylococcus aureus is a pathogenic bacterium for both humans and animals, causing dermatitis, septicemia, pneumonia, osteomyelitis, endocarditis and potentially death (Ayliffe, 1997). Although penicillin has been used as the first broad-spectrum antibiotic against many bacteria, excessive and incorrect usage contributed to increasing bacterial resistance, including *S. aureus*, leading to failure of antibiotic treatment in human cases. *S. aureus* has developed resistance to the β-lactam antibiotics group, which includes penicillin, methicillin and oxacillin, and such resistant bacteria are now referred to as methicillin-resistant *S. aureus*, or MRSA (Barber, 1964; Boyce, 1990).

MRSA infection of humans is hos-

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pital-acquired (nosocomial). Hospital-associated MRSA, or HA-MRSA, represents a growing public health concern, as it far more resistant to antibiotics than other MRSA strains (Williams, 1959; Voss *et al*, 1994; Struelens *et al*, 1996; Fluckiger and Widmer, 1999). MRSA is also found in veterinary hospitals and, as a result, veterinary staff and owners of MRSA-infected pets are a high-risk group for carrying MRSA, despite having no direct human hospital link (Loeffler *et al*, 2012). An MRSA infection has also been found in other animals, including pigs, birds, cattle, horses, and zoo animals and is known as livestock-associated MRSA, or LA-MRSA (OIE, 2011). Other types of MRSA have been found recently in community settings, called community-acquired MRSA (CA-MRSA), which is found in children (Herold *et al*, 1998), athletes (Kazakova *et al*, 2005), prison inmates (CDC, 2003), conscripts (Campbell *et al*, 2004) and people with tattoos (CDC, 2006). However, while resistant to many drugs, CA-MRSA strains do not pose as large a human health risk as HA-MRSA.

The antibiotic resistance mechanism of MRSA is mediated through expression of *mec*A, encoding a penicillin-binding protein PBP2a that has a low affinity for β-lactam antibiotics (Fischetti *et al*, 2000). M*ec*A is a component of the Staphylococcal chromosomal cassette *mec*, or SCC*mec* in the *S. aureus*. Five types of SCC*mec* exist: type I, II, III, IV and V (Zhang *et al*, 2005). SCC*mec* type IV consists of four subtypes, namely, IVa, IVb, IVc and IVd. HA-MRSA contains SCC*mec* type I or III, while CA-MRSA mainly contains SCC*mec* type IV, but also SCC*mec* type V (Zhang *et al*, 2005). For LA-MRSA, SCC*mec* type V is the most common, but SCC*mec* type IV has also been found (Zhang *et al*, 2005). It is believed that MRSA originally came from humans, but some studies have reported animal origins (Loo *et al*, 2007; Alam *et al*, 2011). Thus, more data on the relationship between human- and animal-originated MRSA is needed, as well as environment-originated MRSA.

The prevalence of MRSA is 1% among university students in Thailand (Kitti *et al*, 2011). Jariyasethpong *et al* (2010) showed that the burden of MRSA nosocomial infection is high in governmental tertiary hospital in Thailand. The data of Song *et al* (2011) indicated that various MRSA clones have spread between the community and hospital as well as between Asian countries. The study of Mekviwattanawong *et al* (2006) showed that the prevalence of CA-MRSA infection among hospitalized patients at Siriraj Hospital, Mahidol University, Bangkok, Thailand was uncommon and these patients probably acquired HA-MRSA.

The objectives of this study were to investigate at a small animal hospital the prevalence and distribution of MSRA associated with veterinary medicine, including veterinary staff, dogs and the environmental sources, and identify SCC*mec* subtypes by multiplex PCR. This information will enable better control and reduce risk of nosocomial MRSA infections, both in humans and animals.

MATERIALS AND METHODS

Study location

This study was conducted at the Small Animal Hospital, the teaching hospital of the Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand. The Small Animal Hospital is one of the largest animal hospitals in northern Thailand providing general and specialized medical services for approximately 200 cases per day.

Sample determination and collection

Win Episcope 2.0 software program was used to estimate the sample size with 50% expected proportion and a confidence level of 95% (Thrusfield *et al*, 2001).There were three target groups: veterinary staff including technicians and veterinarians (n = 30), environment (n = 30), and dogs, which were divided into three subgroups, namely, dogs with skin infections (n = 148), dogs admitted for surgery (n = 108) and healthy dogs, defined as any dog that visited the hospital for routine health check, such as for vaccination or deworming (n = 100). Veterinarians and technicians were invited to participate in the study. Samples from both sides of participants' external nares were collected using sterile cotton swabs. Environmental surface swab samples were arbitrarily collected from the door knobs, computer keyboards and mouses in the physical examination and infection ward. All samples were collected using sterile cotton swabs and transferred to the Central Laboratory, Faculty of Veterinary Medicine, Chiang Mai University within 24 hours for further investigation.

Bacterial identification

All samples were cultured in brain heart infusion (BHI) broth with 7% sodium chloride and incubated at 37ºC for 24 hours. Aliquots from BHI were subcultured on mannitol-salt agar and incubated at 37ºC for 24 hours. Three suspected single colonies of *S. aureus* from each sample were selected and identified by Gram staining and catalase test (Bartelt, 2000). Colonies were re-streaked on tryptic soy agar plates (TSA) overnight and a coagulase test was conducted. Positive samples were further screened for methicillin resistance by disc diffusion using oxacillin (1 µg), with an inhibition zone diameter < 10 mm being considered MRSA (Ferraro *et al*, 2000).

Antimicrobial susceptibility testing

Disk diffusion test was performed with the following drugs: amoxicillin-clavulanic acid (20/10 µg), cephazolin (30 µg), cefoxitin (30 µg), ceftriaxone (30 µg), chloramphenicol (30 µg), clindamycin (2 µg), cloxacillin (5 µg), doxycycline (30 µg), gentamicin (10 µg), oxytetracycline (30 µg), tetracycline (30 µg), trimethoprim-sulfamethoxazoles (25 µg) and vancomycin (30 µg). The results were interpreted following the standards of the Clinical and Laboratory Standards Institute (Ferraro *et al*, 2000).

Multiplex PCR

DNA extraction was performed as described previously (CPLS, 2008). In brief, bacterial cultures were sedimented and placed in 200 µl of Chelex® 100 buffer (Biorad, Hercules, CA). DNA was extracted by incubating the tubes at 80ºC for 30 minutes and then boiling for 10 minutes. Multiplex PCR was applied to test for the presence of *mec*A and to classify the SCC*mec* type and subtype. PCR mixture (50 µl total volume) contained 0.5 µl of DNA, 50 mM $MgCl_2$, 5 µl of 10X buffer, 0.2 mM dNTPs, 1.0 U *Taq* DNA polymerase (Vivantis, Selangor Darul Ehsan, Malaysia) and primers (sequences and concentrations are shown in Table 1). Amplification (PTC 200 Thermal Cycler; Biorad, Hercules, CA) was carried out as follows: 94ºC for 5 minutes; followed by 10 cycles of 94ºC for 45 seconds, 65ºC for 45 seconds and 72ºC for 90 seconds; followed by 25 cycles of 94ºC for 45 seconds, 55ºC for 45 seconds and 72ºC for 90 seconds; followed by a final heating at 72ºC for 10 minutes. Amplicons were separated by 2% agarose gel-electrophoresis, stained with ethidium bromide and recorded under UV light.

Table 1
Multiplex PCR primer sequences, amplicon sizes and primer concentrations.

Primer	Sequence (5′- 3′)	Conc (μM)	Amplicon size (bp)	MRSA SCC*mec* subtype
Type I-F	GCTTTAAAGAGTGTCGTTACAGG			
Type I-R	GTTCTCTCATAGTATGACGTCC	0.048	613	SCC*mec* I
Type II-F	CGTTGAAGATGATGAAGCG			
Type II-R	CGAAATCAATGGTTAATGGACC	0.032	398	SCC*mec* II
Type III-F	CCATATTGTGTACGATGCG			
Type III-R	CCTTAGTTGTCGTAACAGATCG	0.04	280	SCC*mec* III
Type IVa-F	GCCTTATTCGAAGAAACCG			
Type IVa-R	CTACTCTTCTGAAAAGCGTCG	0.104	776	SCC*mec* IVa
Type IVb-F	TCTGGAATTACTTCAGCTGC			
Type IVb-R	AAACAATATTGCTCTCCCTC	0.092	493	SCC*mec* IVb
Type IVc-F	ACAATATTTGTATTATCGGAGAGC			
Type IVc-R	TTGGTATGAGGTATTGCTGG	0.078	200	SCC*mec* IVc
Type IVd-F	CTCAAAATACGGACCCCAATACA			
Type IVd-R	TGCTCCAGTAATTGCTAAAG	0.28	881	SCC*mec* IVd
Type V-F	GAACATTGTTACTTAAATGAGCG			
Type V-R	TGAAAGTTGTACCCTTGACACC	0.06	325	SCC*mec* V
*Mec*A147-F	GTG AAG ATA TAC CAA GTG ATT			
*Mec*A147-R	ATGCGCTATAGATTGAAAGGAT	0.046	147	*mec* A

Table 2
Antimicrobial susceptibility MRSA isolates from the Small Animal Hospital, Faculty of Veterinary Medicine, Chiang Mai University.

Antimicrobial agent	Percent antimicrobial susceptibility (*n*) %		
	Susceptible	Intermediate	Resistant
Vancomycin	100 (13)	0	0
Cephazolin	69 (9)	8 (1)	23 (3)
Chloramphenicol	46 (6)	8 (1)	46 (6)
Gentamicin	62 (8)	0	39 (5)
Cefoxitin	54 (7)	0	46 (6)
Clindamycin	31 (4)	31 (4)	39 (5)
Cloxacillin	39 (5)	6 (1)	54 (7)
Amoxicillin-clavulanic acid	54 (7)	0	46 (6)
Trimethoprim-sulfamethoxazoles	31 (4)	0	69 (9)
Doxycycline	15 (2)	0	85 (11)
Ceftriaxone	8 (1)	31 (4)	62 (8)
Oxytetracycline	8 (1)	0	92 (12)
Tetracycline	8 (1)	0	92 (12)

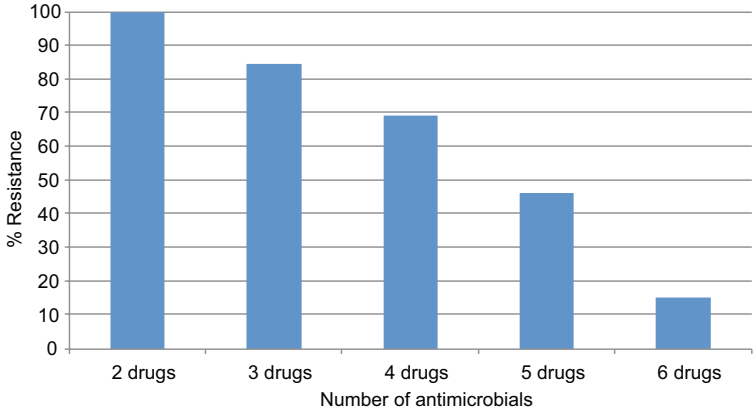

Fig 1–Percent multidrug-resistant MRSA isolates from the Small Animal Hospital, Faculty of Veterinary Medicine, Chiang Mai University. (2 drugs= Betalactams+Cephalosporins and Tetracyclines+ Trimethoprim-sulfamethoxazoles; 3 drugs= Chloramphenicol+Clindamycin+Tetracyclines and Cephalosporins+Tetracyclines+ Trimethoprim-sulfamethoxazoles; 4 drugs: Chloramphenicol+Gentamicin+ Tetracyclines+ Trimethoprim-sulfamethoxazoles; Betalactams +Chloramphenicol+ Tetracyclines+ Trimethoprim-sulfamethoxazoles; and Betalactams +Cephalosporins+ Gentamicin+Tetracyclines; 5 drugs= Cephalosporins+Chloramphenicol+Clindamycin+ Tetracyclines+ Trimethoprim-sulfamethoxazoles; Betalactams+ Chloramphenicol+Clindamycin+ Tetracyclines+ Trimethoprim-sulfamethoxazoles; Betalactams+Clindamycin+Gentamicin+ Tetracyclines+ Trimethoprim-sulfamethoxazoles; and Betalactams+ Cephalosporins Chloramphenicol+Clindamycin + Tetracyclines; 6drugs= Betalactams +Cephalosporins +Clindamycin+Gentamicin+Tetracyclines + Trimethoprim-sulfamethoxazoles).

RESULTS

The total prevalence of MRSA in the Small Animal Hospital, Chiang Mai University from March-September 2012 was 3 % (13/416), including 10% (3/30) among veterinary staff, 2% (7/356) among dogs and 10% (3/30) from environment. Forty-one of the 416 samples (10%) were methicillin-susceptible *S. aureus* (MSSA).

All MRSA isolates (100%) were susceptible to vancomycin, 69% to cephazolin and 62% to gentamicin. Almost all MRSA isolates were resistant to the tetracycline group (oxytetracycline 92%, tetracycline 92% and doxycycline 85%) (Table 2). All MRSA isolates were multidrug resistant (MDR)-MRSA, with resistance up to 6 types of antimicrobial drugs (Fig 1).

When MRSA isolates were classified into SCCmec subtypes, those from veterinarians were type II (n =1), from dog's skin lesions: types I (n =1), I + IVa (n =1), I + V (n = 1), III + V (n = 2) and IVa (n = 1), and from environment: types I (refrigerator handle, n = 1), II (computer keyboard in the diagnostic room, n = 1) and IVb (doorknob in the infection ward, n = 1) (Fig 2). All MRSA samples showed a band of 147 bp, confirming that all harbored *mec*A. Three MRSA isolates (1 from a normal dog's skin and 2 from dog's skin lesions) could not be typed using the method employed.

DISSCUSSION

To the best of our knowledge, this is the first preliminary report of MRSA epidemiology among animal, human and environment inter-relationships in an animal hospital setting in Thailand. A study in an animal hospital in England (Loeffler *et al*, 2005) found a higher MRSA preva-

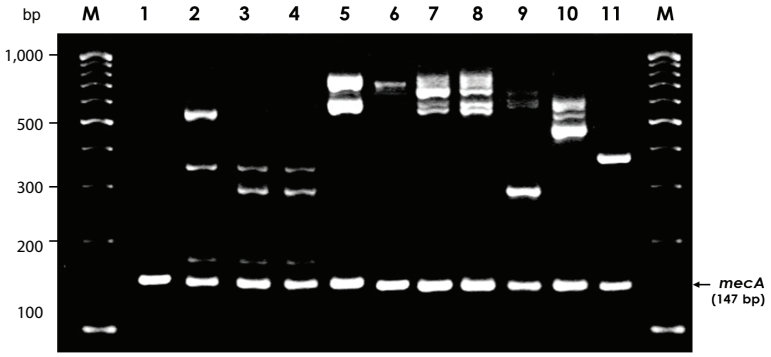

Fig 2–Multiplex PCR profiles of MRSA SCC*mec* subtypes from the Small Animal Hospital, Faculty of Veterinary Medicine, Chiang Mai University. Primers and PCR conditions are described in Materials and Methods and in Table 1. Lanes M, 100-bp DNA ladder molecular size markers; lane 1, untypeable; lane 2, SCC*mec* type I and V; lanes 3 and 4, SCC*mec* type III and V; lane 5, SCC*mec* type I and IVa; lane 6, SCC*mec* type IVa; lanes 7 and 8, SCC*mec* type I; lane 9, SCC*mec* type III; lane 10, SCC*mec* type IVb; lane 11, SCC*mec* type II.

lence in staff (17.9%), similar in environment samples (10%) and higher in dogs (9%) compared to our study. This might be the reason that *S. aureus* was identified and characterized by a combination of an automated bacterial identification and antimicrobial susceptibility testing system in their study.

HA-MRSA in Thailand and Asian countries generally is of SCC*mec* types I, II and III (Song *et al,* 2011). The presence in an animal hospital of CA-MRSA SCC*mec* type IVb must have come from humans and/or sick animals, although this subtype was not detected from both sources. However, the finding of CA-MRSA reflects less than ideal sanitation and hygiene practices. Better cleaning of the hospital and equipment, and better hygiene of staff and dog owners could help address this situation. Different subtypes of SCC*mec* from among staff (type II) and dogs (types I, III, IVa and V) showing that MRSA probably was not transmitted between humans and animals. No MRSA or MSSA was found in the operative wounds of any dogs, reflecting proper hygiene and sterile techniques in the operating room.

The detection of two different MRSA SCC*mec* subtypes from canine skin lesions is similar to the findings of Zhang *et al* (2005), who found co-existence of SCC*mec* type I and II. Inability to assign specific SCC*mec* subtypes in three isolates from canine skin lesions may be due to rearrangements and recombinations of *mec* or to a new SCC*mec* subtype (Zhang *et al*, 2005), which requires further studies.

All MRSA isolates were resistant to penicillin and oxacillin, characteristics of MRSA. However, all MRSA isolates were susceptible to vancomycin, suggesting that there are no vancomycin-intermediate (VISA) and vancomycin-resistant *S. aureus* (VRSA). The majority (39%) of MRSA were susceptible to clindamycin, in contrast to Rich *et al* (2005), who found that MRSA can develop resistance to clindamycin. Assays against first to third generation cephalosporins, namely, cephazolin, cefoxitin and ceftriaxone, revealed that MRSA isolates were 23, 46 and 62% resistant, respectively. These results confirm the characteristics of cephalosporins, with the first generation more effective in killing gram-positive bacteria than the second and third genera-

tion antibacterials, which were developed for killing gram-negative bacteria. A study by Lulitanond *et al* (2010) demonstrated that MRSA isolates from a university hospital in Thailand are resistant to at least 5 antimicrobial agents including cefazolin, erythromycin, gentamicin, ofloxacin and tetracycline, but vancomycin sensitivity is 100%.

The MRSA isolates were resistant to the tetracycline antimicrobial group, including oxytetracycline, tetracycline and doxycycline. Therefore, drugs in this group are not recommended for treatment of MRSA infection, but cephazolin and chloramphenicol should be prescribed. Vancomycin should be the last choice, as it may induce development of VISA and VRSA.

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