METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) ISOLATED FROM PET ANIMALS AND PET AND NON-PET OWNERS

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SUMMARY

This study was done to determine the occurrence of methicillin-resistant *Staphylococcus aureus* (MRSA) among cats and dogs, the pet owners and in persons who did not keep pets and to determine the antibiotic susceptibility of the isolates. MRSA was isolated from 7 (11.7%), 18 (30%) and 8 (13.3%) of the 60 samples each collected from 30 cats and dogs, 30 pet owners and 30 persons who did not keep pets respectively. Twenty percent (6/30) of the cats and dogs, 30% (9/30) of pet owners and 20% (8/30) of persons who did not keep pets were positive for MRSA. The *S. aureus* isolates showed resistance to four other antibiotics, namely ampicillin, erythromycin, tetracycline and streptomycin; all isolates were sensitive to gentamicin. The zoonotic and anthroponotic potentials of MRSA in animals were reviewed.

Keywords: Methicillin-resistant, *Staphylococcus aureus*, pet animals, pet owners, non-pet owners

INTRODUCTION

Staphylococcus aureus causes several diseases in animals, including mastitis, arthritis and urinary tract infections; in humans *S. aureus* causes diseases such as pneumonia, endocarditis, post-operative wound infections, toxic-shock syndrome and is an important cause of food-borne intoxication. The emergence and dissemination of antibiotic resistance amongst *S. aureus* is an important problem in human and veterinary medicine. *Staphylococcus aureus*, particularly those of human origin, are frequently found to be resistant to penicillins but susceptible to penicillinase-resistant penicillins, such as oxacillin and methicillin. Strains that are oxacillin- and methicillin-resistant are referred to as methicillin-resistant *Staphylococcus aureus* or MRSA. Methicillin resistance in staphylococci is mediated by the *mecA* gene which encodes the penicillin-binding protein 2a (PBP2a). This protein has a reduced affinity for penicillinase-resistant penicillins like methicillin, oxacillin and all other beta-lactam antibiotics, including cephalosporin and carbapenem (Lee, 2003; Shopsin and Kreiswirth, 2001).

MRSA is recognised as one of the most prevalent human nosocomial pathogens worldwide. It was first reported in 1961 and has since spread and become endemic in many countries. In US and in some European countries, MRSA accounts for 10 to 40% of all *S.aureus* isolates (Louie *et al.*, 2000). Today, MRSA is found not only in hospital settings but has emerged and become established in the community, including veterinary and laboratory settings. Transmission of MRSA is believed to occur primarily from colonised or infected persons to other persons; however, environment, animals and food products are found to contribute to MRSA transmission (Lee, 2003).

Few reports are available on MRSA in animals; MRSA infections have been reported in cats, dogs, horses, cattle and chickens where the organism has been isolated in low numbers (Lee, 2003; van Duijkeren *et al.*, 2004).

The objectives of the present study are to determine the occurrence of MRSA in cats and dogs and to isolate MRSA from pet owners as well from people who did not have any contact with pet animals during the period of study.

MATERIALS AND METHODS

Collection of samples

The samples for this study were collected from pet cats and dogs in Serdang and Petaling Jaya. Animals were randomly selected. For each animal, two samples were taken - the skin at the abdominal region (a surface area of at least 100cm^2) and the outer surface of the ear were swabbed using moistened sterile swabs. A total of 30 cats and dogs were swabbed. Swabs were also taken from the palms of the hands and the nostrils of the pet owners (30) and from persons who did not own any pet animals (30). All the samples were transported immediately to the laboratory.

Isolation and identification of MRSA

Each swab was streaked onto Oxacillin Resistance Screening Agar Base (ORSAB) supplemented with ORSAB Selective Supplement (Oxoid). ORSAB Selec-

tive Supplement consisted of oxacillin (2mg/l) to inhibit methicillin sensitive *S. aureus* and polymixin B (50,000 i.u./l) to suppress other bacteria that are able to grow at such a high salt concentration. The inoculated agar plates were incubated at 37°C for 24 hours and if there was no growth, the plates were re-incubated for a further 24 hours. Typical colonies of oxacillin resistant *S. aureus*, which are presumptive of MRSA, are intense blue in colour on a colourless background. The colonies were subjected to coagulase test and to Dryspot Staphytect Plus (Oxoid), a latex slide agglutination test kit to differentiate between staphylococci which possess clamping factor (Protein A) and certain capsular polysaccharides (found only in MRSA strains) and those which do not.

Antibiotic susceptibility test

The colonies of MRSA were picked to make a suspension with a turbidity of 0.5 McFarland standard. A sterile swab was dipped into the suspension and then streaked onto the surface of Mueller Hinton agar. Using an automatic antibiotic dispenser, six different antibiotic discs, namely gentamicin (10μg), ampicillin (10μg), erythromycin (15μg), tetracycline (30μg), streptomycin (10μg) and methicillin (5μg) were placed on the streaked agar surface. The agar plates were incubated at 37°C for 24 hours. The zones of inhibition were measured

RESULTS

Of the 60 samples from cats and dogs, seven (11.7%) were positive for methicillin-resistant *S. aureus*; six of the isolates were from the skin of the ears and one from the skin of the abdomen. Methicillin-resistant *S. aureus* were isolated from six (20%) of the cats and dogs. Eighteen (30%) of the 60 samples were from pet owners – nine isolates each from nostrils and palms –taken from nine (30%) pet owners were positive for MRSA.

Among those who did not keep pet animals, MRSA was isolated from eight (13.3%) of the 60 samples, six from nostrils and two from palms, from six (20%) persons (Table1).

All the 33 methicillin-resistant *S. aureus* isolated from ORSAB were positive to coagulase test and the latex agglutination test. The isolates showed resistance to four other antibiotics: ampicillin, erythromycin, tetracycline and streptomycin; all isolates were sensitive to gentamicin as shown in Table 2.

DISCUSSION

Household pets can be reservoirs of important human bacterial species with resistant genes such as MRSA, vancomycin-resistant enterococci (VRE) and multidrug-resistant *Salmonella typhimurium* DT104 (Guardabassi *et al.*, 2004). MRSA has become a widespread problem in human populations in many countries, causing outbreaks of nosocomial infections in human hospitals. There is very little information on the prevalence of MRSA infections in animals, even more so on the epidemiological aspects of nosocomial infections in animal hospital and laboratory settings (Seguin *et al.*, 1999). The occurrence of MRSA in a dog was first described in 1994 and canine infection with MRSA has since then been reported in many countries, including the UK, Canada, South Korea and The Netherlands.

Table 1: Occurrence of *S. aureus* and MRSA in cats and dogs, pet owners and persons who did not keep pets

Sampling sites	Cats & dogs (30)		Pet owners (30)		Persons not keeping pets(30)	
	Ears	Abdomen	Hands	Nostrils	Hands	Nostrils
S.aureus	30 (100%)	30 (100%)	30 (100%)	30 (100%)	30 (100%)	30 (100%)
MRSA per site	6 (20%)	1 (3.3%)	9 (30%)	9 (30%)	2 (6.7%)	6 (20%)
MRSA / 60 samples	7 (11.7%)		18 (30%)		8 (13.3%)	
MRSA / animal or man	6 (20%)		9 (30%)		6 (20%)	

Table 2: Percentage of *S. aureus* isolated from dogs and cats and man resistant to methicillin and five other antibiotics

Antibiotics	Cats and dogs	Pet owners	Persons not keeping pets
	Percentage (%) of antibiotic resistance		
Methicillin	11.7	60.0	26.6
Ampicillin	38.3	96.7	48.3
Erythromycin	35.0	33.3	23.3
Tetracycline	35.0	31.7	25.0
Streptomycin	13.3	23.3	8.3
Gentamicin	0	0	0

In this study, 11.7% MRSA was isolated from cats and dogs. Other reports in Malaysia, include (i) a study on the seven MRSA isolated from 364 cats and dogs with four isolated from cats and three from dogs (Ng *et al.*, 2005); according to the authors, this was the first published report on the isolation of MRSA in animals in Malaysia and PCR was used to detect the *mec*A gene in the isolates; (ii) another study was by Mohamed Jaralla (2004) who isolated one (4.8%) MRSA from a cat hospitalised in a veterinary teaching hospital (from November – December 2003); and (iii) Kersenernayer (2004) reported the occurrence of MRSA in cats and dogs at a veterinary teaching hospital seeking treatment and from stray animals; 19.6% of the 46 *S. aureus* isolated were from animals seeking treatment while 17.4% of those isolated from stray animals were resistant to methicillin. Studies outside Malaysia, include that of van Duijkeren *et al.* (2004) in which 2 (9.5%) MRSA were isolated from 21 dogs and none (0%) from 28 cats, and Gortel *et al.* (1999) who reported that 9 (39.1%) of the 23 *mec*A-positive staphylococci in dogs were identified as *S. aureus*.

MRSA are also found to be frequently resistant to most of the commonly used antimicrobial agents, which include the aminoglycosides, macrolides, chloramphenicol, tetracycline and fluoroquinolones; in addition, MRSA is considered to be resistant to all cephalosporins, cephems and other bata-lactams (Lee, 2003). Ng *et al.* (2005) reported that two of the seven MRSA isolates were resistant to four other antibiotics and two and three isolates were resistant to three and two other antibiotics, respectively; they were mainly resistant to tetracycline (71.4%) and clindamycin and lincomycin (42.9%). The present study found *S. aureus* isolated from pet animals as well as the pet owners were multi-resistant, being sensitive only to gentamicin.

A high number of MRSA was isolated from pet owners (30%) compared to those not in contact with pet animals (20%). Similar findings were observed in the work of Kersenernayer (2004) who also reported that the number of *S. aureus* resistant to methicillin was somewhat higher in man with pets (26.1%) compared to those who did not keep pets (19.6%). Could it be possible that interspecies transmission of MRSA occurs, with the spread being not only from humans to humans but also from humans to animals and animals to humans? In the work of Seguin *et al.* (1999), the biochemical profile and antibiogram of the isolates suggest that they were from a common source. To determine the relatedness of animal MRSA isolates to human isolates, molecular typing methods are usually employed such as random amplified polymorphic DNA (RAPD) and pulsed-field gel electrophoresis (PFGE).

There is increasing evidence on the transmission of MRSA among human patients, their family members and dogs living in the same household, although sometimes the discriminatory ability of the typing methods used are not sufficient to make such an inference. According to Guardabassi *et al.* (2004), MRSA is more likely to be transmitted from humans to pets, that is, more of an anthroponotic potential rather than a zoonotic potential. Oughton *et al.* (2001) studied 14 cases of MRSA in horses, dogs and cats and reported that the cases provided evidence to suggest that MRSA in animals possibly arose from contact with MRSA-positive owners or other human sources at high risk for MRSA colonisation. In the investigation of a MRSA outbreak in Michigan State University's Veterinary Teaching Hospital involving 11 equine patients, the workers suggested that the members of the hospital staff were the primary source of the infections in horses (Seguin *et al.*, 1999). A study by Weese *et al.* (2005) on MRSA in horses (4.7%) and in humans who work with those horses (13%) suggest that residing in a farm that housed >20 horses was the only factor significantly associated with MRSA colonisation in horses while for those humans, regular contact with >20 horses was the only identified risk factor in the MRSA colonisation.

Pet animals can play an important role as secondary reservoirs of MRSA within family members in the same households. Among the studies reported was a case of persistent MRSA infection in a human couple where the source of infection was traced to the nares of their pet dog (Mania, 2003). Cefai *et al.* (1994) isolated MRSA having identical phage type from the nose of a male nurse, his wife and their dog. Recent reports in UK suggest that MRSA is prevalent in small animal veterinary practices and that MRSA can be a hazard to owners (Guardabassi *et al.*, 2004).

It is of interest to note here what Waller (2005) suggested, "that MRSA infection is no longer restricted to humans and is now emerging as an important zoonotic and veterinary disease". An important point to note is that in many studies on the presence of the *mec*A gene which encodes for methicillin resistance, it was reported that the gene is not only found in *S. aureus* but also in other staphylococci and appeared to be more widespread among coagulase-negative staphylococci (van Duijkeren *et al.*, 2004). *Staphylococcus intermedius*, a coagulase-negative staphylococci, is normally rare in humans. However, the organism appears to be common in veterinary staff who are in constant contact with dogs and owners of dogs with atopic dermatitis which poses a risk should resistant genes get transferred from methicillin-resistant *S. intermedius* (MRSI) to human pathogenic staphylococci such as *S. aureus* (Waller, 2005; Guardabassi *et al.*, 2004).

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