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Full Length Research Paper

Prevalence of SCC*mec* types in methicillin resistant *Staphylococcus intermedius* in healthy pets from Southeastern United States

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Methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin resistant *Staphylococcus intermedius* (MRSI) are both major causes of skin and wound infections in humans and domesticated animal populations respectively. In order to investigate the colonization rates in pets, nose, mouth, or skin swabs were taken from 74 healthy pets (61 dogs and 13 cats) in south Louisiana, USA. *Staphylococci* were isolated by routine culture, speciated based on biochemical tests and confirmed by polymerase chain reactions. Our results show a 93% (69/74) colonization rate of domesticated pets by coagulase positive *Staphylococci*, with 50% (37/74) of animals harboring *S. aureus,* 43% (32/74) being colonized with *S.intermedius* and 5.4% (4/74) of pets carrying MRSI. Two of the MRSI contained Staphylococcal cassette chromosome *mec* type III while one isolate contained a type V genetic element. The fourth MRSI isolate did not show any of the SCC*mec* types I - V tested. All of the 4 *mecA* positive strains were isolated from dogs. Antibiotic susceptibility patterns were determined for the MRSI. Our results indicate that pets harbor pathogens that have zoonotic capability and suggest that domesticated animals have the potential to serve as vectors for the transfer of methicillin resistance.

Key words: SCC*mec*, *Staphylococcus aureus*, *Staphylococcus intermedius*, methicillin resistant, dogs, cats.

INTRODUCTION

Staphylococci are gram positive cocci that are found as transient normal flora of the skin and mucosal surfaces of mammals and birds and hence are easily spread to humans by contact and through fomites (Foster, 2005). *Staphylococcus aureus* is by far the most common cause of all *Staphylococci* infections with about 20% of the human population being long-term carriers of this organism and between 60 - 90% of the population being transiently colonized by this species (Foster, 2005). *S. aureus* is an opportunistic pathogen that causes various infections including skin lesions, abscesses, endocarditis, septicemia and toxic shock syndrome with some strains producing Staphylococcal entertoxins that are involved in food borne poisoning outbreaks (Jarraud et al., 2002).

Until the isolation and discovery of *Staphylococcus intermedius*, all coagulase positive testing *Staphylococci* were identified as *S. aureus* (Hajek, 1976). *S. intermedius* is commonly found as a transient colonizer in dogs and has been reported in several animals including cats, minks, foxes, horses and pigeons (Abraham et al., 2007; Epstein et al., 2009; Fazakerley et al., 2009; Wakita et al., 2002). While rarely found in humans, *S. intermedius* is an opportunistic animal pathogen that has zoonotic capabilities (Mahoudeau et al., 1997).

Until recently clinical differentiation of coagulase positive Staphylococcal species causing human infection was not routinely performed. There are reports that the similarities between the species has led to their misidentification in the clinical setting (Pottumarthy et al., 2004). Several biochemical tests can distinguish between *S.aureus* and *S.intermedius*. *S. intermedius* tests positive for PYR (L-pyrroglutamyl-aminopeptidase) and is sensitive to polymixin B (Beheme et al., 1996). More recently, polymerase chain reaction (PCR) amplification of the thermonuclease gene (*nuc*) has been used to distinguish

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the two species (Baron et al., 2004).

Methicillin resistant *Staphylococci* (MRS) strains harbor the *mecA* gene which encodes the modified penicillin binding protein 2A and are also frequently resistant to aminoglycosides, macrolides and fluoroquinolones (Jones et al., 2007). Methicillin resistance is transferable horizontally between Staphylococcal species via the Staphylococcal cassette chromosome (SCC) element that contains the *mecA* gene (Laplana et al., 2007). Typing of SCC*mec* helps identify MRSA that are typically nosocomial or health care associated (HA-MRSA) versus strains that are prevalent in the community (CA-MRSA) (Deurenberg and Stobberingh, 2009). The smaller SCC*mec* elements types IV and V are mainly harbored by CA-MRSA while larger types I - III have been found in HA-MRSA strains (Deurenberg and Stobberingh, 2009). In addition, another marker used to distinguish CA-MRSA from HA-MRSA is the Panton Valentine Leukocidin (PVL) gene which has been detected in almost all CA-MRSA isolates to date (Laplana et al., 2007). While studies involving SCC*mec* typing are well described using *S.aureus*, there have been very few reports on the characterization of SCC*mec* elements in methicillin resistant *S. intermedius* (Campanile et al., 2007). Previous studies have shown the transmission of MRS between animals and humans (Boost et al. 2008; Lloyd 2007). Therefore in the emergence of MRS as a public health crisis, there exists the possibility that in some clinical cases of MRS, household pets could serve as a reservoir for reinfections. To assess the carriage rates of MRS and in particular *S. aureus* / *S. intermedius*, nose, mouth and skin swabs from 74 household pets (dogs and cats) were cultured for *Staphylococcus*. The bacterial isolates were speciated and antibiotic susceptibility patterns, presence of *mecA* and SCC*mec* type was determined.

MATERIALS AND METHODS

Bacterial strains

The following control strains were used in this study. Methicillin sensitive *S. aureus* (ATCC 25923), *S. intermedius* (ATCC 29663), Methicillin resistant *S. aureus* (ATCC 43300).

Sample collection

Swabs (Fisher Scientific, Pittsburgh, USA) of external nares, oral cavities, or skin were collected from 61 dogs and 13 cats at Metairie Small Animal Hospital in New Orleans, Louisiana and Ridgefield Animal Hospital in Thibodaux, Louisiana. The swabs were then incubated in 2 ml of Luria-Bertani broth (LB) (Becton Dickinson, Franklin Lakes, USA) at 37°C for 24 h.

Bacterial culture and characterization

Twenty-four hour LB broth cultures were streaked on mannitol salt agar plates (MSA) (Becton Dickinson) for the isolation of putative *S. aureus* and *S. intermedius*. All gram positive cocci cultures were subjected to catalase and tube coagulase test by standard clinical methods. Up to 5 catalase positive colonies were analyzed from each individual animal. Individual animal colonization by *S. aureus* / *S. intermedius* was determined by having any one of the five colonies test positive for tube coagulase. Coagulase positive colonies were tested for L-pyrroglutamyl-aminopeptidase (PYR) by using the Dry Slide PYR Kit (Becton Dickinson) according to manufacturer's instructions to distinguish between *S. aureus* and *S. intermedius*. In addition, isolates were also tested for resistance to polymixin B (300U, Becton Dickenson; R ≤ 8 mm) by Kirby Bauer method. *Staphylococcus aureus* is resistant to polymixin B whereas, *S. intermedius* is susceptible to this antibiotic. Methicillin resistant *Staphylococci* were identified based on resistance to oxacillin (1 mcg) disk by Kirby Bauer disc diffusion method and confirmed with oxacillin screening plates (Becton Dickinson). Susceptibility to clindamycin (2 mcg), erythromycin (15 mcg), gentamycin (10 mcg), trimethoprim-sulfamethoxazole (1.25 - 23.75 mcg) and vancomycin (30 mcg) (Becton Dickenson) was performed by Kirby Bauer disc diffusion method.

DNA extraction and PCR

Pure cultures of bacteria were grown in 3 ml Luria Bertani broth (Becton Dickenson) for 18 h at 37°C, centrifuged at 10,000 x g for 5 min. Total DNA was extracted from bacterial pellets using the Fast ID Genomic DNA Extraction Kit (Genetic ID, Fairfield, IA) according to manufacturer's instructions. The final elution volume was 100 mcl and 1 mcl of DNA was used in a 50 mcl PCR. The primer pairs used to differentiate *S.aureus* from *S.intermedius* were based on amplification of thermonuclease *nuc* according to Baron (2004). Primers used to amplify *mecA* (McClure et al., 2006) and PVL (Laplana et al., 2007) was according to previous reports. SCC*mec* type I - V PCRs were performed as individual reactions using the primers of Zhang (2005). Each PCR was performed in a 50 mcl reaction volume containing 0.5 units of *Taq* with Thermopol buffer (NEB, Cambridge, MA), 200 mcM of each deoxynucleotide triphosphate (dATP, dTTP, dGTP, dCTP) (Fisher Scientific) and 1 mcM of each primer. Amplification was performed in a GeneAmp 2700 thermal cycler (Applied Biosystems, Foster City, USA) beginning with an initial denaturation step at 94°C for 5 min followed by 30 cycles of 94°C for 30 s, appropriate annealing temperature for 30 s, 72°C for 30 s and ending with a final extension step at 72°C for 7 min followed by a hold at 4°C, unless mentioned otherwise in the specific published protocol. Ten microliters of the PCR was electrophoresied on a 2% Tris-acetate-EDTA agarose gel at 100 v, stained with ethidium bromide and visualized under UV light.

RESULTS

Seventy four animals (61 dogs and 13 cats) were cultured for *Staphylococcus*. Ninety three percent (69/74) of pets tested harbored coagulase positive *Staphylococci* isolates (Table 1). Based on PYR tests and resistance to Polymixin B, our results showed that 50% (31/61) of dogs and 46% (6/13) of cats harbored *S.aureus* while 42% (26/61) of dogs and 46% (6/13) of cats were colonized with *S. intermedius*. Five dogs and one cat were colonized with both species of *Staphylococci*. None of these animals had MRS. As seen in Figure 1, lanes 1 - 7, polymerase chain amplification of the thermonuclease (*nuc*) gene confirmed species identification. The *S.intermedius nuc* PCR product migrated at 125 bp, while the *S.aureus nuc* PCR product migrated at 420 bp (data not shown) as

Table 1. Summary of Isolates.

Test	Dogs	Cats	Total
Number Screened	61	13	74
Coagulase Positive[1]	57 (93%)	12 (92%)	69 (93%)
S. intermedius[2]	26 (42%)	6 (46%)	32 (43%)
S.aureus[2]	31 (50%)	6 (46%)	37 (50%)
Methicillin Resistant *S. intermedius*[3]	4 (6.5%)	-	4 (5.4%)

[1]Five dogs and one cat were colonized with both *S.aureus* and *S.intermedius*
[2]Speciation was determined by PYR, polymixin B sensitivity and confirmed by PCR amplification of thermonuclease *nuc*.
[3]Methicillin resistance was determined by resistance to oxacillin and confirmed by PCR amplification of *mecA*.

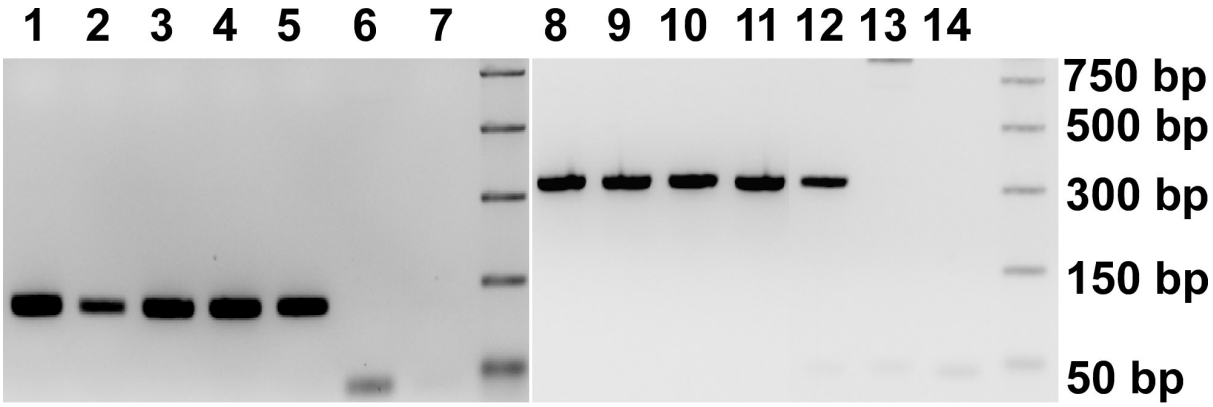

Figure 1. PCR confirmation of *Staphylococcal* species and methicillin resistance. Lanes 1-7 depict PCR results to detect *S.intermedius* thermo nuclease *nuc* (125 bp). Lanes 8-14 depict PCR results to detect *mecA* (310 bp). Lanes 1 and 8, isolate 59A; lanes 2 and 9, isolate 75B; lanes 3 and 10, isolate 94; lanes 4 and 11, isolate 95C; lane 5, *S.intermedius* ATCC 29663; lanes 6 and 12 MRSA ATCC 43300, lanes 7 and 14, no template control; lane 13, *S.aureus* ATCC 25923; PCR molecular weight marker (Promega) is shown.

previously reported by Baron (2004). Methicillin resistance among coagulase positive isolates was tested by growth on oxacillin screening plates and confirmed by polymerase chain amplification of the *mecA* gene. As seen in Figure 1, lanes 8 - 14 the *mecA* PCR product migrated at 310 bp consistent with prior reports (McClure et al., 2006). Of the 69 coagulase positive isolates, 4 isolates harbored the *mecA* gene and were resistant to oxacillin (Table 1) indicating a 5.4% (4/74) rate of methicillin resistance among pets screened. All of the 4 MRS were isolated from dogs and identified as methicillin resistant *S.intermedius* (MRSI). In order to determine if the MRSI isolates shared characteristics with HA-MRSA and CA-MRSA, SCC*mec* typing and detection of the presence of *PVL* was performed by PCR. None of the MRSI isolates contained PVL(data not shown). As seen in Figure 2, of the four MRSI, isolates 94 and 95C harbored SCC*mec* type III PCR products migrating at 280bp while isolate 59A contained SCC*mec* type V PCR product migrating at 325bp consistent with published reports (Zhang et al., 2005). Isolate 72B did not produce PCR amplicons for SCC*mec* types I - V tested. Antibiotic susceptibility patterns show the two SCCmec type III isolates 94 and 95C to have similar antibiograms (Table 2). While all the MRSI isolates were sensitive to gentamycin and vancomycin, they were resistant to erythromycin. These results show that both *S.aureus* and *S.intermedius* were recovered at similar rates from dogs and cats. Furthermore, the presence of SCC*mec* types III and V indicate that these isolates contain genetic elements similar to those found in human strains showing that pets could serve as carriers of MRS.

DISCUSSION

The prevalence of *Staphylococci* species in the domesticated pet is important because of the potential for zoonotic infections and the possibility of resistance gene transfer. Typically in cases of MRS infections, all human members of the patient family are subject to treatment and de-colonization guidelines to eradicate any MRS carriage within the household. Currently there are no policies to include household pets in these regimens

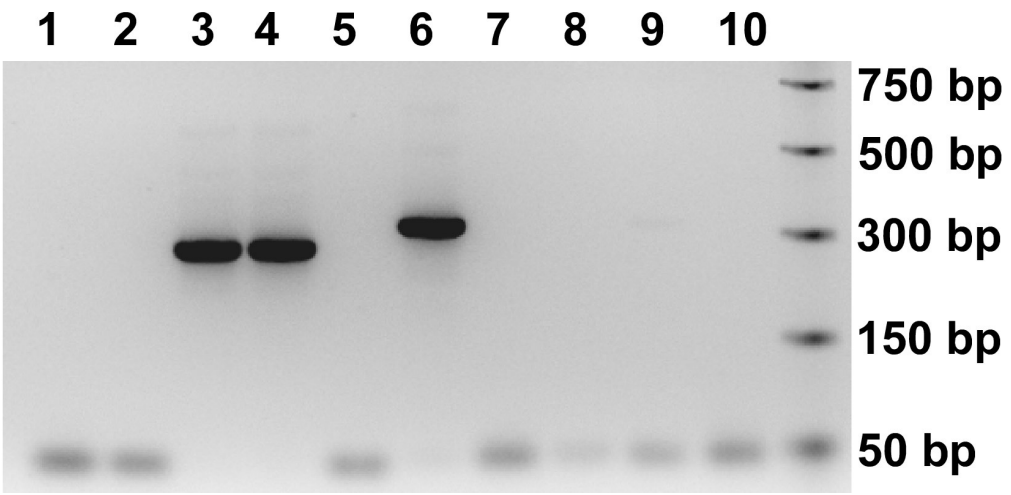

Figure. 2 SCC*mec* characterization by PCR. SCC*mec* type III PCR is shown in lanes 1-5. SCC*mec* type V PCR is shown in lanes 6-10. Lane 1 and 6, isolate 59A; lane 2 and 7, isolate 72B; lane 3 and 8, isolate 94; lane 4 and 9, isolate 95C; lane 5 and 10, no template control. Molecular weight PCR marker (Promega) is shown.

Table 2. Characterization of MRSI Isolates.

Test	59A	72B	94	95C
mecA	+	+	+	+
SCC*mec* Type	V	uk	III	III
PVL	-	-	-	-
Clindamycin	R	R	I	I
Erythromycin	R	R	R	R
Gentamycin	S	S	S	S
Oxacillin	R	R	R	R
Trimethoprim-Sulfamethoxazole	R	S	S	I
Vancomycin	S	S	S	S

R - resistant; S- sensitive; I - intermediate;
uk - unknown and is not types I - V.

(Ammerlaan et al., 2009). Subsequently, MRS carriage in the household pet becomes a potential source of recurrence of the infection if the patient comes in contact with the MRS colonized pet. Resistance to methicillin is conferred by the activity of the *mec*A gene. It is thought that *S.aureus* acquired *mecA* from commensal coagulase negative *Staphylococci* (Deurenberg et al., 2009). In this study, 5 dogs and 1 cat harbored both *S. aureus* and *S. intermedius*, lending support to the claim that colonization of pets with strains harboring *mecA* could serve as vectors for transference of this and other genes. However none of these animals harbored MRSI. In this study, of the 74 pets sampled, 93% of the animals harbored coagulase positive *Staphylococci*, a finding which is within the normal range of colonization for healthy pets (Epstein et al., 2009; Jones et al., 2007). While Gorwitz reported MRSA colonization rates to be 1.5% of the healthy human population (2004), Epstein reports a 17% rate of MRS colonization in healthy pets (2009). Our data indicate a 5.4% (4/74) rate of MRS carriage in healthy pets in general and a 6.5% (4/61) rate when analyzing dogs alone. In this study, none of the cats harbored MRS; however other reports have shown colonization (Abraham et al., 2007). While the presence of *S. intermedius* in pets is in itself not surprising or significant, the presence of MRSI is worthy of note. Since *mecA* can be transferred horizontally between *Staphylococci* (Deurenberg and Stobberingh, 2009), the colonization of companion animals such as dogs and cats with *S.aureus* and *mecA* harboring strains should be closely monitored to prevent MRSI aiding and abetting the already rising problem of MRSA as a public health concern. In this study, PCR was used to identify SCC*mec* types I - V. Typically, HA-MRSA harbor types I-III, while CA-MRSA has been found to have types IV and V genetic elements (Deurenberg and Stobberingh, 2009). In addition, the PVL gene has been associated with CA-MRSA but not other Staphylococcal species to date. Our results show isolates 94 and 95C to be most similar to HA-MRSA, while isolate 59A was negative for PVL, yet harbored the SCC*mec* V type cassette. Isolate 72B though did not show any of the SCC*mec* types I - V could harbor other types of this element. The use of pulse field gel electrophoresis (PFGE) on *S.aureus* isolates has been well established to determine strain relatedness and to identify evolutionary patterns as those seen in the epidemic outbreaks involving emergent HA-MRSA and CA-MRSA types (McClure et al., 2006; Tenover et al., 2008). It would be informative to perform PFGE analysis on all 4 MRSI isolates to address these questions.

In conclusion, we have found MRS isolates among healthy household pets. These isolates share many com-

mon characteristics with human MRSA strains. Routine surveillance of healthy animals can help predict trends in emergent outbreaks and serve as an important tool in preventive medicine. The inclusion of all pets in the initial screening and decolonization strategies for patient households could help decrease recurrent MRS infections.

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