Screening for Methicillin-Resistant *Staphylococcus* spp. Dog Isolates Capable of Transferring *mec*A.

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an antibiotic-resistant strain of the bacterium *Staphylococcus aureus* that is responsible for many community and hospital-acquired infections world-wide. A survey of the dogs at the local Veterinary Hospital was conducted to indicate the relative presence of Methicillin-Resistant *Staphylococcus* spp. (donor strains). We used Mannitol Salt Agar (MSA) with oxicillin (4µg/ml) to collect 67 bacterial samples from 39 dogs. Of these, 38 samples displayed characteristics of MRSA and were designated as potential methicillin-donors. PCR analysis however, determined only one of these donors to be MRSA while the rest appear to be other staphylococcal species. In addition, the MRSA isolate was determined to contain plasmid. All the donors were screened for their ability to transfer the methicillin-resistance gene (*mec*A) to a methicillin-sensitive, streptomycin and spectinomycin resistant *Staphylococcus aureus* recipient (SAS 850). To determine the ability of the isolates to transfer the *mec*A gene, a series of conjugation experiments were conducted with potential donors and recipient. The resulting transconjugants (*S. aureus* SAS850 with methicillin resistance) were selected for on Columbia Blood Agar (CBA) plates containing streptomycin, spectinomycin, and oxicillin. Oxicillin resistant transconjugants were analyzed by PCR and coagulase test to determine the samples to be *S. aureus*. To date, 28 of the 38 donor strains have been tested and we have not been able to detect the transfer of *mec*A.

Introduction

Staphylococcus aureus are gram-positive cocci able to ferment mannitol. *S. aureus* is normally found in the nasal cavities of humans. Recently there have been concerns about the spread of antibiotic resistances in bacteria, more specifically methicillin resistant *S. aureus* (MRSA). MRSA was first reported in 1961, shortly after the introduction of methicillin and has become increasingly more prevalent in recent years. There are two general strains of MRSA, a strain acquired by nosocomial infections (hospital acquired) and a community acquired strain. The CDC reported in 2005, that there were 94,000 MRSA cases in the United States, and of those cases 19,000 resulted in death. Approximately 85% of MRSA cases in 2005 were the result of nosocomial infections while the remaining 15% were as a result of community acquired infections. The presence of a mobile staphylococcal cassette chromosome (SCCmec) in *S. aureus* has been shown to encode methicillin-resistance (*mec*A) along with a number of other antibiotics.

We looked at the incidence of MRSA in the dog population at a local animal hospital. Additionally we tested methicillin-reistant strains for their ability to transfer the *mec*A by conugation.

Table 1. Sample Results

MSA (0xicillin) Positive	Gram-positive cocci	% Gram – positive cocci	Bactistaph positive
67	38	56.7	12

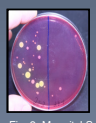

Fig. 2. Mannitol Salt Agar

Fig. 3 Agglutination Test

Fig 4. Catalase Test

Table 2. Analysis of Bactistaph positive samples

Catalase positive	% catalase positive	Coaggulase positive	% coaggulase positive
12	5.0	1	0.08%

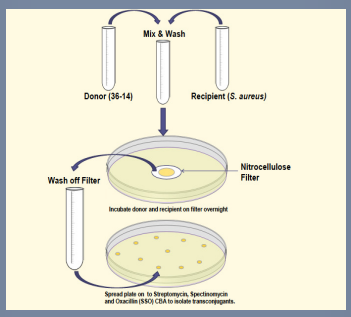

Fig. 1 Filter-mating procedure

Table 3. Minimum inhibitory concentrations (mg/ml)

Strain	Erm	Cam	Tet	Oxi	Amp	Van	Spec	Str
36-14	S	S	S	2500	12,500	S	S	2343
98-6-1	S	S	S	S	380	S	S	S
D3-1-1	312	S	S	1250	12,500	S	> 50,000	S

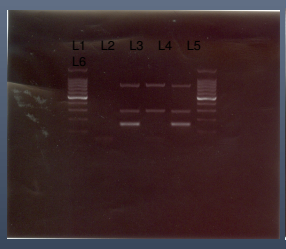

Fig.5 PCR Analysis. Lane (1) 100kb standard ladder , Lane (2) negative control, Lane (3) MRSA, Lane (4) *S. aureus*, Lane (5) D3-1-1,Lane (6) 100kb standard ladder.

Fig. 6 Plasmid extraction. Lane (1) lambda DNA standard, Lane (2) 36-14, Lane (3) D3-1-1 Lane (4) 98-6-1, Lane (5) 95-1, Lane (6) 98-6A

Materials & Methods

Sampling. Sterile cotton-tipped swabs were used to sample the upper nasal passage of dogs . The cotton swab was then streaked onto Mannitol Salt Agar (MSA) (Difco MI) plate containing 4ug/mL oxicillin. Plates were then incubate at 37C for up to 48 hours prior to counting colonies.

Filter-mating. Filter Mating procedure was followed as diagramed in Fig. 1. Donors were the isolates and the recipients were *S. aureus* SAS850 (Str^r,Spec^r).

Presumptive *S. aureus* tests. Mannitol-fermenting colonies were selected from MSA containing 4ug oxicillin/mL and streaked for isolation. A Gram-stain and Catalase test were performed to screen for Gram-positive, catalase-positive cocci.

Agglutination Test. We used the BactiStaph (Remel, Lenexa,KS) to test for the presence of coagulase and protein A associated with *S. aureus* strains.

Coagulase Test. We incubated 1ml overnight broth samples with 3ml rabbit plasma to test for the presence of coaggulase.

Polymerase Chain Reaction

A standard PCR master mix was prepared with forward and reverse primers for amplification of *mec*A, *fem*B and 16S rRNA gene identifying oxicillin-resistance, *Staphylococcus* and *aureus* strains, respectively.

Antibiotic Resistance Test. Serial 2-fold dilutions of *S. aureus* grown in Todd-Hewitt Broth (THB) (Difco MI) were performed to determine the minimum inhibitory concentration (MIC) of Methicillin (oxicillin) and other antibiotics.

Results/Discussion

-We screened 39 dogs and collected 67 potential MRSA.**(Table 2)**
-We confirmed only 1 (1.5%) isolate as MRSA by PCR. **(Fig. 5)**
-The MRSA isolate (D3-1-1) was resistant to erythromycin (312µg/ml), oxicillin (1250µg/ml), ampicillin (12,500µg/ml) and spectionmycin (> 50,000µg/ml). **(Table 3)**
-D3-1-1 was demonstrated to contain plasmids **(Fig. 6**)
-Conjugation experiments with the 12 Bactistaph isolates as donors did not produce transconjugants with oxicillin transfer.

Future Project

-Try different conjugation approach
-Determine the species of other isolates
-Determine the identity of the plasmid

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