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

Courtney Schauer, Kayla Showsh, Emily VerHaag and † Faculty Mentor Dr. Sasha Showsh † Biology † University of Wisconsin-Eau Claire

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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 a local veterinary hospital was conducted to indicate the relative presence of Methicillin-Resistant *Staphylococcus* spp. (donor strains). Mannitol Salt Agar (MSA) with oxicillin (4ug/mL) was used 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 (*mecA*) to a methicillin-sensitive, staphylococcus strain resistant *Staphylococcus aureus* receptor (SAS 850). To determine the ability of the isolates to transfer the *mecA* 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 the transfer of *mecA* has not been detected.

**Materials & Methods**

**Sampling.** Sterile cotton-tipped swabs were used to sample the upper nasal passage of dogs. The cotton swab was streaked onto Mannitol Salt Agar (MSA) (Difco MI) plate containing 4ug/mL oxicillin (Figure 1). Plates were incubated at 37 degrees Celsius for up to 48 hours prior to counting colonies.

**Catalase Test.** One drop of hydrogen peroxide was placed onto a colony of Gram-positive cocci bacteria to determine the presence of the enzyme catalase (Figure 2).

**Filter-Mating.** Filter-Mating procedure was followed as diagrammed in Figure 4. Donors were the isolates and the recipients were *S. aureus* SAS850 (Str, Spec).

**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.** BactiStaph (Remel, Lenexa, KS) was used to test for the presence of coagulase and protein A associated with *S. aureus* strains (Figure 3).

**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**

- Of the 39 dogs screened, 67 potential MRSA samples were collected. (Table 2)
- By PCR, 1 isolate (1.5%) was identified as MRSA. (Figure 5)
- The MRSA isolate (D3-1-1) was resistant to erythromycin (312ug/mL), oxicillin (1250ug/mL), ampicillin (12,500ug/mL) and spectinomycin (> 50,000ug/mL). (Table 3)
- D3-1-1 was demonstrated to contain plasmids (Figure 6)
- Conjugation experiments with 12 BactiStaph isolates as donors did not produce oxicillin transconjugants.

**Future Project**

- Attempt different methods of conjugation
- Determine the species of other isolates
- Determine the identity of the plasmid

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