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Isolation of MRSA from the Oral Cavity of Companion Dogs

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Abstract

There is currently a great deal of concern about the methods of transfer of methicillin resistant *Staphylococcus aureus* (MRSA) in the community and in healthcare facilities. Household dogs have repeatedly been shown to be carriers of MRSA. Most studies have identified colonization by sampling the nares of dogs but there is little data on the presence of the pathogen in the oral cavity. It seems more likely that a companion dog will lick the skin or face of an owner as a show of affection rather than rub its nose on the owner. A total of 63 companion dogs were sampled by swabbing the mouth. Two dogs were found to have been colonized by MRSA for a 3.2% positive rate. This finding may be significant when compared to some studies and insignificant when compared to others as the colonization rate in the nares of dogs varies widely from 0% to 9% depending on the setting and the geographical location of the study.

Abbreviations

MRSA: methicillin resistant *Staphylococcus aureus*; CA-MRSA: community acquired or community associated MRSA; HA-MRSA: Healthcare acquired MRSA; MSA: mannitol salt agar

Introduction

The development and widespread use of a variety of new generation antibiotics has given rise to some strains of bacteria that have become resistant to many currently used antimicrobials [1]. Of particular concern are strains of *Staphylococcus aureus* that have become resistant to beta-lactam antibiotics over the past few years [2]. Known as methicillin resistant *Staphylococcus aureus* (MRSA), these bacteria are becoming a significant source of morbidity in the healthcare setting. MRSA isolates have been shown to be responsible for osteomyelitis, pneumonia, skin infections, arthritis, endocarditis, gastroenteritis, abscesses, and in some cases, even necrotizing faciitis [3-8].

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Originally traced to colonization in healthcare settings, it has become apparent that colonization also occurs outside of healthcare settings in the community [9]. Colonization occurring in the healthcare setting is referred to as healthcare-associated MRSA (HA-MRSA) while MRSA acquired outside the healthcare setting is referred to as community-acquired MRSA (CA-MRSA). It has been documented that community transmission of MRSA can occur by sharing soap, razors, towels, and sports equipment [10].

Because many households have pet dogs, it may be prudent to determine the number or percentage of pet dogs that might be oral MRSA carriers. This CA-MRSA source would be a serious risk factor for persons returning home from the hospital, persons that are non-ambulatory or persons that have some type of immunodeficiency that are living at home. A MRSA colonized therapy dog (a well behaved pet dog taken into healthcare facilities to meet and greet patients or residents) might also be a source of HA-MRSA infection for debilitated residents confined to a healthcare facility or long term care facility.

There have been several studies that have identified dogs as carriers of MRSA but most of these studies cultured the nares (openings of the nasal passage). One study looked at the mouth [11] as sources of MRSA. It is unknown if substances in a dog's saliva will reduce or eliminate MRSA while it remains viable in the nares. Also, results on dogs and other domestic animals like horses and cats have varied widely so there may be significant regional differences in carrier rates. For dogs, one study showed 1.1% carriage rate in Ireland [12] while another in the United Kingdom showed a carriage rate of 8.9% [11] and yet another study in Slovenia showed no evidence of MRSA in 60 household dogs that were sampled [13].

Some dogs will lick their human companions as a show of affection, and thus it would be enlightening to see if companion dogs actually carry MRSA in their mouths as that could potentially be a direct source of transmission. Even if the pet owner was the source of colonization for the dog, the animal would continue to be a possible source of infection if the owner was treated and resolved the infection. Additional studies of this nature could further define the risk factors associated with the transfer of these resistant bacteria from household pets to humans [14]. In the absence of conclusive evidence to the contrary, it is suspected that the carriage rate in the mouth might be lower than that carried in the nares as digestive enzymes in the dog's saliva might inhibit bacterial growth.

Materials and Methods

Approval for this study was granted by the Institutional Animal Care and Use Committee at Texas State University, San Marcos, Texas using the protocol IACUC201554499. Approximately 60 pet dog owners were recruited for this study.

To prevent injury to the animal by forcing a swab into the dog's mouth, the owners were instructed to remove a sterile swab (BBL Culture Swab™ kit containing Amies, Stuart, and Cary-Blair media) from the package and dab a little food product from a freshly opened can of dog food onto the swab. The dog was encouraged to lick the dog food off of the sterile swab. Pet owners were given an instruction sheet that explained the collection procedure. They were instructed not to touch the swab to anything but the food and the dog's tongue. The swab was to be carefully replaced in the sterile tube containing transport media.

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Prior to this, the principle investigator tested five different randomly selected brands of canned dog food for bacteria and found all to be sterile showing no growth after 48 hours incubation on Remel TSA with sheep blood agar™ . The blood agar plate was used to see if there was contamination of the dog food by any species of bacteria.

Swabs were returned to the microbiology section of the laboratory for streaking. Testing for all pathogens was out of the scope of this investigation. As this study only focused on isolating MRSA, the initial streak for isolation was done on BBL mannitol salt agar™ (MSA) to selectively grow and differentiate *Staphylococcus* species. Suspected yellow *Staphylococcus* colonies were then tested for coagulase using the BBL Staphyloslide Latex Test™. Coagulase positive colonies were subcultured onto a BBL MRSA CHROMagar II™ plate that differentiates MRSA by a mauve colored colony. Mauve colonies were then subcultured onto a BBL Mueller Hinton™ plate. A standard BBL™ 1 µg oxcacillin disc for susceptibility to beta lactam antimicrobials was then placed on the plate and the plate was incubated for 48 hours. Resistant colonies were transported to Central Texas Medical Center, San Marcos, Texas and were tested on bioMerieux's Vitek 2™ for confirmation of MRSA. A cassette is inoculated with the sample and is placed in the instrument for analysis. This automated instrument completes identification and susceptibility testing on each sample and reports the results.

Results and Discussion

As Table 1 illustrates, a total of 63 oral samples from different companion dogs were collected in central Texas. Of the 63 total samples, two were confirmed to be MRSA for a 3.2% positive MRSA rate. There were 25 coagulase positive samples that were presumptively *Staphylococcus aureus*. Of the 25 coagulase positive samples, 8% were confirmed as MRSA.

Table 1. Prevalence of MRSA in Dogs

Country	Source	Investigators	Total Sampled	Positive for MRSA	Percent Positive
U.S.	Oral	Patterson et al	63	2	3.2%
U.K.	Oral	Loeffler et al	45	4	8.9%
Ireland	Nares	Abbott et al	2864	32	1.1%
Slovenia	Nares	Vengust et al	60	0	0%
TOTAL			3032	38	1.3%

For statistical analysis, the results were compared to three other similar studies [11, 12, 13]. One study in the United Kingdom by Loeffler et al, sampled the oral mucosa of dogs and found four positive MRSA isolates out of a total of 45 dogs that were sampled for a positive rate of 8.9% [11]. This is a much higher rate than the 3.2% positive rate this study found. Using an α level of 0.05, the chi-square ($X^2 = 4.01$) analysis suggests the difference between the two rates was statistically significant ($p = 0.05$)

When the colonization rate is determined by sampling the nares, the rates are lower. In a four-year retrospective study (2003-2006) in Ireland by Abbott et al, investigators found only 32 MRSA positive dogs out of 2,864 total for a 1.1% colonization rate [12]. A Slovenian study by Vengust et al found no MRSA isolates out of a total of 60 dogs that were sampled [13]. When this oral study is compared to the combined results of both nares studies, the chi-square ($X^2 = 4.06$) analysis also suggests a significant difference ($p = 0.05$).

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A quick analysis of Table 1 suggests that sampling the oral cavity of dogs results in a higher recovery rate of MRSA than sampling the nares. This finding does not support the idea that there should be less colonization in the mouth due to the presence of lysozyme and perhaps other digestive enzymes that might inhibit bacterial growth in the dog's saliva. Perhaps the bacteria are better able to survive in the mouth due to the presence of food particles and plaque that adhere to the dog's teeth.

It can also be seen that the MRSA carriage rates vary widely by location as might be expected with strains of bacteria being more common in different geographical areas. The studies cited in this investigation and the information gathered by this study suggest that there is a significant difference in MRSA colonization rates of companion dogs depending on geographical location.

> Are the dogs being colonized by their owners or by some other source?

This study only investigated colonization rates. What remains to be discovered is the source of the colonization. Are the dogs being colonized by their owners or by some other source? Also, are the owners being colonized by their pets? It might be enlightening to sample companion dogs and their owners in a single study to determine if one or both are colonized and if they are colonized by the same strain of MRSA.

Conclusion

Three previous studies have shown that the prevalence of MRSA in companion dogs varies widely from 0% to 8. 9% depending on geographical location. Combining these previous studies shows a total positive rate of 1.2%. Although the sample size was smaller than the combined previous investigations, this study found a significant difference in colonization rates. This study found that 3.2% of companion dogs sampled in central Texas were colonized with MRSA. Further studies could investigate the dynamics of transfer of MRSA from owner to pet or from pet to owner.

It is impractical to culture all household dogs but it may be advisable for those persons that are highly susceptible to infection to take extra precautions to minimize the possibility of contracting MRSA from their pet. Washing the hands and exposed skin after handling the dog, not allowing the dog to lick or play bite, and observing the dog for signs of skin infection like redness, swelling, and the presence of pus might be advisable.

Also, therapy dogs that travel to retirement homes, long term medical treatment facilities, and places of that nature might be screened for MRSA. Many residents in these facilities are often elderly and disabled and are at high risk for infection. The spread of MRSA can be minimized if all of the sources of infection are considered and logical steps are taken to prevent the spread of these resistant, virulent bacteria.

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