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Short Communication

Anatomical patterns of colonization of pets with staphylococcal species in homes of people with methicillin-resistant *Staphylococcus aureus* (MRSA) skin or soft tissue infection (SSTI)

S.A. Iverson [a,*], A.M. Brazil [a], J.M. Ferguson [a], K. Nelson [b], E. Lautenbach [c], S.C. Rankin [d], D.O. Morris [d], M.F. Davis [a]

[a] Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, 615 North Wolfe Street, Baltimore, MD 21205, USA
[b] Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, 615 North Wolfe Street, Baltimore, MD 21205, USA
[c] School of Medicine, University of Pennsylvania, 3620 Hamilton Walk, Philadelphia, PA 19104, USA
[d] School of Veterinary Medicine, University of Pennsylvania, 3800 Spruce Street, Philadelphia, PA 19104, USA

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ABSTRACT

Methicillin-resistant strains of *Staphylococcus aureus* (MRSA), *Staphylococcus pseudintermedius* (MRSP), and other pathogenic staphylococci can cause infections in companion animals and humans. Identification of colonized animals is fundamental to research and practice needs, but harmonized methods have not yet been established. To establish the optimal anatomic site for the recovery of methicillin-resistant coagulase positive staphylococci (CPS), survey data and swabs were collected from 196 pets (dogs, cats, reptiles, birds, fish and pocket pets) that lived in households with an MRSA-infected person. Using broth-enrichment culture and PCR for speciation, *S. aureus* was identified in 27 of 179 (15%) pets sampled at baseline and 19 of 125 (15%) pets sampled at a three-month follow-up home visit. *S. pseudintermedius* was isolated from 33 of 179 (18%) pets sampled at baseline and 21 of 125 (17%) of pets sampled at follow-up. The baseline MRSA and MRSP prevalence was 8% and 1% respectively from 145 mammalian pets. The follow-up MRSA and MRSP prevalence was 7% and <1% respectively from 95 mammalian pets. The mouth was the most sensitive single site sampled for isolation of *S. aureus* and *S. pseudintermedius* in mammals. In a subset of pets, from which all available isolates were identified, dual carriage of *S. aureus* and *S. pseudintermedius* was 22% at baseline and 11% at follow-up. These results identify the mouth as the most sensitive site to screen for pathogenic staphylococci and suggest that it should be included in sampling protocols.

1. Introduction

Staphylococcus aureus and *Staphylococcus pseudintermedius* (formerly *S. intermedius*) are receiving attention in both human and veterinary medicine because of increased reports of methicillin resistance. Methicillin resistant *S. aureus* (MRSA) infections have been shown to occur in

* Corresponding author. Present address: USDA-APHIS-NVSL-FADDL, PO Box 848, Greenport, NY 11944, USA. Tel.: +1 631 323 3399; fax: +1 631 323 3366.
E-mail address: sallyann.iverson@aphis.usda.gov (S.A. Iverson).

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animals, and pets are increasingly considered as potential MRSA reservoirs in cases of refractory or recurrent human infections (Loeffler and Lloyd, 2010). Furthermore, methicillin-resistant *S. pseudintermedius* (MRSP) colonization has been reported occasionally in humans (Guardabassi et al., 2004; Frank and Loeffler, 2012). These observations underscore the need for a one health approach to monitor pathogenic staphylococci in both research and clinical settings.

Despite the need for standardized methods to screen companion animals for staphylococcal carriage, particularly animals with known pathogen exposure such as those living with MRSA-infected owners, lack of harmonization in sampling and culture methodologies has limited comparability of the studies published to date. Several studies have considered anatomic site patterns of *S. pseudintermedius* carriage in dogs (Harvey and Noble, 1998; Hartmann et al., 2005; Griffeth et al., 2008; Rubin and Chirino-Trejo, 2011; Paul et al., 2012) and cats (Abraham et al., 2007). However, fewer studies that detail canine and especially feline anatomic carriage site patterns of *S. aureus* are available (Abraham et al., 2007; Griffeth et al., 2008; Fazakerley et al., 2009; Davis et al., 2014). The primary aim of this study was to systematically test the sensitivity of different anatomic sites for the recovery of *S. aureus* and MRSA among companion animals living in the home of a person recently treated for MRSA skin or soft tissue infection (SSTI). The secondary aim was to evaluate anatomic site sensitivity for recovery of *S. pseudintermedius* and other coagulase-positive staphylococci (CPS) among this community population of companion animals not associated with a veterinary healthcare setting. We also explored whether randomization of all humans residing in the household to a decolonization protocol between home visits impacted the prevalence and sensitivity of staphylococcal recovery from pets.

2. Materials and methods

2.1. Household recruitment and questionnaire administration

Households were recruited as part of a randomized-controlled trial (RCT) that targeted human outpatients treated at one of five participating institutions in the United States: two urban adult acute care hospitals, an adult community hospital, an urban children's hospital, and a rural adult and pediatric hospital. Inclusion in the RCT was based on a laboratory-confirmed MRSA skin or soft tissue infection (SSTI) in a human household member. Any household (with or without pets) enrolled in the RCT between January and December of 2012 was invited to participate in the nested study of the household environment and resident animals described here. Participating households were visited twice, approximately three months apart, and as part of the RCT protocol all human household members were randomized to a one-week decolonization treatment, which consisted of twice-daily nasal mupirocin and a chlorhexidine body wash and occurred between visits. Verbal questionnaires were conducted at each visit using an iFormBuilder (iFormBuilder, Herndon, VA) application for iPad (Apple, Cupertino, CA) and data regarding pet-related characteristics were collected.

2.2. Companion animal sampling

At each household visit, all pets were sampled under the supervision of a veterinarian using dry culture swabs with transport media (BBL™ Culture Swabs). Four swabs were collected from each pet from the nares, mouth, inguinal region, and perineum. The swab tip was inserted into the nares, or the nasal planum was swabbed when necessitated by poor patient tolerance or small size of the nares. For the mouth, the tongue, gingiva, or hard palate was swabbed. For inguinal and perineal samples, the swab was rubbed gently against the skin of the appropriate region.

2.3. Bacterial culture

Culture swabs were transported to the laboratory and culture-based laboratory methods consisting of two parallel enrichment arms (optimized for methicillin-susceptible (MS) or methicillin-resistant (MR) isolates) were used for recovery of CPS as previously described (Davis et al., 2012a). Swabs were enriched in a Mueller-Hinton broth + 6.5% NaCl and then (for MR only) a Tryptic Soy Broth + 2.5% NaCl + 3.5 mg/L cefoxitin + 10 mg/L aztreonam. Broths were incubated at 37 °C for 16–20 h and then a 10 μl aliquot was subcultured to BBL™ Columbia CNA agar with 5% sheep blood; plates were incubated at 37 °C for 16–20 h. Presumptive staphylococcal colonies were identified on CNA and all phenotypically unique colonies were subcultured to Baird-Parker (BP) agar. All presumptive CPS (based on BP phenotype and additional tube coagulase testing for MS isolates that did not demonstrate lecithinase activity on BP) were archived to Microbank™ tubes (Pro-Lab Diagnostics, Canada) and held at −80 °C. *S. aureus* ATCC43300, *S. pseudintermedius* ATCC49444, and *S. schleiferi* VHUP1939-05 were used as positive controls for culture and PCR.

2.4. PCR speciation of isolates

A single isolate per visit from each animal was selected by one member of the study team (M.F.D.) for speciation by PCR based on blood agar phenotype. Specifically, hemolytic, gold colonies (presumptive *S. aureus*) from the methicillin-resistant-selective culture arm were chosen over non-hemolytic, non-gold colonies not selected for resistance. To identify *S. aureus*, *S. pseudintermedius*, or *S. schleiferi*, a multiplex PCR assay that amplifies species-specific segments of the nuclease gene (*nuc*) was performed as previously described (Sasaki et al., 2010). Methicillin-resistant isolates (MRSA, MRSP) were determined by presence of a universal *mecA/C* sequence, with ATCC43300 and LGA251 used as *mecA* and *mecC* positive controls respectively (Garcia-Álvarez et al., 2011).