Veterinary Microbiology 160 (2012) 443–448

Contents lists available at SciVerse ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Nasal carriage of *Staphylococcus schleiferi* from healthy dogs and dogs with otitis, pyoderma or both☆

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ARTICLE INFO

Article history:
Received 14 December 2011
Received in revised form 16 June 2012
Accepted 18 June 2012

Keywords:
Methicillin resistance
Nasal carriage
Staphylococcus schleiferi
Pyoderma
Bacterial otitis
Skin disease
Dermatology

ABSTRACT

In veterinary medicine, *Staphylococcus schleiferi* was previously assumed to be an inhabitant of carnivore skin, however, more recently, it has been repeatedly documented in the literature as both an inhabitant and as a pathogen. In order to determine the frequency of nasal carriage, and the methicillin susceptibility pattern of *S. schleiferi* from healthy dogs as well as dogs with otitis and/or pyoderma, a prospective study including 24 dogs with healthy ears and skin, 27 dogs with healthy ears and pyoderma, 15 dogs with otitis without pyoderma and 20 dogs with both otitis and pyoderma was performed. Specimens were obtained and cultured and isolates were identified as *S. schleiferi* based on growth and biochemical characteristics. *S. schleiferi* was isolated from the nares of 1 healthy dog, 3 dogs with recurrent pyoderma, 2 dogs with recurrent otitis, and 1 dog with both recurrent otitis and pyoderma. One of the *S. schleiferi* isolates was methicillin resistant. Nasal carriage of *S. schleiferi* does occur in healthy dogs as well as dogs with otitis and pyoderma. Methicillin resistant and sensitive *S. schleiferi* can be found in the nares of dogs with diseased ears and skin.

1. Introduction

Staphylococcus spp. organisms have been recognized as resident members of the cutaneous microflora of humans and animals (Lee et al., 2003; Scott et al., 2001); in humans, methicillin-resistant and methicillin-susceptible *S. aureus* are the most frequently isolated pathogenic Gram-positive bacteria. In companion animals, methicillin-resistant and methicillin-susceptible *S. pseudintermedius* (Devriese et al., 2005) are the most frequently implicated Gram-positive pathogens, however, *Staphylococcus schleiferi* (Frank et al., 2003; May et al., 2005) and other coagulase-negative staphylococcal (CoNS) species are also of concern. The coagulase variable species *S. schleiferi* (*S. schleiferi* subsp. *coagulans* and *S. schleiferi* subsp. *schleiferi*) and other CoNS in particular, are of importance as they have been implicated as emerging pathogens in companion animals and humans, (Kloos and Bannerman, 1994; Scott et al., 2001; Bes et al., 2002) and most importantly, because historically coagulase negative staphylococcal species were considered non-pathogenic and discounted when isolated (von Eiff et al., 2002; Piette and Verschraegen, 2009). Nosocomial infections in humans have been linked to both subspecies of *S. schleiferi* and other CoNS, and their isolation is now recognized as pertinent, considering the frequency with which multidrug resistance is noted in association with isolates recovered (Koksal et al., 2009; Morris et al., 2006).

Studies have attributed acquisition of resistance to bacterial exposure by means of food sources (Wegener et al., 1999), as well as indirect transfer of genes coding for antimicrobial resistance (Kruse, 1999). Because of close contact among humans and pets, it is logical to suspect human and pet bacteria share the same environment. The

☆ Presented in abstract form at the 21st Annual North American Veterinary Dermatology Forum, Palm Springs, CA, April 2006.

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http://dx.doi.org/10.1016/j.vetmic.2012.06.020

most common carriage site for *S. aureus* in humans is the anterior nares (Wertheim et al., 2005) and it is known that both dogs and cats appear to be capable of supporting carriage of methicillin-resistant *S. aureus* after exposure to infected humans (Manian, 2003; Morris et al., 2012). Thus, the purpose of the study reported here was to determine the frequency of isolation from the nares as well as clinical lesions, and to include the methicillin susceptibility pattern of *S. schleiferi* from clinically normal dogs as well as dogs with ear disease and/or pyoderma, with or without previous antibiotic exposure.

2. Materials and methods

2.1. Dogs

A total of 86 client-owned dogs greater than 1 year of age were entered into the study, with the informed consent of their owners. The protocol was reviewed and approved by the Institutional Animal Care and Use Committee. Dogs were assigned to 1 of 4 groups: 24 dogs with healthy ears without otitis and without pyoderma, 27 dogs with healthy ears with pyoderma, 15 dogs with otitis but without pyoderma, and 20 dogs with both otitis and pyoderma. Dogs allocated to the healthy group could not have any history of ear or skin disease for at least 12 months prior to enrollment, and could not receive any medications other than routine heartworm, flea and/or tick preventatives for the same 12 months prior. Dogs were chosen for the study based on presence or absence of otitis and otic cytology findings. All dogs had otic cytology performed for yeast and bacteria on both ears as well as otoscopic examination. To be included in a group without otitis, dogs were required to have normal otic cytology results and normal otoscopic examinations without clinical symptoms associated with otitis for at least 12 months prior to enrollment. All dogs had at least 1 ear cultured, using a micro tip culturette[1] gently inserted to the level of the junction between the vertical and horizontal ear canal and rotated for a minimum of 3 s. In the case where cytology varied greatly between ears (different proportions of cocci and rods), both ears were cultured. Dogs allocated to a pyoderma group were diagnosed with pyoderma by a boarded dermatologist and had clinical lesions consistent with pyoderma (papules, crusted papules, pustules or epidermal collarettes). Demodicosis was ruled out with a minimum of 3 deep skin scrapings, trichogram evaluations were not suggestive or supportive of dermatophytosis, and lesion cytology revealed degenerate neutrophils with the presence of intracellular cocci. If dogs were diagnosed with pyoderma concurrently, skin culture was performed to determine if ear and skin pathogens were similar. Briefly, a minimum of 3 lesions consistent with bacterial pyoderma (papule, crusted papule, pustule or epidermal collarette) were selected and sampled utilizing one sterile 25-gauge needle per lesion to gently prick the lesion or lift the scale or crust, if present, and the expressed material was lightly swabbed with a micro tip culturette[2]. Cytology of each site sampled was performed following collection to ensure bacterial lesions were selected for culture. Any previous topical or oral treatments, specifically those including antimicrobials, but including all administered for 3 months prior to enrollment, were recorded. Infections were defined as recurrent if compatible clinical signs existed and there had been treatment previously. In addition, all dogs had both nares sampled for culture, utilizing the same culturette swab[3] for both nares. The swab was gently inserted into the dorso-medial aspect of the nostril, rubbed gently against the mucosal surface for 3 s, and then removed. The second nares were sampled in identical fashion.

2.2. Identification of S. schleiferi

Standard methods for bacterial culture were used; all staphylococcal species isolated were recorded. Organisms obtained through bacterial culture were initially identified as staphylococci on the basis of growth and colony characteristics on primary plating medium. Colonies considered typical of *S. schleiferi* were opaque, off-white, $\geq$1 mm in diameter after 24 h of incubation, and usually surrounded by a double zone of hemolysis on blood agar medium. All visually distinct colony types were selected for identification. Colonies were identified biochemically as *S. schleiferi coagulans* on the basis of positive coagulase and Voges-Proskauer test results and negative maltose, trehalose, and lactose fermentation test results (Chanchaithong and Prapasarakul, 2011). Isolates with results matching those expected for *S. schleiferi coagulans*, except for negative coagulase test results, were identified as *S. schleiferi schleiferi* with a commercial biochemical identification system[2], however, results were not reported to the level of subspecies here, owing to the challenges associated with differentiating between them (Cain et al., 2011a). Genotyping of isolates was not performed; therefore isolates from dogs that were phenotypically consistent with *S. intermedius* were identified as *S. pseudintermedius*, as recommended (Sasaki et al., 2007; Devriese et al., 2009; Hermans et al., 2010; Bond and Loeffler, 2012).

2.3. Methicillin susceptibility determination

Oxacillin was used as the isoxazolyl penicillin class representative for indirectly judging methicillin susceptibility. Oxacillin susceptibility testing was performed, according to National Committee for Clinical Laboratory Standards guidelines, using the disk diffusion method or an automated broth microdilution susceptibility testing system.[3] Interpretive breakpoints used were those established for human isolates of coagulase-negative *Staphylococcus* spp. ($\leq$0.25 μg/mL for the broth microdilution

[1] BBL CultureSwab™ Collection and Transport System, Becton Dickinson and Company, Sparks, MD.

[2] BBL Crystal Gram Positive ID System/GP, Becton Dickinson Microbiology Systems, Cockeysville, MD.

[3] Sensititre COMEQ2F plate, Trek Diagnostics Inc., Cleveland, OH.