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BRIEF REPORT

Carriage of *Staphylococcus pseudintermedius* by clinically normal dogs in Canterbury, New Zealand

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Abstract

Aims: To investigate the frequency of carriage of methicillin-susceptible and methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) in a population of clinically normal dogs within the Christchurch and wider Canterbury region, an area in which MRSP has been detected.

Methods: Buccal and perianal swabs were collected from 126 clinically normal dogs presenting at veterinary clinics in the Christchurch/Canterbury region for de-sexing or routine vaccination. *S. pseudintermedius* was isolated by selective culture. Isolates were tested for susceptibility to 12 antimicrobials by disc diffusion.

Results: *S. pseudintermedius* was isolated from 92/126 (73.0 (95% CI = 64.4–80.5)%) dogs, with 38/126 (30.2 (95% CI = 22.3–39.0)%) positive dogs carrying *S. pseudintermedius* at both sampled sites. More animals (78/126; 61.9; (95% CI: 52.8–70.4)%) had positive mouth cultures than positive perianal region cultures (52/126; 41.3 (95% CI: 32.6–50.4)%). No MRSP was isolated from clinically normal dogs. However, resistance to penicillin (106/130 (85.1%) swabs) and tetracycline (33/130 (25.4%) swabs) was seen.

Conclusions: The majority of the dogs in this sample were carriers of *S. pseudintermedius*. However none of these isolates were MRSP.

Clinical relevance: While most clinically normal dogs in the studied region are likely to be carriers of *S. pseudintermedius*, only a small proportion, if any, are likely to be carriers of MRSP. Antibiotic stewardship practices may be important to maintain low-level circulation of drug-resistant bacterial lineages.

Keywords: *Staphylococcus pseudintermedius*; dog; carriage; antimicrobial resistance

Abbreviations: MRSP: Methicillin-resistant *Staphylococcus pseudintermedius*; MSSP: Methicillin-susceptible *Staphylococcus pseudintermedius*

Introduction

Staphylococcus pseudintermedius has been described as 'the most common bacterial pathogen in dogs' (Moodley *et al.* 2014). *S. pseudintermedius* may cause pyoderma (Bryan *et al.* 2012), otitis externa (Bugden 2013) and other infections, which may be clinic/hospital-associated (Lehner *et al.* 2014). *S. pseudintermedius* may also be a zoonotic pathogen (Van Hoovels *et al.* 2006; Lozano *et al.* 2017). Reported carriage rates of *S. pseudintermedius* in healthy dogs are very broad, ranging between 16% in dogs from the United Kingdom (Harvey and Noble 1994) and 87% in Canadian dogs (Rubin and Chirino-Trejo 2011). In dogs with *S. pseudintermedius*-associated infections, the infecting strain has been reported as being genetically very similar to that being carried (Pinchbeck *et al.* 2006). Methicillin-resistant and multi-drug resistant (defined as an isolate with resistance to antibiotics of three or more different classes) *S. pseudintermedius* have been reported globally (McCarthy *et al.* 2015), including from clinically normal dogs at rates of approximately 3% (Grönthal *et al*. 2015; Kjellman *et al.* 2015). There is no data on rates of carriage of *S. pseudintermedius* in New Zealand. However, our previous study on *S. pseudintermedius* isolated from diagnostic samples from clinically affected dogs in New Zealand showed that only 40% of isolates were susceptible to all the tested antibiotics and that 38% were methicillin-resistant due to carriage of the *mecA* gene (Nisa *et al.* 2019). The antibiotic resistance status of an infecting *S. pseudintermedius* isolate is suggested to be unrelated to the virulence of the organism (Bryan *et al*. 2012); however, resistance to antibiotics, especially to those used as first- and second-line treatments, is a risk for treatment failure and can result in poor clinical outcomes (Bryan *et al.* 2012; Bell *et al*. 2016).

The aim of this study was to investigate the carriage of methicillin-susceptible (MSSP) and methicillin-resistant (MRSP) *Staphylococcus pseudintermedius* in clinically normal dogs in Canterbury, New Zealand, where significant levels of MRSP have been detected (Nisa *et al.* 2019). We hypothesised that clinically normal dogs would carry *S. pseudintermedius* and the proportion of MRSP would be similar (3%) to that seen in international studies (Grönthal *et al.* 2015; Kjellman *et al.* 2015).

Materials and methods

Convenience sampling of dogs

All veterinary practices in the Canterbury region of New Zealand (including the large urban centre, Christchurch, and smaller urban and rural areas) for whom contact details could be obtained were asked to invite owners of clinically normal dogs presenting for de-sexing or routine vaccination at veterinary clinics to participate in the study. Veterinarians were asked not to sample dogs with signs of skin infection or multiple dogs from the same household. The dog's name, age and breed data were collected and two swabs (flocked swabs with liquid Amies; Copan, Brescia, Italy) were taken by the veterinarian or veterinarian paraprofessional, one from the mouth (buccal) and one from the perianal region. Swabs were shipped at ambient temperature to mEpiLab, (School of Veterinary Science, Massey University, Palmerston North, NZ). This was a cross-sectional survey with sampling taking place between July and November 2018. The study was approved by Massey University Animal Ethics Committee protocol number 18/12.

Isolation and identification of S. pseudintermedius

All incubations were in aerobic conditions at 35°C. Swabs were incubated overnight in mannitol salt broth (Fort Richard Laboratories, Auckland, NZ) and the broth was then subcultured to Chromagar Staph and Chromagar MRSA plates (Fort Richard Laboratories) overnight. Colonies resembling *S. pseudintermedius* (pink or pink-tinged on the Chromagar) were subcultured on Columbia horse blood agar (Fort Richard Laboratories) and frozen in 15% glycerol broth at -80°C. Up to four colonies (two from each plate if present) from each swab were subcultured. Isolates were identified by MALDI-TOF as previously described (Nisa *et al.* 2019).

Antimicrobial susceptibility testing of S. pseudintermedius

One *S. pseudintermedius* isolate from each positive swab was tested against a panel of 12 antimicrobials by disc (Oxoid, Hampshire, UK) diffusion on three Mueller-Hinton agar plates (Fort Richard Laboratories) using CLSI methodologies (CLSI 2019). As recommended by CLSI (2019), the results of the oxacillin disc were taken as a proxy for the presence/absence of resistance to methicillin.

Statistical analysis

Prevalence was calculated by binomial CI normal approximation. Difference and agreement of carriage rates between sites were determined by applying the χ^2 test and the kappa statistic. All statistical and exploratory analyses were performed in R v4.2.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Characteristics of practices and dogs

Fourteen veterinary practices were approached and nine took part in this study. Six practices were in Christchurch and suburbs ('urban') while three were in smaller towns/villages ('rural'). The urban practices provided swabs from 51 dogs while the rural practices sent swabs from 75 dogs. The most frequently sampled breeds were Labrador/Labrador crosses (17/121), followed by Bichon Frisé/Bichon Frisé crosses (8/121), Huntaway/Huntaway crosses (8/121) and cross-breeds (8/121). The median age was 5 years (min 9 weeks, max 15.25 years).

Isolation and identification of S. pseudintermedius

Staphlococcus pseudintermedius was isolated from 92/126 (73.0 (95% CI = 64.4–80.5)%) dogs, with 38/126 (30.2% (95% CI = 22.3–39.0)%) positive dogs carrying *S. pseudintermedius* at both sampled sites. Carriage rates were significantly different when comparing buccal and perianal swab sites ($p<0.001$), with more animals (78/126; 61.9 (95% CI = 52.8–70.4)%) having positive mouth cultures than positive perianal region cultures (52/126; 41.3 (95% CI = 32.6–50.4)%). The swab site test similarity kappa score was 0.177 (95% CI: =0.021–0.333) suggesting minimal agreement between sites. Most (239/240) *S. pseudintermedius* isolates were made from Chromagar Staph plates while one *S. pseudintermedius* isolate was made from the Chromagar MRSA plates.

Antimicrobial susceptibilities of isolates

Of the 130 isolates of *S. pseudintermedius* tested for antimicrobial susceptibility, including the single isolate from Chromagar MRSA plates, none (sampled population proportion 0 (95% CI =0–2.9)%) showed the resistance to oxacillin that would be expected for MRSP. MSSP was isolated from 92/126 (73.0 (95% CI = 64.4–80.5)%) sampled dogs.

Isolates resistant to penicillin (106/130; 85.1%), tetracycline (33/130; 25.4%), trimethoprim (2/130; 1.5%), clindamycin (3/130; 2.3%), chloramphenicol (1/130; 0.8%), gentamicin (1/130; 0.8%) and erythromycin (3/130; 2.3%) were identified. No resistance to enrofloxacin, ciprofloxacin, cefpodoxime or rifampicin was detected. Two isolates were resistant to antibiotics from three different classes, thus were classified as multi-drug resistant.

Discussion

Canine pyoderma is the condition for which antimicrobials are most frequently used in small animal clinical practice (Guardabassi *et al.* 2008). We found no resistance to oxacillin or to the third-generation cephalosporin cefpodoxime in the 130 isolates of *S. pseudintermedius* isolated from 126 clinically normal dogs. This means that the first-line antimicrobials amoxicillin-clavulanate and cephalosporins, which are often used empirically, should be clinically efficacious in clearing infections caused by these wild-type *S. pseudintermedius.* However, the pervasiveness of resistance to penicillin (85%) means that use of this antimicrobial is unlikely to be clinically

successful, while the frequency of resistance to tetracycline (25%) suggests antimicrobial susceptibility testing should be performed before use.

Although direct comparison of two populations of staphylococci separated temporally and by clinical condition is beyond the scope of this study, our findings suggest that the population of *S. pseudintermedius* carried by dogs without frank skin disease may not be the same as those isolated from dogs diagnosed with *S. pseudintermedius*-associated disease (Nisa *et al.* 2019). However, a clinical history of antibiotic therapy within the last 6 months has been associated with the isolation of antimicrobial resistant staphylococci from dogs (Huerta *et al.* 2011), and genes associated with resistance to tetracycline may be co-located within the methicillin-resistance SCC*mec* cassette (Li *et al.* 2011), suggesting that antibiotic treatment may select for MRSP with co-selection for other antimicrobial resistance. The clinical history of dogs presenting with potential *S. pseudintermedius*-associated infections must be considered by veterinarians, and culture with antimicrobial susceptibility testing performed if there may have been selection for antimicrobial resistance.

To further understand the mechanisms of MRSP-associated disease in dogs, a prospective case-control study with long-term follow up of dogs with pyoderma, from their first presentation, is proposed, collecting multiple isolates and antimicrobial and other treatment data.

The cross-sectional design, geographical constraints, biases involved with healthcare-seeking owners, possibility that dogs came from the same household, and the relatively low sample number are all limitations of this study, reducing the generalisability of the conclusions to dogs throughout New Zealand. Nevertheless, our findings on the carriage of *S. pseudintermedius* provides baseline data and supports the current advice in relation to antimicrobial stewardship for veterinarians. Additionally, MSSP can infect humans (Lozano *et al.* 2017), and the wide-spread carriage of these organisms by canine companion animals means vulnerable sub-populations such as the elderly may be at risk. A ‘One Health’ approach encompassing surveillance of *S. pseudintermedius* carriage and disease in humans and companion animals is required for analysis of this risk.

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