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First report of a *mecA*-positive multidrug-resistant *Staphylococcus pseudintermedius* isolated from a dog in New Zealand

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Abstract

CASE HISTORY: A 14-year-old neutered male Sealyham terrier was referred for assessment of a persistent pyoderma. It had experienced numerous episodes of dermatitis involving pododermatitis, pyoderma and otitis over the previous 6 years.

CLINICAL FINDINGS: Superficial, focally deep and mucocutaneous pyoderma were present, with yellow mucoid exudate on both nares and the lower lips crusted with haemopurulent exudate. Epidermal collarettes were present on the dorsal and lateral trunk. There were peri-anal crusts and mild erythema was present on the concave aspect of both pinnae.

MICROBIOLOGICAL FINDINGS: Culture and microbiological testing identified *Staphylococcus pseudintermedius* as the infecting organism. Kirby-Bauer disc susceptibility testing revealed the isolate was resistant to numerous antimicrobials including oxacillin. PCR testing of the isolate identified the presence of the *mecA* gene which confers resistance to β-lactam antimicrobials. Pulsed field gel electrophoresis typing suggested the isolate was not related to the methicillin-resistant *S. pseudintermedius* that had been reported to be associated with canine infections in Western Australia.
DIAGNOSIS: Superficial, deep and mucus membrane pyoderma associated with a multi-drug resistant *S. pseudintermedius*.
CLINICAL RELEVANCE: This is the first recorded case of canine pyoderma involving methicillin-resistant multidrug-resistant *S. pseudintermedius* in New Zealand. Treatment of such cases is difficult because the number of effective and available antimicrobials is limited. This finding should raise the awareness of the veterinary and medical professions to the presence of such organisms in New Zealand and stimulate a discussion about possible biosecurity barriers, treatment strategies and prevention of zoonotic and nosocomial infections.
KEY WORDS: *Staphylococcus pseudintermedius*, MRSP, canine pyoderma, mecA, multi-drug resistant

Introduction

Approximately 50% of healthy dogs are colonised by *Staphlococcus pseudintermedius* (Saijonmaa-Koulumies and Lloyd 1995; Harvey and Noble 1998). Although primarily isolated from the oral, nasal and peri-anal regions, transient colonisation of other sites may occur. *S. pseudintermedius* is an opportunistic pathogen and may cause canine skin and ear infections. The *S. pseudintermedius* strain causing an infection is usually the same as the colonising strain (Pinchbeck *et al*. 2006). Antimicrobial treatment may include the use of oral and topical antimicrobials, and biocides. In New Zealand β-lactam antimicrobials such as amoxicillin-clavulanic acid and cephalosporins are empirically used.

Microbiological culture is reported to not be routinely performed for suspected canine pyoderma and is reserved for cases that respond poorly to antimicrobials or relapse frequently (Pleydell *et al*. 2012). Isolates of *S. pseudintermedius* resistant to methicillin were initially reported in the early to mid 2000s in the United States of America, Europe and Asia, and have subsequently become a significant problem in veterinary medicine (Frank and Loefffler 2012). Similar to methicillin resistance in *Staphylococcus aureus,* resistance in *S. pseudintermedius* is mediated by the production of a modified penicillin binding protein (PBP2a) which is produced by the *mecA* gene. *MecA* is located on a chromosomal mobile element called the staphylococcal chromosomal cassette which can be transferred between staphylococci (Harrison *et al.* 2014). Regional clonal spread of methicillin-resistant *S. pseudintermedius* (MRSP) has been reported in several countries including Australia (Perreten et al. 2010; Siak *et al.* 2014). Globally, MRSP are usually resistant to multiple classes of antimicrobials and can be resistant to all veterinary-licensed antimicrobials (van Duijkeren *et al*. 2011). There is no evidence that MRSP are more virulent than methicillin-susceptible *S. pseudintermedius,* but successful antimicrobial treatment of canine pyoderma associated with MRSP can be difficult.

Here we report the first isolation of *mecA*-positive multidrug-resistant *S. pseudintermedius* in New Zealand.

Case History

A 14-year-old, castrated, Sealyham terrier dog, from the Waikato region of New Zealand had experienced numerous episodes of dermatitis involving pododermatitis, pyoderma and otitis over 6 years, commencing prior to 2006.

Dermatological investigations included food trials over 6–8 weeks using a hydrolysed diet. No change in pruritus resulted. Microbiological culture of an ear exudate in November 2011 identified *Staphylococcus intermedius* group bacteria assumed to be *S. pseudintermedius*. The organism was resistant to neomycin, framycetin and oxacillin. Atopic dermatitis was thought to underlie the predisposition to pyoderma.

Treatment of the skin and ear problems included 10 mg per day oral prednisone (Apo-Prednisone, Apotex NZ Ltd, Auckland, NZ) and antimicrobials; including 600 mg oral cephalexin twice daily, 625 mg twice daily amoxycillin/clavulanate (Vetamox, Ethical agents, Auckland, NZ) and on one occasion 75 mg once daily oral enrofloxacin (Baytril, Bayer NZ Ltd, Auckland, NZ). Episodes of otitis were treated topically with antimicrobial/steroid/antifungal proprietary preparations. When ear cytology indicated overgrowth *Malassezia* spp. 100 mg oral ketoconazole (Nizoral, Janssen-Cilag, Auckland, NZ) was prescribed. A good response was obtained to the treatments although signs recurred after variable intervals.

Mucocutaneous pyoderma became a major feature in late 2013 and did not resolve following several courses of oral cephalexin, each >2 weeks in duration, over several months. Culture of the nasal exudate in January 2014 revealed a *S. intermedius* group isolate that was sensitive to oxacillin, cephalothin, erythromycin, tetracycline, clindamycin, enrofloxacin and trimethoprin/sulpha. The pyoderma failed to resolve and the dog was subsequently referred to the Allergy and Dermatology Clinic, Auckland.

Clinical findings

The dog appeared well on presentation at the Allergy and Dermatology Clinic on the 2 May 2014. The owner reported good exercise tolerance, normal appetence, consistent weight and unchanged water intake.

On examination, a yellow mucoid exudate was present about both nares which were crusted dorsally. The lower lips were crusted with haemopurulent exudate. Epidermal collarettes were present on the trunk with more lesions dorsally and laterally than ventrally. There were peri-anal crusts. Mild erythema was present on the concave aspect of both pinnae. The feet were normal. Peripheral lymph nodes were normal size.

Cytology, performed on the nasal exudate and skin lesions, revealed neutrophils and numerous cocci. Red blood cells were also present in exudate from the lower lip lesions. Trichograms involving approximately 200 hair shafts were negative for *Demodex* mites.

A diagnosis of mucocutaneous pyoderma, superficial spreading pyoderma and focal deep pyoderma was made. Interim treatment was 100 mg cefpodoxime (Simplicef, Zoetis, Auckland, NZ) given orally once per day.

Microbiological findings

Staphylococcus pseudintermedius was isolated from the nasal exudate. Colony morphology, when cultured aerobically on blood agar was typical for staphylococci. The colonies were catalase-positive, DNase-positive, hyaluronidase-negative and polymyxin B-sensitive, indicating a *Staphylococcus* species from the intermedius group. Identification as *S. pseudintermedius* was performed using matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF-MS) (Decristophoris *et al.* 2011), using a Bruker Daltonik GmbH microflex, version 1.3 (Bruker Daltonik GmbH, Bremen, Germany) with the dervied spectra compared to a validated reference database (Maldi biotyper version 3.1; Bruker Daltonik GmbH).

Antimicrobial susceptibility testing was performed using Kirby-Bauer disc diffusion. Zone diameters were interpreted according to the Clinical Laboratory Standards Institute (Anonymous 2008, 2010). The isolate was resistant to numerous antimicrobials including oxacillin (Table 1). Resistance to oxacillin (a surrogate for methicillin) can sometimes occur in isolates without the *mecA* gene (Frank and Loeffler 2012), so the isolate was referred to the Institute of Environmental Science and Research (Porirua, NZ). The presence of the *mecA* gene was confirmed by PCR according to the method of Maes *et al.* (2002).

Swabs of the owner's nose were collected by their medical practitioner and were negative for *S. pseudintermedius* when tested in a medical laboratory. Swabs of the nose, lips and anus of the other dog in the household were similarly negative when tested by Gribbles Veterinary Pathology (Hamilton, NZ).

Genetic relatedness of the isolate to MRSP isolated in Western Australia (Siak *et al.* 2014) was determined using pulsed field gel electrophoresis (PFGE). The PFGE was carried out in the same laboratory and under the same conditions as described by Siak *et al.* (2014), and used *Sma*I (Roche

Diagnostics, Auckland, NZ) and *cfr*91 (ThermoFisher Scientific, Scoresby, Victoria, Australia) restriction enzymes as described by Perreten *et al*. (2013). The pulse times were 5–40 seconds over 18 hours and 20–25 seconds over 5 hours. Chromosomal patterns were examined visually and scanned with a Quality One device (Bio-Rad Laboratories Pty Ltd, Auckland, NZ). The unweighted pair group method with arithmetic mean was used to assess relatedness, with settings for tolerance and optimisation of 1.25% and 0.5%, respectively. Isolates with ≥80% similarity were considered the same pulsotype. The PFGE pulsotype of the isolate from the case described here was found not to be related to the Western Australian isolates, on the basis of <80% similarity. The latter strain was related to the internationally known sequence type (ST)68 (Siak *et al.*2014).

Treatment

Oral antimicrobial treatment was changed on the 10 May 2015 to 100 mg per day rifampicin (Rifadin, Aventis, Auckland, NZ) given orally for 2 weeks. The choice of rifampicin was based on the results of Kirby-Bauer sensitivity tests, the first author's 20-year experience of its use in deep pyoderma, its ability to achieve effective concentrations in neutrophils (Nielson and Black 1999) and evidence that it has the potential to kill staphylococci in carriage sites (Mollema *et al.* 2010). It was planed to be used in 2-week-course separated by a 2-week interval to minimise the risk of hepatoxicity. A topical cream containing mupirocin (Bactroban Cream, GlaxoSmithKline Ltd, Auckland, NZ) was applied to the nose, lips and anus 2–3 times daily. Weekly washing with a chlorhexadine shampoo (Pyohex, Zoetis) was also carried out.

After the 2-week course of rifampicin, treatment with 100 mg per day cefpodoxime was initiated. A clinical response to the first 2 weeks of rifampicin treatment was observed, but the change to cefpodoxime resulted in exacerbation of the lesions after 1 week. The choice of cefpodixime was based on the intermediate Kirby-Bauer sensitivity to cefitoxin and was made before the PCR result was available. It was substituted on 13 June 2014 with approximately 13 mg/kg enrofloxacin (Baytril, Bayer NZ Ltd) given once daily. Toward the end of this phase of treatment, the nasal, skin and anal lesions had resolved although some crusting remained on the lower lips.

Inappetance and lethargy were observed at this time and blood and urine tests were performed. The urine specific gravity was 1.047, with high protein concentrations and moderate blood present. A complete blood count showed a moderate anaemia that was strongly regenerative. Further urine testing showed a urine protein:creatinine ratio of 1.0 (>0.5 is considered consistent with renal disease). The dog collapsed the next day with a temperature of 41°C. Ultrasonographic examination detected an abnormality of one kidney. The owner declined further testing and requested euthanasia on 24 June 2014. Post-mortem examination was not permitted.

Discussion

Globally, infection with MRSP is a major clinical problem for dogs, owners and veterinarians. Clonal spread has been a feature in many regions with ST71 the most frequently identified clone in Europe and ST68 in North America (Perreten *et al*. 2010). In addition to *mecA*, most MRSP strains carry resistance genes to several different classes of antimicrobials (Perreten *et al*. 2010).

While nosocomial infections do occur, transmission is primarily from asymptomatic carriers to infection-vulnerable dogs (Pinchbeck *et al*). Dogs may be colonised by MRSP for >12 months after infection (Windahl *et al*. 2012). MRSP does not appear to be more virulent than methicillin-susceptible *S. pseudintermedius*. Treatment of MRSP carriers is not recommended in countries where MRSP has become established. (Frank and Loeffler 2012). However in countries with low MRSP prevalence, decolonisation treatment may be a reasonable strategy to limit its transmission.

Strategies need to be adapted to particular environments to prevent the transmission of MRSP in low prevalence countries. In New Zealand there are many opportunities for dogs to interact freely; dog competitions, day-care facilities and boarding kennels all present particular risks for exchange of *S. pseudintermedius*. Veterinary clinics are a risk because they will have more infection-vulnerable dogs than in the wider environment. Preventive measures may include scheduling appointments at veterinary clinics for pyoderma-affected dogs distant from debilitated or pyoderma-prone dogs, in addition to hygiene measures within the facility.

Because MRSP is frequently multidrug resistant, treatment of MRSP-associated pyoderma can be difficult. For this reason it would seem reasonable for an island nation to create barriers to the importation of overseas clones that have spread epidemically. In the case described here the organism was not detected in the carriage sites of the owner or companion dog, and no further MRSP were isolated in the following 15 months (C. Douglass, unpublished data), suggesting that this isolate was unlikely to be a successful clone. One major veterinary pathology laboratory in New Zealand has been using oxacillin Kirby-Bauer discs routinely for >2 years (C. Douglass, unpublished data) and the other reported no evidence of multidrug resistant *S. pseudintermedius* (G. D'Amours, pers. Comm[1]. It is unlikely that successful MRSP clones are present in New Zealand currently. This contention is supported by data from both laboratories which together culture approximately 60 *S. intermedius* group isolates weekly.

Identification of this single MRSP isolate over a 2-year period contrasts with the dramatic emergence of MRSP in dogs internationally (Beck *et al.* 2012). The emergence of MRSP in Australia is typified by the experience of a dermatology referral practice in Melbourne which first isolated a canine MRSP in May 2013 and subsequently recorded 95 cases in a 3-month period in late 2014 (Robson 2015). The history of New Zealand having predominantly international strains of MRSA (Heffernan and Bakker 2011), rather than local strains, and the finding that international travel plays a significant role in transmission of MRSA with carrier or infected humans (Zhou *et al.* 2014) would support the use of border controls to detect dogs carrying MRSP.

The unavailability of oral chloramphenicol in New Zealand limits the choice of antimicrobials for treating MRSP infections. Although rifampicin and enrofloxacin were effective in this dog, rifampicin is difficult to use because of potential hepatotoxicity and both rifampicin and fluoroquinalones have been associated with the emergence of antimicrobial resistance. Topical antimicrobials used with shampoos have also been shown to be useful but resistance to some topical antimicrobials has been reported, and topical treatment of deep pyoderma is considered adjunctive rather than primary (Miller *et al.* 2013).

The detection of MRSP in New Zealand is a public health concern, and should prompt increased vigilance in assessing response to antimicrobial treatment of canine pyoderma and the use of microbiological investigation, including susceptibility testing to oxacillin when response is doubtful.

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Notes

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Table 1. Results for antimicrobial susceptibility, determined using Kirby-Bauer disc diffusion, of an isolate of *Staphylococcus pseudintermedius* from the nasal exudate of a Sealyham terrier dog diagnosed with superficial, focally deep and mucocutaneous pyoderma.

Antimicrobial	**Disc strength (µg)**	**Result**
Amoxicillin/clavulanic acid	30	Resistant
Cephalothin	30	Intermediate
Erythromycin	15	Resistant
Oxacillin	1	Resistant
Penicillin	10	Resistant
Tetracycline	30	Resistant
Clindamycin	2	Resistant
Enrofloxacin	5	Sensitive
Trimethoprim/sulphonamide	25	Resistant
Rifampicin	5	Sensitive
Mupirocin	5	Sensitive