Antimicrobial susceptibility of methicillin-resistant *Staphylococcus pseudintermedius* isolated from veterinary clinical cases in the UK

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ABSTRACT

Staphylococcus pseudintermedius is a leading aetiologic agent of pyoderma and other body tissue infections in dogs and cats. In recent years, an increased prevalence of methicillin-resistant *S. pseudintermedius* (MRSP) has been reported. Isolation of MRSP in serious infections poses a major therapeutic challenge as strains are often resistant to all forms of systemic antibiotic used to treat *S. pseudintermedius* -related infections. This study investigates the occurrence of MRSP from a total of 7183 clinical samples submitted to the authors' laboratories over a 15-month period. Identification was based on standard microbiological identification methods, and by *S. pseudintermedius*-specific *nuc* polymerase chain reaction (PCR). Methicillin resistance was confirmed by PBP2a latex agglutination and *mecA* PCR. Susceptibility against non-β-lactam antibiotics was carried out using a disc-diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. In addition, susceptibility to pradofloxacin – a new veterinary fluoroquinolone – was also investigated. SCC*mec* types were determined by multiplex PCR. *Staphylococcus pseudintermedius* was isolated from 391 (5%) samples and 20 were confirmed as MRSP from cases of pyoderma, otitis, wound infections, urinary tract infection and mastitis in dogs only. All 20 isolates were resistant to clindamycin and sulphamethoxazole/ trimethoprim. Nineteen were resistant to chloramphenicol, enrofloxacin, gentamicin, marbofloxacin and pradofloxacin; additionally, seven isolates were resistant to tetracycline. Fifteen isolates carried SCC*mec* type II–III, four isolates had type V and one harboured type IV. To date, only a few scientific papers on clinical MRSP strains isolated from the UK have been published, thus the results from this study would provide additional baseline data for further investigations.

KEY WORDS: Dogs.
Drug resistance, microbial.
SCCmec type.

Introduction

Staphylococcus pseudintermedius is an important and a leading aetiologic agent of pyoderma, otitis, wound infections and other body tissue infections primarily in dogs and to a lesser extent in cats. Previously thought to be caused by *S. intermedius*, multilocus sequencing and DNA-DNA hybridisation has revealed that *S. pseudintermedius* is in fact the most common cause of pyoderma in dogs.[1] The emergence of methicillin-resistant *S. pseudintermedius* (MRSP) has been reported increasingly in dogs, and in recent years human MRSP infections have been reported.[2] Transmission between dogs and staff has been documented at a veterinary hospital,[3] and 4–5% prevalence of MRSP carriage was reported among veterinary dermatology practitioners in the USA and Italy.[4] It is regarded as an important emerging zoonotic agent and its isolation from serious human infections poses therapeutic challenges.

Biochemically, MRSP can be easily misidentified as MRSA, hence the correct identification of this resistant strain in the laboratory is of primary importance. To date, there are no recent published data regarding the antimicrobial susceptibility of clinical *S. pseudintermedius* isolated from the UK since a study by Lloyd *et al*. in 1996.[5]

This study presents the isolation of MRSP from a total of 7183 clinical samples submitted to the authors' laboratories for microbiological examination from February 2011 to May 2012. The antimicrobial susceptibility profile of these isolates against non-β-lactam antibiotics and to pradofloxacin – a new third-generation veterinary 8-cyano-fluoroquinolone introduced in April 2011 in the European Union – is determined. It also identifies the staphylococcal cassette chromosome *mec* (SCC*mec*) type of each isolate and investigates if there is any correlation between SCC*mec* type and antimicrobial resistance patterns.

Materials and methods

Bacterial isolation and identification

Bacterial isolation was carried out using standard protocols from a total of 7183 clinical samples (e.g., swab, tissue, implant, milk, urine) submitted over a 15-month period (February 2011 to May 2012). Columbia and Columbia CNA agar plates with 5% sheep blood (bioMerieux, Hamspshire, UK) were used for primary isolation. Colonies showing typical staphylococcal morphology were Gram stained and subjected to slide and tube coagulase tests (Pro-Lab Diagnostics, Cheshire, UK), and tested for catalase, acetoin and trehalose fermentation. Isolates were verified to be *S. pseudintermedius* by multiplex PCR targeting the *nuc* locus.[6]

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Suspected MRSP isolates were identified as methicillin-resistant by oxacillin (1 µg; Oxoid, Basingstoke, UK) disc susceptibility, detection of penicillin-binding protein 2a (PBP2a) by latex agglutination (Mast Diagnostics, Merseyside, UK) and *mecA* PCR.[7] Unlike with methicillin-resistant *Staphylococcus aureus* (MRSA), cefoxitin disc diffusion with the resistance breakpoint set at ≤24 mm significantly underestimated the presence of *mecA* in *S. pseudintermedius*, as previously reported, and that oxacillin disc diffusion was a much better predictor of methicillin resistance.[8]

Antimicrobial susceptibility testing

All MRSP isolates were tested for their antimicrobial susceptibility by a disc-diffusion method according to the Clinical Laboratory Standards Institute (CLSI) guidelines.[9] As there remains no approved breakpoints for pradofloxacin, the cut-off values determined by the distribution of inhibition zone diameters, recommended by Bayer was used (S: ≥32 mm; I: 20–31 mm; R: ≤19 mm). Non-β-lactam antibiotics tested included chloramphenicol (10 µg), clindamycin (2 µg), enrofloxacin (5 µg), fusidic acid (10 µg), gentamicin (10 µg), sulphamethoxazole/trimethoprim (25 µg) and tetracycline (10 µg) (Oxoid, Basingstoke, UK); marbofloxacin (5 µg; Vetoquinol, Lure Cedex, France); and pradofloxacin (5 µg; Mast Diagnostics, Merseyside, UK). *Staphylococcus aureus* NCTC 6571 and *Escherichia coli* NCTC 10418 (National Collection of Type Cultures, Public Health England, Salisbury, UK) were included as control organisms.

SCC*mec *typing

SCC*mec* typing was determined by a previously described multiplex PCR.[10] In order to assign SCC*mec* types, only M-PCR1 and M-PCR2 were performed to determine the types of recombinase (*ccr*) genes, along with the class of the *mec* gene. The presence of SCC*mec* II–III was demonstrated by the absence of the cadmium resistance operon.[11]

Results and Discussion

A total of 7183 clinical samples were submitted for microbiological examination from February 2011 to May 2012. *Staphylococcus pseudintermedius* was isolated from 391 (5%) samples and 20 (5%) were confirmed as MRSP by resistance to oxacillin, positive PBP2a agglutination and presence of *mecA*. All MRSP were isolated from dogs only. Isolates were recovered from cases of pyoderma, otitis, wound infections, urinary tract infections and mastitis (Table 1).

Antimicrobial susceptibility against non-β-lactam antibiotics showed that all isolates were resistant to clindamycin and sulphamethoxazole/trimethoprim. Except for MRSP isolate number 10, the remaining isolates (95%) were resistant to chloramphenicol, enrofloxacin, gentamicin and marbofloxacin. Seven out of 20 (35%) isolates were additionally resistant to tetracycline. All MRSP isolates were susceptible to fusidic acid only (Table 1).

These findings demonstrated that methicillin resistance in *S. pseudintermedius* is associated with multidrug resistance, as previously demonstrated.[11] In a previous study on the antimicrobial susceptibility of canine staphylococci isolated from the UK, a total of 2296 coagulase-positive staphylococci, 99% of which were presumptively identified as *S. intermedius*, were investigated.[5] In contrast to the results presented here, all surveyed isolates were susceptible to cephalexin and sulphamethoxazole/trimethoprim, and none of the isolates was resistant to amoxicillin/clavulanic acid, enrofloxacin, oxacillin and methicillin. Although MRSP was reported to be susceptible to amikacin, rifampicin, vancomycin, teicoplanin and linezolid, none of these antibiotics are licensed for systemic use in pets.[11] Fusidic acid, a topical antibiotic, is the only active antibiotic against MRSP that falls outside the critically important category set by the World Health Organization (WHO) Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR).[12]

Pradofloxacin is a new third-generation veterinary 8-cyano-fluoroquinolone introduced and approved for use in the European Union in 2011 for the treatment of bacterial infections in dogs and cats. This antibiotic has exhibited enhanced activities against Gram-positive bacteria and more rapid killing of anaerobic bacteria.[13] Recently, the minimum inhibitory concentration (MIC) distribution to pradofloxacin of canine and feline *S. pseudintermedius* was investigated.[14] Only 6/177 isolates, including two MRSP, had MICs >0.25 µg/mL and had single point mutations in *gyrA* and *grlA*. Based on inhibition zone diameter and the cut-off values provided by Bayer, the manufacturer of pradofloxacin, the present results reveal that, except for isolate number 10, which had intermediate pradofloxacin susceptibility, the remaining isolates were resistant. This is alarming because this antibiotic has been indicated for use in dogs for the treatment of wound infections, superficial and deep pyoderma. It should be noted that the cut-off values provided by Bayer are epidemiological values and not clinical break points.

This study also identified the SCC*mec* type of the isolates and determined if there is any correlation between SCC*mec* type and antimicrobial resistance pattern. As there are no published data regarding the SCC*mec* type for *S. pseudintermedius* isolated from the UK, the results were compared with those reported in other European countries.[11,15] Based on these previous studies, it was reported that SCC*mec* type II–III is found in the majority of MRSP strains isolated from dogs and cats in Europe. However, one isolate with an SCC*mec* type IV was reported in Denmark and in The Netherlands, and one isolate with an SCC*mec* type V was reported in Germany and Portugal.[11,16]

The present results follow this pattern as 15/20 isolates carried SCC*mec* type II–III. Interestingly, four isolates harboured SCC*mec* type V and one isolate carried a SCC*mec* type IV (Table 1). SCC*mec* type V was reported to be the predominant SCC*mec* type among MRSP isolated from the USA and Canada.[11] Except for MRSP strains 4 and 16, all strains with SCC*mec* type II–III were only susceptible to tetracycline and fusidic acid. However, all four MRSP carrying SCC*mec* type V, together with strains 4 and 5, were only susceptible to fusidic acid. The MRSP strain with SCC*mec* type IV was more susceptible to antibiotics compared to strains carrying SCC*mec* types II–III and IV, and was only resistant to clindamycin and tetracycline (Table 1).

To the authors' knowledge, this is the first report on the antimicrobial susceptibility and SCC*mec* types of clinical MRSP strains isolated from the UK. Further molecular epidemiological investigations will prove useful as there is a strong association between MRSP clonal types and geographical origin.[11,16] The results from this study will provide additional baseline data for further investigations of this important canine zoonotic pathogen. □

Table 1. Antimicrobial susceptibility of MRSP strains isolated from February 2011 to May 2012.

MRSP No.	Clinical condition	SCC*mec* type	PRAD* (mm)	Resistance pattern†
1	Surgical wound infection	II–III	16	DA-C-ENR-CN-MAR-SXT-PRAD
2	Infected implant	II–III	17	DA-C-ENR-CN-MAR-SXT-PRAD
3	Left ear otitis externa	II–III	15	DA-C-ENR-CN-MAR-SXT-PRAD
4	Pyoderma (toes left hock)	II–III	17	DA-C-ENR-CN-MAR-SXT-TE-PRAD
5	Surgical wound infection	II–III	17	DA-C-ENR-CN-MAR-SXT-PRAD
6	Pyoderma	V	18	DA-C-ENR-CN-MAR-SXT-TE-PRAD
7	Wound breakdown	II–III	17	DA-C-ENR-CN-MAR-SXT-PRAD
8	Pyoderma	V	17	DA-C-ENR-CN-MAR-SXT-TE-PRAD
9	Urinary tract infection	II–III	16	DA-C-ENR-CN-MAR-SXT-PRAD
10	Otitis externa	IV	25	DA-SXT-TE
11	Fracture wound	II–III	17	DA-C-ENR-CN-MAR-SXT-PRAD
12	Infected screws	II–III	17	DA-C-ENR-CN-MAR-SXT-PRAD
13	Fracture wound tissue	II–III	14	DA-C-ENR-CN-MAR-SXT-PRAD
14	Eczema (bridge of nose)	II–III	17	DA-C-ENR-CN-MAR-SXT-PRAD
15	Wound breakdown	II–III	15	DA-C-ENR-CN-MAR-SXT-PRAD
16	Left otitis externa	II–III	17	DA-C-ENR-CN-MAR-SXT-TE-PRAD
17	Pyoderma	V	18	DA-C-ENR-CN-MAR-SXT-TE-PRAD
18	Urinary tract infection	II–III	17	DA-C-ENR-CN-MAR-SXT-PRAD
19	Pyoderma	V	17	DA-C-ENR-CN-MAR-SXT-TE-PRAD
20	Mastitis	II–III	17	DA-C-ENR-CN-MAR-SXT-PRAD

*Pradofloxacin inhibition zone diameter distribution (S: ≥32; I: 20–31; R: ≤19).

†AMP: ampicillin; AMC: amoxycillin/clavulanic acid; CL: cephalexin; DA: clindamycin; C: chloramphenicol; ENR: enrofloxacin; CN: gentamicin; MAR: marbofloxacin; PRAD: pradofloxacin; SXT: sulphamethoxazole/trimethoprim; TE: tetracycline.

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