Evaluation of methicillin resistance in *Staphylococcus intermedius* isolated from dogs

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Abstract Methicillin and multi-drug resistance were investigated in 136 *Staphylococcus intermedius* strains of canine origin. The large majority of isolates were found to be *mecA*-negative by polymerase chain reaction, whereas only four strains were positive for the *mecA* gene. All *mecA*-positive strains were confirmed as methicillin-resistant by complementary tests, except for oxacillin disk diffusion, which yielded one false-negative result. A significantly higher resistance to fusidic acid, lincosamides, and cotrimoxazole was observed in methicillin-resistant *S. intermedius* (MRSI) compared with methicillin-susceptible strains. Although the prevalence of MRSI in dogs appeared to be low, methicillin resistance was confirmed to be associated with multi-drug resistance, suggesting the importance of antimicrobial susceptibility testing of canine *S. intermedius* strains.

Keywords Antimicrobials · Dog · Methicillin resistance · Staphylococci

Abbreviations

MRSI methicillin-resistant *Staphylococcus intermedius*
MSSI methicillin-susceptible *Staphylococcus intermedius*
PBP2a penicillin-binding protein 2a
PCR Polymerase Chain Reaction

Introduction

Staphylococcus intermedius is responsible for canine pyoderma, a widespread inflammatory skin disease (Jones et al. 2007). Although antimicrobial agents such as cephalosporins, aminopenicillins, and fluoroquinolones have proven their efficacy in the treatment of infections caused by *S. intermedius*, an increase in multidrug resistance has been observed

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in recent years, mainly due to the rapid spread of methicillin-resistant *S. intermedius* (MRSI) (Sasaki et al. 2005; Hauschild and Wójcik 2007). Methicillin resistance in staphylococci is linked to the presence of the *mecA* gene that encodes the penicillin-binding protein 2a (PBP2a) with a reduced affinity for penicillins. Polymerase chain reaction (PCR)-based molecular techniques for *mecA* detection are generally considered reference techniques (Van Duijkeren et al. 2004; Pedersen et al. 2007); additional methods include the oxacillin agar-screening test, the agar diffusion method with oxacillin or cefoxitin disks, and a latex agglutination test for PBP2a detection. The purpose of the present study was to evaluate the presence of methicillin resistance in *S. intermedius* strains isolated from dogs, comparing the results obtained using three phenotypic techniques with those of a molecular method, and to investigate the presence of multi-resistant strains.

Materials and methods

One hundred thirty-six *S. intermedius* strains isolated from dogs referred to the Department of Veterinary Clinics, University of Pisa, previously identified using a PCR-based method, were included in this study (Jousson et al. 2007). The presence of the *mecA* gene was detected by a PCR-based technique as described by Zhang et al. (2004), and results were confirmed by the following complementary tests: growth on oxacillin-containing media (2 mg/L), agar diffusion with oxacillin disks (5 µg), and latex agglutination for PBP2a detection (MRSA Slidex Detection, bioMérieux, Marcy l'Etoile, France). Sensitivity against 19 antimicrobial agents (fusidic acid, amikacin, amoxicillin/clavulanic acid, cephalexin, cefoperazone, clindamycin, chloramphenicol, cotrimoxazole, enrofloxacin, erythromycin, fosfomycin, gentamicin, lincomycin, marbofloxacin, moxifloxacin, norfloxacin, penicillin G, pristinamycin, and rifampicin) was evaluated using the disk diffusion method on Muller Hinton agar plates (Oxoid, Milano, Italy). Strains were classified as susceptible or resistant to antimicrobials tested in accordance with breakpoints proposed by the Comité de l'Antibiogramme de la Société Française de Microbiologie (CASFM). The proportion of multi-resistance between MRSI and methicillin-susceptible *S. intermedius* (MSSI) was compared using the Fisher's exact test (differences were considered significant for $P < 0.05$).

Results

A total of 132 out of 136 *S. intermedius* strains (97.1%) were *mecA*-negative, whereas only 4 out of 136 strains (2.9%) were positive for the *mecA* gene (Table 1). All *mecA*-positive

Table 1 Methicillin resistance in 136 *S. intermedius* strains isolated from dogs (PCR vs. phenotypic methods)

		Number of strains with indicated results				
		Oxacillin agar screening test		Oxacillin disk diffusion test		Latex agglutination test
		Growth	No growth	Resistant	Susceptible	Positive Negative
<i>mecA</i> PCR						
positive ($n=4$)		4 (100) ^a	0	3 (75) ^a	1	4 (100) ^a 0
negative ($n=132$)		0	132 (100) ^b	1	131 (99.2) ^b	1 131 (99.2) ^b

^a Sensitivity

^b Specificity

Table 2 Susceptibility of 136 *S. intermedius* strains against 19 selected antimicrobials

Antimicrobial	Susceptibility (%)		Statistical analysis ^a
	MRSI (<i>n</i> =4)	MSSI (<i>n</i> =132)	
Fusidic acid	75	100	0.029
Amikacin	100	100	–
Amoxicillin/Clavulanic acid	0	100	<0.0001
Cephalexin	0	100	<0.0001
Cefoperazone	0	100	<0.0001
Clindamycin	50	93.2	0.032
Chloramphenicol	75	87.9	0.41
Cotrimoxazole	50	97	0.009
Enrofloxacin	50	100	0.0007
Erythromycin	50	83.3	0.14
Fosfomycin	100	99.2	1
Gentamicin	100	98.5	1
Lincomycin	15	89.4	0.0061
Marbofloxacin	50	100	0.0007
Moxifloxacin	100	100	–
Norfloxacin	50	100	0.0007
Penicillin G	0	37.9	0.29
Pristinamycin	100	100	–
Rifampicin	100	99.2	1

^a Fisher's exact test (significant differences for $P<0.05$)**Table 3** Multiple resistance in 25 *S. intermedius* strains

Multi-resistance pattern	MRSI	MSSI	Total
BLs + FQs + LAs + MLs + SAs	2	0	2
BLs + LAs + MLs + APs + RMs	0	1	1
BLs + LAs + MLs + SAs + AGs	0	1	1
BLs + LAs + APs + FD	1	0	1
BLs + LAs + MLs + AGs	0	1	1
BLs + LAs + MLs + APs	0	6	6
BLs + LAs + APs	0	1	1
BLs + LAs + MLs	0	5	5
BLs + MLs + APs	0	6	6
BLs + MLs + SAs	0	1	1
Total	3 ^a	22 ^a	25

BLs = β -lactams, FQs = fluoroquinolones, Las = lincosamides, MLs = macrolides, Sas = sulfonamides, Aps = amphenicols, Ags = aminoglycosides, FD = fusidic acid, RMs = rifamycins

^a $P=0.019$

strains were confirmed as MRSI by the oxacillin agar-screening test and the latex agglutination test, while the oxacillin disk diffusion test gave a false-negative result. Among the *mecA*-negative isolates, one strain resulted positive for PBP2a detection and a second strain was found resistant by the disk diffusion method. Susceptibility tests showed significantly lower rates of susceptibility in MRSI compared with MSSI to fusidic acid, amoxicillin/clavulanic acid, cephalixin, cefoperazone, clindamycin, cotrimoxazole, enrofloxacin, lincomycin, marbofloxacin, and norfloxacin (Table 2). Multidrug resistance (resistance to at least three different antimicrobial classes) was detected in 25 out of 136 *S. intermedius* strains (18.4%), resulting in significantly more frequent MRSI (75%) than MSSI (16.7%) (Table 3).

Discussion

All phenotypic tests used in the present study showed high sensitivity and specificity compared with *mecA*-PCR; nevertheless, the obtained results indicated that the performance of different methods is preferable compared with a single test to discriminate between MRSI and MSSI. MRSI strains were more frequently associated with multiple resistance compared with MSSI, primarily for antimicrobials used to treat canine pyoderma, such as cephalosporins, amoxicillin/clavulanic acid, and fluoroquinolones, confirming the association between methicillin resistance and multiple resistance previously reported (Sasaki et al. 2005; Hauschild and Wójcik 2007). In conclusion, even though a very low prevalence of methicillin-resistant *S. intermedius* was observed, the results suggest the need for continuous monitoring of *S. intermedius* antimicrobial susceptibility, underlying the importance of prudent use of antimicrobials in companion animals.

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