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Short communication

Prevalence and characterization of methicillin-resistant *Staphylococcus pseudintermedius* in pets from South China

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ABSTRACT

The aim of this study was to determine the presence of and characterize methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) isolated from pets in South China. From 2007 to 2009, 898 samples were collected from 785 pets in Guangdong Province. The identity of staphylococcal species and the presence of methicillin resistance were confirmed by phenotypic and genotypic assays. The genetic relationships of MRSP isolates were determined by multilocus sequence typing (MLST), PFGE and *spa* typing. SCC*mec* elements and antimicrobial resistance genes profiling were characterized by PCR amplification. A total of 144 *S. pseudintermedius* isolates were recovered from the dogs and cats tested, and 69 (47.9%) of these isolates were identified as MRSP. Most of the MRSP isolates exhibited simultaneous resistance to four or more different antimicrobial agents. However, valnemulin showed robust activity against MRSP (MIC_{90} = 1 μg/ml). Integron 1, 2 and 3 were not detected in MRSP isolates. Twenty-four different multilocus sequence types were found among the MRSP isolates, with ST4 (n = 9), ST5 (n = 8), and ST95 (n = 7) being dominant sequence types. In addition, 8 new sequence types (ST134, 135, 136, 137, 138, 139, 140 and 148) were identified. Of the 69 MRSP isolates, SCC*mec*V was the most prevalent type (n = 33), followed by SCC*mec*VII (n = 13), SCC*mec*II–III (n = 7), and SCC*mec*III (n = 4). This study demonstrates for the first time that the occurrence of MRSP in healthy pets in China and shows that MRSP in South China has high genetic diversity.

1. Introduction

Staphylococcus pseudintermedius is a new coagulase-positive staphylococcal species identified in 2005 (Devriese et al., 2005). It has been recognized as an opportunistic pathogen in many kinds of animals, especially in dogs and cats (van Duijkeren et al., 2011a). It is associated with canine pyoderma, otitis exerna, wound infections, urinary tract infections, and other kinds of infections in pets (Ruscher et al., 2009). Some recent reports indicated that *S. pseudintermedius* could occasionally cause human infections and be human colonizer (Stegmann et al., 2010; van Duijkeren et al., 2011b), suggesting that *S. pseudintermedius* is a zoonotic pathogen and public health issue.

In recent years, occurrence of methicillin-resistant *S. pseudintermedius* (MRSP) increases greatly (van Duijkeren et al., 2011a). Furthermore, MRSP is usually resistant to various antimicrobial agents used in veterinary medicine, and appears to be a reservoir that accumulates different

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antimicrobial resistant genes (Perreten et al., 2010). Consequently, treating MRSP infections is a new challenge in veterinary medicine because of the very limited therapeutic options. Prevalence of MRSP colonization or contamination has been studied in various pet populations in different countries, including Canada, Japan, Germany, USA, Korea, and so on (van Duijkeren et al., 2011a; Onuma et al., 2012). However, in China, only MRSP isolated from canine with pyoderma has been reported (Wang et al., 2012). The aim of this study was to characterize MRSP and measure its occurrence in both healthy and diseased pets from South China.

2. Materials and methods

2.1. Sampling and bacterial identification

From November 2007 to December 2009, a total of 898 samples were collected from skin, ear, nare, mouth, eye, wound, anus, pudendum, and urinary tract of healthy (416) or diseased (482) dogs ($n = 612$) and cats ($n = 173$) in 10 animal hospitals located in Guangzhou ($n = 463$) and Shenzhen ($n = 435$), two large cities of Guangdong Province in China. All animals sampled upon admission to the veterinary hospital, and the diseased animals were hospitalized. One or two samples from different parts of body were collected from each animal. All samples were cultivated on Columbia agar with 5% sheep blood, and were incubated at 37 °C for 18–24 h. Presumptive *S. pseudintermedius* isolates were firstly identified by colony morphology, hemolysis, tube coagulase reaction, clumping factor test, and API-Staph system (BioMerieux, France). Isolates were further validated by PCR amplification and sequencing of 16S rRNA and *hsp60* genes (Bannoehr et al., 2007). Restriction fragment length polymorphism (RFLP) based on MobI digestion of an internal fragment of *pta* gene was carried out to confirm these isolates as *S. pseudintermedius* (Bannoehr et al., 2009).

2.2. Antimicrobial susceptibility testing and identification of MRSP

Determination of minimum inhibitory concentration (MIC) of 18 antimicrobial agents (Table 1) was carried out by agar dilution method according to Clinical and Laboratory Standards Institute (CLSI) (2008, 2010). Susceptibility of four antimicrobial agents, namely trimethoprim-sulfamethoxazole (1.25/23.75 μg), teicoplanin (30 μg), quinupristin–dalfopristin (15 μg), and linezolid (30 μg), was determined by disk diffusion method as guided by CLSI (2008, 2010). *mecA*-positive but oxacillin-susceptible isolates were further tested for cefoxitin susceptibility by disk diffusion method. *Staphylococcus aureus* ATCC29213 and ATCC25923 were used as quality control strains. Isolates were categorized as susceptible, intermediate or resistant, if applicable breakpoints were available in the CLSI documents M31-A3. When breakpoints were not available for *Staphylococcus* of animal origin, human CLSL documents (M100-S20) or other specific bacteria from animals were referred to. However, clinical breakpoints adopted from human medicine are not allowed to be used to predict therapeutic success or failure in animals.

Table 1
MICs of antimicrobial agents against *S. pseudintermedius* and MRSP isolates.

Antimicrobial	Range (μg/ml)	Resistance breakpoints (μg/ml)	*S. pseudintermedius*				MRSP			
			Resistant (%)	Susceptible (%)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Resistant (%)	Susceptible (%)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
Penicillin	0.125 to >128	≥0.25	70.1	29.9	8	128	100	0	32	128
Oxacillin	0.06 to >64	≥4	42.4	57.6	1	4	79.7	20.3	>64	>64
Gentamicin	0.06 to >128	≥16	24.3	41.7	8	32	21.7	34.8	8	16
Amikacin	0.06 to >128	≥64	4.90	91.7	1	4	5.80	92.8	1	2
Erythromycin	0.125 to >128	≥8	84.0	14.6	>128	>128	95.7	4.35	>128	>128
Tylosin	0.25 to >128	≥64	79.2	20.1	>128	>128	88.4	10.1	>128	>128
Tilmicosin	0.5 to >128	≥32	45.1	53.5	4	>128	44.9	53.6	4	>128
Azithromycin	0.06 to >128	≥8	86.8	13.2	>128	>128	95.7	4.35	>128	>128
Clindamycin	0.03 to >64	≥4	83.3	15.3	>64	>64	95.7	4.35	>64	>64
Tetracycline	0.06–128	≥16	86.8	9.72	32	128	92.8	1.45	64	128
Chloramphenicol	1–64	≥32	39.6	53.5	8	64	31.9	56.5	8	64
Florfenicol	1–64	≥16	18.8	66.7	≤1	16	20.3	59.4	≤1	16
Ciprofloxacin	0.06–128	≥4	56.3	36.1	8	64	75.4	20.3	32	64
Enrofloxacin	0.015–128	≥4	53.5	38.9	8	64	69.6	20.3	32	64
Tiamulin	0.06 to >128	≥32	11.8	88.2	0.125	32	10.1	89.9	≤0.06	32
Valnemulin	0.008 to >64	–	–	–	0.06	1	–	–	0.06	0.25
Rifampicin	0.002–32	≥4	6.3	89.6	0.004	2	2.90	97.1	0.004	0.5
Vancomycin	0.06–4	≥16	0	99.3	0.5	1	0	98.6	0.5	1
Trimethoprim-sulfamethoxazole[a]	–	≤10[b]	77.8	7.0	–	–	92.8	7.25	–	–
Teicoplanin[a]	–	≤10[b]	0	100	–	–	0	100	–	–
Quinupristin–dalfopristin[a]	–	≤15[b]	0	100	–	–	0	100	–	–
Linezolid[a]	–	≤20[b]	0	100	–	–	0	100	–	–

[a] The antimicrobial susceptibility was determined by disk diffusion method.
[b] Zone diameter (mm).