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Insertion sequence IS*256* in canine pyoderma isolates of *Staphylococcus pseudintermedius* associated with antibiotic resistance

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ABSTRACT

Staphylococcus pseudintermedius is the most frequent staphylococcal species isolated from canine pyoderma. The control of *S. pseudintermedius* infection is often difficult due to the expanded antimicrobial resistance phenotypes. Antibiotic resistance in staphylococcal pathogens is often associated to mobile genetic elements such as the insertion sequence IS*256* that was first described as a part of the transposon *Tn4001*, which confers aminoglycoside resistance in *Staphylococcus aureus* and in *Staphylococcus epidermidis*. In this study a collection of 70 *S. pseudintermedius* isolates from canine pyoderma was used to investigate antimicrobial susceptibility to 15 antibiotics and the presence of IS*256*, not revealed in *S. pseudintermedius* yet. Antibiotic resistance profiling demonstrated that all *S. pseudintermedius* isolates had a multi-drug resistance phenotype, exhibiting simultaneous resistance to at least five antibiotics; indeed methicillin resistant *S. pseudintermedius* isolates were simultaneously resistant to at least nine antibiotics and all were also gentamicin resistant. PCR analyses revealed the presence of IS*256* in 43/70 *S. pseudintemedius* isolates. The association between the presence of IS*256* and the resistance was particularly significant for certain antibiotics: cefovecin, amikacin, gentamicin and oxacillin (χ^2 p-value < 0.05). However, there was a striking result in frequency of strains resistant to gentamicin and oxacillin, suggesting a specific association between the presence of the IS*256* element and the determinants for the resistance to these antibiotics. To the best of our knowledge, this is the first report showing the detection of IS*256* in *S. pseudintermedius* isolates and its association with antibiotic resistance. Our findings suggest that *S. pseudintermedius* may acquire antibiotic resistance genes through mobile genetic elements which may play a predominant role in the dissemination of multi-drug resistance.

1. Introduction

Staphylococcus (*S.*) *pseudintermedius* is the most frequent staphylococcal species isolated from canine pyoderma. It has been also associated to other severe infections (Perreten et al., 2010) including wound infections, urinary tract infections (Rubin and Gaunt, 2011; Maaland and Guardabassi, 2011) and otitis externa (Bartlett et al., 2011). Infections in humans have also been described and confirmed the role of *S. pseudintermedius* as zoonotic agent (van Hoovels et al., 2006; Chuang et al., 2010; Riegel et al., 2011; Soedarmanto et al., 2011). A recent study from the Netherlands showed that methicillin resistant *S. pseudintermedius* (MRSP) infected dogs and cats can spread the organism not only by contact between pets and humans, but also to the environment in both, households and veterinary clinics (van Duijkeren et al., 2011). Importantly,

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epidemiological studies have revealed that *S. pseudintermedius* and not *S. intermedius* is the commonest causative agent of canine pyoderma (Bannoehr et al., 2007; Devriese et al., 2009). These findings have led to the awareness that many canine isolates previously identified as *S. intermedius* should have been re-classified as *S. pseudintermedius* (Devriese et al., 2009; Ross Fitzgerald, 2009). The control of *S. pseudintermedius* infection is often difficult due to the expanded antimicrobial resistance phenotypes seen among the causative strains. Recent studies reported indeed that multi-drug resistant (MDR) *S. pseudintermedius* (MDRSP) and MRSP strains have emerged as a serious problem in veterinary practice. In particular, methicillin resistance in *S. pseudintermedius* has been increasing over the last years in Europe as well as in North America (Weese and van Duijkeren, 2010). MRSP isolates usually exhibited resistance to almost all classes of antimicrobials used in veterinary medicine (Ruscher et al., 2009; Kadlec et al., 2010; Perreten et al., 2010). It is known that antibiotic resistance in staphylococcal pathogens is often associated to transposons. Some transposons are flanked by insertion sequences such as IS*256* that was first described as a part of the transposon *Tn4001*, which confers aminoglycoside resistance in *Staphylococcus aureus* (Lyon et al., 1987; Byrne et al., 1989). IS*256* is present in many aminoglycoside-resistant (Dyke et al., 1992; Hennig and Ziebuhr, 2008), and nosocomial strains of *Staphylococcus epidermidis* (Kozitskaya et al., 2004). Multiple copies of IS*256* are also present in 2 *S. aureus* strains that display intermediate resistance to vancomycin and are resistant to macrolides, rifampicin, quinolones, tetracycline, clindamycin, aminoglycosides, and low concentrations of chloramphenicol (Nagel et al., 2011). Although the transposable element IS*256* has been characterized in staphylococcal species, it has not yet been reported in *S. pseudintermedius*. In this study, therefore, we investigated the prevalence of antibiotic resistance among a collection of 70 clinical *S. pseudintermedius* isolates from canine pyoderma, the presence of the insertion element IS*256* and its possible association to antibiotic resistance.

2. Materials and methods

2.1. Bacterial isolation and identification

A collection of 70 *S. pseudintermedius* isolates from canine pyoderma were included in this study. Bacteria were isolated from clinical specimens on 5% defibrinated sheep blood agar (Blood Agar Base, Oxoid, Italy) and mannitol salt agar (Oxoid, Italy) and incubated aerobically at 37 °C for 24 h. Suspect colonies were identified using standard techniques: colony morphology, Gram stain, catalase and coagulase and APIStaph System (BioMérieux, Italy). Methicillin resistant isolates were detected by subculture on screening agar base medium (oxacillin (OXA) resistant screening agar base, Oxoid, Italy) incubated at 37 °C for 24 h. *S. pseudintermedius* isolates were identified using Polymerase Chain Reaction (PCR) restriction fragment length polymorphism (RFLP) assay based on the *MboI* digestion pattern of a PCR-amplified internal fragment of the *pta* gene as described (Bannoehr et al., 2009; Table 1). The isolates were stored at −70 °C in

Table 1
PCR primers and conditions.

Gene	Sequence (5′–3′)	PCR program	Size of amplified products (bp)	Reference
pta	pta_f1: AAAGACAAACTTTCAGGTAA pta_r1: GCATAAACAACGCATTGTACCG	95 °C × 5 min, 30 × (95 °C × 60 s, 53 °C × 60 s, 72 °C × 60 s) 72 °C × 7 min	320	Bannoehr et al. (2009)
blaZ	blaZ1: ACTTCAACACCTGCTGCTTTC blaZ2: TGACCACTTTTATCAGCAACC	94 °C × 6 min, 40 cycles × (94 °C × 30 s, 55 °C × 30 s, 72 °C × 60 s), 72 °C × 5 min	173	El Zubeir et al. (2007)
mecA	mecA1: AAAATCGATGGTAAAGGTTGGC mecA2: AGTTCTGCAGTACCGGATTTGC	94 °C × 6 min, 40 cycles × (94 °C × 30 s, 55 °C × 30 s, 72 °C × 60 s), 72 °C × 5 min	532	El Zubeir et al. (2007)
aac(6′)/aph(2″)	Forward: GTATTAGAATTTTATGGTGG Reverse: CCATACATTCTTAATATATC	95 °C × 5 min, 30 cycles × (95 °C × 1 min, 44 °C × 1 min, 68 °C × 1.5 min), 72 °C × 7 min	1184	Kozitskaya et al. (2004)
IS*256*	IS256-P5: AGTCCTTTTACGGTACAATG IS256-P3: TGTGCGCATCAGAAATAACG	94 °C × 5 min, 35 cycles × (94 °C × 60 s, 52 °C × 60 s, 72 °C × 60 s), 72 °C × 7 min	762	Gu et al. (2005)