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Short communication

Heterogeneity of methicillin-sensitive *Staphylococcus pseudintermedius* strains isolated from diseased dogs

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Abstract

Thirty nine canine *S. pseudintermedius* strains were examined for antibiotic susceptibility and genetic polymorphisms. All strains were methicillin-sensitive *S. pseudintermedius* (MSSP). Resistance to penicillin was most prevalent (66.6%), followed by resistance to neomycin (56.4%), erythromycin (53.8%), clindamycin (48.7%), chloramphenicol (48.7%), and tetracycline (46.2%). Pulsed-field electrophoresis (PFGE) showed a high genetic polymorphism in the investigated strains.

Key words: dog, methicillin-sensitive *S. pseudintermedius*, pulsed-field gel electrophoresis

Introduction

Staphylococcus intermedius is an opportunistic bacterial pathogen that causes various diseases in dogs. Staphylococcal strains designated up to now as *S. intermedius* species are currently assembled into the so-called *S. intermedius* group (SIG), consisting of *S. intermedius*, *S. pseudintermedius* and *S. delphini* (Sasaki et al. 2007). From a clinical point of view, the novel species *S. pseudintermedius* is the most significant of the SIG, being an important canine opportunistic pathogen often isolated from dermatitis, otitis and other secondary infections (Sasaki et al. 2007). The antimicrobial resistance of *S. pseudintermedius* strains, particularly resistance to methicillin, has emerged as an important problem in canine infections (Yoo et al. 2010).

The aim of this study was to determine the antimicrobial resistance of *S. pseudintermedius* isolated from diseased dogs and to characterise their genetic relationships by pulsed field gel electrophoresis.

Materials and Methods

In total, 39 *S. pseudintermedius* strains were collected from diseased dogs of both sexes from 10 veterinary practices, between June 2007 and October 2008, in three cities of Northern Poland (Gdansk, Gdynia, Pruszcz Gdanski). The samples were taken only from dogs with evident symptoms of infection: superficial pyoderma (n=17), abscess (n=6), external otitis (n=6), deep pyoderma (n=5), conjunctivitis (n=2), rhinitis (n=2), and cystitis (n=1). *S. inter-*

medius group (SIG) species were identified using an API ID32 Staph-system (BioMeriux) according to the manufacturer's instructions.

Molecular identification and molecular detection of methicillin resistance was performed following Bannoehr et al. (2009) and Yoo et al. (2010) respectively.

Antibiotic susceptibility testing was performed by disc diffusion using the following discs: amoxicillin-clavulanic acid, bacitracin, carbenicillin, cephalothin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, fusidic acid, gentamicin, mupirocin, neomycin, oxacillin, penicillin, rifampicin, sulfamethoxazole/trimethoprim and tetracycline. Resistance was defined according to the Clinical and Laboratory Standards Institute (CLSI, 2004).

Pulsed-field gel electrophoresis (PFGE) was carried out as described previously (de Lencastre et al. 1994). PFGE was run in an CHEF-DR II system (BioRad, USA). Association of strains from particular sites and cities with genotyping grouping was verified using Fisher's exact test. A *p* value of less than 0.05 was considered significant.

Results and Discussion

Using the PCR-RFLP method (Bannoehr et al. 2009), all of the investigated strains isolated from the diseased dogs belonged to *S. pseudintermedius*, similar to the results of other studies (Fazakerly et al. 2010, Yoo et al. 2010). In contrast to previous reports, *S. pseudintermedius*, and not *S. intermedius*, appears to be the most common pathogen associated with canine diseases.

Analysis of our strains showed a high level of antimicrobial resistance. Similar to other studies from Europe (Norström et al. 2009), the majority of our investigated isolates were resistant to penicillin (66.6%), neomycin (56.4%), erythromycin (53.8%), clindamycin (48.7%), chloramphenicol (48.7%), and tetracycline (46.2%). Resistance to gentamicin (10.3%) and sulfamethoxazole/trimethoprim (7.7%) was also observed. Most (69%) investigated strains were simultaneously resistant to three-seven antibiotics. All multiresistant strains were isolated from dogs previously antimicrobially treated. All strains showed susceptibility to methicillin (MSSP) with the use of disc diffusion and PCR methods. Methicillin resistance of *S. pseudintermedius* isolated from dogs appears to vary with its geographical origin. Studies from Asia have revealed high levels of methicillin-resistance, (Yoo et al. 2010) in contrast to data from

Europe where the prevalence of methicillin-resistant *S. pseudintermedius* (MRSP) ranges from 0% to 0.5% (Norström et al. 2009), similarly to our results.

Analysis of the PFGE patterns in our *S. pseudintermedius* revealed a high level of strain variability, with 16 distinct profiles (A-P) among 39 *S. pseudintermedius* isolates. PFGE types A-C and O were the most frequent and characterized 20 out of 39 (51.3%) isolates. Isolates from particular sites did not associate with one particular PFGE type ($p > 0.05$). Our findings suggest also that isolates from particular cities (Gdansk, Gdynia, Pruszcz Gdanski, Poland) did not associate with one particular PFGE pattern ($p > 0.05$), thus genotyping grouping of *S. pseudintermedius* is not dependent on their origin. The heterogeneity of *S. pseudintermedius* strains seems to depend of their susceptibility to methicillin. Recent studies have revealed a high level of clonality in methicillin-resistant *S. pseudintermedius* isolates but not in methicillin-sensitive *S. pseudintermedius* (Fazakerly et al. 2010), similarly to the present study.

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