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

K. Garbacz¹, L. Piechowicz¹, S. Żarnowska², K. Haras¹, M. Dąbrowska-Szponar³

¹ Department of Medical Microbiology, Medical University of Gdansk, Gdansk, Poland
² Private Veterinary Practice, Gdansk, Poland

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-
S. pseudintermedius group (SIG) species were identified using an API ID32 Staph-system (BioMériux) 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 (Bio-Rad, 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 (Fazakerley 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 meticillin (MSSP) with the use of disc diffusion and PCR methods. Meticillin resistance of S. pseudintermedius isolated from dogs appears to vary with its geographical origin. Studies from Asia have revealed high levels of meticillin-resistance, (Yoo et al. 2010) in contrast to data from Europe where the prevalence of meticillin-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 meticillin. Recent studies have revealed a high level of clonality in meticillin-resistant S. pseudintermedius isolates but not in meticillin-sensitive S. pseudintermedius (Fazakerley et al. 2010), similarly to the present study.

References


