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Clonal distribution of methicillin-resistant *Staphylococcus pseudintermedius* isolates from skin infection of dogs in Korea

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ABSTRACT

Bacterial infection by methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) is challenging in a small animal practice. Zoonotic transmission may occur. The aim of this study was to investigate the genotypic profiles of MRSP isolated from bacterial infections of canine skin in Korea and to compare their molecular lineages with dominant strains from other countries.

Sixty MRSP isolates were obtained from the lesions of canine pyoderma and otitis externa. Their genetic diversity was assessed by multilocus sequence typing (MLST), staphylococcal protein A (*spa*) typing and direct-repeat unit (*dru*) typing. Staphylococcal cassette chromosome *mec* (SCC*mec*) elements were characterized by multiplex PCR.

Thirty-nine different sequence types (STs) were detected. Among them, 21 STs were identified as internationally new sequence types. Fourteen *dru* types (dts) were detected, and the major types were dt11a and dt11y. *spa* typing characterised 21 isolates (35%, 21/60), including *spa* types t02 (n = 8), t05 (n = 5), t06 (n = 6), and t15 (n = 2). Two clonal complexes, CC568 and CC677, were revealed by MLST; this result differed from the dominant STs detected in MRSP isolates from Europe, North America, and other Asian countries. SCC*mec* type V was the major type (27/60. 45%), and 30 (50%) isolates were non-typeable by conventional classifying method.

This is the first report about the clonal lineage of MRSP isolated from Korea. MRSP isolated from dogs in Korea displays independent lineage from other countries. Surveillance is needed to confirm cross-national disseminating patterns.

1. Introduction

Staphylococcus pseudintermedius was reclassified from *S. intermedius* by biochemical features and DNA–DNA hybridizations in 2005 (Devriese et al., 2005) and is traditionally regarded as the main organism isolated from canine pyoderma and otitis. In dogs, *S. pseudintermedius* is part of the normal microbiota and colonizes the skin and mucocutaneous sites like mouth, nose, and anus (Allaker et al., 1992). Most canine bacterial infections are caused by *S. pseudintermedius* (Devriese et al., 2009).

Methicillin-resistant *S. pseudintermedius* (MRSP) emerged very rapidly and poses a challenge for infection control in veterinary medicine because of the limited antibiotic options available for its treatment (Ruscher et al., 2009). The methicillin-resistance of *S. pseudintermedius* is mediated by *mecA*-modifying penicillin-binding protein (PBP), similar to methicillin-resistant *S. aureus* (Weese and van Duijkeren, 2010). Since 2000, MRSP isolates harboring *mecA* have been reported globally across North America, Europe, and Asia, with various clonal distributions. Recent studies have revealed the increased (20%–47%) prevalence of MRSP collected from dogs and cats (Beck et al., 2012; Feng et al., 2012). In this study, we describe the clonal characteristics of MRSP isolates in Korea and compare their molecular lineage with dominant strains from other countries.

2. Materials and methods

2.1. Sample collection and identification

A total of 186 clinical isolates were collected aseptically from infection sites of 186 dogs (pyoderma in 136 and otitis externa in 50) from 2011 to 2015. All animals were owner-owned dogs visiting the Veterinary Medical Teaching Hospital of Seoul National University.

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Each lesion site was sampled using a cotton swab and cultivated overnight at 37 °C on blood agar. All isolates were identified using the Vitek 2 system (Biomerieux, Lyon, France). Isolates identified as *S. pseudintermedius* were subjected to molecular identification using PCR targeted *nuc* (Sasaki et al., 2010) and *mecA* (Kondo et al., 2007). All PCR products were sequenced using an ABI PRISM 3730xl apparatus (Applied Biosystems, Foster City, CA, USA) to confirm the species identification.

2.2. Antimicrobial susceptibility testing

Susceptibility testing was performed using the disc diffusion method by the new edition of CLSI guidelines published in 2016 (CLSI, 2016). The antibiotics examined included penicillin, oxacillin, amikacin, gentamicin, erythromycin, clindamycin, tetracycline, minocycline, ciprofloxacin, norfloxacin, trimethoprim/sulfamethoxazole, chloramphenicol, and rifampicin.

2.3. Multilocus sequence typing (MLST), mec-associated direct repeat unit (dru) typing, and staphylococcal protein A (spa) typing

Sequence types (ST) were determined by MLST using seven housekeeping genes (*tuf*, *cpn60*, *pta*, *purA*, *fdh*, *ack*, and *sar*) (Solyman et al., 2013). MLST sequences were compared with allele sequences in the *S. pseudintermedius* MLST database (http://pubmlst.org/spseudintermedius) to determine the allele number. Clonal relationship of ST was predicted by eBURST analysis (Feil et al., 2004). New ST numbers were assigned by the database curator (vincent.perreten@vetsuisse.unibe.ch). Direct repeat unit (*dru*) variable number tandem repeat (VNTR) region adjacent to IS*431* in SCC*mec* was sequenced and characterized as previously described (Goering et al., 2008). Sequenced tandem repeats were founded (Benson, 1999) and analyzed using the *dru* database website (http://dru-typing.org). Novel *dru* types (dts) were assigned by the curator (richardgoering@creighton.edu). A minimum spanning tree was generated using BioNumerics v7.6 (Applied Maths, Austin, TX, USA). *spa* typing was conducted using previously described primers and condition (Moodley et al., 2009). *S. pseudintermedius spa* database was provided by Arshnee Moodley (asm@sund.ku.dk) with an agreement concerning sharing of the database.

2.4. Characterization of SCCmec

SCC*mec* type was determined using two multiplex PCR methods (M-PCR 1 and 2) (Kondo et al., 2007). M-PCR 1 detected *mecA* and identified the *ccr* complex. M-PCR 2 distinguished classes A, B, and C *mec*. The presence of *ccrA5/B3* in SCC*mec* type VII-241 cassettes was screened using primers 5′-GCCAAAATTTCTTTCGAGACC-3′ and 5′-TACGTGCGAGTCGATTTGTT-3′ (Perreten et al., 2010). The presence of SCC*mec* II–III was distinguished from SCCmec III by detecting the absence of the cadmium resistance operon (Perreten et al., 2010).

3. Results

3.1. Identification and antimicrobial susceptibility testing

Vitek 2 identification revealed *S. pseudintermedius* (n = 143, 77%) as the major species, followed by *S. schleiferi* (11%) and *S. aureus* (7%). Other *Staphylococcus* spp. (all n = 1) included *S. hyicus*, *S. chromogenes*, *S. sciuri*, *S. simulans*, *S. parasanguinis*, *S. haemolyticus*, and *S. hominis*. All *S. pseudintermedius* isolates were positive in PCR identification and sequence of the PCR product was confirmed. In the 143 *S. pseudintermedius* isolates, 60 MRSP strains were evident, as indicated by PCR detection of *mecA* and resistance to penicillin and oxacillin (Table 1).

Resistance rates of MRSP to the other eight antibiotics was high (≥40%). Tetracycline 86.6% (52/60), Trimethoprim–sulfamethoxazole 85% (51/60), Erythromycin 83.3% (50/60), Clindamycin 81.6% (49/60), Ciprofloxacin 60% (36/60), Norfloxacin 58.3% (35/60), Chloramphenicol 46.6% (28/60), and Gentamicin 45% (27/60) were major 8 antibiotics resistant rate is high in MRSP. Resistant rates of Rifampicin 3.3% (2/60), Amikacin 1.6% (1/60), and Minocycline 1.6% (1/60) were very low.

3.2. Molecular characteristics of MRSP

MLST revealed 39 different STs (Fig. 1). Among them, 21 new international STs were detected and assigned as novel STs in the *S. pseudintermedius* MLST database (ST563, ST565, ST566, ST567, ST568, ST573, ST574, ST575, ST578, ST580, ST581, ST584, ST585, ST586, ST587, ST588, ST674, ST677, ST708, ST710, and ST712). There was no dominant ST; six isolates displayed the identical ST.

In *dru* typing, 14 dts were detected (dt11a, dt11av, dt11ax, dt11ca, dt11cj, dt11i, dt11p, dt11x, dt11y, dt10a, dt10dh, dt9a, dt9bo, and dt9bs), with no *dru* detected in three isolates. Three unclassified allele combinations were found and added as new types (dt11av, dt10dh, and dt9bs) to the dt database. The major types were dt11a (n = 19) and dt11y (n = 16); they accounted for 35 (61.4%) of the 57 isolates (Fig. 2).

spa typing could characterize only 21 (35%) of 60 isolates. Presence of *spa* type t02 (n = 8), t05 (n = 5), t06 (n = 6) and t15 (n = 2) was revealed. The predominant SCC*mec* was type V (45%, 27/60). Thirty isolates were non-typeable because of a combination of SCC*mec* components that did not match any other SCC*mec* types confirmed in previous studies (Table S1). Three isolates contained only *mecA*; no other SCC*mec* components were detected using multiplex PCR. All sixty MRSP strain information and molecular characteristics in this study were shown in Table 1.

4. Discussion

The overall 41.9% (60/143) prevalence rate of MRSP in this study is markedly higher than the rates found in the previous studies in Europe and North America (Griffeth et al., 2008; Gronthal et al., 2017; Hanselman et al., 2008; Maluping et al., 2014). However, elsewhere in Asia, a high prevalence range of about 30%–60% has been reported in Japan, Thailand, and China (Chanchaithong et al., 2014; Feng et al., 2012; Kawakami et al., 2010). These high prevalence ranges highlight the rapid emergence of MRSP in Asian countries. Compounding the problem, the resistance rates to other antibiotics were also high. Multidrug-resistant (MDR) bacteria are a serious global concern and virtually all MRSP (58/60) in this study were defined as MDR strains (Magiorakos et al., 2012). In Korea, an official veterinary antibiotic stewardship program does not exist. Therefore, dissemination of MDR-MRSP among owner-owned dogs could be common in the problem with antibiotic medication at veterinary clinics.

The genetic pool of MRSP in Korea showed independent lineage. MLST revealed 21 novel STs, which accounted for 53.8% (21/39) among the total STs found in this study. Dominant STs were not detected. Almost STs had 1 or 2 isolates and only ST365 and ST584 had multiple isolates identical with STs (n = 5 and n = 6). The population of MRSP strain was highly diverse, and many different STs acquired methicillin resistance. CCs 568 and 677 were detected in the Korean MRSP population (Fig. 1). The 40% (24/60) MRSP isolates composed CC568 (n = 14) and CC677 (n = 10). These CCs do not belong to any previously reported CCs predominant in Europe, North America and Asia (Pires dos Santos et al., 2016). Also, CCs 568 and 677 showed different tendency about SCC*mec* type. Non-typeable SCC*mec* was very dominant in CC568 (10/14, 71.4%), but almost CC677 isolates belonged to SCC*mec* type V (9/10, 90%). Except for CCs, single STs were not clustered by any of other characteristics (source, *dru* type, *spa* type, and SCC*mec* type).

Presence of *spa* type t02 (n = 8), t05 (n = 5), t06 (n = 6) and t15 (n = 2) was confirmed. The presence of t15 is a novel finding in Asia. Nevertheless, *spa* typing was not appropriate for the molecular analysis