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Short communication

Evidence of new sequence types of methicillin-resistant *Staphylococcus pseudintermedius* in Italy

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Abstract

Staphylococcus pseudintermedius (SP) and methicillin-resistant SP (MRSP) is one of the most important veterinary pathogens in the dog. Herein, from a total of 126 *S. pseudintermedius* strains, 23 MRSP (18%) were identified. Multi-Locus Sequence Typing (MLST) revealed that most of MRSP strains belonged to ST71 (26%), which have been already reported in Italy and other countries. Interestingly, nine new sequence types (39%), from 1053 up to 1061, were described for the first time. Moreover, the isolated MRSP strains showed relevant antibiotic resistance profiles. This report highlights the circulation of new sequence types of MRSP in Italy and underlines the need of a global epidemiological surveillance to limit the increasing spread of multidrug-resistant MRSP strains worldwide, since they may represent a considerable concern for dog's health.

Key words: methicillin-resistant *S. pseudintermedius*, canine skin diseases, Multi-Locus Sequence Typing, new sequence types

Introduction

Staphylococcus pseudintermedius (SP), a member of the *Staphylococcus intermedius* Group (SIG), is an important opportunistic pathogen causing various infections in dogs (Bannoehr and Guardabassi 2012). Indeed, SP canine infections are reported in many European countries and their therapeutic management has become a relevant challenge; particularly, methicillin-resistant SP (MRSP) represents a growing concern in small animal veterinary practice and its incidence in dogs has been increased from year to year (van Duijkeren et al. 2011, Garbacz et al. 2013, Moodley et al. 2014, De Martino et al. 2016, Kizerwetter-Świda et al. 2017). In the last decade, the spread of MRSP sequence types, mainly the sequence type ST71, as well as its zoonotic potential, have been frequently described worldwide (Stegmann et al. 2010, Ventrella

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et al. 2017). Furthermore, clonal spread of non-ST71 MRSP variants, exhibiting multidrug resistance profiles, was also demonstrated (Worthing et al. 2018). In this study, MRSP isolates were identified and their genetic diversity was defined by Multi-Locus Sequence Typing (MLST). Moreover, their antimicrobial susceptibility profiles were also evaluated.

Materials and Methods

During the years 2015-2017, 400 clinical samples were collected from dogs suffering from otitis externa and pyoderma in Naples and Latina cities. The specimens were processed at the Bacteriology Laboratory of the Department Veterinary Medicine and Animal Production, University of Naples "Federico II", Italy. All the swabs were cultured and streaked both on Columbia CNA Agar and Mannitol Salt Agar (MSA) plates (Liofilchelm, Italy). The isolates were identified by matrix-assisted laser desorption ionization-time of flight/ mass spectrometry (MALDI-TOF/MS) (Bruker Daltonik, Germany). Antibiotic resistance profiles of SP strains were evaluated by disk diffusion method according to CLSI (2015) and EUCAST (2017) guidelines. For streptomycin and vancomycin, breakpoints employed were those recommended by the French Society for Microbiology (http://www.sfm-microbiologie.fr) (2017). The tested antimicrobials (Oxoid, Italy) are listed in Table 1. Molecular profiling, using species-specific thermonuclease *nuc* gene (Sasaki et al. 2010) and β-haemolysin *hlb* gene (Kmieciak et al. 2016), was performed to confirm the identification of species. Genetic profiles of methicillin resistance were carried out by PCR to assess the detection of *mecA* gene (Chovanová et al. 2016). Moreover, SP strains showing phenotypic resistance to tetracycline were subjected to multiplex PCR to confirm, genotypically, the presence of the tetracycline resistance genes: *tet*K, *tet*M, *tet*L, *tet*O (Ullah et al. 2012). SP isolates phenotypically erythromycin-resistant were analyzed with multiplex PCR to detect the presence of *erm*A, *erm*B and *erm*C genes (Sutcliffe et al. 1996).

The genetic diversity of the MRSP isolates was determined by Multi-Locus Sequence Typing (MLST) of seven genes (*tuf*, *cpn*60, *pta*, *pur*A, *fdh*, *sar*, and *ack*), as described previously (Solyman et al. 2013). Alleles and STs were identified using the scheme available on PubMLST.org.

Results and Discussion

Between the years 2015-2017, 259 staphylococcal strains responsible for canine skin disorders were isolated from routine bacteriological analysis of clinical samples. The phenotypic bacterial identification by MALDI-TOF/MS defined 126 (49%) *S. pseudintermedius* strains confirmed also by the presence of the species-specific *nuc* and *hlb* genes. Amongst the 126 *S. pseudintermedius* strains, 23 strains (18%) were MRSP being positive to *mec*A gene. In this study, the most frequently identified MRSP lineage was ST71, with 6/23 strains (26%), displaying no correlation with canine breeds (Table 1). Nine new sequence types (39%) were identified, as reported in Table 1. The newly described sequence types (STs) named from ST1053 up to ST1061, which were submitted and assigned by the curator of the PubMLST database (https://PubMLST.org/spseudintermedius/), were for the first time reported in Italy and worldwide. MRSP ST71, the most frequently isolated ST here, represents the largely predominant ST in Europe (Osland et al. 2012, Haenni et al. 2014, Damborg et al. 2016, Ventrella et al. 2017) and has diffused worldwide (Pires dos Santos et al. 2016). ST71 was the dominating MRSP lineage also in Poland, at least until 2015, but later, in 2016, the other lineage ST551 emerged (Kizerwetter-Świda et al. 2017). In this study we did not found ST551, but other sequences types as ST45, ST118, ST181, ST258 that were already reported in Italy or other countries, particularly ST258 was described for the first time in Southern Italy by Ventrella et al. (2017).

Furthermore, here we report the first finding of ST496 in Italy, already described only in Australia (Worthing et al. 2018). Moreover, all MRSP strains showed interesting antibiotic resistance profiles (Table 1). Precisely, most of them (91%) were multidrug-resistant strains (resistant to at least 3 different antibiotic classes) and only the isolates belonging to the new STs 1058 and 1059 had a low degree of resistance. Only two ST71 strains, one from Naples and one from Latina, presented the same antibiotic resistance profile. The highest resistance rates were observed for erythromycin (91%), tetracycline (87%), sulfamethoxazole/trimethoprim (78%) and streptomycin (78%). However, no resistance was observed to linezolid and vancomycin, that are considered 'antibiotics of last resort' in human medicine. Interestingly, the new sequence types were also associated with carriage of the erythromycin resistance gene *erm*B and tetracycline resistance genes, *tet*M and *tet*K alone or together (Table 1). In conclusion, the results of this study underline the circulation of different and new MRSP lineages among clinically diseased dogs in Italy. Considering that scarce information on genetic lineages and clonal spread of MRSP is currently available, these findings justify the need of a constant epidemiological monitoring of MRSP.

Table 1. Anamnestic information, MLST results, resistance phenotype and *erm* and *tet* genotype of MRSP isolates (n=23) from canine cutaneous infections.

Geographical origin	Canine breeds	Type of infection	MLST	Resistance phenotype	Resistance erm and tet genotype
Naples	Labrador retriever	otitis externa	45	AMC, AMP, FOX, KF, CAZ, CRO, CD, E, CN, K, IMI, OX, P, S, SXT, TE	*erm*B, *tet*M
Naples	Cocker spaniel	otitis externa	71	AMC, AMP, FOX, KF, CAZ, CRO, ENR, E, CN, K, IMI, OX, P, S, SXT, TE	*erm*B, *tet*K
Naples	Half-breed	otitis externa	71	AMC, AMP, FOX, CRO, ENR, E, CN, OX, P, S, SXT	*erm*B
Naples	Beagle	otitis externa	71	AMC, AMP, FOX, KF, CAZ, CRO, CD, CIP, ENR, E, K, IMI, OX, P, S, SXT, TE	*erm*B, *tet*M
Naples	Shar Pei	pyoderma	71	AMC, AMP, FOX, KF, CAZ, CRO, CD, CIP, ENR, E, CN, K, IMI, OX, P, S, SXT, TE, TOB	*erm*B, *tet*M
Naples	Poodle	otitis externa	71	AMC, AMP, FOX, KF, CAZ, CRO, CIP, ENR, E, CN, IMI, OX, P, S, SXT, TE	*erm*B, *tet*K
Naples	English setter	otitis externa	118	AMC, AMP, CAZ, CN, K, OX, P, S, SXT, TE	*tet*K
Naples	Half-breed	otitis externa	118	AMC, AMP, FOX, KF, CAZ, CRO, CD, E, K, OX, P, S, SXT, TE	*erm*B, *tet*K, *tet*M
Naples	Cocker spaniel	otitis externa	181	AMC, AMP, KF, CAZ, CRO, CD, CIP, ENR, E, CN, K, IMI, OX, P, S, SXT, TE	*erm*B, *tet*M
Naples	Half-breed	otitis externa	181	AMC, AMP, CAZ, CRO, CD, CIP, ENR, E, CN, K, IMI, OX, P, S, SXT, TE	*erm*B, *tet*M,
Naples	Half-breed	otitis externa	258	AMC, AMP, CD, ENR, E, OX, P, SXT, S, TE	*erm*B, *tet*M
Naples	English bulldog	pyoderma	496	AMC, AMP, E, IMI, OX, P, SXT	*erm*B
Naples	**Shar Pei**	**pyoderma**	**1053**	**AMC, AMP, CIP, ENR, E, K, OX, P, S, SXT, TE**	***erm*B, *tet*M**
Naples	**Half-breed**	**otitis externa**	**1060**	**AMC, AMP, FOX, CAZ, CD, CIP, ENR, E, K, OX, P, SXT, TE**	***erm*B, *tet*M**
Naples	**Poodle**	**otitis externa**	**1061**	**AMC, AMP, CD, E, CN, K, OX, P, S, SXT, TOB**	***erm*B**
Latina	Golden retriever	pyoderma	71	AMC, AMP, FOX, KF, CAZ, CRO, CD, CIP, ENR, E, CN, K, IMI, OX, P, S, SXT, TE, TOB	*erm*B, *tet*M
Latina	German Sheperd	otitis externa	862	AMC, AMP, CRO, CD, ENR, E, CN, K, IMI, OX, P, S, SXT, TE, TOB	*erm*B, *tet*M
Latina	**Cocker spaniel**	**otitis externa**	**1054**	**AMC, AMP, FOX, CAZ, CD, CIP, ENR, E, K, IMI, OX, P, S, SXT, TE**	***erm*B, *tet*K**
Latina	**English setter**	**otitis externa**	**1055**	**AMC, AMP, FOX, CAZ, CD, CIP, ENR, E, K, OX, P, S, TE**	***erm*B, *tet*K**
Latina	**German sheperd**	**otitis externa**	**1056**	**AMC, AMP, FOX, CAZ, CD, CIP, E, K, OX, P, S, TE**	***erm*B, *tet*K**
Latina	**Beagle**	**otitis externa**	**1057**	**AMC, AMP, CAZ, CD, E, CN, K, IMI, OX, P, S, TE**	***erm*B, *tet*K, *tet*M**
Latina	**Half-breed**	**otitis externa**	**1058**	**AMC, AMP, E, OX, P, TE**	***erm*B, *tet*M**
Latina	**Half-breed**	**otitis externa**	**1059**	**AMC, AMP, FOX, CAZ, OX, P, TE**	***tet*M**

The new isolated sequence types are written in bold.
Antimicrobials: AMC = amoxicillin + clavulanic acid (20/10 µg), AMP = ampicillin (10 µg), OX = oxacillin (1 µg), P = penicillin (10 IU), CRO = ceftriaxone (30 µg), CD = clindamycin (2 µg), CIP = ciprofloxacin (30 µg), ENR = enrofloxacin (5 µg), E = erythromycin (15 µg), CN = gentamicin (10 µg), S = streptomycin (10 µg), TOB = tobramycin (10 µg), IMI = imipenem (10 µg), LNZ = linezolid (30 µg), SXT = sulfamethoxazole-trimethoprim (1.25/23.75 µg), TE = tetracycline (30 µg), VA = vancomycin (30 µg).

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