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# Prevalence and characterization of methicillin-resistant *Staphylococcus pseudintermedius* from symptomatic companion animals in Northern Italy: Clonal diversity and novel sequence types

Maria Luisa Menandro[a], Giorgia Dotto[a], Alessandra Mondin[a], Marco Martini[a], Letizia Ceglie[b], Daniela Pasotto[a,*]

[a] Department of Animal Medicine, Productions and Health, University of Padua, Viale dell'Università, 16-35020, Legnaro, PD, Italy
[b] Istituto Zooprofilattico Sperimentale delle Venezie, Viale dell'Università, 10-35020, Legnaro, PD, Italy

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ABSTRACT

The aim of this study was to assess the prevalence, the genotypic diversity, the antimicrobial resistance traits of canine and feline clinical methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) isolates in a diagnostic laboratory in Italy during 2015-2016.

All isolates were characterized by multilocus sequence typing (MLST), staphylococcal cassette chromosome (SCC)-*mec* typing and staphylococcal protein A (*spa*)-typing. The resistance profiles were assessed by antimicrobial susceptibility testing and confirmed genotypically by the detection of *mecA* gene and by microarray analyses.

The prevalence of MRSP isolates was high (31.6%). All the strains were multidrug resistant and the most frequent clone was ST71-SCC*mec* type II-III. These results confirm a high prevalence of MRSP amongst clinical samples from pets in Italy. These isolates show multidrug resistance features that are of concern both in veterinary and human medicine for clinical and epidemiological reasons.

## 1. Introduction

*Staphylococcus pseudintermedius* is a common inhabitant of the normal microbiota of dogs and cats, but it is also frequently involved in these animals in a wide range of conditions such as pyoderma, otitis, infections of the urinary tract or of surgical site [1]. Its zoonotic potential should not be underestimated since cases of infection of healthy and diseased humans have been reported especially among veterinarians working at small animal hospitals and pet owners [1,2,3].

Over the past decade, methicillin-resistant *S. pseudintermedius* (MRSP) has emerged worldwide as a concern in small animal clinics, for its antimicrobial resistance (AMR) and virulence profiles [4]. Methicillin-resistant staphylococci are considered resistant to all β-lactam agents, i. e. penicillins, -lactam/ -lactamase inhibitor combinations, cephems and carbapenems [5]. Moreover, they are often accompanied by multidrug-resistance (MDR).

The two major epidemic clones found across Europe and North America are sequence type (ST)71 and ST68, respectively [6]. Recently, other clones, such as those belonging to the Clonal Complex (CC)258, although usually less virulent and antimicrobial-resistant, have been described in several EU Countries. Simultaneously, highly resistant clones are emerging, such as ST496 described by Bergot et al. suggesting a global evolution of the population structure of MRSP and a progressive decline of the ST71 Lineage [7,8,9]. However, previous studies have reported the occurrence of MRSP in specific animal populations in Italy, but no comprehensive studies of the characterization of resistance genes of MRSP isolates from dogs and cats, have been published [10,11,12]. The goal of this study was to assess the prevalence, the genotypic diversity and the phenotypic and genotypic AMR traits of clinical MRSP from dogs and cats isolated in a diagnostic laboratory in Italy over a two-year period (2015–2016).

*Abbreviations:* AMR, antimicrobial resistance; CLSI, Clinical and Laboratory Standards Institute; CC, Clonal Complex; MDR, multidrug resistance; MLST, multilocus sequence typing; MRSP, methicillin-resistant *Staphylococcus pseudintermedius*; PCR, polymerase chain reaction; ST, sequence type; SCC*mec*, Staphylococcal Cassette Chromosome mec

* Corresponding author at: Department of Animal Medicine, Productions and Health, University of Padua, Viale dell'Università, 16-35020, Legnaro, PD, Italy.
*E-mail addresses:* marialuisa.menandro@unipd.it (M.L. Menandro), giorgia.dotto@unipd.it (G. Dotto), alessandra.mondin@unipd.it (A. Mondin), marco.martini@unipd.it (M. Martini), lceglie@izsve.it (L. Ceglie), daniela.pasotto@unipd.it (D. Pasotto).

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## 2. Materials and methods

### 2.1. Samples, bacterial isolation and identification

Three hundred forty-two microbiological samples from independent canine and feline clinical cases referred to the Veterinary Teaching Hospital of the University of Padova (VTH-UP), were processed at the Department of Animal Medicine, Productions and Health, from January 2015 to December 2016.

Samples were collected from various sources: skin lesions (pyoderma or cutaneous fistulae), otitis, conjunctivitis, urinary tract or bone infections, prostatitis, surgical wounds and post-surgical infections.

Samples were plated onto *Columbia Agar* supplemented with 5% sheep blood, *MacConkey Agar* and *Mannitol Salt Agar* (Oxoid, Basingstoke, United Kingdom) and incubated under aerobic conditions at 37 °C for 24 h. *S. pseudintermedius* suspected colonies, were confirmed using the macro-method standard procedure (colony morphology, Gram staining and catalase test) for genus confirmation, followed by ID 32 STAPH identification kit (bioMérieux SA, Mercy l'Etoile, France). The species identification was confirmed by amplification and nucleotide sequencing of a portion of the 16S rRNA and *hsp*60 genes as previously described [13]. Comparative analyses of nucleotide sequences with those published in the National Centre for Biotechnology Information database were performed (www.ncbi.nlm.nih.gov/blast/Blast.cgi).

The isolates were confirmed as MRSP by their phenotypic resistance to oxacillin and by detection of the *mec*A gene [14,15].

### 2.2. Antimicrobial susceptibility testing

The susceptibility of the isolates to antimicrobials of interest in veterinary and human medicine was assessed via the agar disk diffusion method according to the recommended Clinical and Laboratory Standards Institute guidelines [5,15]. The antimicrobials tested were: oxacillin (1 μg), penicillin (1 U.I.), trimethoprim/sulphamethoxazole (1.25 + 23.75 μg), tetracycline (30 μg), chloramphenicol (30 μg), enrofloxacin (5 μg), ciprofloxacin (5 μg), erythromycin (15 μg), gentamicin (10 μg), tobramycin (10 μg), mupirocin (5 μg), clindamycin (2 μg) (Table 1). Breakpoints were interpreted following the CLSI susceptibility criteria [5,15] and following Fuchs et al., 1990 [16]. *S. aureus* ATCC 25923 was used as a quality control strain.

The isolates were defined as multidrug resistant if they exhibited resistance to at least three different classes of antimicrobials.

**Table 1**
Number of MRSP showing phenotypic resistance to the antimicrobial agents tested.

| Class of antimicrobial agents | Antimicrobial agents | Disk concentration | MRSP (n 19) number of resistant strains |
|---|---|---|---|
| Beta-lactams | oxacillin | 1 μg | 19 |
| | penicillin | 1 U.I. | 19 |
| Macrolides | erythromycin | 15 μg | 17 + 2[a] |
| Lincosamides | clindamycin | 2 μg | 18 |
| Fluoroquinolones | enrofloxacin | 5 μg | 17 |
| | ciprofloxacin | 5 μg | 17 |
| Folate pathway inhibitors | trimethoprim/sulphamethoxazole | 1.25 + 23.75 μg | 17 |
| Aminoglycosides | gentamycin | 10 μg | 15 |
| | tobramycin | 10 μg | 11 + 1[a] |
| Tetracyclines | tetracycline | 30 μg | 13 |
| Phenicols | chloramphenicol | 30 μg | 7 + 3[a] |
| Monoxycarbolic Acid | mupirocin | 5 μg | 2 |

[a] = intermediate antimicrobial susceptibility profile.

### 2.3. DNA microarray analysis

The StaphyType kit (Alere Technologies, GmBH) was used for DNA microarray assays following the manufacturer's instructions (http://www.alere-technologies.com). This microarray kit includes a chip consisting of probes designed to detect a set of antimicrobial-resistance genes, SCC*mec* elements and virulence-associated genes, as well as of other markers. Since this kit was developed for *S. aureus*, we considered only the results of antimicrobial-resistance genes and SCCmec elements due to the high homology of these between *S. aureus* and *S. pseudintermedius*. Briefly, *S. pseudintermedius* strains were sub-cultivated onto *Brain Heart Infusion Agar* (Oxoid, Basingstoke, United Kingdom). Genomic DNA was lysed and purified using DNeasy blood and tissue kit (Qiagen, Hilden, Germany). DNA samples were subjected to a linear multiplex primer elongation using one primer per target and amplicons labelled by incorporation of biotin-16-dUTP. Single stranded DNA amplicons were hybridized to the probes of the microarray and, after washing, horseradish-peroxidase-streptavidin precipitation reaction was performed, resulting in grey/black spots in case of positivity. The array images were automatically analysed using a reader and the Arraymate software (Alere Technologies GmbH).

### 2.4. Strain typing

Molecular typing of the isolates was determined using the *Staphylococcus pseudintermedius* multilocus sequence typing (MLST) scheme as described by Solyman et al., 2013 [17]. The allelic profiles and the sequence types (STs) were obtained by submitting the allele sequences to the MLST web site (available at http://spseudintermedius.mlst.net/; last access on December 2017). New STs were assigned by the curator of the MLST database, Dr. Vincent Perreten. An arbitrary definition of clonal complexes (CCs) was used and it included major group founders (with more than 3 single-locus links) from the MLST database and single and double locus variants (SLVs and DLVs) as previously described by Damborg et al. (2016) [18]. The software Phyloviz (http://www.phyloviz.net/goeburst/) was used on the entire *S. pseudintermedius* MLST database to determine the relatedness between STs described in it and in this study.

According to the scheme developed by Moodley et al. (2009) [19], *spa*-typing was performed to amplify and sequence the variable, tandem repeat region (X-region) of the *spa* gene.

## 3. Results

During the two-year period, at the microbiology laboratory were performed 342 microbiological analyses from independent clinical cases (275 from dogs and 67 from cats). Seventy-nine (23.1%) *Staphylococcus* spp. were isolated, 63 and 16 from canine and feline clinical samples, respectively. Sixty (76%) out of the 79 isolates were *S. pseudintermedius* (55 from dogs and 5 from cats), 19 (31.6%) of which MRSP and *mec*A-positive. Most MRSP originated from canine clinical case (18/19; 94.7%) and only 1 (5.3%) from feline. The strains derived from otitis (7/19; 36.8%, 1 of which of feline origin), pyoderma (4/19; 21%), cutaneous fistulae (3/19; 15.8%), followed by conjunctivitis (2/19; 10.5%), post-surgical infections (2/19; 10.5%) and prostatitis (1/19; 5.3%).

All MRSP isolates were multidrug-resistant, irrespectively of clone type (Table 2). Besides oxacillin, all MRSP isolates were resistant or with a reduced susceptibility to erythromycin and all except one to clindamycin. More variations were observed in the resistance to trimethoprim/sulphamethoxazole (17/19, 89.5%), enrofloxacin (17/19; 89.5%), ciprofloxacin (17/19; 89.5%), gentamicin (15/19; 79.0%), tetracycline (13/19; 68.4%), tobramycin (12/19; 63.2%), chloramphenicol (10/19; 52.6%) and mupirocin (2/19; 10.5%) (Table 1).

MLST analysis revealed a predominance of the CC71: ST71 (14/19), which accounted for 73.7% of the MRSP isolates and the novel related

**Table 2**
Site of infection, antimicrobial resistance traits and Multilocus Sequence Type (MLST) of the MRSP isolates.

| Strain ID | Origin | Sample | MLST (CC)-*spa* type | SCC*mec* type | Resistance phenotype | Resistance genotype |
|---|---|---|---|---|---|---|
| 2 | dog | pyoderma | ST71 (71)-t02 | SCC*mec*II-III | β-lact, Lin, Ma[c], Am, Te, C, Q, Sul | *mecA, blaZ, blaI, blaR, ermA, ermB, aacA-aphD, aphA3, sat, tetK, cat*[b] |
| 3 | dog | otitis | ST71 (71)- t02 | SCC*mec*II-III | β-lact, Lin, Ma, Mup | *mecA, blaZ, blaI, blaR, ermA, ermB, aacA-aphD, aphA3, sat,* |
| 4 | dog | otitis | ST748 (71)- t02 | SCC*mec*II-III | β-lact, Lin, Ma, Am, Q, Sul | *mecA, blaZ, blaI, blaR, ermB, aacA-aphD, aphA3, sat* |
| 5 | dog | conjunctivitis | ST71 (71)- t02 | SCC*mec*II-III | β-lact, Lin, Ma, Am, Mup, Te, Q, Sul | *mecA, blaZ, blaI, blaR, ermB, aacA-aphD, aphA3, sat, mupA, tetK, cat*[b] |
| 6 | dog | pyoderma | ST301 (258)-t02 | SCC *mec* IV | β-lact, Ma, Am, Te, Sul | *mecA, blaZ, blaI, blaR, ermB, aacA-aphD, aphA3, sat, tetM* |
| 8 | dog | implant screw | ST71 (71)-t19 | SCC*mec*II-III | β-lact, Lin, Ma[c], C, Q, Sul | *mecA, blaZ, blaI, blaR, ermA, ermB, aacA-aphD, aphA3, sat* |
| 9 | dog | cutaneous fistula | ST71 (71)- t02 | SCC*mec*II-III | β-lact, Lin, Ma, Am, Te, C, Q, Sul | *mecA, blaZ, blaI, blaR, ermB, aacA-aphD, aphA3, sat, tetK, cat*[b] |
| 10 | dog | otitis | ST71 (71)-t06 | SCC*mec*II-III | β-lact, Lin, Ma, Am, C, Q, Sul | *mecA, blaZ, blaI, blaR, ermB, aacA-aphD, aphA3, sat, cat*[b] |
| 11 | cat | otitis | ST71 (71)-t23 | SCC*mec*II-III | β-lact, Lin, Ma, Am, Te, C[c], Q, Sul | *mecA, blaZ, blaI, blaR, ermB, aacA-aphD, aphA3, sat, tetK, cat*[b] |
| 13 | dog | otitis | ST71 (71)-t06 | SCC*mec*II-III | β-lact, Lin, Ma, Am[c], Te, Q, Sul | *mecA, blaZ, blaI, blaR, ermB, aacA-aphD, aphA3, sat, tetK, cat*[b] |
| 17 | dog | otitis | ST261 (258)-t19 | SCC *mec* IV | β-lact, Lin, Ma, Te, Q | *mecA, blaZ, blaI, blaR, ermB, aphA3, sat, tetM* |
| 26 | dog | sperm | ST71 (71)-t19 | SCC*mec*II-III | β-lact, Lin, Ma, Am, C, Q, Sul | *mecA, blaZ, blaI, blaR, ermB, aacA-aphD, aphA3, sat* |
| 28 | dog | conjuntivitis | ST71 (71)-t17 | SCC*mec*II-III | β-lact, Lin, Ma, Am, Te, C, Q, Sul | *mecA, blaZ, blaI, blaR, ermA, aacA-aphD, aphA3, sat, tetK, cat*[b] |
| 33 | dog | otitis | ST261 (258)-t19 | SCC *mec* IV | β-lact, Lin, Ma, Te, Q, Sul | *mecA, blaZ, blaI, blaR, ermB, aphA3, sat, tetM* |
| 36 | dog | cutaneous fistula | ST71 (71)-t19 | SCC*mec*II-III | β-lact, Lin, Ma, Am, Q, Sul | *mecA, blaZ, blaI, blaR, ermA, ermB, aacA-aphD, aphA3, sat* |
| 45 | dog | pyoderma | ST71 (71)-t02 | SCC*mec*II-III | β-lact, Lin, Ma, Am, Te, C, Q, Sul | *mecA, blaZ, blaI, blaR, ermB, aacA-aphD, aphA3, sat, tetK, cat*[b] |
| 54 | dog | pyoderma | ST341 (45)-t47 | NT[a] | β-lact, Lin, Ma, Am, Te, C[c], Q, Sul | *mecA, blaZ, blaI, blaR, ermB, aacA-aphD, aphA3, sat, tetM* |
| 304-7 | dog | bone | ST71 (71)-t19 | SCC*mec*II-III | β-lact, Lin, Ma, Am, Te, C[c], Q, Sul | *mecA, blaZ, blaI, blaR, ermB, aacA-aphD, aphA3, sat, tetK* |
| 1483 | dog | cutaneous fistula | ST71 (71)-t19 | SCC*mec*II-III | β-lact, Lin, Ma, Am, Te, Q, Sul | *mecA, blaZ, blaI, blaR, ermB, aacA-aphD, aphA3, sat, tetK* |

Legend: β-lact = beta-lactams, Lin = Lincosamides, Ma = Macrolides, Am = Aminoglycosides, Mup = Monoxycarbolic acid, Te = Tetracyclines, C = Phenicols, Q = Fluoroquinolones, Sul = Folate pathway inhibitor.
[a] = not typable [class*Cmec* (*mec*A, *ugp*Q), *ccr*AA, *ccr*C *ccr*AB1].
[b] = *cat* total/pC221.
[c] = intermediate antimicrobial susceptibility profile.

singleton ST748 that is a SLV of ST71 since it differed in 1 point mutation in the allele *cpn*60 (T to C substitution at nucleotide position 337) (Table 2). The remaining isolates belonged to CC258 (ST261, n = 2 and ST301, n = 1) and ST341 (n = 1).

SCC*mec* typing highlighted the presence of two SCC*mec* types: 15 (79%) of the 19 isolates harboured SCC*mec* type II-III, confirmed by the absence of the cadmium resistance operon, and the remaining 4 isolates (21%) were found to carry SCC*mec* type IV. All the isolates belonging to the CC71 (ST71 and ST748) carried cassette type II-III, those belonging to CC258 (ST261 and ST301) were associated with type IV. The isolate 54 showed a non-typeable SCC*mec,* since it carried unusual combinations of SCC*mec* genes [class*Cmec* (mecA, ugpQ) & *ccr*AA, *ccr*C & *ccr*AB1].

Further characterisation of the MRSP isolates showed a predominance of *spa*-type t02 among the CC71-SCCmecII-III (ST71-t02-II-III and ST748-t02-II-III) (6/15; 40%) and ST71-t19-II-III (5/15; 33.3%), followed by ST71-t06-II-III (2/15; 13.3%), ST71-t17-II-III and ST71-t23-II-III (1/15; 6.7% each).

*spa*-type t19 and t02 were also found in CC258 (ST261 and ST301 respectively), whereas the unique strain belonged to CC45 with NT-SCC*mec* displayed *spa*-type t47.

DNA microarray analysis confirmed the presence of a wide range of antimicrobial resistance genes in our MRSP population. In accordance with the phenotypic resistance to penicillin all the isolates carried the *bla* resistance operon (*blaZ, blaI* and *blaR*) that codifies for beta-lactamase. The resistance to macrolide is related to the presence in all strains of *erm*A and/or *erm*B gene, codifying for erythromicyn ribosome methylase.

Tetracycline resistance (13 isolates) was conferred by *tet*K (9 isolates) and *tet*M (4 isolates). All except one tetracycline resistant MRSP belonging to CC71 carried *tet*K gene, while those belonging to CC258 were *tet*M positive. None of the isolates harboured both *tet*K and *tet*M genes. Mupirocin-resistance was confirmed by the presence of *mup* gene in 1 of the 2 phenotypically resistant strains.

All the isolates except one carried aph(3′)III and most of the isolates the *aac*(6′)Ie-*aph*(2″)Ia gene (16 isolates) encoding resistance to aminoglycosides. However, the correlation between gentamicin-resistant genotype and phenotype was not complete, as 2 of the 16 *aac*(6′)Ie-*aph* (2″)Ia positive strains, were phenotypically susceptible to this drug. Similarly, 3 out of 5 chloramphenicol-resistant strains harboured the *cat* gene, together with 3 of the 12 susceptible or partially susceptible isolates. Conversely, none of the trimethoprim/sulphamethoxazole-resistant isolates harboured *dfr*A gene.

## 4. Discussion

Many studies report the occurrence and distribution of MRSP worldwide. However, recent comprehensive data on prevalence and characterization of MRSP isolates from clinical routine in veterinary settings are limited. This study provides new information about MRSP clinical isolates of canine and feline origin diagnosed in our laboratory during the 2 year period (2015–2016), with particular regard to the abundancy of resistance genes and SCCmec elements undergoing the phenotypic MDR profiles.

Our data show that the MRSP prevalence among *S. pseudintermedius* isolates (31.6%) was similar to that reported by another investigation in Italy (33%), whereas the MRSP isolation frequency in the samples tested (5.6%) was lower when compared to those reported in the same study (12%) [12]. Our MRSP prevalence was higher than previously reported in other countries, such as in The Netherlands, United Kingdom, Finland, Norway and Germany [7,20,21,22,23]. However, comparison between different studies cannot be easily made since the occurrence of MRSP is not only related to the geographic origin but also on the sample type and population studied. Data on the prevalence might depend on study design, identification methods, sampling, animal health status and other factors.

Antimicrobial exposure may be a promoting factor of the diffusion of MRSP. A complete history of the past antimicrobial treatments of the animals considered in this work is not available, but most of them have been hospitalized and treated with antimicrobials in their past.

MLST analyses identified different clones and the most frequent was ST71. This finding is in agreement with has been reported worldwide in dogs [6,8,11,20,24]. In this study ST71 was mainly associated with *spa* type t02, but also with other *spa* types such as t19, t06 and t23, as

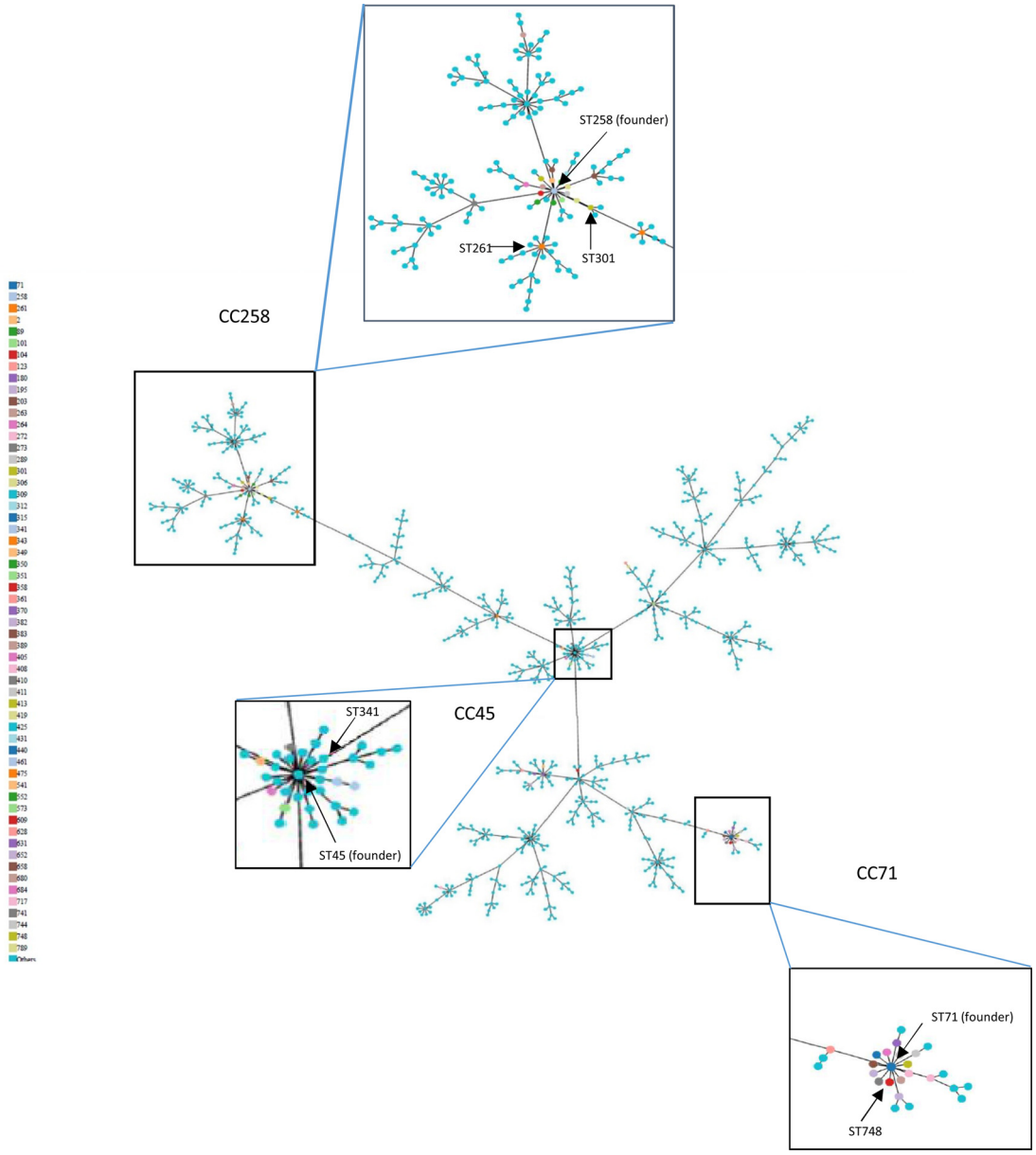


**Fig. 1.** Clonal relationships among the isolates of the entire *S. pseudintermedius* MLST database. STs detected in this study, together with the CCs (and ST founder) they belong to, are emphasised in the squares.

reported in previous studies [6,24]. The high prevalence of MRSP harbouring the SCC*mec* II-III cassette indicates that the typical European clonal type is largely responsible for the dissemination of MRSP also in Italy.

Phyloviz analysis of all STs present in the *S. pseudintermedius* MLST database confirmed that the isolates belonged to 3 different CCs (CC71, CC258 and CC45), with a higher prevalence of CC71.

As shown in Fig. 1, the new ST748 belonged to the clonal complex CC71 (with founder ST71). ST261 (from 2 cases of otitis) and ST301 (from a case of pyoderma) belonged to the same clonal lineage, CC258 (with founder ST258), one of the three most prevalent MRSP clonal group [8]. This clonal group has been previously reported in different European countries such as The Netherlands, Denmark, Norway, Finland, United Kingdom, Germany, France and also in Italy. It is the largest clonal lineage with more than 120 STs as also described in the pubMLST database [7,9,12,18,21,22,23,25]. The presence of so many closely related variants of the founder ST258 may indicate that this clone has a higher evolutionary rate than other STs, or that some unknown factors have favoured its diffusion [16].

ST341 belonged to CC45 (with founder ST45) that, as CC71 and CC258, is common in Asia, Australia and Europe [8,26,27,28]. Although rarely detected, ST 341 has been previously isolated, again from a canine pyoderma, in the Netherlands [7]. The global emergence of MRSP, the widespread distribution of most clonal lineages in distant countries and the changes in their population structure raised questions about the mechanisms driving MRSP genetic diversity and the success of certain clones [6].

Antimicrobial susceptibility testing, indeed, highlighted the multidrug resistant nature of MRSP isolates. Only the susceptibility to the topical drug mupirocin (all except 2 strains) has been generally observed as possible consequence of the limited use of this drug in veterinary practice (Table 1).

In contrast with other recent studies underlying a general higher resistance of isolates belonging to CC71 compared with those of the clonal complex CC258, no evident correlation between antimicrobial resistance profile and ST/CC was observed, except for the infrequent

resistance to aminoglycosides, fluoroquinolones and chloramphenicol of strains belonging to CC258 [7,8,18,22]. Common resistance profiles to almost all antimicrobial classes were identified, without any evident difference in the occurrence of resistance among isolates of different clonal lineage (Table 2).

As shown in a recent review, distribution of SCC*mec* types usually vary significantly between the different MRSP clonal groups [8]. Accordingly, SCC*mec* type analyses showed the association of SCC*mec* type III with CC71 and of type IV with CC258 [8,26]. SCC*mec* was non-typeable (NT) only in the MRSP isolate belonging to CC45 (ST341) (Table 2). NT-SCC*mec* elements are very common among MRSP of this CC and a pseudo SCC*mec* element (ΨSCCmec57395) has been discovered in it [18,26]. The variability of these SCC*mec* types in staphylococcal species seems to be a consequence of insertions and deletions of elements arranged in tandem. This is the case of SCC*mec* elements that carry *mec*A and *ccr* genes in separated genetic structures [26].

The phenotypic differences in the AMR profiles found among MRSP isolates should reflect variations in the carriage of resistance genes, probably due to truncations of mobile elements that carry these genetic determinants as shown recently by McCarthy et al. (2015) [29].

A substantial concordance between AMR gene profiles and antimicrobial susceptibility testing of the isolates was observed (Table 2).

In staphylococci, the transposon structure Tn5405 found on the chromosomes, has been shown to harbor various resistance genes including aph(3′)III and aadE conferring kanamycin and streptomicin resistance, respectively and gene *sat*4 conferring streptothricin resistance [6,30]. Tn5405 has been also found linked to an erm(B) gene cluster in different isolates of S. pseudintermedius [31,32]. All our isolates carried aph(3′)III as well as *sat*4 and all but one the *erm*B gene, suggesting the probable presence of these clusters and of the transposon.

All but two isolates carried the *aac*(6′)Ie-*aph*(2″)Ia gene codifying for aminoglycoside adenyl-phosphotransferase, responsible of gentamicin-tobramycin resistance.

The susceptibility of the two MRSP strains (ID 3 and ID 8) to gentamicin and tobramycin is in concordance with previous studies highlighting how carriage of resistance genes does not necessarily result in a resistance phenotype, since lack of necessary genetic elements, such as promoters, hampers their expression [33]. Tetracycline resistance was explained by carriage of *tet*M and *tet*K. Although *tet*M is usually the most common tetracycline resistance determinant, in these species, all the resistant isolates belonging to CC71 carried *tet*K gene. This atypical feature of this clone has been confirmed in previous studies, suggesting a strong association between CC71 and *tet*K gene [6,8,34].

## 5. Conclusions

In conclusion, this study provides new insight into phenotypic and genotypic features of MRSP isolates from clinical cases of animal origin, especially dogs. The high prevalence of different clones with MDR traits among MRSP population highlights the importance of preventing the spread of MRSP in the dog population, i.e. implementing antimicrobial stewardship and surveillance programmes globally [35].

Infections due to virulent MDR strains rise in severity and may increase concern about their potential transmission to humans, especially among veterinarians and pet owners.

The potential consequences of the use in veterinary medicine of critically important antimicrobials for treatment of infections in humans should not be underestimated and suggest to enforce the surveillance of extra-label drug use, as suggested by other Authors [36].

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