Genomic insights into the emergence and spread of methicillin-resistant *Staphylococcus pseudintermedius* in veterinary clinics

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**ABSTRACT**

*Staphylococcus pseudintermedius* is a common cause of skin and soft tissue infections in dogs but can also cause infections in cats and humans. The frequency of methicillin-resistant *S. pseudintermedius* (MRSP) strains is increasing worldwide. Here, we obtained 43 MRSP isolates from dogs (*n* = 41), one cat (*n* = 1) and the small animal clinic environment (*n* = 1) in Slovenia from the period 2008–2018, which underwent whole-genome sequencing (WGS) and antimicrobial susceptibility testing. Five sequence types (STs) were identified, with ST71 (32/43) and ST551 (8/43) being the predominant. In Slovenia, ST551 was first detected in 2016, whereas a decrease in the frequency of ST71 was observed after 2015. All isolates were multidrug-resistant and most antimicrobial-resistant phenotypes could be linked to acquisition of the corresponding resistance genes or gene mutations. Core-genome multilocus sequence typing (cgMLST) revealed several potential MRSP transmission routes: (i) between two veterinary clinics by a single MRSP-positive dog, (ii) between the environment of a veterinary clinic and a dog, and (iii) between a canine and a feline patient through the contaminated environment of a veterinary clinic. Of the six dogs that were additionally sampled from 14 days to five months after the initial sampling, each harbored the same MRSP strain, suggesting a limited within-host diversity of MRSP in symptomatic dogs. The present results highlight the importance of MRSP-positive dogs in the spread of veterinary care-associated MRSP infections and call for the implementation of strict control measures to reduce MRSP contamination in veterinary clinic environments originating from animal-contact surfaces.

1. Introduction

*Staphylococcus pseudintermedius* is part of the commensal microbiota of the skin and mucous membranes in companion animals but can also cause opportunistic infections mainly affecting skin and soft tissues (Bannoehr and Guardabassi, 2012). Over the past decade, an increase of methicillin-resistant *S. pseudintermedius* (MRSP) in both asymptomatic and symptomatic dogs has been observed (Bannoehr and Guardabassi, 2012). Risk factors for MRSP carriage and infections include frequent veterinary visits, surgery, hospitalization and antimicrobial use (Lehner et al., 2014). When inspecting antimicrobial resistance (AMR) in MRSP isolates, multidrug resistance (MDR, i.e. resistance to ≥3 antimicrobial groups; Magiorakos et al., 2012) has been frequently reported, greatly complicating therapeutic interventions (Perreten et al., 2010; Windahl et al., 2016).

*S. pseudintermedius* has an epidemic clonal population structure, with certain successful lineages, i.e. clonal complexes (CCs) or sequence types (STs), emerging and rapidly spreading within a weakly clonal background (Pires dos Santos et al., 2016). The predominant MDR MRSP clones vary with geographic location. In Europe, ST71 is the most prevalent but seems to be challenged recently by other successful clones such as ST258, ST496 and ST551 (Kizerwetter-Swida et al., 2017; Bergot et al., 2018). In Australia and New Zealand, ST71 and ST496 are frequently reported (Worthing et al., 2018a; Nisa et al., 2019). In North America, CC68 is the predominant clone, whereas CC45 and CC112 are frequent in Asia (Perreten et al., 2010; Pires dos Santos et al., 2016).

Whole-genome sequencing (WGS) is the current method of choice for typing of bacterial pathogens. Although WGS has been employed to determine the population structure of MRSP and its genetic characteristics (Bergot et al., 2018; Worthing et al., 2018a, Worthing et al., 2018c; Nisa et al., 2019; Smith et al., 2020; Zukancic et al., 2020), only a limited number of studies have used it for epidemiological investigations to elucidate MRSP transmission routes (Windahl et al., 2016; Corro et al., 2018). Moreover, the genomic epidemiology of MRSP in Slovenia has
not yet been examined. The aims of the present study were to determine the clonal relationship between MRSP isolates from seven small animal clinics in Slovenia obtained over a 10-year period and to compare phenotypic and genotypic AMR patterns.

2. Materials and methods

2.1. Selection of isolates

MRSP isolates were selected from the *S. pseudintermedius* strain collection of Veterinary Faculty, Institute of Microbiology and Parasitology. A total of 43 clinical MRSP isolates originating from seven small animal clinics (designated A–G) located in three different geographical regions of Slovenia from the period 2008–2018 were included in the study; only isolates with extensive epidemiological data were selected. For each isolate, the corresponding metadata (date of collection, sample type, clinic and animal owner) were obtained (Supplementary file 1). Canine (*n* = 41) and feline (*n* = 1) MRSP isolates were obtained from different sample types, mainly swabs (e.g., skin, wound, nose and implant) and urine, but also synovial fluid and callus tissue. One isolate (SP6) was obtained from the veterinary clinic environment (clinic A). Of the 41 canine isolates, 13 originated from six dogs (SP8/SP9/SP10; SP15/SP16; SP17/SP18; SP28/SP29; SP34/SP35; SP46/SP50) that were additionally sampled from 14 days to five months after the initial sampling, and two (SP31/SP33) originated from a single dog that visited two different veterinary clinics within a 20-day period.

All isolates were confirmed as *S. pseudointermedius* by the matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometer using the Biotyper 3.1 software (Bruker Daltonics, Germany) according to the manufacturer’s instructions. The MRSP phenotype was determined as resistant to oxacillin. Susceptibility to oxacillin was determined on Mueller–Hinton agar (Merk, Germany) and performed according to the recommendations of Clinical and Laboratory Standard Institute (CLSI, 2020). Resistance to oxacillin was defined as resistant to oxacillin. Susceptibility to oxacillin was determined using the CLSI scheme consisted of 2887 loci. After the removal of 14 paralogous and 2 neutral clusters, the sequence of the strain HKU10-03 (NCBI accession number CP080156) was performed using a NextSeq500 system (Illumina, USA) to a minimum coverage of 140 × . Sequencing data were submitted to the NCBI Sequence Read Archive (SRA) database under the BioProject accession number PRJNA635146.

Quality of the obtained reads was assessed using FastQC v0.11.5 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/); reads passing the ‘basic statistics’, ‘per base sequence quality’ and ‘per sequence quality scores’ were included in further analyses. Raw reads were assembled using SPAdes v3.13.1 (Bankevich et al., 2012) / Shovill v1.18 (https://github.com/tseemann/shovill) by applying the default parameters and the ‘-trim’ option. The assembly quality was assessed using Quast v5.0.2 (Gurevich et al., 2013); only the genomes with N₅₀ > 20,000 bp, number of contigs longer than 1,000 bp < 500 and total assembly length of 2.6 Mb with allowed deviation of ± 20 % were included in further analysis.

2.4. Analysis of genome sequences

Analysis included in silico 7-gene multilocus sequence typing (MLST), SCCmec (staphylococcal chromosomal cassette mec) typing and core-genome multilocus sequence typing (cgMLST). The content of virulence-associated and antimicrobial resistance genes was also determined. To gain further insight into the genetic diversity of ST551, including its virulence-associated and antimicrobial resistance genes, all ST551 genomes (*n* = 3) from a previously published collection of 622 *S. pseudintermedius* genomes (Zukancic et al., 2020) were included in the analysis for comparison.

2.4.1. MLST and SCCmec typing

In silico MLST was performed using the ‘Sequence query’ tool implemented in the *S. pseudintermedius* BIGSdb website (https://pubmlst.org/spseudintermedius/). SCCmec type was determined by a BLASTn tool to compare a custom BLAST database using a cut-off of ≥ 90 % identity and ≥ 60 % coverage; the custom BLAST database consisted of six SCCmec types previously described in *S. pseudintermedius* (Worthing et al., 2018c). When no BLAST hit was obtained, SCCmec type was predicted by read mapping using Genoieus read mapper v6.0.3 implemented in Genoieus v11.1.5 (Biomatters Ltd.).

2.4.2. Determination of virulence-associated and antimicrobial resistance genes

The presence of previously described 67 *S. pseudintermedius* virulence-associated genes (Bergot et al., 2018; Zukancic et al., 2020) and six qac genes conferring resistance to the quaternary ammonium compounds (Worthing et al., 2018b) was determined by BLASTn using a cut-off of ≥ 90 % identity and ≥ 70 % coverage (Supplementary file 2). The presence of AMR genes was determined using ResFinder 3.2 (Zankari et al., 2012) by applying the default cut-off settings of 60 % coverage and 90 % identity. The hits where alignment length was shorter than database resistance gene length were reported. To determine the genetic background of fluoroquinolone resistance in *S. pseudintermedius*, the presence of mutations in gyrase (Ser84Leu in gyrA) and topoisomerase IV (Ser80Ile in parC) genes (Bergot et al., 2018) was analyzed using BLASTn. To this aim, the reference gyrA and parC genes sequences from the strain CCUG 49543 (NCBI accession numbers AM262968 and AM262971, respectively) were queried against a custom BLAST database consisting of all the studied genome assemblies.

2.4.3. cgMLST

The construction of an ad hoc cgMLST scheme for *S. pseudintermedius* was performed using the blast2MMACA v2.1.0 by applying the default parameters (Silva et al., 2018). The Shovill assemblies were used for the scheme construction and genome of the strain HKU10-03 (NCBI accession number NC_014925.1) was used as a training file for gene prediction using Prodigal (Hyatt et al., 2010). The initial whole-genome MLST scheme consisted of 2887 loci. After the removal of 14 paralogous and
780 non-core loci, the final cgMLST scheme (defined as loci present in at least 95% of the studied genomes) consisted of 2093 loci. The minimum-spanning tree (MST) was constructed using Grapetree v1.5.0 (Zhou et al., 2018) with the MSTreeV2 algorithm; all loci with missing values were removed from each pairwise comparison. The neighbor-joining cgMLST dendrogram was constructed using Grapetree and visualized and annotated using iTol v4.4.2 (Letunic, and Bork, 2019).

3. Results

3.1. Antimicrobial susceptibility testing

All the tested isolates showed a MDR phenotype regardless of their MLST type; they were resistant to five (1/41; 2.4%), six (18/41; 43.9%), seven (19/41; 46.3%) or eight (3/41; 7.3%) antimicrobial groups (Fig. 1). The latter (SP34, SP35 and SP42) were isolated in 2010 or 2011 and belonged to ST71. All the tested isolates were resistant to oxacillin, penicillin, clindamycin, erythromycin, and trimethoprim, but susceptible to rifampicin, fusidic acid, vancomycin, linezolid, mupirocin, quinupristin/dalfopristin, and sulfamethoxazole (Fig. 1). Non-wild-type MICs for streptomycin and kanamycin were observed in all isolates (MIC > 32 and > 64, respectively). For tiamulin, all isolates showed wild-type MIC ≤ 0.5. Furthermore, 40/41 (97.6%); all isolates except SP25 belonged to ST307) isolates were resistant to ciprofloxacin, 38/41 (92.7%) to gentamicin, 15/41 (36.6%) to tetracycline and 13/41 (31.7%) to chloramphenicol. All ST551 isolates were resistant to tetracycline and susceptible to chloramphenicol, whereas this pattern was rarely observed in ST71 isolates (Fig. 1).

3.2. Whole-genome sequencing

3.2.1. MLST typing

All genomes passed the basic quality metrics for the read sequencing data and assemblies. In silico MLST analysis revealed that 32/43 (74.4%) isolates belonged to ST71 and 8/43 (18.6%) to ST551; three STs (ST25, ST307 and a novel ST1095) were each represented by a single isolate (SP49, SP25 and SP12, respectively) (Fig. 1, Supplementary file 1).

3.2.2. SCCmec typing

All ST71 isolates harbored SCCmec type II–III, whereas all ST551 isolates SCCmec type V (Fig. 1). The latter was also found in ST25 and ST1095 isolates (SP49 and SP12, respectively), whereas the ST307 isolate (SP25) harbored SCCmec type IVg. The SCCmec type was conserved within a given MLST type (Fig. 1).

3.2.3. Determination of virulence-associated genes

Fig. 2 shows the distribution of the 67 virulence-associated genes divided into eight functional groups (cell division protein, exoenzyme, genetic regulator, metallo-beta-lactamase, protease, signal transduction, surface protein and toxin). The majority (56/67; 83.6%) of the genes were present in at least one of the investigated genomes; since 42/67 (62.7%) were present in all the studied genomes, this indicates they constitute part of the core genome. All isolates harbored genes encoding the bicomponent leukotoxin Luk-I (lukF-I and lukS-I), exofoliatative toxin

Fig. 1. cgMLST tree of 43 MRSP isolates showing the distribution of AMR phenotypes and genotypes and qac genes. The neighbor-joining cgMLST tree is based on the allele profiles of 2093 cgMLST loci. SCCmec and MLST type are added to the tree. *Isolates SP32 and SP44 could not be revived from frozen stocks for antimicrobial susceptibility testing after performing WGS. AMR: antimicrobial resistance. The following antimicrobial groups were tested: aminoglycosides (GEN: gentamicin, KAN: kanamycin*; STR: streptomycin*), beta-lactams (OXA: oxacillin, PEN: penicillin), fluoroquinolones (CIP: ciprofloxacin), lincosamides (CLI: clindamycin), macrolides (ERY: erythromycin), phenicols (CHL: chloramphenicol), tetracyclines (TET: tetracycline) and trimethoprim (TMP).

* For KAN and STR, epidemiological cut-off values were used; only non-wild-type phenotypes are reported.
SIET (siet) and enterotoxin Se-int (se-int), but none the exfoliative toxin genes expA and expB or canine type C enterotoxin gene sec canine. Among the ‘non-core’ virulence-associated genes, mazG, mbl, spsD, spsP and spsQ and virE genes were generally present in ST71 isolates but absent in all ST551 isolates, whereas coa and spsK genes were absent in all ST71 isolates (Fig. 2). No virulence-associated gene was found exclusively in ST551 isolates.

3.2.4. Determination of resistance genes

In total, 10 AMR genes were identified, conferring resistance to: (i) aminoglycosides (aac(6)-aph(2'), aph(3')-III and ant(6)-Ia); (ii) beta-lactams (blaZ and mecA); (iii) macrolide, lincosamide and streptogramin (MLS) group (erm(B)); (iv) tetracyclines (tet(K) and tet(M)); (v) trimethoprim (dfrG); and (vi) chloramphenicol (catPC221) (Fig. 1). In addition, two chromosomal point mutations conferring fluoroquinolone resistance were identified in gyrA (Ser84Leu) and grlA (Ser80Ile). ST551 isolates showed a conserved pattern of resistance genes, whereas ST71 isolates were more heterogeneous. The presence of tet(K) and tet(M) genes and the absence of chloramphenicol resistance gene were observed in all ST551 isolates, whereas this pattern was observed in a single ST71 isolate (SP47). Regarding the comparison of AMR phenotype–genotype, high levels of concordance were observed with few exceptions. In general, 100 % concordance was observed for 16 antimicrobials tested (including oxacillin), whereas discordances were observed for chloramphenicol (1/41; catPC221 gene present but phenotypically susceptible), tetracycline (2/41; tet(K) and tet(M) genes present

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**Fig. 2.** cgMLST tree of 43 MRSP isolates showing the distribution of 67 virulence-associated genes. The neighbor-joining cgMLST tree is based on the allele profiles of 2093 cgMLST loci. The virulence-associated genes are classified into eight functional categories. MLST type and clinic are added to the tree.

**Fig. 3.** Minimum-spanning tree of 43 MRSP isolates based on the allele profiles of 2093 cgMLST loci. The numbers on the connecting lines denote allele distances and the lines are scaled logarithmically.
but phenotypically susceptible) and gentamicin (2/41; *aac(6')-aph(2')* gene present but phenotypically susceptible). The *qac* genes conferring resistance to the quaternary ammonium compounds were always absent, except in the isolate SP49 (ST25) with *qacG* (Fig. 1).

### 3.2.5. cgMLST-based transmission analysis

The cgMLST analysis revealed two genetically homogeneous groups of isolates corresponding to ST71 and ST551 (Fig. 3). The median pairwise distance between ST71 isolates was 39 cgMLST alleles (range, 0–76) and between ST551 isolates 25 cgMLST alleles (range, 0–38) (Supplementary file 3). Isolates SP5 (canine abscess swab) and SP6 (veterinary clinic environment), both originating from clinic A, differed in a single cgMLST allele (Fig. 3); environmental swab was collected ten days after the canine patient had visited the clinic and harbored the same strain as the abscess swab. In all the six cases where more than one isolate per dog was collected, the isolates from the same dog (SP6/SP9/SP10, SP15/SP16, SP17/SP18, SP28/SP29, SP34/SP35 or SP46/SP50; Fig. 3) were genetically closely related with a median pairwise distance of 2 cgMLST alleles (range, 0–8), indicating the within-host persistence of the same MRSP strain for up to five months. Moreover, the isolates SP31 and SP33, originating from the same dog that visited two different animal clinics (Supplementary file 1), differed in 5 cgMLST alleles, indicating a potential for transmission of MRSP between different animal clinics. The canine isolates SP8, SP9 and SP10 were isolated from a wound of the same dog within a 1-month period. During the same period, the isolate SP11 was obtained from a feline wound at the same clinic (clinic B; Supplementary file 4). All four isolates differed in ≤ 1 cgMLST allele (Fig. 3, Supplementary file 3). The feline isolate SP11 was obtained ten days after the canine isolate SP9 was collected, suggesting a transmission of the MRSP clone from a dog to a cat through the veterinary clinic environment. The feline patient visited the clinic due to a broken leg and underwent osteosynthesis. Subsequently, SP11 was isolated from the feline wound, which did not respond to the empirical antibiotic treatment. Five other presumable transmission clusters of closely related isolates (≤ 9 cgMLST allele differences) obtained within a 1-year period from the same clinic were identified (Fig. 3, Supplementary file 3), but incomplete epidemiological data precluded identification of transmission routes or common sources of infection (e.g., persistent strains in veterinary clinics).

### 3.2.6. Phylogeny of ST551 clone

The cgMLST analysis revealed that all ST551 isolates were fairly clonal, regardless of the country of isolation (Supplementary file 4). In addition, their resistance and virulence-associated genes were generally conserved (Supplementary file 4).

### 4. Discussion

In the present study, most MRSP isolates belonged to ST71, which is also the most frequently reported MDR clone in Europe (Perret et al., 2010; Pires dos Santos et al., 2016) and increasingly reported worldwide (Worthing et al., 2018a; Nisa et al., 2019; Smith et al., 2020). ST551, the second most frequent clone in Slovenia, was only encountered in the last 2010; Pires dos Santos et al., 2016) and increasingly reported worldwide observed over time. Both prevailing clones were encountered in three years of the study (2016–2018), whereas a decrease of ST71 was observed over time. Both prevailing clones were encountered in three geographically distant regions in Slovenia, suggesting they are widely distributed across the country. The present results indicate that ST551 may be an emerging MDR clone that will gradually replace ST71 in Slovenia, as reported in Poland (Kizerwetter-Swiđa et al., 2017). In France, other emerging clones (ST258 and ST496) were observed (Bergot et al., 2018). ST551 was reported as a rare clone in a large global collection of *S. pseudintermedius* genomes (3 out of 622 genomes; 0.5%) (Zukancic et al., 2020). We showed that ST551 isolates are fairly clonal, regardless of their country of origin. Further studies are needed to assess the genetic diversity of ST551 when additional genomes are available. Most virulence-associated genes were generally conserved across different STs including ST551. Nonetheless, some lineage-associated virulence genes were also identified, as described previously (Zukancic et al., 2020). In our study, the virulence-associated genes coa (coagulase) and spsK (cell-wall anchored surface protein SpSK) were found in all ST551 isolates, but not in ST71 isolates, which may contribute to the success of this clone with regard to canine colonization/infection. The study conducted by Lee and Yang (2020) showed that ST551 shows a high *in vitro* resistance to canine cathelicidin (K9CATH), a cationic antimicrobial peptide, which may also explain in part its successful adaptation to the canine host. None of the known virulence-associated genes were found exclusively in ST551 isolates, which may suggest that other, as yet undescribed, genetic traits underlie the host adaptation and virulence of this successful MRSP clone. Further genotypic and phenotypic studies are needed to better understand the successful adaptation of ST551 to the canine host. In addition to the sole presence of virulence-associated genes, their expression rates may also vary between different MRSP clones, affecting their virulence (Couto et al., 2016).

The *qac* genes, which are associated with increased tolerance to the quaternary ammonium compounds and other cationic biocides, were found in the ST25 isolate (SP49), contrary to a previous study in which ST71 strains were significantly more likely to harbor *qac* genes compared with other MRSP clones (Worthing et al., 2018b). Regarding the antimicrobial resistance, all ST71 and ST551 isolates were MDR in the present study, which may contribute to the success of these MRSP clones in the veterinary clinics where antimicrobial selection pressure is present.

The present results suggest that ST551 is one of the emerging MDR clones in Slovenia, whereas in France and in the Northern Europe, the reported emerging clone is ST258, which shows a higher susceptibility to the veterinary-licensed antimicrobials compared with ST71 (Bergot et al., 2018). In this study, the detected AMR genes were similar to those reported previously in canine MRSP isolates (Wegener et al., 2018; Worthing et al., 2018a; Nisa et al., 2019; Pérez-Sancho et al., 2020). Similar to the previously published studies, we observed high levels of concordance between the AMR phenotype and genotype patterns (Corrò et al., 2018; Wegener et al., 2018). All discordances observed in this study were due to the presence of acquired AMR genes, but none could be explained by mutations in these genes. These results are consistent with the findings of Wegener et al. (2018), who also reported the presence of acquired AMR genes in tetracycline-, chloramphenicol- and gentamicin-susceptible isolates, and more than half of these discrepancies could not be explained by mutations in these genes. Discrepancies could be explained by the modulation of gene expression, which is difficult to detect using WGS-based antimicrobial susceptibility testing (Bortolaia et al., 2020). In addition, the observed MIC values for chloramphenicol and gentamicin were close to the breakpoint and may thus be misinterpreted due to the lack of breakpoints specific for *S. pseudintermedius* or may be related to the limitations of the MIC method.

In this study, antimicrobials used exclusively in human medicine were also tested and all isolates were susceptible. Therefore, these antimicrobials could be used in human MRSP infections, which have also been reported in the past (Stegmann et al., 2010; Starlander et al., 2014). Due to the zoonotic potential of MRSP and horizontal transfer of AMR genes, AMR surveillance should not be limited to antimicrobials for veterinary use only.

In WGS-based typing, pairwise genetic relatedness (i.e. the number of pairwise allele or single nucleotide polymorphism (SNP) differences) between the isolates is essential to confirm their microbiological association (clonal origin), but the use of a fixed threshold may lead to erroneous conclusions because the observed genetic distances are species- and context-dependent (Pighiting et al., 2018). Previous studies revealed a small genetic distance between pairs of epidemiologically related *S. pseudintermedius* isolates, with ≤ 7 cgMLST alleles and ≤ 5 SNPs in vertical transmission of *S. pseudintermedius* in dogs (Corrò et al.,...
removing MRSP and veterinary staff can also be transiently colonized. Disinfection of the animal clinic environment is often ineffective in these settings can occur relatively easily and the surfaces and equipment can be contaminated suggesting that contamination of the environment in the veterinary clinic A and in a canine patient. Furthermore, a pair of epidemiologically related and genetically closely related isolates originating from a single dog treated at different clinics indicates that dogs can transmit MRSP between different clinics and may thus represent a potential source of veterinary care-associated MRSP infections. Other clusters, particularly those observed among ST551 isolates, suggest that small animal clinics may be the source of MRSP infections in dogs, but more isolates should undergo WGS to gain further insight into the genetic diversity of the ST551 clone.

Certain limitations of the study need to be acknowledged. First, the studied isolates were obtained from animals during their routine examination and/or treatment. Hence, the veterinary care-associated transmission of MRSP could not be reliably confirmed because detailed data on patient’s visits or hospitalizations prior or at the time of MRSP isolation were not available. Second, a single MRSP isolate per animal was analyzed at a given sampling time and a maximum of three isolates per single animal were analyzed over time. Thus, the within-host diversity of MRSP was not extensively assessed herein. In Staphylococcus aureus, the within-host diversity of a single clone can be as high as 40 SNPs (Golubchik et al., 2013); however, the maximum within-host diversity of MRSP remains understudied. For the latter, we reported 8 cgMLST alleles, although it should be noted that the isolates were not obtained at the same time. Because MRSP is susceptible only to a limited number of veterinary-licensed antimicrobials, the within-host persistence of MDR MRSP strains in dogs may be explained by unsuccessful antibiotic treatment.

Transmission of MRSP between animals is common, especially in animals in contact with symptomatic MRSP patients (van Duijkeren et al., 2011). We obtained one of the isolates from the elbow callus, suggesting that contamination of the environment in the veterinary settings can occur relatively easily and the surfaces and equipment can be the source of MRSP for other dogs, especially if underlying skin problems are present. Felleti et al. (2018) reported that environmental MRSP isolates were found in the dog ward areas, waiting- and triage-rooms, where animal traffic is frequent. In the veterinary clinics, these areas can lead to the veterinary care-associated MRSP infections, and more frequent clinic visits and hospitalizations are associated with a higher risk of MRSP infection (Lehner et al., 2014). Moreover, regular disinfection of the animal clinic environment is often ineffective in removing MRSP and veterinary staff can also be transiently colonized with MRSP, indicating a potential public health risk (van Duijkeren et al., 2011). Of note, ST71 clone has a propensity to cause large nosocomial MRSP outbreaks in cats and dogs, and rigorous control measures are needed to control such outbreaks (Grönhôl et al., 2014). Due to the limited therapeutic options for MDR MRSP infections in animals, strict hygiene practices are of utmost importance. In addition to general recommendations for hygiene and infection control in veterinary practice, MRSP-specific control measures should be considered, such as risk-based classification of animal patients and isolation of high-risk patients, screening of at-risk patients, contact tracing and early detection of a potential outbreak (Grönhôl et al., 2014; Morris et al., 2017).

5. Conclusion

The present study shows a regional spread of two successful and genetically homogeneous MDR MRSP clones (ST71 and ST551) in veterinary settings in Slovenia. Transmission of MRSP strain from a canine to a feline patient through the contaminated environment of a veterinary clinic was identified. In addition, the same MRSP strain was obtained from a dog upon its visit to two different veterinary clinics. Because both symptomatic and asymptomatic dogs can carry MRSP and contaminate the veterinary settings, implementation of appropriate control measures is needed to prevent veterinary care-associated infections. Implementation of a national and international routine surveillance system for MRSP in companion animals, veterinary clinics and veterinary personnel is necessary to reduce the burden of this important veterinary pathogen, with particular emphasis on detection of emerging MDR MRSP clones such as ST551.

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Declaration of Competing Interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary material related to this article can be found in the online version, at doi:https://doi.org/10.1016/j.vetmic.2021.109119.

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