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Genome Note

Draft genome of an extremely drug-resistant st551 *Staphylococcus pseudintermedius* from an Italian dog with otitis externa

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ABSTRACT

Objectives: To determine the draft genome sequence and analyse the genetic features of a *Staphylococcus pseudintermedius* clinical isolate according to the main typing schemes available, with a special focus on antibiotic resistance.
Methods: The strain was isolated from a case of otitis externa in a dog. Its identity and pattern of antibiotic susceptibility were determined using an automated system. The genome was sequenced using an Illumina platform. MLST, SCCmec typing, resistome, and mobile genetic elements were derived by comparative analysis using available specific databases.
Results: S. pseudintermedius CAM1 isolate has a chromosome size of 2 652 610 bp. It showed a wide pattern of phenotypic resistance, comprising beta-lactams, macrolides and lincosamides, aminoglycosides, fluoroquinolones, tetracyclines, and trimethoprim-sulfamethoxazole. The genetic determinants of the underlying mechanisms were all found by in silico analysis of the genome. The *mec*A gene for methicillin resistance was harboured by the Vc type of the SCCmec. MLST of the strain was st551.
Conclusion: By comparison with the MLST database of *S. pseudintermedius* and data from published molecular epidemiology studies, CAM1 is the first st551 strain recorded in Italy and, in the context of an already extremely wide antibiotic resistance pattern, it harbours also the *tet*K gene, the prevalence of which is rare in MDR *S. pseudintermedius*.

Staphylococcus pseudintermedius (SPI) is an opportunistic pathogen in dogs, commonly found on the nasal and pharyngeal mucosa, where it can actually be considered resident, and on the skin, where it represents the main saprophytic microorganism. It is often associated with otitis when the ear is affected [1].

S. pseudintermedius CAM1 was isolated at the end of 2017 from a dog with a recurrent and difficult to treat otitis externa. Identity was determined by automated clinical diagnostics (Vitek 2) and successively confirmed by comparison of the draft genome with the Genome Taxonomy Database (GTDB) and the rMLST database (Table 1). CAM1 is a methicillin-resistant SPI (MRSP) – although resistant to beta-lactams – with a wide array of resistance to many other antibiotics belonging to macrolides, lincosamides, streptogramin B, aminoglycosides, fluoroquinolones, tetracyclines, and trimethoprim-sulfamethoxazole, and it showed an intermediate susceptibility towards fusidic acid (Supplementary Table 1S).

The NGS pipeline prior to last quality assessments and annotation was conducted with BaseClear B.V. (The Netherlands) and is outlined in Supplementary Table 2S. Briefly, paired-end sequence reads were produced using the Illumina HiSeq2500 system. FASTQ sequence files were generated using bcl2fastq2 version 2.18. Initial quality assessment was based on data passing the Illumina Chastity filtering. Subsequently, reads containing PhiX control signal were removed using an in-house filtering protocol. In addition, reads containing (partial) adapters were clipped (up to minimum read length of 50 bp). A second quality assessment was based on the remaining reads using the FASTQC quality control tool. The final quality score per sample was 37.87 and the genome coverage was 380×. The quality of Illumina reads was improved using a read error correction tool. Assembly into contigs followed using SPAdes. The order of contigs, and the distances between them, were estimated using the insert size information derived from an alignment of the paired-end reads to the draft assembly.

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Table 1
Main genotypic signatures based on in silico analysis of the draft genome.

Category	Name	Gene or type
Classification	Objective taxonomic classification[a]	*Staphylococcus pseudintermedius* (99.33%)
	rMLST[b]	
General typing	MLST[c]	ST551
	SCCmec type[d]	Vc
Resistance[e]	Beta-lactams	*bla*Z, beta-lactamase
		*mec*A, methicillin resistant PBP2
	Aminoglycosides	APH(2″)-AAC(6′)
		ANT(6)
		APH(3′)
	Tetracyclines	TetM, ribosomal protection protein
		TetK, MFS antibiotic efflux pump
	Diaminopyrimidines	*dfr*, dihydrofolate reductase
	macrolides; lincosamides; streptogramin B	*erm*B, 23S ribosomal RNA methyltransferase
	Fluoroquinolones*	Ser84 → Leu in GyrA
	Fusidic acid	H457 → Y in FusA
	Nucleosides	SAT, streptothricin acetyltransferase
Mobile genetic element	Plasmid[f]	rep7a
		repUS43
	Insertion sequence[g]	IS431mec (IS6 family)
		IS256
		ISCco2 (IS1595 family)

Software and databases used in the analysis.
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* Mutations in gyrA, gyrB, parC, and parE genes were searched by standard alignment using BLASTn and BLASTp tools available at NCBI.

Consequently, contigs were linked together and placed into scaffolds. Using Illumina reads, gapped regions within scaffolds were (partially) closed. Finally, assembly errors and the nucleotide disagreements between the Illumina reads and scaffold sequences were corrected.

The annotated draft genome was 2 652 610 bp long and was predicted to express 2542 genes (2444 proteins) (GenBank accession no. JADFWN000000000.1). It was inspected for several specific genes and to derive common typing schemes.

CAM1 is an MLST sequence type 551 strain according to the 7 loci scheme [2]. The PubMLST database contains 12 records of *S. pseudintermedius* st551 strains isolated between 2015 and 2018, from different geographical locations and animal hosts (https://pubmlst.org/, last accessed 5 February 2021): six from Poland (two from dogs and four from cats), three from Switzerland (one from a dog, one from a human patient, and one from an unspecified source), two from dogs in Sweden, and one from a dog in the USA. They were isolated in these countries in a period of circulation of the st551 clone, that was likely to emerge in Poland between 2016 and 2017 [3]. CAM1 was in fact isolated in 2017, evidence of the spread of st551 throughout Europe at the time. The comprehensive genomic analysis by Zukancic et al. considered 660 SPI isolated worldwide and recorded three st551 strains only, all isolated in the USA (GenBank accession ns. SRR8206956, SRR8864267, SRR10076036) [4]. In addition, the genome sequence of one strain from India was submitted to GenBank recently (GenBank accession no. CP054206). Isolation of no other st551 was apparently reported before 2015 in the literature [5]. The comparison of CAM1 with the genomic sequences of the cited four st551 strains revealed that it is spa-negative with the spa-region classified as type VI according to the classification proposed by Zukancic et al. [4]. Also, the virulence gene profiles of CAM1 in the st551 strains from USA were similar, presenting one copy of the gene coding for the surface protein SpsR (94% similarity) and one for the exotoxin Sec (54% similarity).

CAM1 is resistant to tetracyclines not only from presence of the *tet*M gene, which is well known in SPI, but also from *tet*K, adding tetracycline efflux to ribosomal protection. *tet*K is far less frequent in SPI [4]. BLAST search of the *tet*K nucleotide sequence against the SPI reference database set (taxid:283734) hit only two deposited plasmid sequences (GenBank accession nos. MN612109 and CP016075) related to two st71 strains, namely G3C4 and 081661 (GenBank accession nos. CP032682 and CP016073). These plasmids shared similarity to a sequence in contig28 of the SPI CAM1 scaffolding, which contained both the rep7a CDS found by PlasmidFinder analysis (Table 1) and the *tet*K gene itself. Plasmid-mediated circulation in SPI isolates might have contributed to the increase in *tet*K-positive isolates observed very recently in Italy in a contest of an apparent expansion of new STs [6].

A literature survey showed that the wide resistance pattern of CAM1, enriched by the presence of *tet*K, is rare among SPI characterized so far. Studies conducted in the USA and Italy have detected some *tet*K-positive multidrug-resistant MRSP strains, however, belonging to different STs [6,7].

The genomic data of CAM1, belonging to a clone reported as spreading and causing infections in dogs in Europe, permits further comparative genomic analyses and gives new insights into the molecular epidemiology and biological characteristics of multi-drug-resistant *S. pseudintermedius*.

Ethical approval

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Competing interests

None declared.

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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.jgar.2021.02.025.

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