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Letter to the Editor

High genetic diversity among methicillin-susceptible *Staphylococcus pseudintermedius* in dogs in Europe

Sir,

Staphylococcus pseudintermedius is a commensal and opportunistic pathogen of cats and dogs, mostly causing skin and soft-tissue infections and post-operative complications. Since its emergence in 1999, the proportion of methicillin-resistant *S. pseudintermedius* (MRSP) has been on an increasing trend in Europe, ranging from 2.0% in Finland, 16.9% in France and up to 33% in Italy [1,2]. In addition to being resistant to β-lactams, MRSP also present numerous additional antimicrobial resistances that impair antibiotic treatment. Specific lineages of MRSP are disseminating worldwide, with sequence type 71 (ST71) being the dominant lineage in Europe [3]. In parallel to MRSP, the population structure of methicillin-susceptible *S. pseudintermedius* (MSSP) isolates is poorly known and only a few studies reported a large genetic diversity [1,2,4]. This is an obvious knowledge gap, since the majority of infections caused by *S. pseudintermedius* remain susceptible to methicillin.

To document both the antimicrobial susceptibility and the population structure of MSSP in Europe, in this study a collection of 97 *S. pseudintermedius* clinical isolates sampled between 2012 and 2016 in five European countries (France, n = 31; Germany, n = 32; Switzerland, n = 1; the Netherlands, n = 12; and Great Britain, n = 21) was characterised (Supplementary Table S1). All isolates were collected from dog otitis prior to any antibiotic treatment. For each animal, only one sample was taken in order to collect epidemiologically unrelated isolates, and chronically infected animals were excluded from the study. Whole-genome sequencing of all isolates was performed using Illumina NextSeq technology (BioProject no. **PRJNA574615**). Genomes were assembled using SPAdes 3.12 after subsampling reads to 80× coverage. STs were determined in silico using the database from the Center for Genomic Epidemiology, and antimicrobial resistance genes were identified using the ResFinder database (both available at http://www.genomicepidemiology.org). A total of 95 isolates were confirmed as MSSP, whilst the 2 remaining isolates were ST71 MRSP isolated in Germany in 2013 (isolate 13-01330) and Great Britain in 2014 (isolate 14-00872) (Supplementary Table S1). This suggests that MRSP belonging to the major European lineage can be responsible for otitis in untreated dogs (2/97; 2.1%). Among the 95 MSSP isolates, 17 (17.9%) presented no antimicrobial resistance genes, which was consistent with the phenotype determined by disk diffusion according to the breakpoints of the Antibiogram Committee of the French Society for Microbiology (https://www.sfm-microbiologie.org/). The *blaZ* gene responsible for resistance to penicillin G was observed in 75/95 (78.9%) MSSP isolates, of which 2 were susceptible to penicillin G, potentially due to an inducible phenotype [5]. Genes encoding the following antimicrobial resistances were detected among the MSSP isolates: tetracyclines (*tetM*) (33/95; 34.7%); macrolides–lincosamides (*ermB*) (17/95; 17.9%); streptomycin [*ant(6)-Ia*] (19/95; 20.0%); kanamycin [*aph(3′)-III*] (16/95; 16.8%); chloramphenicol (*cat*) (12/95; 12.6%); gentamicin [*aac(6′)–aph(2″)*] (3/95; 3.2%); and trimethoprim (*dfrG* or *dfrK*) (2/95; 2.1%). The canonical mutations in GyrA (Ser84→Leu or Gly88→Glu) and GrlA (Ser80→Ile or Asp84→Asn) were found only in the four isolates displaying high resistance to the veterinary-approved drug marbofloxacin [minimum inhibitory concentration (MIC) ≥ 4 mg/L], i.e. the two multidrug-resistant MRSP ST71 isolates and the MSSP ST610 and ST1155 isolates. Among the nine isolates with a low level of resistance to marbofloxacin (MIC = 0.5 mg/L), five were solely mutated in GrlA (Ser80→Ile or Asp84→Tyr). This confirms that isolated mutations in GrlA lead to low-level resistance whilst accumulation of mutations in GrlA and GyrA allows high-level resistance to fluoroquinolones. The proportions of antimicrobial-resistant *S. pseudintermedius* observed here are globally consistent with those reported in Italy and in France, but are much higher than those detected in Australia where MSSP isolates were susceptible to nearly all antibiotics tested [1,2,4]. This study indicates that MSSP isolates retrieved from untreated animals are frequently resistant to tetracyclines, macrolides–lincosamides and aminoglycosides.

Using a homemade python script (https://github.com/bvalot/pyMLST), the population structure was characterised by core genome multilocus sequence typing (cgMLST) analysis based on an allelic comparison of 1881 genes (carried by ≥93 of the 97 isolates) out of the 2432 genes carried by the *S. pseudintermedius* HKU10-03 reference strain (accession no. **NC_014925.1).** cgMLST revealed that the MSSP population was highly diverse, with the 95 isolates distributed into 90 STs (Fig. 1). Only four pairs of clonal MSSP isolates were retrieved, with no obvious epidemiological link for each pair. Two ST978 isolates were detected from the Netherlands (retrieved 8 months apart from two different dogs), two ST442 from Germany (retrieved 15 months apart from two different dogs), two ST1111 from Great Britain (retrieved 9 months apart from two different dogs) and two ST241, one coming from France and the second from the Netherlands. We confirm that the population structure of MSSP is highly heterogeneous and contrasts with that of MRSP [1,2,4]. Interestingly, no MSSP isolate belonging to the major MRSP clones (ST71, ST258 or ST496) was detected.

In conclusion, this study shows that *S. pseudintermedius* from dog otitis can harbour several antimicrobial resistance genes even in the absence of antibiotic treatment. The resistances observed further imply that treatment with penicillin G, for example, may no

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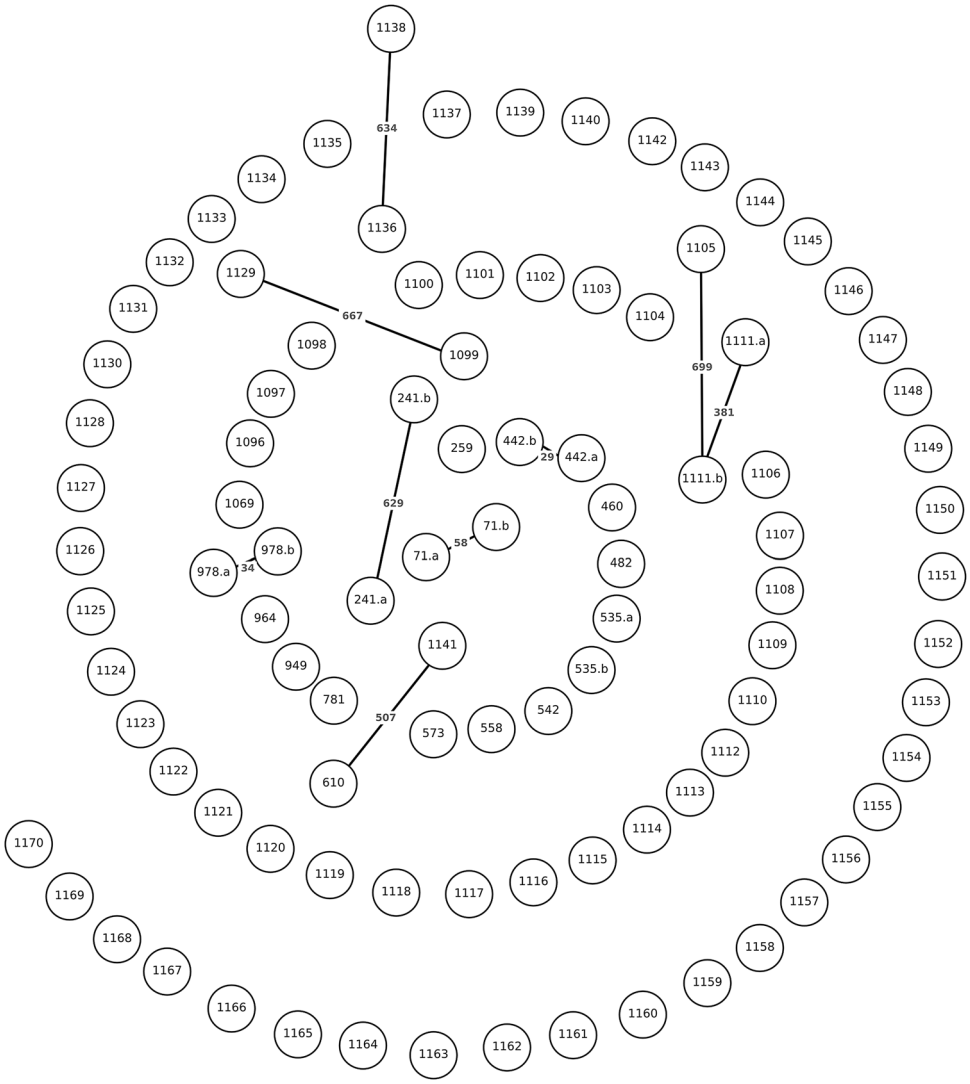

Fig. 1. Minimum spanning tree of a collection of 97 *Staphylococcus pseudintermedius* isolates [95 MSSP and 2 MRSP (71.a and 71.b)]. The tree was built with GrapeTree [6] using core genome multilocus sequence typing (cgMLST) distances inferior to 800 genes. Each isolate is named by its sequence type (ST). When two isolates belonged to the same ST, the letters 'a' and 'b' were added after the ST number. MSSP, methicillin-susceptible *S. pseudintermedius*; MRSP, methicillin-resistant *S. pseudintermedius*.

longer be a treatment option for MSSP, mirroring the situation of *Staphylococcus aureus* in humans. The population structure is highly heterogeneous despite the fact that all isolates came from the same pathology. Only studies performed at a national or even local level may document whether any clonality could be found between MSSP isolates.

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Competing interests

FEG and CM are employees of Vetoquinol S.A. (France).

Ethical approval

Not required.

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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.2020.02.016.

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Marisa Haenni*
Unité Antibiorésistance et Virulence Bactériennes, Université de Lyon–ANSES Laboratoire de Lyon, Lyon, France

Farid El Garch
Christine Miossec
Vetoquinol S.A., Scientific Division, Lure, France

Jean-Yves Madec
Unité Antibiorésistance et Virulence Bactériennes, Université de Lyon–ANSES Laboratoire de Lyon, Lyon, France

Didier Hocquet[a,b,c]
[a]*Laboratoire d'Hygiène Hospitalière, Centre Hospitalier Régional Universitaire, Besançon, France*

[b]*UMR 6249, Laboratoire Chrono-Environnement, Centre National de la Recherche Scientifique–Université de Bourgogne Franche-Comté, Besançon, France*

[c]*Bioinformatique et big data au service de la santé, UFR Santé, Université de Bourgogne Franche-Comté, Besançon, France*

Benoit Valot
UMR 6249, Laboratoire Chrono-Environnement, Centre National de la Recherche Scientifique–Université de Bourgogne Franche-Comté, Besançon, France

* Corresponding author.
E-mail address: marisa.haenni@anses.fr (M. Haenni).

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