Staphylococcus caledonicus sp. nov. and Staphylococcus canis sp. nov. isolated from healthy domestic dogs

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Abstract

Two strains, H8/1¹ and H16/1A¹, of Gram-stain-positive, coagulase-negative staphylococci were isolated from separate healthy domestic dogs in Scotland. Both strains were genome sequenced and their inferred DNA–DNA hybridisation indicates that H8/1¹ and H16/1A¹ represent two novel species of the genus Staphylococcus. On the basis of the results of genome sequence analysis (genome BLAST distance phylogeny and single nucleotide polymorphism analysis) H8/1¹ is most closely related to Staphylococcus devriesei and H16/1A¹ most closely related to Staphylococcus felis. Also, average nucleotide identity distinguished H8/1¹ and H16/1A¹ from S. devriesei and S. felis as did minor phenotypic differences. On the basis of these results, it is proposed that H8/1¹ and H16/1A¹ represent novel species with the respective names Staphylococcus caledonicus and Staphylococcus canis. The type strain of S. caledonicus is H8/1¹ (=NCTC 14452¹=CCUG 74789¹). The type strain of S. canis is H16/1A¹ (=NCTC 14451¹=CCUG 74790¹).

At the time of writing the genus Staphylococcus [1, 2] of Gram-stain-positive, coccus-shaped bacteria consists of 55 species (https://lpsn.dsmz.de/genus/staphylococcus, accessed 20th March 2020) [3]. Typically, staphylococci are found as commensal inhabitants of the skin and mucous membranes in a wide range of animal hosts, particularly in humans, other mammals and birds. The genus includes several important human and veterinary opportunistic pathogens, such as Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus lugdunensis and Staphylococcus pseudintermedius. Two canine staphylococcal strains H8/1¹ and H16/1A¹ have been characterised, for which the respective names of Staphylococcus caledonicus sp. nov. and Staphylococcus canis sp. nov. are proposed.

S. caledonicus H8/1¹ and S. canis H16/1A¹ were isolated in 2018 from multisite swabs (a single swab sampling the nares, axilla, groin and perineum) from separate healthy adult dogs at the Royal (Dick) School of Veterinary Studies, University of Edinburgh, Scotland, UK. These samples were collected as part of an epidemiological study to isolate and genome sequence canine commensal bacteria. The dogs had no clinical signs or history of skin disease and had not received antimicrobial treatment for at least 12 months prior to sampling. Swabs were cultured initially by salt broth enrichment, statically at 37 °C for 24 h [tryptone soya broth (Oxoid) plus 6.5 % w/v sodium chloride] before plating onto mannitol salt agar (Oxoid) and then incubation at 37 °C for 24 h. Both isolates are Gram-stain-positive, catalase-positive cocci and were whole-genome sequenced using HiSeq technology (Illumina) with 2×250 bp paired-end reads, read trimming and assembly (performed by Microbes NG, Birmingham, UK). Reads were trimmed using Trimmomatic version 0.30 [4], using a sliding window quality cut-off of 15. Genome assembly was done de novo using SPAdes version 3.7 [5], with default parameters for 250 bp Illumina reads. Assemblies were annotated using the NCBI Prokaryotic Genome Annotation Pipeline [6]. To identify these two strains to the species level their genome sequences were uploaded onto the Type (Strain) Genome
The results of this genome-based analysis support the proposal of the two strains as each representing a novel species of the genus *Staphylococcus*. The TYGS phylogenetic tree generated by the Genome blast Distance Phylogeny approach (GBDP) indicated that H8/1\textsuperscript{T} is most closely related to, but distinct from *Staphylococcus devriesei* CCUG 58238\textsuperscript{T} with H16/1A\textsuperscript{T} most closely related to, but distinct from *Staphylococcus felis* DSM 7377\textsuperscript{T}.
Fig. 2. 16S rRNA gene phylogeny of all species of the genus *Staphylococcus*. Tree generated using the Type (Strain) Genome Server (TYGS) (https://tygs.dsmz.de) [7] and inferred with FastME 2.1.6.1 [22] from GBDP distances calculated from 16S rRNA gene sequences. *Staphylococcus caledonicus* H8/1T and *Staphylococcus canis* H16/1A T are highlighted in bold type. The branch lengths are scaled in terms of GBDP distance formula $d = \ldots$. The numbers below branches are GBDP pseudo-bootstrap support values >50% from 100 replications. *Macrococcus canis* KM45013T was included as the outgroup to root the tree. Accession numbers for all isolates in the analysis are provided in Table S1.
Table 1. Genome and gene-based comparisons of *S. caledonicus* H8/1¹ and *S. canis* H16/1A¹ with their nearest-related species

| Strain comparison | Gene/method (thresholds for circumscribing strains as the same species shown in brackets) |
|-------------------|-----------------------------------------------------------------------------------------|
|                   | dDDH (>70%) [12] | ANIb (>95–96%) [13] | ANIm (>95–96%) [13] | Tetra* (>99%) [13] | 16S rRNA (>98.7%) [23] | dnaJ (>88.8%) [17] | tuf (>98%) [18] | sodA (>97%) [19] | rpoB (>93.6%) [20] |
| *S. caledonicus* H8/1¹ compared with *S. devriesei* DSM 25293⁷ | 50.6% | 92.7% | 93.2% | 99.7% | 99.8% | 95.0% | 99.1% | 97.8% | 97.3% |
| *S. canis* H16/1A¹ compared with *S. felis* DSM 7377⁷ | 22.0% | 77.3% | 84.5% | 95.0% | 99.7% | 84.9% | 95.0% | 84.4% | 87.5% |

Accession numbers of analysed sequences: *S. devriesei* DSM 25293⁷; genome (for dDDH, ANI and tetranucleotide signature correlation index), GCF_002902625; 16S rRNA, UHCZ01000002; dnaJ, JX174277; tuf, FJ389248; sodA, MF679044; rpoB, FJ389232. *S. felis* DSM 7377⁷; genome (for dDDH, ANI and tetranucleotide signature correlation index), GCA_002902185; 16S rRNA, D83364; dnaJ, AB234071; rpoB, HM35294; sodA, AJ343908; rpoB AF325878.

* tetranucleotide signature correlation index.

(Fig. 1). A 16S rRNA gene phylogeny, produced by TYGS, also indicated that H8/1¹ is most closely related to *S. devriesei* and H16/1A¹ most closely related *S. felis* (Fig. 2). Although, 16S rRNA gene sequences often lack sufficient variation to differentiate between species of the genus *Staphylococcus* in pairwise comparisons [8–11]. Indeed, neither H8/1¹ nor H16/1A¹ could be differentiated from *S. devriesei* or *S. felis* type strains respectively on the basis of 16S rRNA gene sequence, Table 1. Nonetheless, designation of both H8/1¹ and H16/1A¹ is most closely related to, but distinct from, *Staphylococcus devriesei*; with H16/1A¹ most closely related to, but distinct from, *Staphylococcus felis*. Before the advent of accessible whole-genome sequencing, the partial sequences of various housekeeping genes such as dnaJ [17], tuf [18], sodA [19] and rpoB [20] had been proposed to discriminant staphylococcal species. Whereas each of these single-gene approaches could distinguish H16/1A¹ from *S. felis* DSM 7377⁷, none of them could be used to separate H8/1¹ from *S. devriesei* CCUG 58238⁷, Table 1. The draft genome of H8/1¹ is 2503367 bases in length with a DNA G+C content of 33.6 mol%, while the draft genome of H16/1A¹ is 2229149 bases in length with a DNA G+C content of 34.8 mol%. These values are similar to those of their nearest relatives and consistent with the average and range values of the rest of the members of the genus *Staphylococcus* (Table 2).

Phenotypic characterisation of H8/1¹ and H16/1A¹ was performed using the API Staph system (bioMérieux) according to the manufacturer’s instructions alongside the type strains of *S. devriesei* DSM 25293⁷ and *S. felis* DSM 7377⁷ (Table 3). H8/1¹ is distinguished from the related *S. devriesei* by the inability of the former to ferment lactose while the lack of arginine dihydrolase activity differentiates H16/1A¹ from the related *S. felis*, (Table 3). Additionally, H8/1¹ and H16/1A¹ were tested for clumping factor and coagulase activity using rabbit plasma (with EDTA) and for DNAse activity using DNAse agar (Oxoid). In each case, both H8/1¹ and H16/1A¹ tested negative for these activities.

Antimicrobial sensitivity testing was performed using the Vitek 2 system (bioMérieux) according to the manufacturer’s instructions. Using the AST-GP80 card and applying the CLSI 2017 interpretations for coagulase-negative staphylococci.
Fig. 3. Maximum likelihood phylogeny of the genus *Staphylococcus* based on single-nucleotide polymorphisms. Tree generated using CSI Phylogeny 1.4 [16] with *S. aureus* DSM 20231\(^T\) as the reference genome with *M. canis* KM45013\(^T\) included as the outgroup to root the tree. *Staphylococcus caledonicus* H8/1\(^T\) and *Staphylococcus canis* H16/1A\(^T\) are highlighted in bold type. The analysis comprised 19637 nucleotide positions. Bootstrap values of \(>50\%\) are shown. Accession numbers for all isolates in the analysis are provided in Table S1.
both H8/1T and H16/1A T were susceptible to all the antimicrobials tested, which were as follows: amoxicillin/clavulanic acid, benzylpenicillin, cefovecin, cefoxitin (screen), ceftiofur, chloramphenicol, clindamycin, doxycycline, enrofloxacin, erythromycin, gentamicin, inducible clindamycin resistance, kanamycin, marbofloxacin, neomycin, nitrofurantoin, oxacillin, pradofloxacin, tetracycline and trimethoprim/sulfamethoxazole. No known antimicrobial resistance genes (perfect and strict hits) were identified in either H8/1 T and H16/1A T on using The Comprehensive Antibiotic Resistance Database (CARD) (https://card.mcmaster.ca/) [21].

**DESCRIPTION OF STAPHYLOCOCCUS CALEDONICUS SP. NOV.**

*Staphylococcus caledonicus* (ca.le.do’ni.cus. L. masc. adj. caledonicus, from Caledonia (Scotland), the country where the type strain was isolated).

Gram-stain-positive, non-spore forming, facultative anaerobe, forms non-pigmented, smooth, circular colonies about 1–2 mm in diameter with entire margins on Columbia horse blood agar after 18 h incubation at 37°C. Able to produce acid from d-glucose, d-fructose, maltose, trehalose, d-mannitol and sucrose but not from d-mannose, lactose, xylitol, melibiose, raffinose, d-xylene, methyl α-d-glucopyranoside or N-acetylglucosamine. Has arginine dihydrolase activity and is able to reduce nitrates to nitrites. Catalase-positive and negative for clumping factor, coagulase and DNase. The type strain of *S. caledonicus*, H8/1 T (=NCTC 14452 T=CCUG 74789 T), was isolated from a healthy dog in Scotland during 2018. The draft genome of H8/1 T is 2503367 bases in length with a DNA G+C content of 33.6 mol%, it comprises 38 contigs with an average coverage of approximately 85-fold. The genome sequence data from H8/1 T is available under these accession numbers: BioSample, SAMN15065541; Sequence Read Archive, SRR11909362; and assembly, JABTXV00000000.0.

**DESCRIPTION OF STAPHYLOCOCCUS CANIS SP. NOV.**

*Staphylococcus canis* (ca’nis L. gen. masc./fem. n. canis, of a dog, in reference to the host from which the type strain was isolated).

Gram-stain-positive, non-spore forming, facultative anaerobe, forms non-pigmented, smooth, circular colonies about
1–2 mm in diameter with entire margins on Columbia horse blood agar after 18 h incubation at 37°C. Able to produce acid from d-glucose, d-fructose, d-mannose, maltose, lactose, trehalose and d-mannitol but not from xyitol, melibiose, raffinose, d-xylene, sucrose or methyl α-d-glucopyranoside. Has alkaline phosphatase and urease activity and is able to reduce nitrates to nitrates. Catalase-positive and negative for clumping factor, coagulase and DNase.

The type strain of *Staphylococcus canis*, H16/1A^T^ (=NCTC 14451^T^=CCUG 74790^T^), was isolated from a healthy dog in Scotland during 2018. The draft genome of H16/1A^T^ is 2229149 bases in length with a DNA G+C content of 34.8 mol%, it comprises 143 contigs with an average coverage of approximately 92-fold. The genome sequence data from *Staphylococcus canis* H16/1A^T^ is available under these accession numbers: BioSample, SAMN14548534; Sequence Read Archive, SRR11498036; and assembly, JABANU0000000000.

Funding information
L. L. N. was supported by a PetSavers (British Small Animal Veterinary Association) Masters Degree by Research award (MDR 05.18).

Acknowledgements
Genome sequencing was provided by MicrobesNG (http://www.microbesng.uk).

Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
Samples were taken with the informed consent of owners and approved by the institutional Veterinary Ethical Review Committee (approval reference number 92.17).

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