Staphylococcus cornubiensis sp. nov., a new member of the *Staphylococcus intermedius* Group (SIG)

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Abbreviations: ANI= Average Nucleotide Identity, SIG= *Staphylococcus intermedius* Group

Strain deposition: = Strain NW1^T has been deposited in the Public Health England Culture Collection (=NCTC 13950 ^{T}) and in the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (=DSM 105366 ^{T}).

Sequence deposition: The ENA and NCBI accession number for the genome assembly of strain NW1^T is GCA_900183575 (provisionally classified as *S. intermedius*, will be changed during revision. DDBJ deposition is in progress).

Abstract

We here describe a novel species in the *Staphylococcus intermedius* group (SIG) which is phenotypically similar to *S. pseudintermedius* but is genomically distinct from it and other SIG members, with an Average Nucleotide Identity of 90.2% with closest relative *S. intermedius*. The description of *Staphylococcus cornubiensis* sp. nov. is based on Type Strain NW1^T (= NCTC 13950^T, = DSM 105366^T) isolated from a human skin infection in Cornwall, UK. Although pathogenic, NW1^T carries no known virulence genes or mobilizable antibiotic resistance genes and further studies are required to assess the prevalence of this species in humans as well as its potential presence in companion animals.

Introduction

The *Staphylococcus intermedius* group (SIG) as currently defined consists of the species *Staphylococcus intermedius* [1], S. *pseudintermedius* [2] and *S. delphini* [3]. All are opportunistic pathogens associated with a wide range of wild and domesticated animals [4, 5]. *S. pseudintermedius* is a common cause of cutaneous infections in dogs [6] and can cause a variety of infections in humans who have been in contact with canines, and as such has been identified as a potential emerging zoonotic threat [7, 8]. In routine diagnostic bacteriology, SIG isolates are hard to differentiate from other staphylococci and may be misidentified as *S. aureus* or one of the coagulase negative staphylococci, depending on the scheme of phenotypic identification used [9]. Colony morphology may be suggestive of SIG but this is not sufficient to identify them [9]. SIG coagulate rabbit plasma (positive tube coagulase reaction) and are negative for clumping factor and protein A [9]. These latter reactions form the basis of many commercial latex kits for the detection of *S. aureus* [2, 9]. Because of their similar biochemistry, distinction of species within the SIG clade requires the use of molecular methods [4].

As part of a previous study which aimed to improve detection and identification of SIG species in a diagnostic microbiology laboratory, the identities of SIG isolates recovered from human samples were confirmed by sequencing [9]. Partial sequences of the *hsp60* and *sodA* marker genes showed one isolate to be distinct from the other three SIG species [9]. This strain, designated NW1^T, was isolated from the skin of a 64-year old man with cellulitis who attended a primary care setting in Cornwall, UK.

Only a single colony type was observed in the wound culture. Details of dog ownership or contact were not recorded. Colony morphology and standard bacteriological tests did not differentiate NW1^T from 39 *S. pseudintermedius* strains isolated during the study. After overnight incubation at 37°C on sheep blood agar (Oxoid) colonies were white, entire, convex, glistening and 2-3 mm in diameter surrounded by double zone haemolysis (Supplementary Figure 1). The outer, incompletely-haemolysed band developed complete haemolysis after further incubation at 4 °C (hot–cold haemolysis), which is typical of staphylococcal β-haemolysin activity. The organism demonstrated DNase activity (Oxoid agar), positive catalase reaction, positive tube coagulase with rabbit plasma, negative latex agglutination (Prolab StaphXtra) implying absence of protein A and clumping factor, the latter confirmed by "slide coagulase" test with rabbit plasma (BioConnections).

Further biochemical characterization (VITEK2, GP card, Biomerieux) revealed few differences between NW1^T and the three SIG Type strains (Table 1). The antibiotic susceptibility profile (VITEK2, AST-P578 panel, Biomerieux) of NW1^T did not differ significantly from that of the 39 human isolates of *S. pseudintermedius* from the same laboratory with susceptibility to oxacillin, erythromycin, chloramphenicol, ciprofloxacin, clindamycin, gentamicin, linezolid, and rifampin. NW1^T has a lower polymixin MIC (4 mg/liter) than the *S. pseudintermedius* strains (8 to 24 mg/liter) [9] and unlike them it is resistant to fusidic acid.

We found NW1^T to be genetically similar to a canine isolate '2008-01-1056-2' reported in an earlier study from Norway which utilized the same marker genes (nucleotide similarity *hsp60* 98%, 100% *sodA* gene) [10]. This Norwegian isolate was phylogenetically distinct from other SIG species based on four housekeeping genes, leading the authors to hypothesize it represented a novel species. As with strain NW1, 2008-01-1056-2 does not produces pigment, is coagulase-positive, "clumping factor" negative, DNAse positive and displays double haemolysis on sheep blood agar. Biochemically, NW1^T and 2008-01-1056-2 differ in four tests in the VITEK panel (alpha-galactosidase, D-galactosidase, acid production from Methyl- B-D-glucopyronaside and D-maltose) indicating some metabolic variability within the putative species (Table 1). Despite NW1^T being only very weakly divergent phenotypically from *S. pseudintermedius*, the report of a related, genetically distinct SIG isolate prompted us to obtain a whole genome sequence of NW1^T to more comprehensively assess its place in the SIG clade.

DNA isolation, Illumina HiSeq sequencing and basic bioinformatics was performed through the MicrobeNG program in Birmingham, UK (see Supplementary Methods). The NW1^T genome size is 2,677,814 bp, with 2465 ORFs and a GC content of 37.3%, closely resembling other SIG species [11]. Core genes from NW1^T and representative SIG genomes were extracted using Roary v3.8.0 [12] (blast percentage identity of 70%). SNP-sites v2.3.2 [13] found 369,267 SNPs in shared core genes which served as input for RAxML v8.2.8 [14] to construct a core genome phylogenetic tree (Figure 1). The phylogeny shows NW1^T to cluster in the SIG clade but separate from the three described species. Average Nucleotide Identity (ANI) of the core genome was calculated using pANIto v0.0.1 (https://github.com/sanger-pathogens/panito) and revealed strain NW1^T to represent a distinct SIG species [15]. NW1^T is most closely related to *S. intermedius* with a core genome nucleotide similarity of 90.2% (Table 2). A phylogenetic tree based on the three housekeeping genes available for the Norwegian canine '2008-01-1056-2' isolate, NW1 and the three named SIG species suggests that NW1 and '2008-01-1056-2' are very closely related, possibly belonging to the same species (Supplementary Figure 2). SIG species display very high inter-species 16S ribosomal rRNA similarity (>99% [2]) and the same is true when comparing NW1^T with other species (Supplementary Table 1). A tree based on the presence and absence of accessory gene content mirrors the evolutionary relationships between all strains found through nucleotide divergence of the core genomes (Supplementary Figure 3).

The presence of (putative) virulence factors was assessed by applying ARIBA (version 2.9.3) [16] and compared to the core and full VFDB (downloaded 2017-05-10) [17] databases and the VirulenceFinder database (downloaded 2017-05-11) [18]. There were no hits of any significance to any virulence genes using a 90% nucleotide cut-off. It has to be noted that applying this same method, no virulence genes were detected in the other SIG genomes used in this study. Using less stringent criteria (as used in other SIG studies, e.g. [11]), putative virulence genes e.g. fibronectin-binding protein *fnbB*, leukotoxin *lukD* and *lukE*, enterotoxin type C gene *entC1* and the gamma-hemolysin locus *hlgA, hlgB* and *hlgC* (the latter being present in *S. aureus* but not having been reported in SIG genomes [11]) were indicated. No antibiotic resistance genes were detected in NW1^T using ResFinder [19] or ARIBA [16]. NW1^T contains a single CRISPR locus [20] of the Nmeni subtype (Class 2, Type II [21]) which has also be found in *S. intermedius* and *S. pseudintermedius* (with the former also containing a Mtube

subtype) [11]. A single fragment of a *Staphylococcus* beta-like prophage was found using PHASTER [22].

The three named *Staphylococcus intermedius* group (SIG) species, along with *S. hyicus*, *S. lutrae*, *S. schleiferi* and *S. aureus* form the most pathogenic representatives of the coagulase-positive staphylococci [23]. The sequence data presented in this paper demonstrate that NW1^T is a novel member of the *Staphylococcus intermedius* group (SIG), for which we propose the name *Staphylococcus cornubiensis*. It remains to be seen whether *S. cornubiensis* is a mutualist or opportunistic pathogen of companion animals capable of occasional transfer to humans, like *S. pseudintermedius*. The latter possibility is supported by the isolation of a related strain (2008-01-1056-2) from a dog [10]. Although NW1^T was isolated in pure culture from a human infection, is coagulase positive and produces haemolysin, the absence of high similarity hits to known virulence genes means that its pathogenic mechanisms are unclear. Increased detection capabilities will be crucial to routinely differentiate non-*S. aureus* coagulase-positive isolates, including novel species, and to assess their importance in clinical and veterinary settings.

Description of *Staphylococcus cornubiensis* sp. nov.

Staphylococcus cornubiensis (cor.nu.bi.en'sis. M.L. fem. n. *Cornubia* medieval name of Cornwall; N.L. masc. adj. *cornubiensis* pertaining to Cornwall).

Consists of Gram-positive cocci arranged in clusters. Colonies on sheep blood agar are non-pigmented and surrounded by double zone haemolysis typical of staphylococcal β-haemolysin activity. It is catalase-positive, DNase producing and coagulates rabbit plasma. It is clumping-factor negative in the slide coagulase test and does not produce protein A. Negative for acetoin production (Voges-Proskauer test). Automated biochemistry (Vitek2 GP card, Biomerieux) positive alkaline phosphatase, arginine dihydrolase, leucine arylamidase, pyrrolidonyl arylamidase, alanine arylamidase, β-galactosidase and α-glucosidase. Negative for urease, L-aspartate arylamidase, L-proline arylamidase, tyrosine arylamidase, β-galactopyranosidase, α-mannosidase, β-glucuronidase and α-galactosidase. Acid

production from D-ribose, lactose, D-mannitol, D-mannose, sucrose and D-trehalose. No acid production from D-xylose, D-sorbitol, D-galactose, D maltose or D-raffinose.

The Type Strain NW1^{T} (= NCTC 13950^{T}, = DSM 105366 ^{T}) was isolated from the skin of a 64-year old man with cellulitis travelling from the north of England who attended a primary care setting in Cornwall, UK. The G+C DNA content of the Type Strain is 37.3%.

Author Statements

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Conflicts of Interest

We declare no conflicts of interest.

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Figure 1. An unrooted Maximum Likelihood tree (GTRGAMMA model, 100 bootstraps) containing representatives of all three SIG species, outgroup *S. schleiferi* and *S. cornubiensis* sp. nov. NW1^{T}. The tree is based on 1,399 core genes comprising of 1,309,702 bases (covering ~45% of each genome). The scale bar represents the mean number of nucleotide substitutions per site as a function of branch length. GenBank Accession numbers: *S. cornubiensis* sp. nov.: GCA_900183575, *S. schleiferi* 2317-03: GCA_001188915.1, *S. pseudintermedius* NA45: GCA_001682335.1, *S. pseudintermedius* ED99: GCA_000189495.1, *S. pseudintermedius* 063228: GCA_001685665.2, *S. pseudintermedius* E140: GCA_000478385.1, *S. pseudintermedius* 081661: GCA_001682435.2, *S. pseudintermedius* HKU10-03: GCA_000185885.1, *S. delphini* NCTC12225^{T} (no GenBank Accession, raw reads available via Public Health England/Sanger Centre ERS798846), *S. intermedius* NCTC 11048^{T}: GCA_000308095.1.

Table 1. Characteristics of isolate NW1^T , *2008-011056-2* and three type strain SIG species.

Characteristic	**NW1^T**	**S. *intermedius* DSM 20373**	***S. pseudintermedius* DSM 21284**	***S. delphini* DSM20771**	**2008-011056-2**
Pigment	-	-	-	-	-
Coagulase	+	+	+	+	+
Clumping factor	-	-	-	-	-
DNase	+	+	+	-	+
β-Haemolysin	+	+	+	+	+
Mannitol fermentation	+	+	-	+	+
Acetoin	-	-	-	-	-
Pyrrolodonyl Aryalamidase*	+	+	+	+	+
d-Maltose*	-	+	+	+	+
d-Galactose*	+	+	+	+	+
D-Trehalose*	+	+	+	-	+
Sucrose*	+	+	+	+	+

-= negative, += positive, * data based on VITEK2 GP card (Biomerieux)

Table 2. Average Nucleotide Identity between NW1^T (*S. cornubiensis*, the three described SIG species and the related coagulase-positive species *S. schleiferi*)

		S. intermedius	*S. pseudintermedius*	*S. delphini*	*S. schleiferi*
NW1^T (*S. cornubiensis*)	ANI	90.2	88.8	89.1	78.6
S. intermedius	ANI		89.7	90.0	78.3
S. pseudintermedius	ANI			94.6	78.2
S. delphini	ANI				78.3

Strains used: *S. intermedius* LMG 13351^T, *S. pseudintermedius* LMG 22219, *S. delphini* DSM 20771^T and *S. schleiferi* 2317-03.

254 Figure 1

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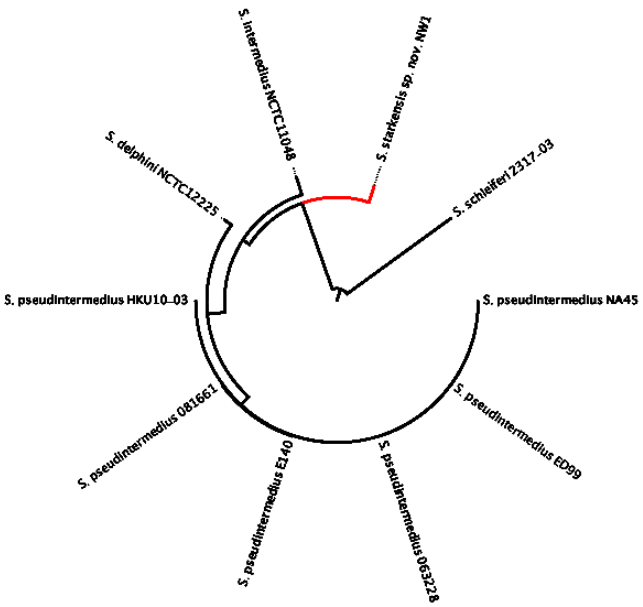

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