

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

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17

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

19

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

23

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

27 **Abstract**

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

36

37 **Introduction**

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

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

55 Only a single colony type was observed in the wound culture. Details of dog ownership or contact were
56 not recorded. Colony morphology and standard bacteriological tests did not differentiate NW1^T from
57 39 *S. pseudintermedius* strains isolated during the study. After overnight incubation at 37°C on sheep
58 blood agar (Oxoid) colonies were white, entire, convex, glistening and 2-3 mm in diameter surrounded
59 by double zone haemolysis (Supplementary Figure 1). The outer, incompletely-haemolysed band
60 developed complete haemolysis after further incubation at 4°C (hot-cold haemolysis), which is typical
61 of staphylococcal β-haemolysin activity. The organism demonstrated DNase activity (Oxoid agar),
62 positive catalase reaction, positive tube coagulase with rabbit plasma, negative latex agglutination
63 (Prolab StaphXtra) implying absence of protein A and clumping factor, the latter confirmed by “slide
64 coagulase” test with rabbit plasma (BioConnections).

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

72 We found NW1^T to be genetically similar to a canine isolate ‘2008-01-1056-2’ reported in an
73 earlier study from Norway which utilized the same marker genes (nucleotide similarity *hsp60* 98%,
74 100% *sodA* gene) [10]. This Norwegian isolate was phylogenetically distinct from other SIG species
75 based on four housekeeping genes, leading the authors to hypothesize it represented a novel species. As
76 with strain NW1, 2008-01-1056-2 does not produce pigment, is coagulase-positive, “clumping factor”
77 negative, DNase positive and displays double haemolysis on sheep blood agar. Biochemically, NW1^T
78 and 2008-01-1056-2 differ in four tests in the VITEK panel (alpha-galactosidase, D-galactosidase, acid
79 production from Methyl- B-D-glucopyronaside and D-maltose) indicating some metabolic variability
80 within the putative species (Table 1). Despite NW1^T being only very weakly divergent phenotypically
81 from *S. pseudintermedius*, the report of a related, genetically distinct SIG isolate prompted us to obtain
82 a whole genome sequence of NW1^T to more comprehensively assess its place in the SIG clade.

83 DNA isolation, Illumina HiSeq sequencing and basic bioinformatics was performed through
84 the MicrobeNG program in Birmingham, UK (see Supplementary Methods). The NW1^T genome size
85 is 2,677,814 bp, with 2465 ORFs and a GC content of 37.3%, closely resembling other SIG species
86 [11]. Core genes from NW1^T and representative SIG genomes were extracted using Roary v3.8.0 [12]
87 (blast percentage identity of 70%). SNP-sites v2.3.2 [13] found 369,267 SNPs in shared core genes
88 which served as input for RAxML v8.2.8 [14] to construct a core genome phylogenetic tree (Figure 1).
89 The phylogeny shows NW1^T to cluster in the SIG clade but separate from the three described species.
90 Average Nucleotide Identity (ANI) of the core genome was calculated using pANItO v0.0.1
91 (<https://github.com/sanger-pathogens/panito>) and revealed strain NW1^T to represent a distinct SIG
92 species [15]. NW1^T is most closely related to *S. intermedius* with a core genome nucleotide similarity
93 of 90.2% (Table 2). A phylogenetic tree based on the three housekeeping genes available for the
94 Norwegian canine ‘2008-01-1056-2’ isolate, NW1 and the three named SIG species suggests that NW1
95 and ‘2008-01-1056-2’ are very closely related, possibly belonging to the same species (Supplementary
96 Figure 2). SIG species display very high inter-species 16S ribosomal rRNA similarity (>99% [2]) and
97 the same is true when comparing NW1^T with other species (Supplementary Table 1). A tree based on
98 the presence and absence of accessory gene content mirrors the evolutionary relationships between all
99 strains found through nucleotide divergence of the core genomes (Supplementary Figure 3).

100 The presence of (putative) virulence factors was assessed by applying ARIBA (version 2.9.3)
101 [16] and compared to the core and full VFDB (downloaded 2017-05-10) [17] databases and the
102 VirulenceFinder database (downloaded 2017-05-11) [18]. There were no hits of any significance to any
103 virulence genes using a 90% nucleotide cut-off. It has to be noted that applying this same method, no
104 virulence genes were detected in the other SIG genomes used in this study. Using less stringent criteria
105 (as used in other SIG studies, e.g. [11]), putative virulence genes e.g. fibronectin-binding protein *fnbB*,
106 leukotoxin *lukD* and *lukE*, enterotoxin type C gene *entCI* and the gamma-hemolysin locus *hlgA*, *hlgB*
107 and *hlgC* (the latter being present in *S. aureus* but not having been reported in SIG genomes [11]) were
108 indicated. No antibiotic resistance genes were detected in NW1^T using ResFinder [19] or ARIBA [16].
109 NW1^T contains a single CRISPR locus [20] of the Nmeni subtype (Class 2, Type II [21]) which has
110 also be found in *S. intermedius* and *S. pseudintermedius* (with the former also containing a Mtube

111 subtype) [11]. A single fragment of a *Staphylococcus* beta-like prophage was found using PHASTER
112 [22].

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

125

126 **Description of *Staphylococcus cornubiensis* sp. nov.**

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

129

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

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

140

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

144

145 **Author Statements**

146

147 Funding Information

148 This work was supported by the Wellcome Trust (grant WT 098051). We acknowledge BBSRC/MRC
149 funding (grant MR/N007174/1) enabling discounted sequencing through the microbesNG program.

150

151 Acknowledgments

152 We thank Steve Merrifield who isolated NW1 and all staff at the Clinical Microbiology unit of the
153 Royal Cornwall Hospital, for their help with SIG isolation.

154

155 Conflicts of Interest

156 We declare no conflicts of interest.

157

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215 Figure 1. An unrooted Maximum Likelihood tree (GTRGAMMA model, 100 bootstraps) containing
216 representatives of all three SIG species, outgroup *S. schleiferi* and *S. cornubiensis* sp. nov. NW1^T. The
217 tree is based on 1,399 core genes comprising of 1,309,702 bases (covering ~45% of each genome). The
218 scale bar represents the mean number of nucleotide substitutions per site as a function of branch length.
219 GenBank Accession numbers: *S. cornubiensis* sp. nov.: GCA_900183575, *S. schleiferi* 2317-03:
220 GCA_001188915.1, *S. pseudintermedius* NA45: GCA_001682335.1, *S. pseudintermedius* ED99:
221 GCA_000189495.1, *S. pseudintermedius* 063228: GCA_001685665.2, *S. pseudintermedius* E140:
222 GCA_000478385.1, *S. pseudintermedius* 081661: GCA_001682435.2, *S. pseudintermedius* HKU10-
223 03: GCA_000185885.1, *S. delphini* NCTC12225^T (no GenBank Accession, raw reads available via
224 Public Health England/Sanger Centre ERS798846), *S. intermedius* NCTC 11048^T: GCA_000308095.1.
225

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

227

228 **Characteristic** **NW1^T** ***S. intermedius*** ***S. pseudintermedius*** ***S. delphini*** **2008-011056-2**

229 **DSM 20373** **DSM 21284** **DSM20771**

230	Pigment	-	-	-	-	-
231	Coagulase	+	+	+	+	+
232	Clumping factor	-	-	-	-	-
233	DNase	+	+	+	-	+
234	β -Haemolysin	+	+	+	+	+
235	Mannitol fermentation	+	+	-	+	+
236	Acetoin	-	-	-	-	-
237	Pyrrrolodonyl Aryalamidase*	+	+	+	+	+
238	d-Maltose*	-	+	+	+	+
239	d-Galactose*	+	+	+	+	+
240	D-Trehalose*	+	+	+	-	+
241	Sucrose*	+	+	+	+	+

242

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

244

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

246

247

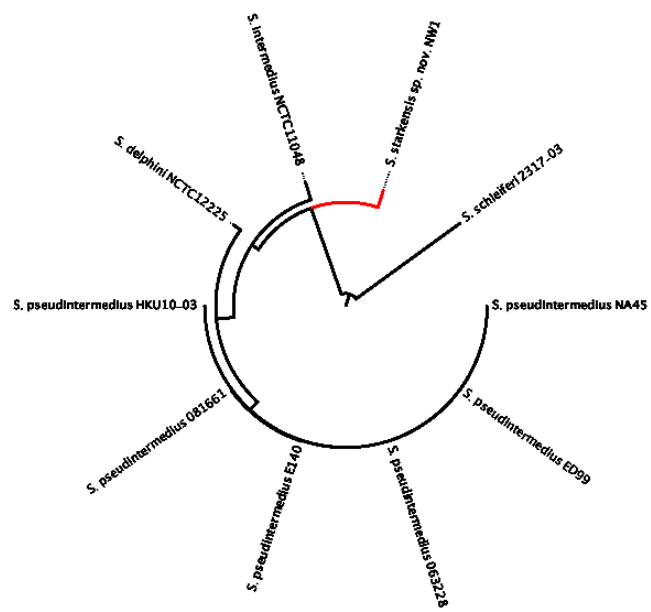
	<i>S. intermedius</i>	<i>S. pseudintermedius</i>	<i>S. delphini</i>	<i>S. schleiferi</i>
248 NW1 ^T (<i>S. cornubiensis</i>) ANI	90.2	88.8	89.1	78.6
249 <i>S. intermedius</i> ANI		89.7	90.0	78.3
250 <i>S. pseudintermedius</i> ANI			94.6	78.2
251 <i>S. delphini</i> ANI				78.3

252

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

254 Figure 1

255



256

0.2