Neisseria wadsworthii sp. nov. and *Neisseria shayeganii* sp. nov., isolated from clinical specimens

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An analysis of 16S rRNA gene sequences from archived clinical reference specimens has identified two novel *Neisseria* species. For each species, two strains from independent sources were identified. Amongst species with validly published names, the closest species to the newly identified organisms were *Neisseria canis*, *N. dentiae*, *N. zoodegmatis*, *N. animaloris* and *N. weaveri*. DNA–DNA hybridization studies demonstrated that the newly identified isolates represent species that are distinct from these nearest neighbours. Analysis of partial 23S rRNA gene sequences for the newly identified strains and their nearest neighbours provided additional support for the species designation. Bayesian analysis of 16S rRNA gene sequences suggested that the newly identified isolates belong to distinct but related species of the genus *Neisseria*, and are members of a clade that includes *N. dentiae*, *N. bacilliformis* and *N. canis*. The predominant cellular fatty acids [16:0, summed feature 3 (16:1 ω 7c and/or iso-15:0 2-OH) and 18:1 ω 7c], as well as biochemical and morphological analyses further support the designation of *Neisseria wadsworthii* sp. nov. (type strain 9715^T = DSM 22247^T = CIP 109934^T) and *Neisseria shayeganii* sp. nov. (type strain 871^T = DSM 22246^T = CIP 109933^T).

In the present study, four clinical isolates received between 2004 and 2008, for which our laboratory was not able to provide a definitive species identification, were further characterized. During our routine reference identification, these isolates did not provide an acceptable percentage match to any available 16S rRNA gene sequence in the MicroSeq (Applied Biosystems) and GenBank (<http://www.ncbi.nlm.nih.gov/entrez/>) databases.

The closest 16S rRNA gene sequence similarities ranged from 96 to 98 %, based on 500 nt in the 5' region of the gene. At the time of identification, strains 9715^T and 2507 were identified as most closely resembling *Neisseria canis*. Strain 871^T was identified as *Neisseria* sp. and strain 12337 was identified as most closely resembling CDC group EF-4b (now named *Neisseria zoodegmatis*; Vandamme *et al.*, 2006).

Abbreviation: CFA, cellular fatty acid.

The GenBank/EMBL/DDBJ accession numbers for the partial 16S rRNA gene sequence of strains 9715^T, 2507, 871^T and 12337 are respectively FJ654662–FJ654665. The accession numbers for the partial 23S rRNA gene sequences of strains 12337, 2507, 9715^T, 871^T, *N. canis* ATCC 14687^T, *N. weaveri* DSM 17688^T, *N. dentiae* DSM 19151^T, *N. animaloris* DSM 21642^T and *N. zoodegmatis* DSM 21643^T are respectively FJ654666–FJ654670 and GU323335–GU323338.

A supplementary table and two supplementary figures are available with the online version of this paper.

To determine whether our isolates represented novel bacterial species, we performed a polyphasic study on these organisms and their phylogenetic neighbours. Identification of closely related species was initially carried out by using the BLAST (Altschul *et al.*, 1997) and megaBLAST (Zhang *et al.*, 2000) programs to compare nearly full-length 16S rRNA gene sequences (1463 nt) of our isolates against the database of prokaryotic type strains with validly published names (Chun *et al.*, 2007). The 50

sequences with the highest scores were then selected for the calculation of pairwise sequence similarity using a global alignment algorithm, which was implemented at the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Previous studies have indicated that organisms with greater than 97 % 16S rRNA gene sequence similarity may represent the same species (discussed by Tindall *et al.*, 2010). Therefore, we acquired all type strains with more than 97 % 16S rRNA gene sequence similarity to our isolates and have included them in our analysis. We employed 16S rRNA (1463 nt) and 23S rRNA (2093 and 2172 nt) gene sequence similarity, DNA–DNA hybridization, analysis of cellular fatty acid (CFA) composition, PFGE and phenotypic characterization to establish that our isolates represent two novel species of *Neisseria*.

The bacterial strains used in this study, their sources and the dates of isolation are listed in Table 1. All strains were grown at 37 °C in a 5 % CO₂ atmosphere on trypticase soy agar (TSA) plates (Becton Dickinson) supplemented with 5 % sheep blood. For CFA studies, the strains were grown in air at 37 °C on trypticase soy broth agar (TSBA) plates without sheep blood.

DNA for sequencing was prepared by heating a 1.0 McFarland standard suspension of the isolate to 95 °C in sterile distilled water for 15–20 min. The 16S rRNA gene sequence was amplified using the forward primer 16S1 (5'-AGAGTTTGGATCTGGCTCAG-3', corresponding to *Escherichia coli* positions 8–27) and the reverse primer rp2 (5'-ACGGCTACCTTGTTACGACTT-3'; 1492–1512). Amplification was performed on an Icyler (Bio-Rad Laboratories) using AmpliTaq polymerase LD (Applied Biosystems) with a single cycle at 95 °C for 9 min followed by 40 cycles of 95 °C for 30 s, 60 °C for 1 min and 72 °C for 1.5 min, followed by a final extension at 72 °C for 7 min and a 4 °C hold. PCR products were prepared for sequencing using ExoSAPIT (USB Corporation). The sequencing primers used were pD (5'-CAGCAGCCGC-GGTAATAC-3'), rpE (5'-CCGTCAATTCATTTGAGTTT-3') and the two amplification primers. Nucleotide identities in each sequence represent two or more unambiguous calls from reads in both the forward and reverse directions. Polymorphisms (found only in isolate 871^T) are indicated by IUPAC code. A phylogenetic tree

was constructed based on sequences retrieved from GenBank and aligned using a progressive alignment algorithm (CLC Main Workbench software 4.0; CLC Bio) with MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003), 5 × 10⁶ generations, using a GTR+I+G substitution model with six rate categories. Two independent analyses were run, each consisting of four chains. Inspection of tree log-likelihood in this sample revealed that stationarity was reached after 2 × 10⁶ generations, with the mean standard deviation of split frequencies stabilizing at ~0.013. These trees were discarded as burn-in, and the phylogenetic tree was constructed using the remaining 3 × 10⁶ generations.

A comparison of 16S rRNA gene sequences for strains 9715^T and 2507 revealed 100 % nucleotide similarity over 1463 nt, with no ambiguous nucleotides. The closest match based on 16S rRNA gene sequences (determined from the EzTaxon database; Chun *et al.*, 2007) was *Neisseria canis* ATCC 14687^T (Berger & Paepcke, 1962), followed by *Neisseria dentiae* V33^T (Sneath & Barrett, 1996), *Neisseria animaloris* LMG 23011^T, *Neisseria zoodegmatis* LMG 23012^T (Vandamme *et al.*, 2006) and *Neisseria weaveri* CDC 8142 (Andersen *et al.*, 1993) (note that *N. weaveri* Holmes *et al.* 1993 has priority over *N. weaveri* Andersen *et al.* 1993 and has a different type strain). The nucleotide similarities ranged from 98.35 % for *N. canis* ATCC 14687^T to 96.25 % for *N. weaveri* CDC 8142 over 1311–1455 nt. Pairwise comparisons of the 16S rRNA gene sequence between strains 871^T and 12337 revealed 99.86 % similarity over 1463 nt. Strain 871^T harboured three polymorphisms: two purine-to-purine substitutions and one pyrimidine-to-pyrimidine substitution. One set of polymorphisms matched the sequence of strain 12337, so these sites are considered a match when the two sequences are compared. The closest type strain to strains 12337 and 871^T was that of *N. canis*; followed by *N. dentiae*, *N. zoodegmatis*, *N. weaveri* and *N. animaloris*, with similarities ranging from 98.55 % for *N. canis* to 96.75 % for *N. animaloris* over 1455–1329 nt. The sequences of strains 9715^T and 2507 were 97.88 and 97.68 % similar to the sequences of strains 871^T and 12337, respectively.

As recommended by Tindall *et al.* (2010), DNA–DNA hybridization was performed between our clinical isolates and type strains exhibiting more than 97 % 16S rRNA gene

Table 1. Strains used in this study

All four strains were isolated in New York State, USA.

Strain	Source	Isolation date
<i>Neisseria wadsworthii</i> sp. nov.		
9715 ^T (=DSM 22247 ^T =CIP 109934 ^T)	Hand wound, 39-year-old male; Rensselaer County	16 Dec 2005
2507 (=DSM 22245 =CIP 109931)	Peritoneal fluid, 36-year-old female; Monroe County	20 Mar 2005
<i>Neisseria shayegani</i> sp. nov.		
871 ^T (=DSM 22246 ^T =CIP 109933 ^T)	Sputum, 77-year-old female; Schenectady County	7 Feb 2008
12337 (=DSM 22244 =CIP 109932)	Arm wound, 47-year-old female; Westchester County	9 Sep 2004

sequence similarity. Spectroscopic DNA–DNA hybridization was performed in duplicate on DNA that was isolated using a French pressure cell (Thermo Spectronic) and purified by chromatography on hydroxyapatite (Cashion *et al.*, 1977). DNA–DNA hybridization was carried out as described by De Ley *et al.* (1970) under consideration of the modifications described by Huß *et al.* (1983) using a model Cary 100 Bio UV/Vis spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with *in-situ* temperature probe (Varian). The hybridization values, in duplicate, between strains 9715^T and 871^T and to type strains showing more than 97 % 16S rRNA gene sequence similarity are shown in Table 2. Based on a cut-off of 70 % hybridization for defining species (Wayne *et al.*, 1987), strains 9715^T and 871^T represent distinct species and, furthermore, do not belong to the same species as any of the tested type strains. By contrast, strains 9715^T and 2507 yielded hybridization values greater than 70 %, indicating they are members of the same species, as did strains 871^T and 12337.

To obtain further support for the species designations of these clinical isolates, we obtained partial sequences of the 23S rRNA gene for each isolate and *N. canis* ATCC 14687^T, *N. weaveri* DSM 17688^T, *N. dentiae* DSM 19151^T, *N. animaloris* DSM 21642^T and *N. zoodegmatis* DSM 21643^T. The 23S rRNA sequence was amplified using the forward primer 129F (5'-CCGAATGGGGAAACC-3'; *E. coli* positions 115–129) and the reverse primer 2241R (5'-ACCGCCCCAGTCAAAC-3'; 2241–2257) (adapted from Hunt *et al.*, 2006). Amplification was performed using AmpliTaq polymerase Gold (Applied Biosystems) with a single cycle at 95 °C for 9 min followed by 40 cycles of 95 °C for 30 s, 55 °C for 1 min and 72 °C for 2 min, with a final extension at 72 °C for 7 min and a 4 °C hold. The sequencing primers used were 129F, 2241R, 1602F (Ludwig *et al.*, 1994), 473tR (5'-CTTTCCTCACGGTACT-3'; Hunt *et al.*, 2006) and 1399F (Van Camp *et al.*, 1993). Product sizes ranged from 2091 to 2172 bp.

Table 2. DNA–DNA relatedness of the novel strains and related type strains

Values in parentheses represent duplicate measurements. ND, Not done; NA, not applicable.

Strain	DNA–DNA hybridization (%)	
	9715 ^T	871 ^T
2507	96.3 (94.0)	ND
871 ^T	37.6 (40.2)	NA
12337	ND	84.9 (87.9)
<i>N. canis</i> DSM 18000 ^T	51.6 (52.8)	17.1 (20.5)
<i>N. dentiae</i> DSM 19151 ^T	33.9 (40.3)	4.2 (13.8)
<i>N. animaloris</i> DSM 21642 ^T	41.8 (33.8)	ND
<i>N. zoodegmatis</i> DSM 21643 ^T	ND	12.2 (21.8)
<i>N. weaveri</i> DSM 17688 ^T	ND	28.9 (34.0)

The 23S rRNA gene sequences of strains 9715^T and 2507 were identical over 2093 nt, while the sequences of strains 871^T and 12337 were 99.81 % similar over 2172 nt (see Supplementary Table S1, available in IJSEM Online). Strain 871^T had a single polymorphism, a purine-to-purine substitution. Interestingly, strains 871^T and 12337 each had a 78 nt insertion at a site corresponding to *E. coli* position 1179. This insertion was absent from all other *Neisseria* strains for which 23S rRNA gene sequences are available in GenBank: these include strains 9715^T and 2507 and the type strains of *N. canis*, *N. weaveri*, *N. dentiae*, *N. animaloris* and *N. zoodegmatis* sequenced in this study and *Neisseria meningitidis* Z2491 (Parkhill *et al.*, 2000) and *Neisseria gonorrhoeae* NCCP 11945 (Chung *et al.*, 2008). The insertion was omitted when we calculated sequence similarity. The sequences of strains 9715^T and 2507 were most similar to that of *N. canis* ATCC 14687^T (98.38 %). The sequences of strains 871^T and 12337 were most similar to those of strains 9715^T and 2507 (97.23 %). Other similarities are detailed in Supplementary Table S1. Thus, 23S rRNA gene sequence analysis provides additional support that strains 9715^T and 2507 are members of the same species, as are strains 871^T and 12337.

CFA analysis was undertaken from isolates cultured for 24–48 h at 37 °C in air on TSBA plates. Fatty acid methyl esters were prepared according to the manufacturer's instructions (Sherlock Microbial Identification System; MIDI Corporation) and were identified using an Agilent Technologies 6890N gas chromatograph. Only minor differences were observed between strains 9715^T and 2507 and between strains 871^T and 12337 (Table 3). In isolates 9715^T and 2507, the levels of 16:0 were reduced and the levels of 18:1 ω 7c and summed feature 3 (16:1 ω 7c and/or iso-15:0 2-OH) were increased compared with *N. canis* ATCC 14687^T, resulting in a distinctive pattern. However, overall, the three most abundant fatty acids, 16:0, 18:1 ω 7c and summed feature 3, were the same as for *N. canis* ATCC 14687^T and other closely related type strains (those of *N. zoodegmatis*, *N. animaloris*, *N. dentiae* and *N. weaveri*). In contrast, strains 871^T and 12337 had a distinctive pattern of CFA abundance, in which 16:0 comprised over half of the fatty acids, 18:1 ω 7c was greatly reduced and four additional fatty acids, present at similar levels, made up most of the rest of the total fatty acids.

Strains 9715^T and 2507 were indistinguishable based on molecular and phenotypic analysis (see species description). As an aid in distinguishing these isolates from one another, PFGE was performed as described by Popovic *et al.* (2001) (Supplementary Fig. S1). After digestion of DNA with either *Sfi*I, *Spe*I or *Nhe*I, fewer than three band differences were seen between 9715^T and 2507; this level of difference is interpreted as closely related (Tenover *et al.*, 1995). However, differences between strain 9715^T or 2507 and *N. canis* ATCC 14687^T were very pronounced, with few apparent matches.

Based on 16S and 23S rRNA gene sequences, DNA–DNA hybridization, CFA composition and phenotypic analysis

Table 3. Fatty acid compositions of the novel strains and related type strains

Strains: 1, 871^T; 2, 12337; 3, 9715^T; 4, 2507; 5, *N. canis* ATCC 14687^T; 6, *N. dentiae* DSM 19151^T; 7, *N. weaveri* DSM 17688^T; 8, *N. animaloris* DSM 21642^T; 9, *N. zoodegmatidis* DSM 21643^T. Values are percentages of total CFAs; fatty acid methyl esters for which no values were greater than 1 % have been omitted. The three most abundant fatty acids for each isolate are in bold. ND, Not detected.

Fatty acid	1	2	3	4	5	6	7	8	9
12:0	7.97	6.83	4.96	4.95	5.56	6.56	5.21	8.22	6.29
12:0 3-OH	8.71	7.35	3.37	3.54	3.84	4.25	3.15	4.81	4.1
14:0	7.54	11.17	1.99	1.86	6.68	4.24	6.29	8.16	5.61
Summed feature 2*	ND	ND	2.02	2.04	2.77	3.14	2.00	2.56	2.34
Summed feature 3*	11.07	14.22	28.45	28.00	23.33	22.76	37.24	29.73	30.04
16:1 ω 5c	ND	ND	ND	ND	ND	0.73	2.88	1.4	0.93
16:0	57.15	55.97	33.3	32.83	35.85	30.53	17.89	26.56	32.08
16:0 2-OH	0.51	ND	ND	ND	1.52	3.03	ND	ND	2.26
16:0 3-OH	1.24	1.17	ND	ND	0.41	0.31	ND	ND	0.17
18:1 ω 9c	1.84	ND	ND	ND	ND	ND	ND	ND	ND
18:1 ω 7c	2.45	3.28	24.4	25.15	19.19	23.15	24.27	17.45	15.56
18:0	0.87	ND	1.07	1.13	0.65	0.36	ND	0.28	0.18

*Summed features consist of groups of two or three fatty acids that cannot be separated using the MIDI System. Summed feature 2 is composed of iso-16:1 I and/or 14:0 3-OH; summed feature 3 is composed of 16:1 ω 7c and/or iso-15:0 2-OH.

(see species description and Table 4), we propose that strains 9715^T and 2507 are representatives of a single species, *Neisseria wadsworthii* sp. nov. Similarly, 12337 and 871^T represent a single species, *Neisseria shayegani* sp. nov. Strain differences for *N. wadsworthii* sp. nov. were revealed only by PFGE, while the *N. shayegani* sp. nov. strains could be distinguished based on 16S rRNA gene sequence divergence of 0.14 %, a level which is consistent with intraspecies variation for *Neisseria* species (Clarridge, 2004). For the designated type strains of both *N. wadsworthii* sp. nov. and *N. shayegani* sp. nov., DNA–DNA hybridization levels to type strains with more than 97 % 16S rRNA gene sequence similarity were well below the species threshold of 70 % (Wayne *et al.*, 1987), justifying the designation of both isolates as representatives of novel species.

Additional features that help to distinguish *N. wadsworthii* sp. nov. and *N. shayegani* sp. nov. from one another and from other closely related taxa characterized in this study are shown in Table 4 and can be summarized as follows: (i) production of acid from D-glucose in Gordon base medium by *N. wadsworthii* sp. nov. (see species description); (ii) production of acid from peptone water base with 1 % L-arabinose by *N. canis*, but not by either *N. wadsworthii* sp. nov. or *N. shayegani* sp. nov.; (iii) reaction patterns for API NH and API ZYM strips (bioMérieux), for which the alkaline phosphatase and valine arylamidase reactions are particularly useful; (iv) rod-shaped cells in *N. shayegani* sp. nov. (see species description and Supplementary Fig. S2), a character that is unusual for *Neisseria* and is shared only with *Neisseria elongata*, *N. weaveri* and *Neisseria bacilliformis* (Andersen *et al.*, 1993; Bøvre & Holten, 1970; Han *et al.*, 2006); (v) small, light yellow–grey colonies for *N. shayegani* sp. nov. compared with larger,

yellow colonies for *N. canis* and *N. wadsworthii* sp. nov. (Supplementary Fig. S2); (vi) a 78 nt insertion in the 23S rRNA gene sequence of strains of *N. shayegani* sp. nov. that is absent from all other *Neisseria* strains examined so far; and (vii) the distinctive CFA composition of *N. shayegani* sp. nov. compared with those of neighbouring taxa.

N. wadsworthii sp. nov. and *N. shayegani* sp. nov. are distinguished from most other species of *Neisseria* with validly published names by the absence of acid production from various sugars and/or the ability to reduce nitrate (Tønjum, 2005). Exceptions are *Neisseria iguanae*, which is distinguished by the absence of anaerobic growth and the presence of α -haemolysis, *N. elongata* subsp. *glycolytica*, which is distinguished by the ability to grow on MacConkey agar, *N. elongata* subsp. *nitroreducens*, which is distinguished by the absence of catalase activity, and *N. bacilliformis*, in which isolates are distinguished by the absence of either catalase activity or nitrate-reducing activity (Han *et al.*, 2006; Weyant *et al.*, 1984). Furthermore, *N. wadsworthii* sp. nov. and *N. shayegani* sp. nov. are distinguished from the type species of the genus, *N. gonorrhoeae*, by phylogenetic analysis of the 16S rRNA gene sequence (Fig. 1), showing that the novel species reside in a separate clade, 23S rRNA gene sequence distances (Supplementary Table S1), different relative abundances of predominant CFAs (Weyant *et al.*, 1984) and the inability of strains of the novel species to produce acid from glucose and their ability to reduce nitrate (Tønjum, 2005).

Characteristics that support inclusion of our isolates in the genus *Neisseria* are Gram-negative staining, catalase and oxidase activity, the absence of motility and CFA profiles (Tønjum, 2005; Weyant *et al.*, 1984). Additionally,

Table 4. Characteristics that distinguish the novel strains from the type strains of neighbouring taxa

Strains: 1, 871^T; 2, 12337; 3, 9715^T; 4, 2507; 5, *N. canis* ATCC 14687^T; 6, *N. dentiae* DSM 19151^T; 7, *N. weaveri* DSM 17688^T; 8, *N. animaloris* DSM 21642^T; 9, *N. zoodegmatis* DSM 21643^T. v, Variable.

Characteristic	1	2	3	4	5	6	7	8	9
Nitrate reduction	+	+	+	+	+	-	-	+	+
Arginine dihydrolase	-	-	+	-	-	-	-	+	-
Acid production from (O-F):									
D-Glucose	-	-	-	-	-	+	-	-	+
D-Mannitol	-	-	-	-	-	+	-	-	-
Sucrose	-	-	-	-	-	+	-	-	-
D-Xylose	-	-	-	-	-	+	-	-	-
D-Fructose	-	-	v	-	-	+	-	-	-
Acid production from (peptone water):									
L-Arabinose	-	-	-	-	+	-	-	-	-
D-Glucose	+	-	-	-	-	+	-	+	+
Sucrose	-	-	-	-	-	+	-	-	-
Acid production from (Gordon):									
D-Glucose	-	-	+	+	-	+	-	+	+
D-Mannitol	-	-	-	-	-	+	-	-	-
Sucrose	-	-	-	-	-	+	-	-	-
D-Fructose	-	-	-	-	-	+	-	-	-
Trehalose	-	-	-	-	-	+	-	-	-
D-Mannose	-	-	-	-	-	+	-	-	-
Acidification of (API NH):									
D-Fructose	-	-	-	-	-	+	-	+	-
Sucrose	-	-	-	-	-	+	-	-	-
L-Ornithine decarboxylase	-	-	-	-	-	-	-	+	-
Hydrolysis of (API NH):									
Proline 4-methoxy- β -naphthylamide	-	+	+	+	+	-	+	+	+
γ -Glutamyl 4-methoxy- β -naphthylamide	+	+	+	-	-	+	-	+	+
API ZYM results									
Alkaline phosphatase	+	+	-	-	+	+	+	+	+
Esterase lipase (C8)	+	+	+	+	+	-	-	-	-
Valine arylamidase	-	-	+	+	+	-	-	+	-
Cystine arylamidase	+	+	+	+	+	-	+	-	+

phylogenetic analysis provides strong support for the assignment of *N. wadsworthii* sp. nov. and *N. shayegani* sp. nov. to a clade containing *N. canis*, *N. dentiae* and *N. bacilliformis* (Fig. 1). A neighbour-joining phylogenetic tree was generally concordant with the Bayesian tree, although the bootstrap support was weaker (not shown). However, the genus *Neisseria* is polyphyletic; therefore, when novel species are assigned to the genus, membership of closely related genera should be excluded based on multiple criteria. *N. wadsworthii* sp. nov. and *N. shayegani* sp. nov. are as distant from some species of *Eikenella*, *Kingella*, *Alysiella*, *Uruburuella*, *Bergeriella*, *Conchiformibium* and *Simonsiella* as they are from some of the other more distantly related *Neisseria* species. The presence of catalase activity and the absence of motility observed in our isolates

exclude membership of the genera *Bergeriella*, *Eikenella* and *Kingella*, which are catalase-negative. These characteristics also exclude from consideration the genera *Simonsiella*, *Alysiella* and *Conchiformibium*, whose members form long filaments and display gliding motility. These genera are further excluded from consideration because they reside in well-supported clades separate from the clade made up by *N. wadsworthii* sp. nov. and *N. shayegani* sp. nov. The single species of *Uruburuella* produces acid from fructose and glucose, a feature that, along with its 16S rRNA gene sequence, clearly distinguishes it from our isolates. At this time, there exist no clear phenotypic grounds on which to distinguish the genus *Uruburuella* from the genus *Neisseria* (Vandamme *et al.*, 2006). Therefore, until characters are described that permit unambiguous differentiation of the two genera, we deem it prudent, based on our analysis, to include our isolates in the genus *Neisseria*.

Description of *Neisseria wadsworthii* sp. nov.

Neisseria wadsworthii (wads.wor'thi.i. N.L. gen. n. *wadsworthii* of the Wadsworth Center, to recognize the service provided to New York State residents by the Bacteriology Laboratory of the New York State Department of Health's Wadsworth Center and its role as a public health resource).

Colonies are small, circular, entire, convex, moist, light yellow to orange and non-haemolytic. Cells are Gram-negative, coccoid, 1.3–1.8 μ m in diameter, and may be present in pairs and chains. Growth is observed between 10 and 42 °C. No growth is observed on MacConkey agar after 5 days. Facultative anaerobe. Cells produce catalase and cytochrome oxidase and reduce nitrate to nitrite. Both known isolates produce acid from D-glucose after 10 days on a Gordon base containing 10 % D-glucose. Negative for acid production using Hugh and Leifson oxidation-fermentation (O-F) base with 1 % D-fructose, D-glucose, lactose, maltose, D-mannitol, sucrose or D-xylose. Negative for acid production in peptone water base with 1 % adonitol, L-arabinose, dulcitol, D-glucose, inositol, lactose, maltose, D-mannitol, raffinose, D-rhamnose, D-salicin, D-sorbitol, sucrose or D-xylose. Negative for acid production (Gordon sugar base containing 10 % carbohydrate) from L-arabinose, cellobiose, dulcitol, D-fructose, D-galactose, glycerol, inositol, inulin, lactose, maltose, D-mannitol, D-mannose, melibiose, raffinose, D-rhamnose, D-salicin, D-sorbitol, sucrose, trehalose or D-xylose. Negative for utilization of Simmons' citrate, hydrolysis of aesculin, urea and gelatin, indole production, decarboxylation of arginine, lysine and ornithine using Moeller's decarboxylase medium and production of H₂S in triple-sugar iron agar (for details of methods, see Forbes *et al.*, 1998; Gordon *et al.*, 1973; Kohlerschmidt *et al.*, 2009). Using the API NH system, both known isolates are positive for proline arylamidase and acidification of D-glucose and negative for penicillinase, acidification of D-fructose, maltose and sucrose, ornithine decarboxylase, urease, lipase, alkaline phosphatase, β -galactosidase and hydrolysis of indole; variable for γ -glutamyltransferase (type strain positive).

Fig. 1. Bayesian phylogenetic tree from a 1307-position 16S rRNA gene sequence alignment, showing the positions of strains of *Neisseria wadsworthii* sp. nov. and *Neisseria shayegani* sp. nov., relative to previously described species and genera. Posterior probabilities of nodes are indicated. The branch for the outgroup *Oligella ureolytica* ATCC 43534^T is shown at 1/10 actual length. Branches with posterior probabilities of less than 50% were collapsed. Bar, 0.1 expected changes per site.

Using the API ZYM system, both known isolates are positive for esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase and negative for alkaline phosphatase, lipase (C14), trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. The predominant CFAs for both known strains are 16:0, summed feature 3 (16:1 ω 7c and/or iso-15:0 2-OH) and 18:1 ω 7c.

The type strain, 9715^T (=DSM 22247^T =CIP 109934^T), was isolated from a hand wound of a 39-year old male in New York, USA. Reference strain 2507 (=DSM 22245 =CIP 109931) was isolated from peritoneal fluid of a 36-year old female in New York, USA.

Description of *Neisseria shayegani* sp. nov.

Neisseria shayegani (sha.ye.ga'ni.i. N.L. gen. n. *shayegani* of Shayegani, to recognize and honour over 40 years of public service and leadership by Dr Mehdi Shayegani in the Bacteriology Laboratory of the Wadsworth Center, New York State Department of Health).

Colonies are small, circular, entire, convex, moist, light yellow to grey and non-haemolytic. Cells are Gram-negative,

rod-shaped and 1.0–1.5 μ m wide by 2.5–5.5 μ m long. Growth is observed between 10 and 42 °C. No growth is observed on MacConkey agar after 5 days. Facultative anaerobe. Cells produce catalase and cytochrome oxidase and they reduce nitrate to nitrite. Negative for acid production using Hugh and Leifson O-F base with 1% D-fructose, D-glucose, lactose, maltose, D-mannitol, sucrose or D-xylose. Except as noted, negative for acid production in peptone water sugar (containing 1% carbohydrate) from adonitol, L-arabinose, dulcitol, D-glucose (positive for strain 871^T), inositol, lactose, maltose, D-mannitol, raffinose, D-rhamnose, D-salicin, D-sorbitol, sucrose or D-xylose. Negative for acid production (Gordon sugars) from L-arabinose, cellobiose, dulcitol, D-fructose, D-galactose, D-glucose, glycerol, inositol, inulin, lactose, maltose, D-mannitol, D-mannose, melibiose, raffinose, D-rhamnose, D-salicin, D-sorbitol, sucrose, trehalose and D-xylose. Negative for utilization of Simmons' citrate, hydrolysis of aesculin, urea and gelatin, indole production, decarboxylation of lysine, ornithine and arginine using Moeller's decarboxylase medium and production of H₂S in triple-sugar iron agar (Forbes *et al.*, 1998; Gordon *et al.*, 1973; Kohlerschmidt *et al.*, 2009). Using the API NH system, both known strains are positive for γ -glutamyltransferase and acidification of D-glucose and negative for penicillinase, acidification of D-fructose, maltose and sucrose, ornithine decarboxylase,

urease, lipase, alkaline phosphatase, β -galactosidase and indole; variable for proline arylamidase (type strain negative). Using the API ZYM system, both known strains are positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, cystine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase and negative for lipase (C14), valine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase and α -mannosidase; variable for α -fucosidase (type strain negative). The predominant CFAs for both known strains are 16:0, summed feature 3 (16:1 ω 7c and/or iso-15:0 2-OH), 12:0, 12:0 3-OH and 14:0.

The type strain, 871^T (=DSM 22246^T =CIP 109933^T), was isolated from sputum of a 77-year-old female in New York, USA. Reference strain 12337 (=DSM 22244 =CIP 109932) was isolated from an arm wound of a 47-year-old female in New York, USA.

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