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Neisseria weaveri sp. nov., Formerly CDC Group M-5, a Gram-Negative Bacterium Associated with Dog Bite Wounds

BJØRG MARIT ANDERSEN,[1]*† ARNOLD G. STEIGERWALT,[2] STEVEN P. O'CONNOR,[3] DANNIE G. HOLLIS,[2] ROBBIN S. WEYANT,[2] ROBERT E. WEAVER,[2] AND DON J. BRENNER[2]

Department of Medical Microbiology, University Hospital of Tromsø, 9000 Tromsø, Norway,[1] and Meningitis and Special Pathogens Branch[2] and Respiratory Diseases Branch,[3] Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333

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CDC group M-5 is a rod-shaped, gram-negative, nonmotile bacterium associated with dog bite wounds. DNA-DNA relatedness and biochemical and growth characteristics were studied for 54 strains from the collection at the Centers for Disease Control and Prevention. One typical M-5 strain, 8142, was further studied by 16S rRNA sequencing. DNA from 40 of 53 strains showed 82 to 100% relatedness (hydroxyapatite method) to labeled DNA from strain 8142. The guanine-plus-cytosine (G+C) content in 8 of the 41 highly related M-5 strains was 50.5 to 52 mol%. These 41 strains were oxidase and catalase positive, nonfermentative, nitrite positive, nitrate negative, weakly phenylalanine deaminase positive, aerobic, and alpha-hemolytic (sheep blood). DNA from the 13 remaining strains showed only 7 to 46% DNA relatedness to strain 8142. These 13 non-M-5 strains differed from the M-5 strains in G+C content, growth characteristics, and biochemical profiles. DNA from M-5 strain 8142 was most closely related to DNA from groups EF-4b (47%) and EF-4a (45%). 16S rRNA sequence analysis placed M-5 strain 8142 in the *Neisseriaceae* cluster of the beta-3 subgroup of the class *Proteobacteria*. It was most homologous (98.4 to 98.8%) to *Neisseria animalis*, *Neisseria flavescens*, *Neisseria canis*, and *Neisseria elongata*. All data are consistent with M-5 being a new species of *Neisseria*, for which we propose the name *Neisseria weaveri*.

From 1960 through 1992, more than 160 isolates of a *Moraxella*-like bacterium, named CDC group M-5, were received for identification in the Special Bacteriology Reference Laboratory, Meningitis and Special Pathogens Branch, Centers for Disease Control and Prevention. M-5 is associated with dog bite wounds and was first described by Tatum, Ewing, and Weaver in 1974 (42).

M-5 is a gram-negative, rod-shaped, nonmotile, oxidase- and catalase-positive, aerobic bacterium. It is biochemically similar to *Neisseria* and *Moraxella* spp. and oxidase-positive, glucose-negative nonfermenters (3, 10, 17, 19, 21, 22, 33, 35, 40, 42, 45). Its cell wall fatty acid composition is similar to that of *Neisseria* species (32, 33).

In this study we have defined M-5 phenotypically, by DNA-DNA relatedness, and by its 16S rRNA sequence. On the basis of these data, M-5 is shown to be a new species in the genus *Neisseria*, for which the name *Neisseria weaveri* is proposed.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The sources of the strains are shown in Table 1. A total of 54 strains (including two colonial variants of one strain) were selected in a manner to span diversity in source, geographic location, year of isolation, and biochemical reactions.

Strains were grown on sheep blood-agar plates (SBAP) (Trypticase soy agar base with 5% defibrinated sheep blood; BBL Microbiology Systems, Cockeysville, Md.) for 24 h at 35°C in a candle jar. A single colony from each strain was selected for preparation of DNA. It was inoculated into 100 ml of brain heart infusion broth at 35°C for 24 h in a shaker. Fifty milliliters of this broth culture was added to each of two 1-liter portions of brain heart infusion broth and incubated for 24 to 72 h under the same conditions. The bacteria were sedimented by centrifugation at 4,200 × *g* for 30 min, and the supernatant was discarded. Cells were frozen until use.

Control strains. The 13 defined control strains studied were *Eikenella corrodens* (ATCC 23834^T, EF-4a ATCC 29858, and EF-4b ATCC 29859), *Neisseria elongata* subsp. *nitroreducens* ATCC 49377^T, *Neisseria canis* ATCC 14687^T, *Neisseria flavescens* ATCC 13120^T, *Moraxella osloensis* ATCC 19976^T, *Kingella kingae* ATCC 23330^T, *Kingella denitrificans* CDC B8312, *Simonsiella muelleri* ATCC 29453^T, *Chromobacterium violaceum* ATCC 12472^T, and Gilardi unnamed rod group 1 strains CDC F6511 and CDC F6512 (18, 33). The control strains were grown identically to the M-5 strains, except for *M. osloensis* and *K. kingae*, which were harvested after growth on SBAP for 2 days.

Biochemical characterization. The biochemical characteristics of all strains (except *K. denitrificans*) were determined by using the standard methods of the Special Bacteriology Reference Laboratory for identification of nonfermenting bacteria (10). In addition, we used sodium acetate, acetamide, serine, and tartrate (36). The biochemical reactions were read after 24 h for oxidase, catalase, and phenylalanine deaminase (growth at 25, 35, and 42°C) and after 48 h and 7 days all other reactions. The gelatin test was also read after 14 days.

The phenylalanine test was performed with a heavy inoculum both from a heart infusion agar slant and from SBAP. Two commercial PPA tests were also used: Minitek (BBL)

* Corresponding author.
† Present address: Centers for Disease Control and Prevention, Building 1, Room 2226, Mailstop D11, Atlanta, GA 30333.

TABLE 1. Sources of CDC group M-5 strains studied

Strain no.	Clinical source	Geographic source	Year received
5838A	Dog bite wound	Washington	1960
8142	Dog bite wound	New York	1962
A202	Cheek abscess	Louisiana	1964
A1420	Dog bite wound	Washington	1964
A3240	Dog bite wound	Colorado	1965
A3399	Wound	Florida	1965
A4682	Dog bite, scratch	Michigan	1966
A8190	Dog bite wound	Florida	1967
A8209	Dog bite wound	Colorado	1967
A9934	Dog bite wound	Delaware	1968
B327	Dog bite wound	New Hampshire	1968
B1203	Dog gums or tonsils	Colorado	1968
B5522	Dog bite wound	Washington	1970
B7744	Dog, tracheal aspirate	Virginia	1971
B9108	Dog, tongue	Georgia	1971
C9145	Dog bite wound	Illinois	1974
C9202	Dog bite wound	Colorado	1974
D1673	Dog, teeth	California	1974
D1674	Dog, mouth	California	1974
D3537	Dog bite wound	Alaska	1975
D5641	Wound, hand	Colorado	1976
D5983	Dog bite wound	Hawaii	1976
D6680	Dog bite wound	Florida	1976
D9355	Dog bite wound	Virginia	1977
E7032	Dog bite wound	Canada	1979
E7557	Dog bite wound	Alabama	1980
E7900	Dog bite wound	Maine	1980
E8000	Dog bite wound	Virginia	1980
E8456	Eye	North Carolina	1980
E9924	Hand wound	New Mexico	1981
F973	Dog bite, hand	Kentucky	1981
F1315	Cheek wound	Finland	1981
F2404	Dog bite wound, finger	New Zealand	1982
F5828	Dog bite wound	Delaware	1984
F8888	Dog bite wound	California	1986
G529	Peritoneal and chest fluid	Maryland	1987
G1139	Multiple dog bites	Lousiana	1988
G3025	Dog bite wound, hand	Colorado	1989
G3199	Dog bite wound, hand	Oregon	1989
G5627	Dog bite wound	Spain	1990
G6750	Unknown	England	1991
B1149	Bronchoscopy	Washington	1968
B8575	Neck wound	Vermont	1971
D637	Dog bite, abdominal wound	Pennsylvania	1974
D1257	Dog bite wound	Missouri	1974
E9111	Lesion	Puerto Rico	1980
E9204	Dog bite, face wound	Montana	1980
E9329	Human bite, hand	Pennsylvania	1980
F714	Neck wound	Colorado	1981
G1018	Blood × 8	California	1988
G1043	Sputum	Nevada	1988
G5129	Dog bite, leg wound	Hawaii	1990
G5415a	Wound, finger	Pennsylvania	1990
G5415b	Wound, finger	Pennsylvania	1990

and the urea-phenylalanine deaminase-disk test (Remel). They were heavily inoculated with bacteria grown on SBAP. *Oligella urethralis* and *Pseudomonas aeruginosa* were used as positive and negative controls, respectively.

DNA-DNA hybridization. The preparation and purification of DNA and the conditions used to determine DNA relatedness by the hydroxyapatite method have been described previously by Brenner et al. (4, 6, 7). DNA from strain 8142 was labeled with [^{32}P]dCTP by using a nick translation kit (GIBCO BRL, Gaithersburg, Md.) as described by the manufacturer. The hybridization reactions were performed at 60°C (optimal reassociation of single-stranded DNA) and at 75°C (stringent reassociation) (6). The optimal temperature of incubation (60°C) allows reassociation of partly complementary sequences, implying the formation of hybrids between sequences which could differ by as much as 1 base in 5. The stringent temperature of incubation (75°C) permits only the reassociation of sequences with a small degree of mismatching. Divergence (unpaired bases within hybridized sequences) was estimated to be approximately 1% per degree of decreased thermal stability in a heterologous reassociated DNA duplex compared with that of the

TABLE 2. Sources and accession numbers for 16S rRNA data

Organism (serogroup)	Strain	GenBank accession no.	Reference or source[a]
Alpha-proteobacteria			
Brucella abortus (a-2)	11-19	X13695	31
Rochalimaea vinsonii (a-2)	ATCC VR-152^T	L01259	11
Beta-proteobacteria			
Rhodocyclus gelatinosus (b-1)	ATCC 17011^T	M60682	47
Pseudomonas testosteroni (b-1)	ATCC 11996^T	M11224	47
Rhodocyclus purpureus (b-2)	6770	M34132	47
Pseudomonas cepacia (b-2)	ATCC 25416^T	M22518	13
Alcaligenes xylosoxidans (b-2)	ATCC 15173^T	M22509	13
Alcaligenes faecalis (b-2)	ATCC 8750^T	M22508	13
Chromobacterium violaceum (b-3)	ATCC 12472^T	M22510	13
Iodobacter fluviatile (b-3)	ATCC 33051^T	M22511	13
Vitreoscilla stercoraria (b-?)	VT1	M22519	13
Spirillum volutans (b-3)	ATCC 19554^T	M34131	47
Nitrosolobus multiformis (b-3)	C-71	M96401	47
Nitrosomonas europaea (b-3)	C-31	M96399	C. Woese
Kingella orale (b-3)	CCUG[b] 30450	L06164	13
Kingella kingae (b-3)	ATCC 23330^T	M22517	13
Simonsiella muelleri (b-3)	ATCC 29453^T	M59071	C. Woese
Eikenella corrodens (b-3)	ATCC 23834^T	M22512	13
Neisseria denitrificans (b-3)	ATCC 14686^T	L06173	F. E. Dewhirst
Kingella denitrificans (b-3)	ATCC 33394^T	M22516	13
Neisseria elongata (b-3)	ATCC 25295^T	L06171	F. E. Dewhirst
Neisseria animalis (b-3)	ATCC 19573^T	L06172	13
Neisseria macacae (b-3)	ATCC 33926^T	L06169	F. E. Dewhirst
Neisseria flavescens (b-3)	ATCC 13120^T	L06168	F. E. Dewhirst
Neisseria polysaccharea (b-3)	ATCC 43768^T	L06167	F. E. Dewhirst
Neisseria gonorrhoeae (b-3)	NCTC 8375^T	X07714	47
Neisseria canis (b-3)	ATCC 14687^T	L06170	F. E. Dewhirst
Gamma-proteobacteria			
Escherichia coli (g-3)	*rrB* cistron	J01695	8
Proteus vulgaris (g-3)	Monteil	J01874	9
Delta-proteobacteria			
Desulfovibrio desulfuricans	ATCC 27774	M34113	34
Myxococcus xanthus	MD 207	M34114	34
Cyanobacteria, *Anacystis nidulans*	Clone pAN4	X03538	44

[a] Unpublished sequences were deposited in GenBank by C. Woese or F. E. Dewhirst (as indicated).
[b] CCUG, Culture Collection of the University of Goteborg.

homologous DNA duplex. Divergence was calculated to the nearest 0.5%. All reactions were done at least twice.

G+C content in DNA. The guanine-plus-cytosine (G+C) content was determined spectrophotometrically by thermal denaturation (27, 28), with *Escherichia coli* K-12 DNA included as a control (6).

16S rRNA sequencing and analysis. The 16S rRNA gene from strain 8142 was amplified enzymatically by polymerase chain reaction (PCR) with primers fD1 and rD1 (46), as described previously (11). The PCR product (template DNA) was purified with streptavidin-coated magnetic beads (Dynabeads M-280; Streptavidin, Dynal, Oslo, Norway) according to a modification of the method of Hultman et al. (24), as previously described (11). The template DNA was denatured by treatment with 0.1 M NaOH for 15 min at room temperature. Each strand was recovered for sequencing (11).

Sequencing reactions were done according to the manufacturer's protocol (Sequenase, version 2.0; United States Biochemical, Cleveland, Ohio). Sequencing primers and conditions used for gel electrophoresis have been described previously (11, 41).

16S rRNA sequences of 32 species were obtained from GenBank (Table 2). The sequences were aligned by the multisequence alignment program Pileup (12). The alignment was edited by removing all positions at which any sequence contained an ambiguous or undetermined nucleotide. Phylogenetic relationships were inferred by using version 3.4 of the PHYLIP software package (16). Evolutionary distance values were determined by the method of Jukes and Cantor (25). A dendrogram was constructed by the distance-based, neighbor-joining method (38) by using the sequence from *Anacystis nidulans* (44) as the outgroup (nonrelated species used to root the tree). The data were also analyzed by maximum parsimony (47). The reproducibility of the tree nodes was analyzed by bootstrapping (15).

Nucleotide sequence accession number. The 16S rRNA sequence for *N. weaveri* strain CDC 8142 (ATCC 51223) has been deposited in the GenBank data base under accession number L10738. Accession numbers and references for all other 16S rRNA sequences used in this study are listed in Table 2.

TABLE 3. Relatedness of labeled DNA of strain 8142 to CDC group M-5 strains and to control strains

Source of unlabeled DNA	% Binding at:		
	60°C	D[a]	75°C
Typical M-5 strains			
8142	100	0.0	100
A3240	100	0.5	
G3199	100	0.5	
A8209	100	1.0	
G6750	100	2.0	
A9934	99	1.0	
B5522	99	1.0	
B7744	99	1.0	
B9108	99	1.5	
C9145	99	1.5	
C9202	99	1.5	95
A3399	99	2.0	
G3025	98	0.5	
A8190	98	1.0	
B327	98	1.0	
E8456	98	1.0	
E9924	98	1.5	92
F1315	98	2.0	94
F8888	98	2.0	
5838A	97	0.5	93
A1420	97	0.5	
A4682	97	0.5	
E7900	97	1.0	
E8000	97	1.0	
D1673	97	1.5	
F973	97	1.5	
F5828	96	0.5	
G529	96	0.5	
F2404	94	0.0	
D5641	94	0.5	91
A202	93	0.5	92
E7557	93	1.0	
D3537	91	0.5	
B1203	89	1.0	80
E7032	89	1.0	
D1674	89	1.5	
G1139	89		
D9355	87	1.0	92
D6680	83	1.5	
D5983	82	0.0	
G5627	82		
Atypical M-5 strains			
E9329	46		
B8575	39		
G5129	39	13.5	
G1043	32	10.5	
F714	31		
E9111	30		
G1018	30		
B1149	25		
D1257	18		
D637	12		
G5415b	10		
E9204	8		
G5415a	7		
Control strains			
EF-4b, ATCC 29859	47	11.0	
EF-4a, ATCC 29858	45	12.5	
N. elongata subsp. *nitroreducens* ATCC 49377^T	40	14.0	
K. denitrificans CDC B8312	26		
E. corrodens ATCC 23834a^T	24		
E. corrodens ATCC 23834b^T	24		
N. flavescens ATCC 13120^T	16		
N. canis ATCC 14687^T	15		
K. kingae ATCC 23330^T	15		
S. muelleri ATCC 29453^T	9		
C. violaceum ATCC 12472^T	9		
Gilardi unnamed rod group 1:			
CDC F6512	8	11.0	
CDC F6511	3		
M. osloensis ATCC 19976^T	3		

[a] D, divergence, the decrease in thermal stability (in degrees Celsius) of heterologous DNA duplexes compared with those of homologous DNA duplexes.

RESULTS

Clinical source and geographic origin of the strains. Most strains were isolated from dog bite wounds (Table 1). Five isolates were obtained directly from dogs. Two isolates were from presumptively sterile sites (blood culture and peritoneal-chest fluid). Most isolates were from the United States, with three from Europe, one from Canada, one from Puerto Rico, and one from New Zealand.

DNA relatedness. Labeled DNA from M-5 strain 8142 was 82 to 100% related to 40 strains in reactions done at 60°C (Table 3). In 75°C reactions, strain 8142 showed 80 to 95% relatedness to 8 strains selected from these 40. The divergence (D), which is unpaired bases within hybridized sequences, was 0 to 2%. These 41 strains are subsequently referred to as M-5 or the M-5 hybridization group and are listed as the first 41 strains in Table 1. Strain 8142 showed 7 to 46% relatedness to 13 other biochemically atypical M-5-like strains, with a divergence of 10.5 to 14% (Table 3). These 13 strains are subsequently referred to an non-M-5.

Among the 13 control strains, strain 8142 showed the highest relatedness (40 to 47%) at 60°C to EF-4b, EF-4a, and *N. elongata* subsp. *nitroreducens*, with a divergence of more than 10% (Table 3). A lower level of relatedness (15 to 25%) was found to *K. denitrificans*, *E. corrodens*, *N. canis*, *N. flavescens*, and *K. kingae*.

G+C content. DNAs from 7 M-5 strains showed a G+C content similar to that of strain 8142, ranging from 50.8 to 52 mol% (Table 4). DNAs from 12 non-M-5 strains had G+C contents that were dissimilar to that of strain 8142. Among the known organisms studied, the G+C content most similar to that of M-5 8142 was observed in strains of groups EF-4a and EF-4b (Table 4).

16S rRNA sequence comparison. We determined 1,487 bases of the 16S rRNA sequence of the M-5 strain 8142, corresponding to *E. coli* sequence positions 51 to 1537 (8). Thirty-two sequences were aligned: 2 alpha, 25 beta, 2 gamma, and 2 delta and 1 *A. nidulans*, which was used as the outgroup (Table 2). 16S rRNA sequences of EF-4a and EF-4b were not available when we made this comparison. The sequences in the alignment were edited to 1,129 positions as described in Materials and Methods. The dendrogram made from the edited alignment by the neighbor-joining method demonstrated that the alpha-, delta-, and gamma-*Proteobacteria* species branched off early and separately, while the beta-*Proteobacteria* species divided into two main

TABLE 4. G+C content of DNA from CDC group M-5 strains compared with those of other species

Source of DNA	G+C mol%		Reference
	This study	Other studies	
Typical M-5 strains			
8142 (type strain)	51.5		
A202	50.8		
A1420	51.5		
A3240	51.1		
A8209	51.9		
D5983	51.2		
D6680	52.0		
B5522	51.5	51.3	37
CCUG 4007		52.0	37
Atypical M-5 strains			
G5415a	68.1		
D1257	65.7		
E9204	61.8		
G1043	61.6		
G5129	61.4		
B1149	61.2		
E9329	57.1		
B8575	56.9		
F714	56.8		
G1018	55.6		
D637	48.5		
E9111	37.2		
Control strains			
C. violaceum ATCC 12472^T	66.8		
C. violaceum		65–68	29
E. corrodens ATCC 23834^T	57.6		
E. corrodens		56–58	30
N. elongata subsp. *nitroreducens* ATCC 49377^T	55.0		
N. elongata subsp. *nitroreducens*		56–58	22
K. denitrificans		54.1–54.8	40
EF-4a, ATCC 29858	53.2	49.3	37
EF-4b, CDC T-194/78		50.5	37
N. flavescens ATCC 13120^T		49.6	37
N. canis ATCC 14687^T		49.6	37
K. kingae		47.3–47.4	40
Gilardi unnamed rod group 1, CDC F6512	47.2		
Gilardi unnamed rod group 1, CDC F6511	46.3		
M. osloensis		43–43.5	3
S. muelleri ATCC 29453^T	42.0	43.0	37
E. coli K-12 (control)	51.5		

branches (Fig. 1). M-5 (*N. weaveri*) was located within the *Neisseriaceae* cluster, which consists of *Neisseria*, *Kingella*, and *Simonsiella* species and *E. corrodens* (13). Specifically, it grouped with a small cluster of species associated with animals, consisting of M-5, *Neisseria animalis*, and *N. canis*.

The consensus tree from 100 replicates obtained by bootstrap analysis (neighbor-joining method) had a topology identical to the dendrogram in Fig. 1, with the exception that the positions of *Pseudomonas cepacia* and *Spirillum volutans* were reversed.

M-5 clustered with *N. animalis* 43 of 100 times; the two came from a common branch with *N. canis* 34 of 100 times, and all three had a common branch with the *Neisseria gonorrhoeae* cluster 50 of 100 times. *N. gonorrhoeae* clustered with *Neisseria polysaccharea* 100 of 100 times, *Neisseria macacae* branched from them 69 of 100 times, and further down *N. flavescens* branched from these three strains 81 of 100 times.

With the maximum parsimony method (data not shown), the results were approximately the same as with the neighbor-joining method, with one exception: *N. canis* branched off much earlier, even before *E. corrodens* and *S. muelleri*. By bootstrap analysis M-5 came out 31 of 100 times together with *N. animalis*. They had a common branch with the *N. gonorrhoeae* cluster only 16 of 100 times. The robustness of the *N. gonorrhoeae* cluster was similar to that obtained by the neighbor-joining method.

A homology matrix was made for strain 8142 and 25 other species in the beta-group of *Proteobacteria* (Table 5). Strain 8142 grouped with species in the family *Neisseriaceae* with highest homology to *N. animalis* (98.8%), *N. flavescens* (98.6%), *N. canis* and *N. elongata* (98.4% each), *N. denitrificans* (98.1%), *N. polysaccharea* (98.0%), *N. macacae* (97.8%), and *N. gonorrhoeae* (97.3%). This homology level was similar to the homology level present between established *Neisseria* species. The degree of homology was also more than 95% to *E. corrodens* (96.8%), *K. denitrificans* (97.5%), *K. kingae* (96.8%), *S. muelleri* (96.4%), and *Vitreoscilla stercoraria* (95.7%).

Biochemical and other phenotypic characteristics of M-5 hybridization group strains. The 41 M-5 hybridization group strains had almost identical biochemical reactions (Table 6). The phenylalanine deaminase test was positive in all 41 M-5 strains when cultures were grown on SBAP, in 6 of 41 (14.6%) strains from cultures grown on heart infusion agar in 20 of 27 (74.1%) strains tested in the Minitek system, and in 0 of 5 M-5 strains in the urea-phenylalanine deaminase-disk test.

Non-M-5 strains. All 13 non-M-5 strains were differentiated from M-5 on the basis of the following characteristics: 7 strains differed in Gram stain, 10 had no alpha hemolysis on SBAP, 10 had a smaller colony size, 2 showed pigmentation, 1 was Simmons citrate positive, 1 was urea positive, 3 were nitrate positive, 4 were nitrite negative, 3 produced gas from nitrate and nitrite, 6 were phenylalanine negative, 2 showed very strong phenylalanine reaction, 4 were gelatin positive, 1 peptonized litmus milk, and 4 were sodium acetate positive. Two strains were identical to M-5 in biochemical reactions but had no alpha hemolysis, had a different picture in Gram stain, and showed a G+C content higher than that of the M-5 strains. Four strains (E9329, B8575, F714, and G1018) were similar, but not identical, to the three subspecies of *N. elongata*, with DNA-relatedness of 30 to 46%. These strains were subsequently shown to be *N. elongata* by DNA hybridization (data not shown). One strain was *O. urethralis* (D637). One strain with a G+C content of 37.2 mol% (E9111) and six strains with a G+C content of 61 mol% or higher were not identified.

DISCUSSION

DNAs from the 41 M-5 bacteria were highly related (82 to 100%). The G+C content of a few selected strains was 50.8 to 52 mol%, which agrees with the findings of Rossau et al. (37). This group of bacteria showed consistent growth and biochemical patterns dominated by strong oxidase and catalase reactions, inability to utilize carbohydrates, a positive nitrite but negative nitrate reaction, and a weak but distinctly positive phenylalanine deaminase reaction, dependent on a

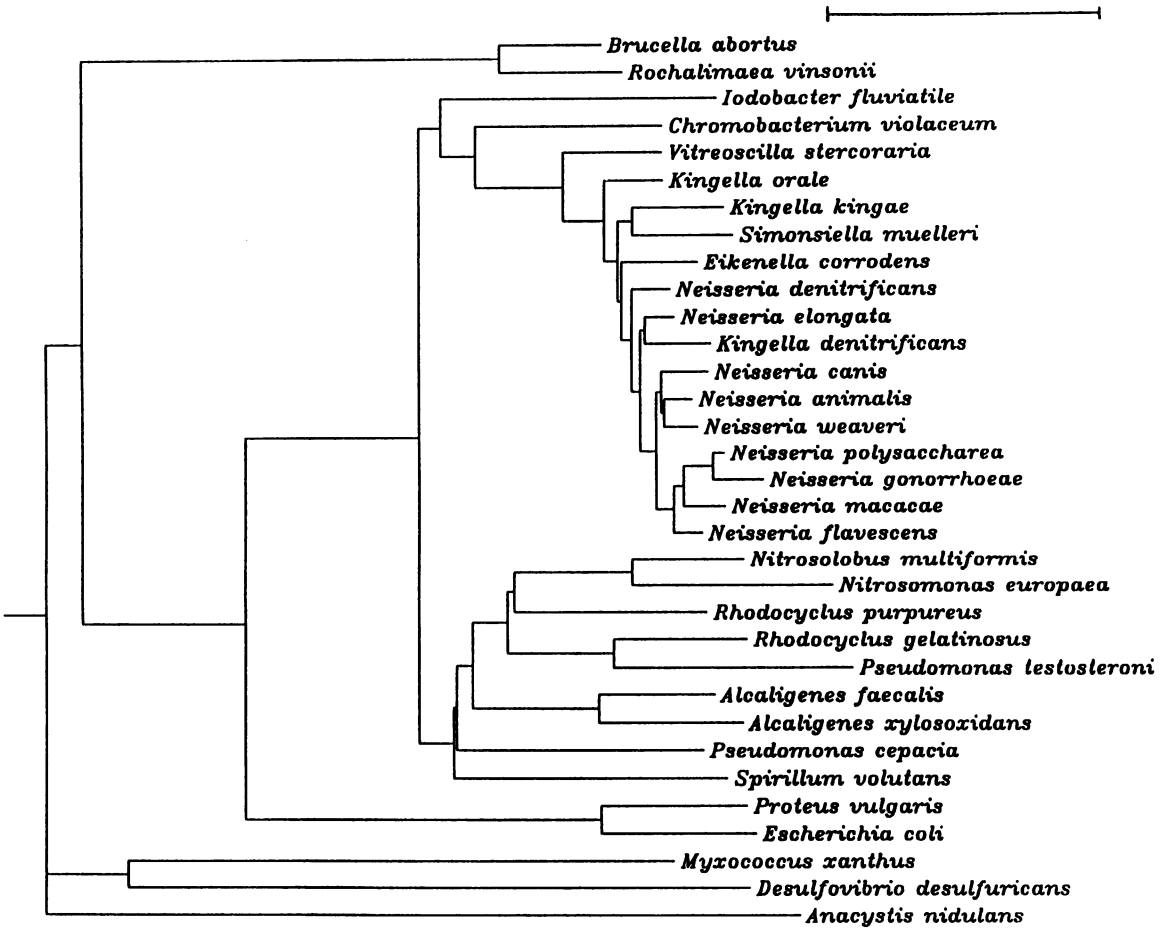

FIG. 1. Phylogenetic neighbor-joining tree for 16S rRNA of CDC group M-5 (*N. weaveri*) and of the species listed in Materials and Methods. Edited sequences are shown. The scale represents a 5% difference in nucleotide sequence.

heavy inoculum from an 18- to 24-h culture on sheep blood-agar.

Various media are used to grow the inoculum used for the phenylalanine deaminase test in microbiological diagnostics (10). The choice of culture medium may influence the test results, as shown in this study. The test is dependent on presence of phenylalanine from phosphoenolpyruvate and erythrose-4-phosphate and deaminase activity (26). The accessibility of these compounds or their precursors may be better in media containing blood.

The 13 atypical M-5-like strains were not sufficiently DNA-related to M-5 strain 8142 (7 to 46%) to be classified as the same species. They were different from M-5 strains in colony morphology, G+C content, Gram stain, and biochemical patterns. This was not unexpected since we purposely included strains from unusual sources (e.g., sputum and bronchoscopy) and with biochemical reactions that were unusual for M-5. Four of the 13 atypical strains were from dog bite wounds. One was *O. urethralis*; the three others are still unidentified.

Differential diagnosis is difficult when working with samples from dog bite wounds. M-5 is a part of the normal canine oral flora (in 10 to 12% of oral flora samples), together with biochemically similar bacteria such as *Weeksella zoohelcum* (38 to 90%); EF-4 (up to 74%); *Capnocytophaga canimorsus* (up to 8%); *Pasteurella multocida* (12 to 60%); other *Pasteurella* spp.; *N. flavescens*; other species of *Neisseria*, *Moraxella*, *Oligella*, *Pseudomonas*, *Simonsiella*, *Alcaligenes*, and *Flavobacterium*; and *Bordetella bronchiseptica* (1, 5, 19–21, 23, 39). They are all oxidase-positive, gram-negative rods or cocci, and some are relatively inert against carbohydrates (Tables 7 and 8). Clinical sources of M-5 are most often dog bite wounds (1, 19–21, 42), as also shown in this study. Of the 41 M-5 strains, 28 (68%) were from dog bite wounds.

The only isolate of an M-5 strain from a deep-seated infection was from peritoneal and chest fluid, sent in from Maryland in 1987. This organism was isolated from a tube culture from a woman who had recently undergone a lobectomy. Graham et al. (21) showed that M-5 is usually not associated with systemic disease. Since we have little clinical information, it is difficult to determine how often M-5 is a pathogen in dog bite wounds.

Four of the 13 non-M-5 strains were similar, but not identical, to *N. elongata*, including the only blood isolate in the actual collection, which is consistent with the pathogenicity of this species (21, 22, 48). *N. elongata* is part of the normal flora in the pharynges of healthy persons and may be associated with human bites, as was one isolate in this study.

M-5 is closely related to EF-4a and EF-4b, as shown in this study by DNA-DNA hybridization (45 and 47%), G+C content, and biochemical and growth studies. This is in accordance with the findings of Rossau et al., who demon-

TABLE 5. 16S rRNA similarity values for *N. weaveri* and related organisms[a]

Organism	*N. weaveri*	*A. faecalis*	*A. xylosoxidans*	*N. europaea*	*N. multiformis*	*P. testosteroni*	*R. gelatinosus*	*R. purpureus*	*N. gonorrhoeae*	*N. polysaccharea*	*N. flavescens*	*N. macacae*	*N. animalis*	*N. elongata*	*N. denitrificans*	*K. orale*	*N. canis*	*K. denitrificans*	*V. stercoraria*	*K. kingae*	*S. muelleri*	*I. fluviatile*	*E. corrodens*	*C. violaceum*	*P. cepacia*	*S. volutans*
N. weaveri (M-5)	100																									
A. faecalis	89.8	100																								
A. xylosoxidans	89.0	95.2	100																							
N. europaea	87.7	89.1	89.1	100																						
N. multiformis	89.1	91.0	91.1	94.3	100																					
P. testosteroni	87.0	89.5	88.9	89.2	90.2	100																				
R. gelatinosus	89.1	90.4	90.9	89.5	90.6	93.3	100																			
R. purpureus	90.2	90.6	90.4	90.9	92.4	90.1	92.3	100																		
N. gonorrhoeae	97.3	89.3	89.4	87.6	88.7	86.6	88.7	89.5	100																	
N. polysaccharea	98.0	89.7	89.6	87.9	89.2	87.1	89.3	89.7	98.8	100																
N. flavescens	98.6	89.8	89.5	88.1	89.4	87.3	89.4	90.3	98.0	98.6	100															
N. macacae	97.8	89.5	89.3	87.7	89.3	87.4	88.9	89.7	97.7	98.5	98.4	100														
N. animalis	98.8	89.6	89.0	87.5	88.8	87.2	88.9	89.9	97.5	98.0	98.3	97.9	100													
N. elongata	98.4	90.2	89.0	87.7	89.1	87.5	89.0	90.1	96.9	97.5	98.2	98.0	98.5	100												
N. denitrificans	98.1	90.3	89.1	87.5	88.7	87.3	89.1	89.6	96.6	97.4	98.0	97.5	98.4	98.3	100											
K. orale	97.3	90.3	89.8	88.1	89.7	87.7	89.7	90.5	95.9	96.8	97.4	96.5	97.0	97.5	97.5	100										
N. canis	98.4	90.0	89.6	88.0	89.5	87.8	89.9	90.9	97.3	98.0	98.1	97.7	98.6	98.0	97.7	98.1	100									
K. denitrificans	97.5	90.4	89.2	87.1	89.0	86.7	88.7	89.5	96.5	97.2	97.2	97.5	97.8	98.2	97.9	96.4	97.4	100								
V. stercoraria	95.7	90.0	89.7	88.7	89.5	88.4	89.9	90.0	94.9	95.8	96.2	95.4	95.6	95.7	96.1	96.4	96.0	94.9	100							
K. kingae	96.8	89.5	89.5	87.2	88.4	86.7	88.6	89.5	95.4	96.0	96.5	95.8	96.6	96.7	96.6	96.9	96.6	96.6	94.8	100						
S. muelleri	96.4	90.1	89.5	86.7	88.6	87.2	88.8	89.0	94.9	95.8	96.1	95.9	93.3	96.4	97.3	96.4	96.0	96.5	95.1	96.5	100					
I. fluviatile	90.8	89.5	88.6	88.3	90.0	88.7	89.5	90.1	89.6	90.2	90.7	90.2	90.4	90.9	90.9	91.1	90.6	90.3	90.6	89.9	90.3	100				
E. corrodens	96.8	90.5	89.5	87.8	89.5	87.7	89.5	89.4	95.8	96.6	97.3	97.1	96.9	97.8	97.8	96.9	96.9	97.3	95.7	96.4	96.7	90.6	100			
C. violaceum	92.6	90.1	89.5	89.3	90.6	88.7	90.4	91.0	91.9	92.6	92.7	92.0	92.1	92.6	92.4	93.2	92.8	92.7	93.6	92.3	92.0	91.6	93.1	100		
P. cepacia	90.3	90.5	90.2	89.1	90.4	89.4	91.5	91.5	89.3	90.0	90.1	89.8	90.2	90.1	90.0	90.6	91.0	89.6	89.8	89.9	89.3	89.8	90.3	90.5	100	
S. volutans	89.6	90.0	90.3	89.5	90.9	87.8	90.0	91.2	88.7	89.4	89.5	89.4	89.5	89.5	89.7	90.3	90.2	89.8	89.7	89.8	90.0	88.9	89.4	89.9	90.6	100

[a] Values are the percent similarity corrected for multiple base changes by the method Jukes and Cantor (25).

TABLE 6. Biochemical and other characteristics of CDC group M-5 and type strains[a]

Test or characteristic	CDC M-5 (n = 41) (%)	Type strain 8142
Morphology	Short and long rods	Short and long rods
Motility	0	−
Oxidase	100	+
Catalase	100	+
Alpha hemolysis on sheep blood[b]	100	+
Colony size on sheep blood (1-2 mm; 24 h)	100	+
Growth on:		
MacConkey agar	0	−
Salmonella-shigella agar	0	−
Acid from D-glucose, D-xylose, D-mannitol, lactose, sucrose, maltose	0	−
Simmons citrate	0	−
Cetrimide	0	−
Urea	0	−
Nitrite reduction	0	−
0.1%	100	+
0.01%	100	+
Gas production from nitrate or nitrite	0	−
Indole	0	−
Phenylalanine deaminase inoculum[b] from:		
Heart infusion agar slant	14.6 (w)	−
Sheep blood	100	+
Triple sugar iron slant and butt, acid	0	−
H_2S (triple sugar iron butt)	0	−
H_2S (lead acetate paper)	100	+
Gelatin	0	−
Esculin	0	−
Litmus milk	78 (wk)	−
Pigment, colony	0	−
Pigment, soluble[c]	100 (w)	+ (w)
Fluorescence	0	−
Growth at:		
25°C	100	+
35°C	100	+
42°C	82.9	+
Growth in nutrient broth:		
0% NaCl	100	+
6% NaCl	4.9 (w)	−
Penicillin diagnostic disk (10 U; zone size, >20 mm)	100	+
Utilization of:		
Sodium acetate	0	−
Serine, acetamide, tartrate	0	−
ONPG[d]	0	−
Lysine, arginine, ornithine	0	−

[a] w, weak reaction; wk, weak alkalinization.
[b] Heavy inoculum.
[c] Weak, yellow, soluble pigment production in Bacto-peptone and proteose peptone media (10).
[d] ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

strated rRNA cistron similarities and DNA relatedness between species within the emended family *Neisseriaceae*, including M-5 (37). The position of the M-5 strain was somewhat uncertain but was located nearest to group EF-4 and *Eikenella*, *Simonsiella*, and *Neisseria* spp. (37). A close relationship between EF-4b and M-5 was recently found by 16S rRNA sequence comparison (12a).

In this study, labeled M-5 DNA was 40% related to *N. elongata*. In a previous study (22), labeled DNA from *N. elongata* subsp. *nitroreducens* was only 1 to 8% related to five M-5 strains, three of which were included in this study. DNA relatedness of *N. elongata* to two group EF-4b strains was also low, 3 and 14% (22). We therefore tested labeled DNA from *N. elongata* subsp. *nitroreducens* and found it to be from 14 to 22% related to three M-5 strains and 21% and 22% related to EF-4a and EF-4b, respectively (data not shown). We directly compared our DNA preparation with that used by Grant et al. (22), finding them to be 94% related. While somewhat low for different DNA preparations from the same strain, this result confirms that both preparations are from *N. elongata* subsp. *nitroreducens*. We are unable to explain the quantitative difference in relatedness obtained in our study and that of Grant et al., although it may be partially the result of a larger genome size in *N. elongata* subsp. *nitroreducens*.

Studies of fatty acids of M-5 strains and *N. elongata* subsp. *nitroreducens* have shown that they are similar and distinctly different from those of other organisms, such as *Moraxella*, *Oligella*, and *Acinetobacter* spp., *Psychrobacter immobilis*, and CDC group EO-2 (33).

16S rRNA sequence analysis demonstrated that M-5 belongs to the β-3 subgroup of *Proteobacteria*, within the *Neisseriaceae* cluster (Fig. 1). This agrees with our hybridization data and previous fatty acid analyses (33). Homology values showed that it was most similar to *N. animalis*, *N. flavescens*, *N. canis*, *N. elongata*, *N. denitrificans*, *N. polysaccharea*, and *N. macacae* (98.8 to 97.8%). Dewhirst found a homology value of approximately 97% between EF-4b and M-5 (12a). We examined the stability of the tree by using both unedited (but excluding 79 bases from the 3′ terminus; data not shown) and edited sequences with a distance method and parsimony analysis, by changing outgroups, and by bootstrap analysis (2, 43). Each of these approaches revealed several interspecies changes within the emended family *Neisseriaceae*, especially concerning the localization of *N. canis*. This may indicate that the precise ordering of *Neisseria* spp., including the position of M-5, will not be accomplished until additional sequences are available. M-5 always occurred, however, within the *Neisseriaceae*. In addition, M-5 16S rRNA possessed an adenine at position 585 and a uracil at position 756 (number relative to the *E. coli* sequence) (8, 13), which is a characteristic signature of members of the family *Neisseriaceae*.

M-5 is a new species on the basis of DNA hybridization, comparison with available 16S rRNA sequences, and biochemical characteristics. It was once considered to be related to *Moraxella* spp. because of its morphologic and biochemical characteristics (42). On the basis of 16S rRNA sequence, the genus *Moraxella* has been placed in the gamma-*Proteobacteria* class. M-5 has been informally called "*Neisseria parelongata*" because it was considered to be similar to *N. elongata* (14, 17, 35). Since both hybridization and biochemical studies demonstrated that M-5 has a closer relationship to other species than to *N. elongata*, we propose the name *N. weaveri* for this new species.

Description of *Neisseria weaveri*, sp. nov. *N. weaveri* (wea'ver · i · M. L. gen. n. *weaveri*), was named in honor of Robert E. Weaver, for his substantial contributions to the characterization, identification, and classification of unusual pathogenic and opportunistic bacteria (5, 10, 11, 21–23, 33, 42). The decision to name M-5 was made by the senior author without R. E. Weaver's knowledge. This species conforms to the description of the emended family *Neisser-*

TABLE 7. Characteristics of some oxidase-positive species similar to M-5 bacteria

Test or characteristic	*N. weaveri*	EF-4a	EF-4b	*N. elongata*[a]	*E. corrodens*	*K. kingae*
Morphology[b]	S+LR	CSR	CSR	MR	SR	CR
Motility	−	−	−	−	−	−
Catalase	+	+	+	v[c]	−[d]	−
Hemolysis on sheep blood (type)	Alpha	v (alpha)	v (alpha)	−	v (alpha)	Beta
Colony size on blood at 24 h (mm)	1–2	0.5–1	0.5–1	1–2	<0.5	0.5–1
MacConkey, growth	−	v	v	−	−	v
D-Glucose, acid	−	+	+ (w)[e]	v	−	+ (w)
Maltose, acid	−	−	−	−	−	+ (w)
Simmons citrate	−	−	−	−	−	−
Urea	−	−	−	−	−	−
Nitrate reduction	−	+	+	v	+	−
Nitrite reduction	+	+	v	v	−	+
Indole	−	−	−	−	−	−
Phenylalanine deaminase[f]	+	v	+	+	−	−
Gelatin	−	v	−	−	−	−
Litmus milk	v (wk)[g]	−	−	−	−	−
Sodium acetate	−	−	+	+	−	−
Arginine	−	v	−	−	−	−
Ornithine	−	−	−	−	+	−
G+C (mol%)	50.8–52	49–53	50.5	55–58	56–58	47.3

[a] *N. elongata* subsp. *elongata* is catalase, glucose, and nitrate negative and nitrite positive. *N. elongata* subsp. *glycolytica* is catalase positive and D-glucose weakly positive. *N. elongata* subsp. *nitroreducens* is catalase negative and D-glucose weakly positive or negative and reduces nitrate and nitrite.
[b] S, short; L, long; R, rod; C, coccus; M, medium long.
[c] v, variable, 11 to 89% positive.
[d] Of the results, 8% were positive.
[e] w, weak reaction.
[f] The phenylalanine test result is dependent on the culture medium used. All species are inactive against D-mannitol, lactose, sucrose, and D-xylose. The data were from references 3, 10, 18, 21–23, 30, 33, 35, 37, 40, 42, and 45.
[g] wk, weak alkalinization.

TABLE 8. Characteristics of additional oxidase-positive species similar to M-5 bacteria

Test or characteristic	*M. osloensis*	*Moraxella atlantae*	*Moraxella bovis*	*O. urethralis*	*W. zoohelcum*	*A. faecalis*	*B. bronchiseptica*	Gilardi unnamed group 1
Morphology[a]	CSR	CSR	CSR	CSR	SCR	SR	CR	LR
Motility	−	−	−	−	−	+ (w)[b]	+	−
Catalase	+	+	v	+	+	+	+	+
Hemolysis on sheep blood	v[c]	−	Beta	−	v	v	v	v (alpha)
Colony size on blood at 24 h (mm)	<0.5	<0.5	<0.5	<0.5	<1.0	0.5–1	<0.5	1–3
MacConkey, growth	v	+	−	+	−	+	+	+
D-glucose, acid	−	−[d]	−	−	−	−	−	−
Maltose, acid	−	−	−	−	−	−	−	−
Simmons citrate	−	−	−	+	−	+	+	+
Urea	−	−	−	−	+	−	+	−
Nitrate reduction	v	−	v	−	−	−	+	−
Nitrite reduction	−	−	−	+	−	+		−
Indole	−	−	−	−	+ (w)	−	−	−
Phenylalanine deaminase[d]	v	−	−	+	+			+
Gelatin	−	−	+	−	+	−	−	−
Litmus milk[e]	−	−	pep	v (k)	v (pep)	k	k	v (k)
Sodium acetate	+	v	+	+	−	+		−
Arginine	−			−	+	−		−
Ornithine	−			−	−	−		−
G+C (mol%)	43–46	46–48	41–45	48.5	35–38	56–59.5[f]	67–69	46–47

[a] S, short; L, long; R, rod; C, coccus; M, medium long.
[b] w, weak reaction.
[c] v, variable, 11 to 89% positive.
[d] The phenylalanine test result is dependent on the culture medium used. All species are inactive against D-mannitol, lactose, sucrose, and D-xylose. The data were taken from references 3, 10, 18, 21–23, 30, 33, 35, 37, 40, 42, and 45.
[e] pep, peptonization; k, alkalinization.
[f] *A. faecalis* varies in G+C contents from 56 to 70 mol%. This species is indole negative, does not use carbohydrates, and grows with flat, spreading edges and umbonated central plateaus or with high convex and circular colonies on blood-agar plates. Often poor to no growth.

iaceae (3, 13) and to the description of the genus *Neisseria* (45). The bacteria are gram-negative, broad, plump, medium-to-large, straight rods of varying length when grown on slants and plates, with a tendency to grow in chains or longer rods in broth cultures. They are nonmotile, aerobic, and non-salt requiring, and grow well between 25 and 35°C; most strains grow at 42°C. Colonies are grey-white with an entire border, flat, somewhat glistening, and smooth and variable in size. They are 1 to 2 mm in diameter after 24 h of incubation at 35°C and 2 to 4 mm after 48 h of incubation on SBAP. A zone of alpha-hemolysis is produced on SBAP in areas of heavy growth. The oxidase and catalase reactions are strongly positive. The bacterium does not utilize carbohydrates; it reduces nitrite but not nitrate and has a weakly positive phenylalanine deaminase reaction from culture grown on SBAP. Additional biochemical reactions are given in Table 6. Biochemical tests useful in differentiating it from other bacteria are given in Tables 7 and 8. The G+C content of DNA is 50.8 to 52.0 mol%. It is found as normal oral flora in dogs and is associated with human wound infections resulting from dog bites.

Description of the type strain. The type strain of *N. weaveri* is CDC 8142 (= ATCC 51223). It shares all the characteristics of the species (Table 6). The G+C content of DNA was 51.5 mol%. It was isolated from an infected dog bite wound.

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