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Neisseria elongata subsp. *nitroreducens* subsp. nov., Formerly CDC Group M-6, a Gram-Negative Bacterium Associated with Endocarditis

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CDC group M-6 is the vernacular name given to a gram-negative, oxidase-positive, aerobic, nonmotile, rod-shaped bacterium. This organism is biochemically similar to *Kingella denitrificans* and displays a cellular fatty acid profile consistent with CDC groups M-5 and EF-4 and with *Neisseria elongata*. Of the 95 M-6 strains referred to the Centers for Disease Control (CDC) for identification, 32 (64%) of the first 50 were from the throat or sputum and only 3 (6%) were from blood; only 5 (11%) of the next 45 isolates were from the upper respiratory tract and 23 (51%) were from blood, with many of these (15 or 65%) being associated with endocarditis. The major characteristics of CDC group M-6 include reduction of nitrate and nitrite with no gas formation; positive reaction for oxidase; negative reactions for catalase, urease, indole, and motility; and no acid production from carbohydrates. Guanine-plus-cytosine content determined spectrophotometrically by thermal denaturation was 55 to 58 mol% for six M-6 strains tested: 56 mol% for the *N. elongata* subsp. *elongata* type strain and for the *N. elongata* subsp. *glycolytica* type strain. By the hydroxyapatite method, DNAs from 24 M-6 strains showed an average of 78% relatedness to M-6 reference strain B1019 in reactions at 60°C and 73% relatedness in reactions at 75°C. M-6 strain B1019 was 79% related to the *N. elongata* type strain at 60°C and was 71% related at 75°C; it was 75% related to the type strain of *N. elongata* subsp. *glycolytica* at 60°C and was 66% related at 75°C. DNAs from CDC group EF-4, *K. denitrificans*, and CDC group M-5 were all less than 14% related to CDC group M-6 at 75°C. The DNA relatedness data showed conclusively that all the M-6 strains belong in the species *N. elongata*. M-6 is different from *N. elongata* subsp. *elongata* in that M-6 reduces nitrate and sometimes weakly acidifies D-glucose, and it is different from *N. elongata* subsp. *glycolytica* in that it reduces nitrate and is negative for glucose and catalase. Because of the apparent clinical significance of M-6 compared with the clinical significance of *N. elongata* subsp. *elongata* and *N. elongata* subsp. *glycolytica* and the ease in distinguishing it biochemically, we propose M-6 as a third subspecies of *N. elongata*, *N. elongata* subsp. *nitroreducens* subsp. nov.

From 1964 through 1988, the Special Bacteriology Reference Laboratory, Meningitis and Special Pathogens Branch, Centers for Disease Control (CDC), received for identification 95 isolates of gram-negative, oxidase-positive, aerobic, nonmotile, rod-shaped bacteria, which were identified as CDC group M-6 (3). This organism is biochemically similar to *Neisseria elongata* and displays a cellular fatty acid profile consistent with CDC groups M-5 and EF-4, *N. elongata*, and other *Neisseria* species (3, 6; C. W. Moss, unpublished data).

Three case reports identifying M-6 as the cause of disease have been published (4, 7, 8). In 1983, Simor and Salit (8) reported a case of endocarditis caused by M-6 that occurred in a 31-year-old woman with known mitral valve prolapse. The infection responded to ampicillin, but valve replacement was required because of progressive left ventricular failure. A second case of M-6-linked endocarditis, this time after a complicated heart catheterization, was reported by Perez (7) in 1986. A case of osteomyelitis caused by M-6 was also reported by Garner and Briant (4) in 1986 in a patient who had major dental surgery 6 months prior to the onset of symptoms.

The purpose of this study was to review the source and clinical significance of M-6, to characterize this group phenotypically and by DNA hybridization to determine whether these strains represented one or more species, and to classify them properly.

MATERIALS AND METHODS

Bacterial strains. A total of 95 M-6 strains were reviewed for data on their clinical origins (Table 1). Clinical isolates submitted to CDC were accompanied by some demographic and clinical information about the patient. Additional clinical information for 15 strains was obtained by contacting the laboratory directors in the state public health laboratories that submitted the isolates and by reviewing copies of the patients' charts. All data were not available for all patients.

DNA relatedness studies were done on 24 of the most recent human M-6 isolates received by CDC, as well as one of the earliest M-6 strains from 1968 (Table 2). The following strains were also used in the DNA relatedness studies: five CDC group M-5 strains (A1420, A4682, A8190, A8290, G2471), two CDC group EF-4B strains (D5986, D9733), *N. elongata* subsp. *elongata* type strain ATCC 25295, *N. elongata* subsp. *glycolytica* type strain ATCC 29315, and *Kingella denitrificans* type strain NCTC B8312.

All strains were grown on plates containing heart infusion agar with 5% rabbit blood. Incubation was done at 35°C for 24 h in a candle extinction jar. For preparation of DNA, an

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TABLE 1. CDC group M-6 strain data

M-6 strain no.	Clinical source	Diagnosis	Sex[a]	Age (yr)	Geographic source, yr received
A512	Throat		M		California, 1964
A2589	Urine		M		Pennsylvania, 1965
A2596	Throat		M		Indiana, 1965
A2612	Throat	Scarlet fever symptoms	M		Pennsylvania, 1965
A5379	Sputum		M		Michigan, 1966
A6486	Throat		M		Colorado, 1966
B467	Throat				Louisiana, 1968
B1019	Blood				South Carolina, 1968
B1252	Wound		F		Delaware, 1968
B1283	Peritoneal cavity		M		Pennsylvania, 1968
B1715	Throat		M		Colorado, 1969
B2562	Stool				Guatemala, 1969
B3374	Throat		F		Georgia, 1969
B3942	Sputum				Colorado, 1969
B4811	Corneal ulcer				Puerto Rico, 1970
B5020	Pericardial fluid		M		Massachusetts, 1970
B5336	Throat		M		Pennsylvania, 1970
B6986	Chest		F		Colorado, 1970
B7631	Throat		M		Montana, 1971
B7797	Sputum				California, 1971
B7810	Foot ulcer		F		Colorado, 1971
B8057	Peritoneal fluid		M		Mississippi, 1971
B8386	Sputum				Colorado, 1971
B8720	Throat		M		North Carolina, 1971
B8922	Sputum		F		Nevada, 1971
C495	Throat				Illinois, 1971
C2218	Sputum		F		Georgia, 1972
C3590	Sputum		F		South Carolina, 1972
C3827	Throat		F		Alaska, 1972
C4500	Trachea		F	15	Colorado, 1972
C4585	Ear		M		Maine, 1972
C4982	Mouth		M		Canada, 1973
C5407	Peritoneal fluid	Appendix removed	F	6	Colorado, 1973
C5843	Throat		F		Montana, 1973
C5980	Throat		F		Hawaii, 1973
C6028	Blood		F		Virginia, 1973
C6121	Throat				Maryland, 1973
C6683	Throat	Fever	M	2	Colorado, 1973
C6796	Sputum		F		Alaska, 1973
C7048	Throat		F	5	Rhode Island, 1973
C7352	Sputum		F	46	Louisiana, 1973
C7481	Mouth		F		Alabama, 1973
C7800	Cervical exudate		F		Ontario, Canada 1973
C9005	Wound (catheter site)		M	63	Connecticut, 1974
C9056	Throat		M		California, 1974
D435	Sputum		M	48	Arizona, 1974
D3205	Right lung autopsy	Jaundice	M	72	Rhode Island, 1975
D3371	Urine		M	30	Delaware, 1975
D3539	Tracheal secretion	Car accident, brain damage	M	17	Pennsylvania, 1975
D3540	Blood		F	46	California, 1975
D6506	Blood		M		Washington, 1976
D7131	Sputum		M		Missouri, 1976
D7951	Abdominal wound		M		Maine, 1976
D7972	Appendectomy	Appendicitis	M	15	New Zealand, 1976
D8695	Throat	Acute pharyngitis	M	17	Pennsylvania, 1977
D8714	Pleural fluid	Respiratory arrest	M		Colorado, 1977
D9764	Appendix exudate	Appendicitis	F	2.5	Connecticut, 1977
D9773	Blood		F	55	Florida, 1977
E565	Wound		M		Maryland, 1977
E1069	Sputum		F	78	Louisiana, 1977
E1807	Foot dorsum	Infected laceration	M	22	Hawaii, 1978

Continued on following page

isolated colony was placed onto 50 ml of brain heart infusion broth and incubated at 36 ± 1°C for 24 h. Two 1-liter portions of brain heart infusion broth were then inoculated with each 50-ml culture and incubated for 24 to 72 h under the same conditions. Cells were pelleted by centrifugation for 35 min, and the supernatant was discarded.

Biochemical tests. Since 1964, the Special Bacteriology Reference Laboratory has received 95 strains of CDC group

TABLE 1—*Continued*

M-6 strain no.	Clinical source	Diagnosis	Sex[a]	Age (yr)	Geographic source, yr received
E2481	Tracheal aspirate		M	68	North Carolina, 1978
E3891	Human bite	Human bite	M	12	New Mexico, 1978
E5696	Blood	Endocarditis	M	49	Iowa, 1979
E6520	Chest drainage		M	22	Kansas, 1979
E6808	Bronchial washing		M	56	Kansas, 1979
E6825	Blood	Endocarditis	F	22	New Zealand, 1978
E7434	Blood	Endocarditis	M	25	California, 1980
E7792	Blood	Viral encephalitis	M	32	Hawaii, 1980
E8494	Blood	Periorbital cellulitis	F	13	Ohio, 1980
F521	Sputum	Endocarditis	M	54	Maryland, 1981
F913	Foot wound	Osteomyelitis	M	37	Pennsylvania, 1981
F3076	Blood	Endocarditis	F	31	Canada, 1982
F3380	Blood	Septicemia	F	64	Rhode Island, 1982
F3521	Blood		F	39	Massachusetts, 1982
F3656	Ear	Ear infection	M	10	Rhode Island, 1982
F3986	Blood	Meningitis	M	51	Canada, 1982
F4104	Blood	Endocarditis	M	34	Georgia, 1983
F4127	Blood	Endocarditis	F		Georgia, 1983
F4434	Blood	Septicemia, possible endocarditis	M	57	California, 1982
F4713	Blood	Endocarditis	M	66	Florida, 1983
F4998	Joint fluid	Osteomyelitis	M	48	New Zealand, 1983
F5360	Blood	Endocarditis	F	56	Pennsylvania, 1984
F5246	Foot exudate	Osteomyelitis	M	46	Washington, 1984
F5663	Blood	Endocarditis	F	60	California, 1984
F6014	Peritoneal fluid	Acute appendicitis	F	13	Hawaii, 1984
F6027	Blood	Endocarditis	M	41	Maryland, 1984
F6169	Blood	Endocarditis	F	42	California, 1984
F7615	Blood	Endocarditis, pulmonary edema	F	71	Canada, 1985
F8112	Blood	Endocarditis	M	50	Federal Republic of Germany, 1986
F9253	Blood	Septicemia	M	Neonate	Rhode Island, 1986
F9924	Blood	Diabetes	F	53	Virginia, 1987
G155	Stool		F		Maryland, 1987
G616	Bronchial washing	Hemoptysis	F	31	Oregon, 1987
G1256	Infected hip wound	Osteomyelitis	F		Illinois, 1988

[a] M, Male; F, female.

M-6 for identification or confirmation. The biochemical properties of these isolates were determined as the cultures were received and were examined at that time in the laboratory, as described previously (3). Oxidation-fermentation base semisolid agar was used to determine utilization of carbohydrates by the organisms. Motility was first observed microscopically and was confirmed on semisolid motility agar. DNA relatedness studies were done with 25 strains. The biochemical test data given in Table 3 are for the 25 strains tested for DNA relatedness.

DNA methods. Guanine-plus-cytosine (G+C) content was determined spectrophotometrically by thermal denaturation (5). The preparation and purification of DNA and the conditions used to determine DNA relatedness by the hydroxyapatite method have been described previously (2). DNA from M-6 strain B1019 was labeled in vitro with [^{32}P]dCTP provided in a nick-translation reagent kit (Bethesda Research Laboratories, Gaithersburg, Md.), as directed by the manufacturer. Hybridization reactions were done at 60°C (optimal reassociation) and 75°C (stringent reassociation) (2).

RESULTS

Clinical significance. The 95 M-6 strains from humans were isolated from the respiratory tract (mostly throat or sputum, 39 strains [41%]), blood (26 strains [27%]), or wounds (12 strains [13%]); and 18 strains came from other sources, including 4 strains from peritoneal fluid; 2 strains each from appendix exudate, urine, ear, and stool; and single strains each from pleural fluid, pericardial fluid, chest drainage, lung tissue, corneal ulcer, and cervical exudate. Diagnoses of the infections caused by 39 of the strains were given. Among these, the most frequent were endocarditis (13 strains from blood plus 1 strain from sputum, for a total of 14 strains, 36% of all diagnoses given), septicemia with possible endocarditis (1 strain from blood, or 2.6% of all diagnoses given), osteomyelitis (4 strains from wound joint fluid and foot exudate, or 10% of all diagnoses given), appendicitis (4 strains from peritoneal fluid and appendix, or 10% of all diagnoses given), and septicemia (3 strains from blood, or 8 % of all diagnoses given). When reported, 49 of the strains were isolated from male patients and 37 of the strains were isolated from female patients. Age was reported for 48 patients. Of these, 10% were <10 years old, 17% were 10 to 19 years old, 8% were 20 to 29 years old, 15% were 30 to 39 years old, 17% were 40 to 49 years old, 17% were 50 to 59 years old, 10% were 60 to 69 years old, and 6% were 70 to 79 years old. The isolates were received from 32 states in the United States, Puerto Rico, Canada, Guatemala, the Federal Republic of Germany, and New Zealand.

Diagnoses of the infections caused by 21 of the 26 M-6 blood isolates were given, as follows: endocarditis (13 strains, 62% of all diagnoses given for isolates from blood);

septicemia with possible endocarditis (1 strain, or 5%); septicemia (3 strains, or 14%); and periorbital cellulitis, meningitis, diabetes, and viral encephalitis (1 strain each, or 5% each). When reported, 11 strains were isolated from male patients and 14 strains were from female patients. Age was reported for 22 of the 26 patients. Of these, 5% were <10 years old, 5% were 10 to 19 years old, 9% were 20 to 29 years old, 18% were 30 to 39 years old, 18% were 40 to 49 years old, 27% were 50 to 59 years old, 14% were 60 to 69 years old, and 5% were 70 years old or older.

The first diagnosis of M-6-associated endocarditis was reported in New Zealand in 1978. It is noteworthy that of the first 50 isolates, 32 (64%) were from throat or sputum and only 3 (6%) were from blood. Endocarditis was not noted until the 64th isolate was received at CDC. Starting in 1979, however, 20 of 31 (65%) cultures were isolates from blood, and endocarditis was diagnosed in 14 patients (45%).

Clinical case summaries of patients infected with isolates of M-6. The following three case reports are presented as examples of the severity of the infections in patients infected with isolates of CDC group M-6 and to give some indication of the range of clinical presentations.

(i) Case 1. A 25-year-old man was admitted to the hospital with a 2-week history of fever, chills, malaise, and anorexia. Symptoms included headache, nausea, vomiting, abdominal pain, and diarrhea. On the day before admission, he was observed to be acting irrationally. He was a resident of a mountain area and was employed in construction work. He had been seen at another hospital 10 days previously and had had similar manifestations and mild leukocytosis but no cardiac murmur.

On admission his temperature was 102.7°F (39.3°C), respiration was 20/min, pulse was 130 beats per min, and blood pressure was 108/70-50 mm Hg. He appeared to be tense and confused. The only positive physical finding was a grade III holosystolic murmur. A chest X ray showed cardiomegaly and increased pulmonary markings. An electrocardiogram showed left ventricular hypertrophy and sinus tachycardia. Six blood samples were drawn on admission, all of which subsequently grew M-6 on culture.

The patient was treated with nafcillin and gentamicin at high dosages for bacterial endocarditis. He was treated with massive doses of penicillin, resulting in improvement for about a week, but he developed left upper quadrant pain, azotemia, and severe heart failure at that time. Cardiac catheterization and angiographic studies indicated that his mitral valve had been destroyed, and he was transferred to another center for valve replacement.

(ii) Case 2. A 31-year-old man was admitted to the hospital for evaluation of possible cerebrovascular accident. There was no known history of hypertension or cerebrovascular accident. Physical examination results were significant for grade 1/6 systolic murmur at the left sternal border and grade 1/6 diastolic murmur at the apex. An electrocardiogram showed left ventricular hypertrophy and early repolarization. Echocardiography showed mitral valve prolapse and an absence of vegetations. Bacteriologic studies revealed gram-negative rods (M-6) from blood samples. Antibiotic coverage was initiated with nafcillin, ampicillin, and gentamicin. One month later, cardiac catheterization showed probable vegetation on the mitral valve, and bacterial endocarditis of the mitral valve was documented. Debridement of vegetations of the mitral valve was accomplished and the valve was left intact. The patient remained in the hospital with antibiotics and was discharged 35 days after surgery.

(iii) Case 3. The patient was an infant born at 37 weeks of gestation to an 18-year-old woman with a history of multiple episodes of urinary tract infection with pyelonephritis. The mother had complied poorly with her prenatal care and had had anemia that required transfusion. She had taken cocaine and crack during the first trimester and smoked two packs of cigarettes per day. The infant was admitted to the hospital to rule out sepsis. Culture of a blood specimen was positive for a gram-negative rod (M-6) at 72 h; the organism was susceptible to ampicillin. The child was treated with ampicillin and gentamicin for 7 days. Otherwise, the child did well throughout the course of its hospital stay.

DNA studies. DNAs from six M-6 strains (F9253, F5360, G155, B1019, F9924, E7434) had G+C contents ranging from 56 to 58 mol%. DNA from *N. elongata* type strain ATCC 25295 and *N. elongata* subsp. *glycolytica* type strain ATCC 29315 both contained 56 mol% G+C.

Labeled DNA from M-6 strain B1019 showed an average of 78% relatedness to 24 other M-6 strains (range, 64 to 86%) in reactions at 60°C and 73% relatedness (range, 60 to 79%) in reactions at 75°C. In all cases, divergence (unpaired bases within hybridized sequences) was 2.5% or less (Table 2). The relatedness of M-6 strain B1019 on the type strain of *N. elongata* was 79% at 60°C and 71% at 75°C, and its relatedness to the type strain of *N. elongata* subsp. *glycolytica* was 75% at 60°C and 66% at 75°C, with 2.5% divergence in each case. CDC group EF-4B, *K. denitrificans*, and CDC group M-5 were all less than 14% related to CDC group M-6 at 75°C.

Biochemical results. Table 3 shows the results of biochemical tests of the 25 human M-6 bacterial strains that were tested by DNA hybridization. Reactions useful for differentiating M-6 from *N. elongata*, CDC groups EF-4b and M-5, and *K. denitrificans* are given in Table 4.

CDC group M-6 was catalase negative, was positive for nitrate and nitrite reduction without the production of gas, and exhibited a weak, variable D-glucose reaction. Both *N. elongata* strains were nitrate negative and nitrite positive, but *N. elongata* subsp. *glycolytica* was catalase positive and showed a weakly positive glucose reaction. *K. denitrificans* exhibited a positive glucose reaction and gas production from nitrate and nitrite reduction; EF-4b was positive for glucose, catalase, and nitrate but gave variable results for nitrite reduction; M-5 also gave variable results for nitrite and was nitrate and glucose negative and catalase positive.

DISCUSSION

DNA relatedness data showed conclusively that CDC group M-6 is a single group within the species *N. elongata*. The G+C contents for all six M-6 strains tested were 56 to 58 mol%; these were close to the value of 56 mol% obtained for both subspecies of *N. elongata*. In 1970, Bøvre and Holten (1) reported the G+C composition of *N. elongata* ATCC 25295 to be 53 to 54 mol%, as calculated from buoyant density in a CsCl gradient. Buoyant density determinations are usually somewhat lower than thermal denaturation determinations.

N. elongata subsp. *elongata* was glucose negative and catalase negative, characteristics that differentiated it from *N. elongata* subsp. *glycolytica* (9) (Table 4). The ability of M-6 to reduce nitrate differentiated it from *N. elongata* subsp. *elongata*. Its positive nitrite reduction, negative catalase reaction, and negative or weakly positive glucose reaction separated it from *N. elongata* subsp. *glycolytica* (Table 4).

All *N. elongata* isolates have been obtained from humans.

TABLE 2. DNA relatedness of CDC group M-6

Source of unlabeled DNA	Relatedness to strain B1019		
	RBR,[a] 60°C	D (%)[b]	RBR, 75°C
CDC group M-6 strains			
B1019	100	0.0	100
G1256	87	2.0	75
D8714	87	2.0	74
F9924	86	1.0	77
F4998	85	2.0	78
F8112	81	0.5	79
F6014	81	1.5	79
E8494	81	1.5	76
F3076	80	1.0	72
E7434	79	0.5	79
F4434	79	1.0	72
F4713	79	1.0	72
F5360	79	1.0	71
F7615	78	1.0	79
F3521	77	1.0	65
F9253	76	0.5	81
G616	76	1.5	64
F4127	75	1.5	73
F6027	74	0.5	70
$F6169_{(1)}$	74	1.0	70
F5246	74	1.0	69
E6825	70	1.5	72
G155	70	2.5	70
F4101	70	1.0	61
F3986	64	2.0	65
N. elongata subsp. *elongata* type strain ATCC 25295	79	2.5	71
N. elongata subsp. *glycolytica* type strain ATCC 29315	75	2.5	66
K. denitrificans B8312			7
CDC group EF-4b strains			
D9733			14
D1420			3
CDC group M-5 strains			
A8290			8
A4682			8
A8190			5
G2471			4
A1420			1

[a] RBR, Relative binding ratio (percent heterologous DNA bound to hydroxyapatite)/(percent homologous DNA bound to hydroxyapatite) × 100.

[b] D, Divergence. Divergence is the decrease in thermal stability (in degrees Celsius) of heterologous DNA duplexes compared with those of homologous DNA duplexes. It can be expressed in percent because each degree of decreased thermal stability is caused by approximately 1% unpaired bases in double-stranded DNA. Values were calculated to the nearest 0.5%.

TABLE 3. Biochemical characteristics of CDC group M-6

Test or substrate	% *N. elongata* subsp. *nitroreducens* positive (n = 25 strains)[a]	Type strain B1019 (ATCC 49377)[b]
Motility	0	−
Hemolysis (rabbit blood)	0	−
Acid from:		
D-Glucose	20w[c]	+w[c]
D-Xylose	0	−
D-Mannitol	0	−
Lactose	0	−
Sucrose	0	−
Maltose	0	−
Growth in:		
Nutrient broth–0% NaCl	88	+
Nutrient broth–6% NaCl	4	−
Catalase	0	−
Oxidase	100	+
Growth on MacConkey agar	20 (32w)	(+w)
Simmons citrate	0	−
Urea, Christensen	0	−
Nitrate reduction	100	+
Nitrite reduction	100	+
Indole	0	−
TSI[d] slant acid	0	−
TSI butt acid	0	−
H_2S in TSI	0	−
H_2S in paper	100	+
Gelatin hydrolysis	0	−
Litmus milk peptonization	0	−
Growth at:		
25°C	60	+w
35°C	100	+
42°C	24	−
Esculin hydrolysis	0	−
Utilization of:		
Acetate	87	−
Acetamide	0	−
Serine	0	−
Tartrate	0	−
Phenylalanine deaminase	0	−
Lysine decarboxylase	0	−
Arginine dihydrolase	0	−
Ornithine decarboxylase	0	−

[a] Test results are expressed as percent positive after 24 to 48 h.

[b] −, Negative reaction; +, positive reaction after 24 to 48 h; parentheses, reaction delayed 3 to 7 days; w, weak reaction.

[c] No change or neutral with an alkaline reaction in the control was interpreted as a weak reaction.

[d] TSI, Triple sugar iron.

Sources include the pharynx of healthy individuals, patients with pharyngitis, some oral abscesses, and the urinary tract (9). There is no evidence of systemic infection. M-6 isolates were also obtained predominantly from these sources until approximately 10 years ago, when isolates from blood that were often associated with endocarditis became the prime source. M-6 has also been associated with osteomyelitis, appendicitis, and meningitis. The reasons for the change in the source of isolation and the increase in severity of disease are unknown, although a number of factors might have contributed to these observed changes. It is possible that one or two clones with increased virulence have emerged. It is also possible that one or a combination of changes in blood culture systems have resulted in an increased ability to isolate the organism from blood and, concomitantly, that more stringent criteria applied to the culturing of sputum samples has resulted in a decreased number of isolates from sputum. The first of these possibilities can and should be tested by comparing isolates from blood and cerebrospinal fluid with isolates from sputum by subtyping, using either multilocus enzyme typing or ribotyping.

CDC group M-6 has been successfully isolated within 24 h by using the BACTEC system and on 5% sheep blood agar (4, 7, 8). Garner and Briant (4) reported that M-6 is susceptible to polymyxin, aminoglycosides, and ampicillin.

Because of the apparent clinical significance of M-6 and the ease in distinguishing it biochemically, it is appropriate to classify it as a third subspecies of *N. elongata*. We propose the name *N. elongata* subsp. *nitroreducens* subsp. nov.

TABLE 4. Biochemical differentiation of CDC group M-6, *N. elongata*, and other biochemically similar groups[a]

Test	M-6	*N. elongata* subsp. *elongata*	*N. elongata* subsp. *glycolytica*	*K. denitrificans*	CDC group EF-4b	CDC group M-5
D-Glucose	− or +[w]	−	w+	+	+	−
Catalase	−	−	+	−	+	+
Nitrate reduction	+	−	−	+, gas	+	−
Nitrite reduction	+	+	+	+, gas	v	v

[a] +, >90% positive within 48 h; −, 10% or less positive within 48 h; − or +[w], most strains negative, about 20% weakly positive after 48 h; v, 10 to 89% positive in 48 h.

Description of *N. elongata* subsp. *nitroreducens* subsp. nov. *N. elongata* subsp. *nitroreducens* subsp. nov. (ni.tro.re. du'cens L.n. *nitrum* nitrate; L.v. *reduco* to bring back to a state or condition; M.L. part. adj. *nitroreducens* nitrate reducing) is proposed for CDC group M-6. The subspecies conforms to the description of *N. elongata* (1, 9). It is different from *N. elongata* subsp. *elongata* in that it reduces nitrate and sometimes causes a weak acidification of glucose media; and it is different from *N. elongata* subsp. *glycolytica* in that it is catalase negative, reduces nitrate, and utilizes D-glucose weakly or not at all.

N. elongata subsp. *nitroreducens* appears to be a rarely occurring but often serious human pathogen. Its association with endocarditis and other systemic diseases differentiates it from the other *N. elongata* subspecies.

The G+C content is from 56 to 58 mol%. Strain B1019 (ATCC 49377) isolated from human blood in South Carolina is the type strain. It has a G+C content of 57 mol%.

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