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Characterization of *Neisseria elongata* subsp. *glycolytica* Isolates Obtained from Human Wound Specimens and Blood Cultures

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Four slightly yellow-pigmented, alpha-hemolytic, gram-negative coccobacilli, three from wound specimens and one from multiple blood cultures of a patient with endocarditis, were identified as *Neisseria elongata* subsp. *glycolytica* on the basis of their overall biochemical and genetic similarities to this subspecies. These strains resembled *N. elongata* in their guanine-plus-cytosine contents (55.6 to 57.1 mol%) and in their overall cellular fatty acid profiles, which are characterized by large amounts of 16:0, 16:1ω7c, and 18:1ω7c fatty acids. Their identities were confirmed by species-level DNA relatedness (hydroxyapatite method) to the type strains of all three *N. elongata* subspecies. The biochemical profiles and cultural characteristics of these strains resembled those of the type strain of *N. elongata* subsp. *glycolytica* except for the production of a weak yellow growth pigment and alpha-hemolysis on sheep blood agar. They differed from *N. elongata* subsp. *elongata* by the production of catalase, by the production of alpha-hemolysis on sheep blood agar, and by acid production from D-glucose. They differed from *N. elongata* subsp. *nitroreducens* by the production of catalase and an inability to reduce nitrate. These studies suggest a pathogenic potential for *N. elongata* subsp. *glycolytica*, usually considered to be a transient colonizer in humans.

Neisseria elongata was first described in 1970 by Bøvre and Holten (5). It now contains three subspecies, *N. elongata* subsp. *elongata*, *N. elongata* subsp. *glycolytica*, and *N. elongata* subsp. *nitroreducens*, that are separated on the basis of biochemical differences. Of these subspecies, only *N. elongata* subsp. *nitroreducens* has been associated with invasive disease (8).

Between 1971 and 1988 the Special Bacteriology Reference Laboratory, Centers for Disease Control and Prevention (CDC), received four similar clinical isolates, three from wound specimens and one that had been isolated in six blood cultures from a patient with subacute bacterial endocarditis. All four of these isolates resembled *N. elongata* but were not identical to type strains of any of the described subspecies. In the present study we confirmed that they are *N. elongata* subsp. *glycolytica*. An association between this subspecies and systemic disease has not been reported previously.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The clinical strains studied were B8575, E9329, F714, and G1018. All were of human origin. Strains B8575 and F714 were isolated from neck wound specimens in 1971 and 1981, respectively. Strain E9329 was isolated in 1980 from a bite wound specimen of the hand inflicted by a human. Strain G1018 was isolated in six blood cultures from a patient with endocarditis associated with aortic valve damage in 1988 (see clinical history below). *N. elongata* subsp. *elongata* ATCC 25295^T, *N. elongata* subsp. *glycolytica* ATCC 29315^T, and *N. elongata* subsp. *nitroreducens* ATCC 49377^T were used as control strains. Strains were grown on sheep blood plates (SBAPs; Trypticase soy agar base with 5% sheep blood; Becton Dickinson Microbiology Systems, Cockeysville, Md.) for 24 h at 35°C in a candle jar.

Cellular fatty acid analysis. Cells were grown for 2 to 3 days on SBAP medium. Cellular fatty acids were determined as described previously (12).

Biochemical characterization. Unless noted otherwise below, biochemical tests were done by the standard methods of the Special Bacteriology Reference Laboratory, Emerging Bacterial and Mycotic Diseases Branch, CDC (7). Sodium acetate, acetamide, serine, and tartrate reactions were done as described by Rarick et al. (13). Tests for oxidase, catalase, phenylalanine deaminase, and growth at 25, 35, and 42°C were read at 24 h, as was colony size. All other tests were read at 48 h and 7 days. The gelatin test was also read after 14 days.

DNA methods. The guanine-plus-cytosine (G+C) content of DNA was determined spectrophotometrically by thermal denaturation (10). *Escherichia coli* K-12 DNA was included as a control. The techniques used for DNA preparation and purification and for DNA hybridization by the hydroxyapatite method have been described previously (6).

Clinical information. Detailed data on the patients from whom the three isolates from wounds were obtained were not available. However, the following case history was obtained for the patient from whom the blood isolate was obtained. A 57-year-old black male was admitted to the hospital on 17 January 1988 with a history of malaise, headaches, and fever of unknown duration. He had no history of previous dental procedures. On examination his temperature was 100°F (37.8°C) and his blood pressure was 130/56 mm Hg. Splinter hemorrhages were noted under his nails. Grade II/VI diastolic and a grade II/VI holosystolic murmurs were audible on chest auscultation (no previous murmur had been noted). Abdominal and neurological examinations were unremarkable. Complete blood profile showed a hematocrit of 34% and a leukocyte count of 8,000. His erythrocyte sedimentation rate was 85 cm H_2O, and serum chemistry was normal. Urinalysis showed 30 mg of protein per dl. Chest radiographs were suggestive of left ventricular enlargement, and electrocardiography showed some left ventricular hypertrophy. Neurological examination was normal, and a computed tomographic brain scan was negative. Six of six blood cultures were positive for a gram-variable bacillus. Initially, the organism was reported as being of the genus *Actinobacillus* and was referred to the Centers for Disease Control and Prevention for species identification. A diagnosis of subacute bacterial endocarditis with aortic insufficiency was made.

Antimicrobial susceptibility test results revealed that the isolate was susceptible to penicillin, and the patient was started on intravenous penicillin G, 24 million units a day, and gentamicin, 80 mg every 8 h. This antibiotic regimen was continued throughout his hospitalization.

The patient rapidly became afebrile following antibiotic therapy. An echocardiogram, performed on 1 February 1988, revealed an abnormal lesion on the ventricular side of the aortic valve compatible with a vegetation. His aortic root and left atrium were normal in size. The mitral valve showed good motion. A repeated chest radiograph showed no abnormal findings.

Clinical improvement continued and the patient was discharged on 2 February 1988 on a home intravenous therapy regimen of 4 million units of penicillin every 4 h and 80 mg of gentamicin every 8 h for 3 weeks with weekly outpatient examination.

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TABLE 1. Cellular fatty acid composition of *N. elongata* and similar species

Species (no. of strains)	Fatty acid (% of total)[a]											
	12:0	3-OH-12:0	14:0	3-OH-14:0	15:0	16:1ω7C	16:0	2-OH-16:0	18:2	18:1ω9C	18:1ω7C	18:0
N. elongata (11)	6	3	4	1	2	25	38	−	4	3	11	2
N. elongata-like (4)[b]	7	5	4	1	1	19	38	−	3	4	10	6
N. weaveri (23)	7	4	6	1	−	27	25	−	2	1	22	1
CDC Group EF-4a (20)	7	4	8	1	2	25	27	−	4	2	15	1
CDC Group EF-4b (21)	7	4	4	1	−	23	30	2	3	2	20	1

[a] The number before the colon is the number of carbon atoms, and the number after the colon is the number of double bonds. 2-OH indicates a hydroxyl group at the 2-carbon; 3-OH indicates a hydroxyl group at the 3-carbon; ω indicates the position of the double bond from the hydrocarbon end of the chain; C indicates the *cis* isomer. Values are percentages of total fatty acids and are given as arithmetic means; the minus signs indicate not detected or less than 0.5% of total fatty acids.
[b] Strains B8575, E9329, F714, and G1018.

RESULTS

The cellular fatty acid compositions of strains B8575, E9329, F714, and G1018, along with those of similar bacteria studied at CDC, are listed in Table 1. Although the results for the other strains presented in Table 1 were obtained from cultures grown on heart infusion 5% rabbit blood agar (BBL), the substitution of Trypticase soy agar base with sheep blood produced no observable profile differences. The four strains of interest were characterized by large amounts of 16:0, 16:1ω7c, and 18:1ω7c carbon acids. Their fatty acid profiles were essentially identical to one another and were similar to the profiles obtained for strains of *N. elongata.*

All test and control strains contained coccal to rod-shaped cells that, with the exception of *N. elongata* subsp. *nitroreducens* ATCC 49377^T, formed small colonies (<0.5 to 1.5 mm in diameter) after 24 h of incubation on SBAP. The type strain of *N. elongata* subsp. *nitroreducens* produced slightly larger colonies. All strains gave positive reactions in tests for oxidase, nitrite reduction, H_2S production (lead acetate paper method), production of a pale yellow soluble pigment, growth at 25 and 35°C, and growth in nutrient broth. All strains gave negative reactions in tests for acid production from lactose, maltose, D-mannitol, sucrose, and D-xylose; Simmons citrate; urea; acid production on triple sugar iron agar slant; litmus milk; growth in nutrient broth containing 6% NaCl; utilization of serine, acetamide, and tartrate; and arginine dihydrolase, lysine decarboxylase, and ornithine decarboxylase.

The differential biochemical characteristics of strains B8575, E9329, F714, G1018, and type strains of the three *N. elongata* subspecies are given in Table 2. Strains B8575, E9329, F714, and G1018 shared a similar biochemical profile, with growth on MacConkey agar (positive for strain F714 and delayed weakly positive for strain B8575) and growth at 42°C (trace growth for B8575, E9329, and F714) representing variable characteristics. These strains resembled the type strain of *N. elongata* subsp. *glycolytica* except for their ability to produce slightly yellow-pigmented alpha-hemolytic colonies on sheep blood agar. The positive phenylalanine deaminase test was observed only with strains grown on sheep blood prior to inoculation of the phenylalanine deaminase slant. These strains differed from the type strain of *N. elongata* subsp. *elongata* in their positive reactions in tests for catalase, alpha-hemolysis of sheep blood agar, and acid production from D-glucose. These strains differed from the type strain of *N. elongata* subsp. *nitroreducens* in their positive reactions in tests for catalase and utilization of sodium acetate and in their inability to reduce nitrate.

Detection of gas production from nitrite was problematic with these organisms. By a standard broth culture method (7), all strains studied reduced 0.1 and 0.01% nitrite to completion

TABLE 2. Biochemical differentiation between the four *N. elongata*-like strains and type strains of the three subspecies of *N. elongata*[a]

Test or characteristic	*N. elongata*-like (four strains)	*N. elongata* subsp. *glycolytica* ATCC 29315^T	*N. elongata* subsp. *elongata* ATCC 29295^T	*N. elongata* subsp. *nitroreducens* ATCC 49377^T
Catalase	+	+	−	−
Colony size on sheep blood agar (mm)	<0.5–1.5	<0.5	0.5–1	1–2
Alpha-hemolysis (sheep blood agar)	+	−	−	−
Growth on MacConkey agar	v (2/4)	+	+	−[b]
Acid from D-glucose[c]	+ (w)	+ (w)	−	+
Nitrate reduction	−	−	−	+
Gas from nitrate	−	−	−	−
Nitrite reduction	+	+	+	+
Phenylalanine deaminase:				
Heart infusion agar inoculum	−	−	−	−
Sheep blood agar inoculum	+	+w	−	
Colony pigment	sl yel	−	−	−
Growth at 42°C	v (3w/4)	+ (w)	−	−
Sodium acetate utilization	+	+	+	−[d]
G+C (mol%)	55.6–57.1	56.0	56.0	55.0

[a] Strains B8575, E9329, F714, and G1018. Abbreviations: +, all strains positive; −, all strains negative; v, variable results, number of positive strains/total strains tested are in parentheses; w, weak reaction; (w), delayed weak reaction; sl yel, slight yellow.
[b] Although the type strain is negative, 52% of DNA hybridization-confirmed strains are positive (8).
[c] King OF base (7).
[d] Although the type strain is negative, 87% of DNA hybridization-confirmed strains are positive (8).

TABLE 3. DNA relatedness of *N. elongata*-like strain G1018

Source of unlabeled DNA	Labeled DNA from strain G1018	
	% Relatedness, 60°C	% Divergence[a]
G1018	100	0.0
N. elongata subsp. *elongata* (type strain)	83	2.0
N. elongata subsp. *glycolytica* (type strain)	83	2.0
N. elongata subsp. *nitroreducens* (type strain)	79	2.0
F714	78	2.0
B8575	76	1.5
E9329	72	2.5

[a] Divergence was measured to the nearest 0.5%. All reactions were done twice.

at 48 h of incubation. In some instances very small gas bubbles could be observed in 0.1% nitrite broths with Durham tube inserts inoculated with E9329, F714, and the type strain of *N. elongata* subsp. *elongata*. However, these results could not be consistently reproduced.

The G+C content in DNAs from strains B8575, E9329, F714, and G1018 ranged from 55.6 to 57.1 mol% (Table 2). The relatedness of labeled DNA from strain G1018 to unlabeled DNAs from strains B8575, E9329, and F714 and to the type strains of *N. elongata* subsp. *elongata*, *N. elongata* subsp. *glycolytica*, and *N. elongata* subsp. *nitroreducens* was 72 to 83%, with 1.5 to 2.5% divergence in DNA hybridization reactions done at 60°C (Table 3).

DISCUSSION

The results of the phenotypic and genotypic studies presented in this report show that strains B8575, E9329, F714, and G1018 are *N. elongata* subsp. *glycolytica*. The cellular fatty acid profiles of these strains were characteristic of those of *N. elongata* and other similar species, including *N. weaveri* and CDC groups EF-4a and EF-4b. The G+C contents of DNAs from these strains varied between 55.6 and 57.1 mol%, which is within the range of the 55 to 58 mol% previously reported for *N. elongata* (8, 14). DNA hybridization studies conclusively demonstrated that these strains were related to *N. elongata* at the species level. Although the biochemical profiles of these strains do not exactly correspond to those of type strains of the three described *N. elongata* subspecies or to that of "*N. elongata* subsp. *intermedium*," a subspecies without standing in nomenclature, they most closely resemble *N. elongata* subsp. *glycolytica* (1–5, 7–9, 14).

These four strains differ from the type strain of *N. elongata* subsp. *nitroreducens* in their ability to produce catalase, in their ability to deaminate phenylalanine, and in their failure to reduce nitrate. Their ability to utilize sodium acetate also differentiated them from the type strain of *N. elongata* subsp. *nitroreducens*; however, other DNA hybridization-confirmed, sodium acetate-positive *N. elongata* subsp. *nitroreducens* strains have been described (8). They differ from the type strain of *N. elongata* subsp. *elongata* in their ability to produce catalase and to produce acid from D-glucose and in their alpha-hemolysis on sheep blood agar. They differ from the type strain of *N. elongata* subsp. *glycolytica* only in their ability to produce a slightly yellow growth pigment and in their alpha-hemolysis on sheep blood agar. Interestingly, under our study conditions, none of the three control strains produced a slightly yellow growth pigment, whereas this character is listed as positive for *N. elongata* in *Bergey's Manual of Systematic Bacteriology* (14). This suggests that pigmentation of these strains may vary with culture conditions or laboratory passage.

Of the three recognized subspecies of *N. elongata*, only *N. elongata* subsp. *nitroreducens*, formerly CDC Group M-6, has been associated with human disease, usually endocarditis (8). *N. elongata* subsp. *elongata* and *N. elongata* subsp. *glycolytica* have been considered transient colonizers of the human upper respiratory tract and urogenital tract (2–5, 9, 11). Although the clinical significance of the wound isolates is not clear, the isolation of *N. elongata* subsp. *glycolytica* from multiple blood cultures of a patient with endocarditis indicates that this subspecies is also capable of producing systemic disease. To our knowledge, this report is the first to document the isolation of *N. elongata* subsp. *glycolytica* from sites other than the human upper respiratory and urogenital tracts, and strain G1018 is the first recognized isolate of a non-*N. elongata* subsp. *nitroreducens* subspecies to be associated with endocarditis.

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