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Characterization of Atypical Aerobic Gram-Negative Cocci Isolated from Humans

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Six atypical aerobic gram-negative cocci, isolated from humans, were classified taxonomically by genetic relatedness (DNA base pairs and DNA homology), fatty acid content, pigment absorption profile, and other physiological and biochemical characteristics. One strain (M448), which produced acid only from glucose, was identified as *Neisseria meningitidis*; one strain (CHI), which produced acid from glucose, maltose, and sucrose, was identified as *N. gonorrhoeae; Branhamella catarrhalis* was confirmed in a case of acute meningitis; *N. canis* was identified in a cat bite wound; and representative isolates of a proposed new pigmented species were found to have genetic affinities with *N. meningitidis* and *N. perflava*.

The taxonomic position of many aerobic, gram-negative cocci has not been established. This is mainly due to the lack of genetic information on established and proposed species and the often disproportionate weight generally given to a few variable biochemical and physiological characteristics. In previous reports (7, 8) we attempted to establish genetic, physiological, and biochemical characteristics of *Neisseria* species which have tentatively been adopted as prototype or reference strains by the Working Group of the Subcommittee on Neisseriaceae of the International Committee on Systematic Bacteriology.

In this study, we have applied the taxonomic criteria used in the previous study (7, 8) to several isolates from humans that were considered atypical or isolated under unusual circumstances.

MATERIALS AND METHODS

Microorganisms. The source of the atypical aerobic gram-negative cocci used in this study is shown in Table 1. The source of reference strains was previously described (7).

Morphological, physiological, and biochemical tests. Characteristics of the atypical strains are shown in Table 2, and those of the reference strains are noted in the text. All atypical strains examined were oxidase-positive, gram-negative cocci that were most commonly in diplococcal arrangement (but often observed in tetrad or single-cell arrangement). In addition to the characteristics listed in Table 2, none of the strains examined grew under anaerobic conditions or hemolyzed erythrocytes (sheep, horse, rabbit, or human). The procedures used for determining the morphological, physiological, and biochemical activity of these strains were as follows.

Cellular and colonial morphologies were determined after 18 h of growth on Mueller-Hinton (MH) base agar (Difco Laboratories). Gram stains were performed by Hucker's modified procedure, using equal parts of acetone and isopropyl alcohol as a decolorizer. Cultures were examined visually for pigment production. Hemolytic activity of cultures was determined on MH base agar containing 5% defibrinated sheep, horse, rabbit, and human blood after 18 h of incubation (37°C).

The growth-promoting effect of CO_2 was examined on duplicate plates of 5% horse blood agar incubated with and without 5% CO_2. Oxygen requirements were examined on duplicate 5% horse blood agar plates incubated for 72 h under aerobic and anaerobic conditions (anaerobe jar, GasPak BBL Microbiology Systems). The optimum growth temperature was determined from 5% horse blood agar plates incubated at 22, 30, 37, and 42°C without supplemented CO_2. Enrichment requirements were examined by incubating organisms on nutrient agar (Difco) and brain heart infusion agar (Difco) (37°C; humidity, 5% CO_2) for 72 h.

Acid formation from glucose, maltose, sucrose, fructose, mannitol, and lactose was tested in 1% cystine Trypticase agar (BBL). Cultures were incubated without supplemented CO_2 (37°C) and examined daily for 7 days. Nitrate reduction was demonstrated in MH broth (Difco) containing 0.1% (wt/vol) KNO_3. Tubes were incubated for up to 5 days at 37°C and examined for gas production. Reduction was demonstrated by adding sulfanilic acid and alpha-naphthylamide. Zinc powder was added to verify the presence of nitrate in negative tests. Denitrification was tested in MH broth containing 0.001% (wt/vol) $NaNO_2$. The cultures were incubated for up to 14 days at 37°C and examined for gas and reduction. The synthesis of polysaccharide from sucrose was determined on 48-h cultures grown at 37°C on brain heart infusion agar containing 5% sucrose. Polysaccharide production was detected by treatment with fresh Lugol's iodine, diluted 1:4, which formed a blue color around positive colonies. Degradation of DNA was detected on DNAase agar (BBL). Organisms were grown on this medium for 3 to 6 days under standard conditions. A

TABLE 1. Isolation site of atypical aerobic gram-negative cocci in humans

Strain designation	Source of isolation
M448	Nasopharynx
M889	Cerebrospinal fluid
M1644	Arm wound
M1713	Nasopharynx
M1715[a]	Nasopharynx
CHI	Cervix

[a] Representative of six isolates. Initially isolated on selective medium (15).

clear zone around the colonies after treatment with 1 N HCl was considered a positive test. Colonies on MH agar were tested for catalase activity by adding freshly prepared 3% H_2O_2, and oxidase reaction was demonstrated by the addition of a freshly thawed 0.1% solution of tetramethyl-*p*-phenylenediamine monochloride.

Pigment extraction, fatty acid analysis, aminopeptidase activity, DNA base pair ratios, transformation, and DNA hybridization techniques were as previously described (7, 8).

Pigment profiles. A modification of the techniques of Ellinghausen and Pelczar (6) was used for pigment extraction. Pigment profiles were determined on the methanol extracts of cells and examined on a Cary model 219 scanning spectrophotometer (Varian Associates, Palo Alto, Calif.) at wave lengths of 200 to 500 μm.

Fatty acid analysis. Cellular fatty acids were extracted by a modification of the procedure recommended by Supelco Inc. (14). The methyl esters of cellular fatty acids were analyzed in a Varian model 2700 aerograph gas chromatograph (Varian Aerography Corp., Walnut Creek, Calif.) equipped with a flame ionization detector. The coiled glass columns (6 ft [ca. 182.8 cm] by 2 mm inside diameter) were packed with 3% SP2100 DOH on 100/200 Supelcoport (Supelco Inc., Bellefonte, Pa.). Routine conditions of analysis were: detector, 275°C; injector, 190°C; oven, 150 to 225°C at 4°C/min and held at this temperature for 10 min; and carrier gas (helium) at a flow rate of 20 ml/min. The bacterial sample size was 2 μl. The retention times of the test samples were compared with those of the standards (Supelco, Inc.), which were included

TABLE 2. Morphological, physiological, and biochemical characteristics of atypical aerobic gram-negative cocci[a]

Tests	Organisms					
	M448	CHI	M889	M1644	M1713	N1715
Colony characteristics						
Pigment	−	−	−	Y	Y	Y
Physiological tests (temp)						
22°C	NG	NG	1–2+	1–2+	NG	NG
30°C	2+	NG	2+	2+	2+	2+
37°C	2+	1+	3+	2+	2–3+	2+
42°C	2+	NG	3+	2+	1–2+	1–2+
Atmosphere						
Air	2+	1+	3+	2+	2–3+	2+
CO_2(5%)	3–4+	1–2+	2–4+	3+	3+	3+
Anaerobic	NG	NG	NG	NG	NG	NG
Culture media						
Nutrient agar	NG	NG	NG	3+	NG	NG
BHI	3–4+	1–2+	3–4+	3+	3+	3+
Carbohydrates (Production of acid)						
Glucose	+	+	−	−	+	+
Maltose	−	+	−	−	+	+
Sucrose	−	V	−	−	V	+
Fructose	−	−	−	−	−	−
Mannitol	−	−	−	−	−	−
Lactose	−	−	−	−	−	−
ONPG	−	−	−	−	−	−
Nitrate	−	NG	+(w)	+(w)	−	−
Nitrite	+	NG	+	−	+	+
DNA	NG	NG	+(w)	+(w)	−	−
Synthesis of polysaccharide from 5% sucrose	−	+(w)	−	−	+	+

[a] Symbols: +, positive reaction; −, negative reaction; V, character inconsistent; +(w), weakly positive; NG, no growth; c, coccal; dc, diplococci; s, singles; t, tetrads; Y, yellow; ONPG, *o*-nitrophenyl-β-galactopyranoside; and BHI, brain heart infusion.

with each run. Standards and test samples were also cochromatographed to corroborate peak identity. Peaks that could not be identified were labeled "X" and were listed by their retention time relative to known major fatty acid peaks. Major peaks were those contributing to at least 3% of the total peak area, whereas less than 3% were reported as trace amounts.

Aminopeptidase activity. Aminopeptidase activity was determined by a modification of the procedure described by D'Amato et al. (2). Growth from 18-h-old cultures on GC agar (Difco, 5 to 10% CO_2; 37°C) was suspended to a concentration of approximately 9×10^8 colony-forming units/ml. *N*-γ-glutamyl-β-naphthylamine (Sigma Chemical Co.) was diluted to 0.004 M (final concentration) in 0.1 M Tris-hydrochloride buffer to pH 7.6. Forty microliters of bacterial suspensions was added to 40 μl of substrate and incubated for 1 h (37°C; 70% humidity). Enzyme activity was detected by coupling fast blue BB (Fast Blue BB Salt; Sigma) with the enzymatically liberated naphthylamide.

DNA base composition. Purified DNA was prepared by the procedure originally described by Marmur (10), modified slightly to include additional RNase (Sigma) and protease (Sigma) treatment of the crude DNA. Thermal melting point (T_m) determinations were made with a Beckman DU spectrophotometer equipped with a Gilford thermal programmer 2527 and thermal cuvettes on DNA diluted to approximately 20 μg/ml in 0.1× SSC (SSC: 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0). The temperature of the DNA was raised at a rate of 0.25°C/min, and the absorbance at 260 nm was recorded on a Moseley X-Y recorder (Hewlett Packard Co.). The equation used to relate T_m to moles percent (mol%) guanine plus cytosine content (G+C) was that described by Snell and Lapage (13): mol% G+C = mol% G+C of reference strain + slope of equation × (T_m of unknown − T_m of reference strain). The value 2.44 (3, 11) was used for the slope of the equation. *Escherichia coli* B (mol% G+C = 50.0) was used as the reference strain. The average T_m from a minimum of three separate determinations was used as the T_m of the unknown. To ensure reproducibility of results, the DNA from the reference strain *E. coli* B was run in parallel with each sample. For the unknown T_m to be considered valid, the T_m of the reference DNA had to be in good correlation (±0.5% with its predetermined value). All T_m determinations performed on a single DNA sample were in good correlation, with a standard deviation of less than ±0.32°C (less than ±0.7 mol% G+C).

Transformation. Transformation procedures were performed by the technique described by Maier et al. (9), with some minor modifications. DNA isolated from streptomycin-resistant organisms was added to 18-h-old recipient cell suspensions (approximately 3×10^8 colony-forming units/ml) to make a final concentration of 1 to 2 μg/ml. The mixture was incubated (30 min; 37°C water bath) before DNase (Sigma) was added at a final concentration of 25 μg/ml to destroy the unbound DNA. Portions of the appropriate dilution of cell suspensions were spread onto triplicate plates containing antibiotic-free GC agar. Controls consisting of cells not treated with DNA were run in parallel to determine the number of spontaneous streptomycin-resistant mutant colony-forming units. The plates were incubated for 5 to 6 h (36°C; 5% CO_2) to allow phenotypic expression of antibiotic resistance. The agar containing the microcolonies was layered on top of 20 ml of GC agar containing 1,000 μg of streptomycin per ml. The double-layered agar plates were incubated for 72 h (36°C; 5% CO_2). At this time, the streptomycin-resistant transformants were counted, and the average count of the three plates was used for the determination of all colony counts. The number of colony-forming units exposed to DNA was determined from plate counts of the same dilutions of cell suspension that were plated on plain GC agar only. The transformation values, reported as the ratio of interspecific to intraspecific transformation, represent the average of two determinations unless otherwise indicated.

DNA hybridization. The extracted purified DNA was diluted to an optical density of 2.0 (260 nm) in 0.1× SSC (ca. 100 μg/ml) and sheared at 21,000 lb/in^2 in a Ribi cell fractionator (model RF-1; Ivan Sorvall, Inc.). Homogenicity was determined by analytical zone centrifugation. (The majority of DNA fragments were approximately 400,000 daltons.) Renaturation rates were determined for hybridization by the technique of De Ley et al. (4). The optimum renaturation rate temperature was based on the base composition of each isolate and was calculated by the formula of De Ley and Tijtgat (5). The average was 74°C, and this temperature was used in all experiments. Renaturation rates were recorded for 30 min on a Gilford spectrophotometer (model 252) with a thermal programmer (model 2527; Gilford Instruments, Oberlin, Ohio). The blank in the thermal cuvettes consisted of a guanine solution (optical density, 2.0; 260 nm), and the instrument was set at a dwell time of 4 s, with a chart speed of 0.5 cm/min. All DNA hybridization results reported represent the average of two individual determinations. All determinations were in good correlation, with a standard deviation of 0.73 to 2.59.

RESULTS

Strain M448 was isolated from the nasopharynx of a heterosexual male during routine screening for *N. meningitidis*. The isolate was originally identified as *N. gonorrhoeae*, based on its ability to produce acid from glucose only and its growth on Thayer-Martin agar. When strain M448 was evaluated in a broader battery of morphological, physiological, and biochemical tests (Table 2), M448 differed from *N. gonorrhoeae* primarily in its ability to reduce nitrite. When genetic data were evaluated (Table 3), strain M448 appeared to be more closely related to *N. meningitidis* than to *N. gonorrhoeae*. The DNA base composition of strain M448 (mol% G+C = 51.3) was closer to *N. meningitidis* (mol% G+C = 52.0) than to *N. gonorrhoeae* (mol% G+C = 53.3). The percent binding of DNA from M448 was higher with *N. meningitidis* (97.65) than with *N. gonorrhoeae* (94.08). Similarly, DNA from strain M448 transformed *N. meningitidis* cells at a much higher frequency than *N. gonorrhoeae* cells. Although the fatty acid and pigment profiles were not helpful in distinguishing strain M448 from either *N. menin-*

TABLE 3. Criteria used for establishing the taxonomic position of atypical gram-negative cocci related to *N. meningitidus* and *N. gonorrhoeae*

Test microorganism	Chemical constituents of cell		Genetic data					Enzyme activity
	Major cellular fatty acids[a]	Pigment profile[b]	DNA base composition (mol% G+C)	Transformation[c] (recipient cells from:)		Degree of DNA-DNA binding (%)		γ-Glutamyl-aminopeptidase
				N. gonorrhoeae	*N. meningitidis*	*N. gonorrhoeae*	*N. meningitidis*	
N. gonorrhoeae	12:0[d], 14:0, 16:1, 16:0, 18:1	270	53.3 (52.9–53.7)[e]	1.0	0.78 (0.45–1.06)			–[f]
CHI	12:0, 14:0, 16:1, 16:0, 18:1	270	53.54 (53.1–54.2)	0.88 (0.57–1.2)	0.892[g]	96.53 (95.2–97.86)	95.56 (94–97.02)	–
M448	12:0, 14:0, 16:1, 16:0, 18:1	270, 398[h]	51.3 (51.0–51.7)	0.19 (0.15–0.23)	1.9 (0.94–2.8)	94.08 (93.56–94.59)	97.65 (96.98–98.32)	+
N. meningitidis	12:0, 14:0, 16:1, 16:0, 18:1	270, 398[g]	52.0 (51.2–52.7)	0.078 (0.02–0.19)	1.0			+

[a] Fatty acids which contribute at least 3% to the total fatty acid content of the cell.
[b] Absorption peaks of methanol extract in micrometers.
[c] Average ratio of interspecific to intraspecific transformation frequency, using DNA preparations from streptomycin-resistant strains of test microorganisms.
[d] The figure before the colon indicates the number of carbons in the fatty acid chain, and the figure after the colon denotes the number of double bonds.
[e] Numbers in parentheses represent the range of values obtained.
[f] Symbols: –, absence of detectable enzyme; +, presence of detectable enzyme.
[g] Value based on one determination only.
[h] Present in trace quantities.

TABLE 4. Criteria used for establishing the taxonomic position of an atypical gram-negative coccus related to *B. catarrhalis*

Test microorganism	Chemical constituents of cell		Genetic data[a]		
	Major cellular fatty acids	Pigment profile	DNA base composition (mol% G+C)	Transformation (recipient cells from:)	
				M889	*B. catarrhalis*
M889	12:0, 16:1, 16:0, α-17:0[b], 18:0, X-8[c]	270, 399	40.0 (39.4–40.2)[d]	1.0	0.313 (0.23–0.4)
B. catarrhalis	12:0, 16:1, 16:0, α-17:0, 18:1, X-8	270, 398–400	41.3 (41.1–41.8)	0.272 (0.16–0.38)	1.0

[a] Percent binding of *B. catarrhalis* with strain M889 by DNA-DNA hybridization = 99.14.
[b] A 17-carbon fatty acid with a retention time similar to methyl-14 methyl hexadecanoate and methyl heptadecanoate.
[c] Unidentified compound with a retention time of 1.23 × 18.0.
[d] Numbers in parentheses represent the range of values obtained.

gitidis or *N. gonorrhoeae*, the production of γ-glutamyl-aminopeptidase was consistent, with M448 being a strain of *N. meningitidis* which produces acid from glucose only.

Strain CHI was isolated from the cervix of a female patient presenting symptoms of acute cervicitis. At low passage (2×), CHI grew as small (0.5-mm) amorphous, viscid colonies on chocolate blood agar and required a nutritionally complex medium (chocolate blood agar) for continual passage. The temperature range for growth was also very limited, and acid was produced from glucose, maltose, and weakly with sucrose at low passage (<3×). The latter reaction was variable at later passages. The strong genetic affinities between CHI and *N. gonorrhoeae*, as indicated by DNA base composition, transformation, and DNA hybridization (Table 3), indicate that strain CHI is a biochemical variant of *N. gonorrhoeae* that possesses the ability to produce acid from glucose, maltose, and possibly sucrose.

Strain M889 was isolated from the cerebrospinal fluid of a patient with clinical meningitis. The isolate was tentatively identified as *Branhamella catarrhalis* because of the following: (i) growth over a wide temperature range (22 to 42°C), (ii) growth on nutritionally simple medium, (iii) ability to reduce both nitrate and nitrite, and (iv) its asaccharolytic nature. Strain M889 contained large amounts of a 17-carbon fatty acid characteristic of *B. catarrhalis* but not *Neisseria* species (8) (Table 4). The high transformation capability with *B. catarrhalis*, the DNA base composition, and the genetic homology confirm that strain M889 is a strain of *B. catarrhalis* that caused acute bacterial meningitis.

Strain M1644 was isolated from an arm wound of a child who was bitten by a cat. The isolate closely resembled *N. canis* in colonial morphology (visible yellow pigment) and biochemical properties (negative synthesis of polysaccharide from sucrose, growth on nutrient agar, reduction of nitrate but not of nitrite, and its asaccharolytic nature). Because there had not been any reports to our knowledge of humans becoming infected with *N. canis*, the isolate was compared with one of the common, yellow-pigmented *Neisseria* species that reside in humans, i.e., *N. perflava*.

The absorption peaks of extracted pigment and the fatty acid content of strain M1644 more closely resembled *N. canis* than *N. perflava* (Table 5). Furthermore, a closer relationship between strain M1644 and *N. canis* was noted by DNA homology (78.3% binding capacity with *N. canis* compared with 57.04% for *N. perflava*). Although we did not have a suitable transformation recipient among our *N. canis* collection, *N. perflava* was poorly transformed by strain M1644. Based on the above, strain M1644 was identified as *N. canis*.

Strains M1713 and M1715 are two of seven human, nasopharyngeal isolates obtained during surveys for *N. meningitidis* in the United States and Belgium. All were identical morphologically and biochemically except for strain M1713, which occasionally produced acid from sucrose (Table 2). Strains M1713 and M1715 both contained pigment absorption peaks typical of the genus *Neisseria* (Table 6). The profile closely resembled *N. lactamica*, but differed from other species by having peak pigment absorption ranges of 328 to 330 nm and 347 to 348 nm. The major fatty acids of strains M1713 and M1715 were similar to other *Neisseria* species. The DNA base compositions of strains M1713 and M1715 (52.6 and 52.7%, respectively) were slightly higher than those of other pigmented species (49.3 and 51.7%). The moderate-to-high levels of transformation and degree of binding of DNA (Table 7) by strains M1713 and M1715 with *N. meningitidis* and *N. perflava* support our

TABLE 5. Criteria used for establishing the taxonomic position of an atypical gram-negative coccus related to *N. canis*

Test microorganism	Chemical constituents of cell		Genetic data				Biochemical data[a]					Degree of DNA-DNA binding (%)	
	Major cellular fatty acids[b]	Pigment profile[c]	DNA base composition (mol% G+C)	Transformation[d] (recipient cells from:)			Synthesis of polysaccharide from sucrose	NA[e]	NO[f]	NO[g]	Sugars[h]	*N. canis*	*N. perflava*
				N. gonorrhoeae	*N. meningitidis*	*N. perflava*							
N. canis	12:0, 14:0, 16:1, 16:0, 18:1	270, 330, 346, 377, 400	49.63 (48.8–50.4)[i]	ND[f]	ND	ND	−	+	+	−	−		
M1644	12:0, 14:0, 16:1, 16:0, 18:1	270, 330, 347, 377, 400	48.61 (48.1–49.3)	0.0011[j]	0.0435 (0.031–0.056)	0.0295[j]	−	+	+	−	−	78.32 (78.26–78.38)	57.04 (56.13–57.96)
N. perflava	12:1, 16:1, 16:1, 16:0, 18:1	340, 357, 377, 408	49.6 (48.9–50.2)	0.009 (0.006–0.012)	0.097 (0.052–0.142)	1.0	+	+	−	+(G)	(+)		

[a] Symbols: +, positive; −, negative; (G), gas produced; +, positive reaction with glucose, maltose, sucrose, and fructose; ND, not done.
[b] Fatty acids which contribute at least 3% to the total fatty acid content of the cell.
[c] Absorption peaks of methanol extract in micrometers.
[d] Average ratio of interspecific to intraspecific transformation frequency, using DNA preparations from streptomycin-resistant strains of test microorganisms.
[e] Growth on nutrient agar.
[f] Reduction of nitrate.
[g] Reduction of nitrite.
[h] Production of acid from glucose, maltose, sucrose, and fructose.
[i] Numbers in parentheses represent the range of values obtained.
[j] Value based on one determination only.

TABLE 6. Criteria used for establishing the taxonomic position of atypical gram-negative cocci (strains M1713 and M1715)

Test microorganism	Chemical constituents of cell	Genetic data				Biochemical data[a]							
	Pigment profile[b]	DNA base composition (mol% G+C)	Transformation[c] (recipient cells from:)			Production of:						Reduction of:	
			N. gonorrhoeae	*N. meningitidis*	*N. perflava*	Polysaccharides from sucrose	Acids from sugars					NO_3	NO_2
							G	M	S	F	L		
N. perflava	340, 357, 377, 408	49.6 (48.9–50.2)[d]	0.0088 (0.006–0.012)	0.097 (0.052–0.142)	1.0	+	+	+	+	+	−	−	+G
N. sicca	337, 357, 377, 404	51.2 (50.5–51.7)	0.0169 (0.024–0.01)	0.231 (0.16–0.301)	0.028[e]	v	+	+	+	+	−	−	+G
N. flava	338, 355, 375, 400	49.3 (49.0–49.4)	0.0085 (0.007–0.01)	0.124 (0.11–0.138)	0.1 (0.009–0.19)	−	+	+	−	+	−	−	+G
N. subflava	398	51.0 (50.0–51.6)	0.009 (0.0089–0.0094)	0.111 (0.081–0.14)	0.014[e]	+	+	+	v	v	−	−	v
N. mucosa	337, 357, 377, 400	51.6 (50.6–52.4)	0.0069[e]	0.073 (0.044–0.102)	0.045[e]	+	+	+	+	+	−	+	+G
N. lactamica	330, 347, 377, 404	51.6 (50.9–52.2)	0.028 (0.018–0.039)	0.21 (0.157–0.262)	0.36[e]	−	+	+	−	−	+	−	+G
M1713	328, 348, 376, 398	52.7 (52.2–53.7)	0.074 (0.061–0.087)	0.342 (0.33–0.35)	0.0946[e]	+	+	+	v	−	−	−	+
M1715	329, 347, 376, 400	52.6 (51.5–53.9)	0.045 (0.0421–0.046)	0.131 (0.09–0.172)	0.123[e]	+	+	+	+	−	−	−	+

[a] Symbols: +, positive; −, negative; v, variable; G, gas produced; G, glucose; M, maltose; S, sucrose; F, fructose; and L, lactose.

[b] Absorption peaks of methanol extract in micrometers.

[c] Average ratio of interspecific to intraspecific transformation frequency, using DNA preparations from streptomycin-resistant strains of test microorganisms.

[d] Numbers in parentheses represent the range of values obtained.

[e] Values based on one determination only.

TABLE 7. DNA-DNA hybridization between strains M1713 and M1715 with other *Neisseria* species

Neisseria species	Degree of DNA-DNA binding (%)	
	M1713	M1715
N. meningitidis	93.5	95.7
N. perflava	82.0	75.5
N. lactamica	76.1	74.4
N. mucosa	71.1	68.7
N. sicca	38.1	39.8

conclusion that these former isolates represent a new species or variant with genetic affinities with *N. meningitidis* and *N. perflava*.

DISCUSSION

In previous studies (7, 8), the roles of genetic data (DNA base composition, DNA hybridization, and intergenic transformation) versus cell composition and biochemical data (whole cell fatty acid analysis, pigment profiles, and aminopeptidase activity) for determining the taxonomic relatedness of a wide variety of aerobic gram negative cocci were compared. We concluded that although the genetic studies should be weighted most heavily when establishing taxonomic relationships, other biochemical and cell composition data can be quite useful in selected cases. In the present study, we have demonstrated the limitations of using a low number of biochemical characteristics for identifying aerobic gram-negative cocci, and have clarified the identity of several of the atypical cocci isolated primarily through the use of genetic data.

Except for the unusual occurrence of the cat bite isolate (strain M1644), the strains used in this study were chosen because they represent the variable biochemical reactions that are often seen with aerobic gram-negative cocci in diagnostic laboratories. The general use of only a few characteristics (e.g., oxidase-positive gram-negative diplococci which produce acid only from glucose) to identify the gonococci presents particular problems with a disease that has social and moral complications. Strain M448 was considered to be a gonococcus by technologists who were experienced in the isolation of these organisms from the nasopharynx of males. The isolate however was shown to be a meningococcus when other taxonomic criteria were applied. Similarly, strain CHI, which may have been the cause of acute cervicitis, would not have been correctly identified as *N. gonorrhoeae*. The aminopeptidase activity (2) would have been useful for the identification of the above isolates in the clinical laboratory. Furthermore, in our experience and as reported by others (12), the least variability among isolates was noted in the nitrate and nitrite reduction tests and by synthesis of polysaccharide from sucrose. Both of these tests are economically practical at the clinical laboratory level.

It is not unusual to have suppurative infections after animal bites. The identification of strain M1644 as *N. canis*, however, raises the question as to whether certain *Neisseria* species can be opportunistic pathogens. *N. canis* has only been reported in dogs (1), but studies currently in progress indicate that *Neisseria* species which reside as normal flora of animals other than humans have a much broader host range than previously suspected.

Meningococcal isolates from the nasopharynx of healthy carriers are often atypical in colonial morphology and show antigenic diversity (16). Strains M1713 and M1715 resembled *N. meningitidis* in some phenotypic traits (e.g., nitrate and nitrite reduction) but differed in pigment, positive production of polysaccharide from sucrose, and production of acid from sucrose. The latter characteristics have been consistent with passage on several different media.

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