Historical Perspectives and Identification of *Neisseria* and Related Species

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INTRODUCTION

Neisseria spp. are pathogens and normal flora in humans (73). Because the diagnosis of gonorrhea in a person may have important social and medicolegal consequences (100), it is important that clinical isolates of *Neisseria* spp. be correctly identified. Rapid tests provide timely laboratory confirmation of a clinical diagnosis of gonorrhea (30). However, problems have occurred with most rapid diagnostic tests which may result in the misidentification of nonpathogenic *Neisseria* spp. as *Neisseria gonorrhoeae* (18, 26, 30, 34).

Strains of nonpathogenic *Neisseria* spp. isolated on selective media for the gonococcus have been misidentified (34, 63) because they can give reactions similar to the gonococcus in rapid tests for the confirmation of *N. gonorrhoeae* (18, 34). Nonpathogenic strains isolated on nonselective

media have been inappropriately tested in rapid diagnostic tests specifically designed to identify strains isolated on selective media (100). Some problems may have resulted because several species either have been combined into a single species (80) or have not previously been described (46, 47) or recognized (63, 96).

This paper reviews the history of the taxonomy of the genus *Neisseria* to provide an historical perspective on the difficulties faced in previous studies of the species, the taxonomic changes that have been made within the genus, and the prevalence of the nonpathogenic species and to discuss the procedures that may be used to identify *Neisseria* and related species. Discussions of other genera are limited to *Kingella denitrificans* and *Branhamella catarrhalis*, which may be misidentified as *N. gonorrhoeae* (100). The bibliography for this review is extensive but selective

and provides key references that will guide the reader to additional readings on this subject.

HISTORICAL PERSPECTIVES ON THE TAXONOMY OF THE FAMILY NEISSERIACEAE

Taxonomy of the Family Neisseriaceae

The genus *Neisseria* belongs to the family *Neisseriaceae* (95), which has undergone many taxonomic changes (14, 15, 19, 74, 75, 80) that are summarized in Table 1. The genus *Neisseria* was assigned to the family *Coccaceae* until 1948 (74), when it was reassigned as the type genus in the family *Neisseriaceae* (75). The family *Neisseriaceae* at that time also contained the strictly anaerobic *Veillonella* spp. (14, 19). The family *Neisseriaceae* now contains the genera *Neisseria*, *Moraxella*, *Acinetobacter*, and *Kingella* (14), which are differentiated from each other by cell morphology, oxidase and catalase reactions, the presence of carbonic anhydrase, the production of acid from glucose, the ability to reduce nitrite, the presence of thymidine phosphorylase, nucleoside deoxyribosyl transferase, and thymidine kinase, and the presence of true waxes in the cell wall (14).

The genus *Neisseria* contains species that are isolated from humans and other animals. The human species have undergone few taxonomic changes. The most notable change in the taxonomy of the family has been a result of genetic studies. These led to the reassignment of *N. catarrhalis* to the genus *Branhamella* (25) and the inclusion of *B. catarrhalis* as a subgenus in the genus *Moraxella* (14, 15). Because subgenus and subspecific epithets are not used (90), strains of *B. catarrhalis* should correctly be called *Moraxella catarrhalis*. However, because *B. catarrhalis* is distinctly different from the *Moraxella* spp. in cell morphology and has recently been recognized as a pathogen (21, 27, 68), the name *B. catarrhalis* is commonly used, although no formal request has been made to have the name conserved taxonomically.

Taxonomy of *Neisseria* spp. and *B. catarrhalis*

The genus *Neisseria* contains 12 species and biovars (95) isolated from humans. They can be identified by many characteristics, including their patterns of acid production from carbohydrates and their ability to reduce nitrate and to produce polysaccharide from sucrose (Table 2). Although several human *Neisseria* species were described in the late 1800s, most were described in 1906, when von Lingelsheim cultured specimens to determine the etiology of meningitis (97). Studies to characterize the *Neisseria* spp. were undertaken to determine the etiology of influenza, colds, and meningitis (36, 38) and to classify the species objectively (104, 105). During these studies, problems in identifying commensal *Neisseria* spp. were noted. It was found that colonial cell morphology could not be used for the classification of *Neisseria* spp. and that reproducible patterns of acid production from carbohydrates could not be obtained from subcultures of the same strain (105) or from strains tested in different media (104). Attempts to classify *Neisseria* spp. were also hampered by a lack of differential tests such as the oxidase (92), nitrate reduction (5), and polysaccharide production (3) tests. Because the oxidase reaction was not used as a differential test, oxidase-negative species were included in the family *Neisseriaceae* until 1974 (19, 86). Consequently, the taxonomy of the genus has been confused and the data in many early publications on *Neisseria* spp.

TABLE 1. Taxonomy of the family *Neisseriaceae*, 1939 to 1984

Genus and species	Edition of <i>Bergey's Manual</i> (yr of publication)				
	5th (1939)	6th (1948)	7th (1957)	8th (1974)	1st (1984) ^a
<i>Neisseria gonorrhoeae</i>	Yes	Yes	Yes	Yes	Yes
<i>N. meningitidis</i> ^b	Yes	Yes	Yes	Yes	Yes
<i>N. catarrhalis</i>	Yes	Yes	Yes	No ^c	No
<i>N. sicca</i>	Yes	Yes	Yes	Yes	Yes
<i>N. perflava</i>	Yes	Yes	Yes	No ^d	No ^d
<i>N. flava</i>	Yes	Yes	Yes	No ^d	No ^d
<i>N. subflava</i>	Yes	Yes	Yes	Yes	Yes
<i>N. flavescens</i>	Yes	Yes	Yes	Yes	Yes
<i>N. discoides</i> ^e	Yes	Yes	No	No	No
<i>N. reniformis</i> ^e	Yes	Yes	No	No	No
<i>N. orbitulata</i> ^e	Yes	Yes	No	No	No
<i>N. haemolysans</i> ^f	No	No	Yes	No	No
<i>N. caviae</i>	No	No	Yes	(Yes) ^g	(Yes) ^h
<i>N. mucosa</i>	No	No	No	Yes	Yes
<i>N. animalis</i>	No	No	No	(Yes)	No
<i>N. canis</i>	No	No	No	(Yes)	Yes
<i>N. cinerea</i>	No	No	No	(Yes)	Yes
<i>N. cuniculi</i>	No	No	No	(Yes)	(Yes) ^h
<i>N. denitrificans</i>	No	No	No	(Yes)	Yes
<i>N. elongata</i>	No	No	No	(Yes)	Yes
<i>N. lactamica</i> ⁱ	No	No	No	(Yes)	Yes
<i>N. ovis</i>	No	No	No	(Yes)	(Yes) ^h
<i>N. suis</i>	No	No	No	(Yes)	No
<i>N. kochii</i> ^j	No	No	No	No	No
<i>N. polysaccharea</i> ^k	No	No	No	No	No
<i>Veillonella</i> ^k <i>parvula</i>	Yes	Yes	Yes	No	No
<i>V. gazogenes</i>	Yes	Yes	Yes	No	No
<i>Branhamella catarrhalis</i> ^c	No	No	No	Yes	No
<i>Moraxella lacunata</i>	No	No	No	Yes	Yes
<i>M. bovis</i>	No	No	No	Yes	Yes
<i>M. nonliquefaciens</i>	No	No	No	Yes	Yes
<i>M. phenylpyruvica</i>	No	No	No	Yes	Yes
<i>M. osloensis</i>	No	No	No	Yes	Yes
<i>M. kingii</i>	No	No	No	(Yes)	No
<i>M. "urethralis"</i>	No	No	No	(Yes)	(Yes)
<i>M. atlantae</i>	No	No	No	No	Yes
<i>Acinetobacter calcoaceticus</i>	No	No	No	Yes	Yes
<i>Kingella kingae</i>	No	No	No	No	Yes
<i>K. indologenes</i>	No	No	No	No	Yes
<i>K. denitrificans</i>	No	No	No	No	Yes

^a *Bergey's Manual of Determinative Bacteriology* was renamed *Bergey's Manual of Systematic Bacteriology* in 1984.

^b *N. meningitidis* was named *N. intracellularis* in the 3rd and 4th editions of *Bergey's Manual of Determinative Bacteriology*.

^c *N. catarrhalis* was transferred to the genus *Branhamella* in 1974. Although this species was reassigned as a subgenus in the genus *Moraxella* in 1984 (14, 15), the name *B. catarrhalis* is still used; no application has been made to have this name retained as nomen conservandum.

^d *N. perflava* and *N. flava* were grouped in the species *N. subflava* in 1974 (80). Although retained in the species *N. subflava* in 1984, they were noted to be biovars (95).

^e *N. discoides*, *N. reniformis*, and *N. orbitulata* were anaerobic species that were removed from the genus *Neisseria* in 1957 (74); they were assigned to the genus *Veillonella* as *V. discoides*, *V. reniformis*, and *V. orbiculus*, respectively (83).

^f *N. haemolysans* was an anaerobic species assigned to the genus *Neisseria* in 1957 (74) and renamed *Gemella haemolysans* (75).

^g (Yes) indicates species that were listed in the genus as species incertae sedis; that is, their taxonomic status was not certain.

^h *N. caviae*, *N. cuniculi*, and *N. ovis* were assigned to the subgenus *Branhamella* (15).

ⁱ *N. lactamica* was first listed in the 8th edition of *Bergey's Manual* as *N. lactamicus*, a species incertae sedis, and was listed as *N. lactamica* in *Bergey's Manual of Systematic Bacteriology* in 1984 (95).

^j *N. polysaccharea* and *N. gonorrhoeae* subsp. *kochii* were described after the publication of *Bergey's Manual of Systematic Bacteriology* (95). *N. polysaccharea* was first named *N. polysacchareae* (82). *N. kochii* has characteristics of both *N. gonorrhoeae* and *N. meningitidis*.

^k The genus *Veillonella* was included in the family *Neisseriaceae* until 1974 (80), when it was assigned as the type genus in the family *Veillonaceae*.

TABLE 2. Characteristics of human *Neisseria* spp., *B. catarrhalis*, and *K. denitrificans*^a

Species	Pig- ment ^b	Super- oxol ^c	Acid produced from:					Polysac- charide from ≥1% sucrose ^d	Reduction of:		DNase	Extra CO ₂ needed ^f	Growth on:		
			Glucose	Maltose	Fructose	Sucrose	Lactose (ONPG)		NO ₃	NO ₂ ^e			MTM, ML, or NYC medium	Chocolate, blood agar at 22°C	Nutrient agar at 35°C
<i>N. gonorrhoeae</i>	—	+	+	—	—	—	—	—	—	—	—	VI	+ ^g	—	—
<i>N. meningitidis</i>	—	—	+	+	—	—	—	—	—	d	—	I	+	—	—
<i>N. lactamica</i>	—	—	+	+	—	—	+	—	—	d	—	d	+	—	—
<i>N. cinerea</i>	—	—	— ^h	—	—	—	—	—	—	+	—	d	— ⁱ	—	—
<i>N. polysaccharea</i>	—	—	+	+	—	—	—	+	—	d	—	d	+	—	+
<i>N. kochii</i>	—	+	+	—	—	—	—	—	—	—	—	No	+	—	+
<i>N. flavescens</i>	+	—	—	—	—	—	—	+	—	—	—	I	+	—	—
<i>N. sicca</i>	d	—	+	+	+	+	—	+	—	+	—	No	—	+	+
<i>N. subflava</i> ^j															
Biovar <i>subflava</i>	+	—	+	+	—	—	—	—	—	+	—	No	—	+	+
Biovar <i>flava</i>	+	—	+	+	+	—	—	—	—	+	—	No	—	+	+
Biovar <i>perflava</i>	+	—	+	+	+	+	—	+	—	+	—	No	— ^k	+	+
<i>N. mucosa</i>	+	—	+	+	+	+	—	+	+	+	—	No	—	+	+
<i>B. catarrhalis</i>	—	—	—	—	—	—	—	—	+	—	+	No	d	+	+
<i>K. denitrificans</i>	—	—	+	—	—	—	—	—	+	—	—	I	+	—	—

^a ONPG, o-Nitrophenyl-β-D-galactopyranoside; DNase, deoxyribonuclease; MTM, modified Thayer-Martin medium; ML, Martin-Lewis medium; NYC, New York City medium. +, Most strains (≥90%) positive; —, most strains (≥90%) negative; d, some strains positive, some strains negative.

^b Pigment observed in colonies on nutrient agar. Strains of *N. cinerea* and *N. lactamica* are yellow-brown and yellow pigmented when growth is harvested on a cotton applicator or smeared on filter paper.

^c All *Neisseria* species and *B. catarrhalis* give a positive catalase test with 3% H₂O₂; *N. gonorrhoeae* strains give strong reactions with 30% H₂O₂ (superoxol), whereas other species are negative.

^d Some strains may be inhibited by 5% sucrose; reactions may be obtained on a starch-free medium containing 1% sucrose. Strains of *N. gonorrhoeae*, *N. meningitidis*, and *N. kochii* do not grow on this medium.

^e Results for tests in 0.1% (wt/vol) nitrite; *N. gonorrhoeae* strains and strains of some other species that are negative in 0.1% nitrite can reduce 0.01% (wt/vol) nitrite.

^f Extra CO₂: VI, very important; I, important for growth; No, not needed for growth.

^g ≥90% of vancomycin-susceptible strains of *N. gonorrhoeae* may not grow on TM or MTM medium.

^h Some strains of *N. cinerea* may give a weak reaction in glucose in some rapid tests for the detection of acid from carbohydrates.

ⁱ Some strains of *N. cinerea* have been isolated on gonococcal selective medium, but are colistin susceptible and will not grow when subcultured on selective media. Colistin-resistant mutants of *N. cinerea* have not been described.

^j Strains of *N. subflava* biovars give consistent patterns of acid production when tested in appropriate media.

^k Some strains of *N. subflava* biovar *perflava* grow on gonococcal selective media in primary culture, are colistin resistant, and grow on selective media on subculture.

must be interpreted cautiously. The problems relating to the classification of *Neisseria* spp. are discussed chronologically and by groups.

The human *Neisseria* spp. can be divided into two major groups. The first group includes *N. gonorrhoeae*, *N. meningitidis*, *N. lactamica*, *N. cinerea*, *N. flavescens*, *N. polysaccharea*, and *N. gonorrhoeae* subsp. *kochii*. Species belonging to this group generally grow as nonpigmented, translucent colonies. The yellow-pigmented species, *N. flavescens*, is the only exception to this rule. The second group of species includes the saccharolytic commensal *Neisseria* species, *N. subflava* (including the *N. subflava* biovars *perflava* and *flava*, which will be referred to as *N. perflava* and *N. flava* in some sections of this review), *N. sicca*, and *N. mucosa*. Colonies of these species are generally opaque, although some strains of *N. perflava* grow as transparent, nonpigmented colonies (unpublished observation). Strains of most other species are yellow pigmented; strains of *N. sicca* are usually described as nonpigmented, as are some strains of *N. mucosa*. *B. catarrhalis* is considered as a third group with the *Neisseria* spp. because of shared phenotypic similarities (14).

Early Studies

Pathogenic *Neisseria* spp. The pathogenic *Neisseria* spp., *N. gonorrhoeae* and *N. meningitidis*, have been studied more intensively than most *Neisseria* spp. However, historically, some nonpathogenic *Neisseria* spp. may have been

misidentified as *N. gonorrhoeae* or *N. meningitidis*. There have been several reports of the isolation of atypical *N. gonorrhoeae* strains from the genitourinary tract (41, 79). Speculation that some of these strains may have been *N. cinerea* (63) was recently confirmed when strains isolated in the early 1950s were retested and identified (I. Lind and J. S. Knapp, unpublished observations). It must be remembered that *N. gonorrhoeae* strains were isolated from clinical specimens on nonselective media until 1965 (61). Strains of *N. cinerea* were likely misidentified as atypical *N. gonorrhoeae* because they were phenotypically similar to gonococcal strains but failed to produce acid from glucose (34, 63).

Elser and Huntoon (36) described an organism which they called "pseudomeningococcus"; strains assigned to this group were indistinguishable from the meningococcus. It is reasonable to speculate that these strains may have been *N. lactamica* or *N. polysaccharea*. Elser and Huntoon did not, however, identify lactose-positive strains, probably because they collected specimens only from adults. Although *N. lactamica* is frequently isolated from children (12, 37), it is rarely isolated from adults (59). Thus, it is possible that some of Elser and Huntoon's strains were *N. polysaccharea*, which can be differentiated from *N. meningitidis* strains by their ability to produce polysaccharide from sucrose (81). Retrospectively, Boquette et al. (13) found that 25% of strains previously identified as nontypable *N. meningitidis* were, in fact, *N. polysaccharea*. It is also possible that, if

TABLE 3. Synonyms of various *Neisseria* spp. and *B. catarrhalis*

Species	Synonym	Author, yr of publication (reference)
<i>N. gonorrhoeae</i>	<i>Micrococcus gonorrhoeae</i>	Elser and Huntoon, 1909 (36)
<i>N. meningitidis</i>	<i>Micrococcus intracellularis</i>	Von Lingelsheim, 1906 (97)
	<i>M. intracellularis meningitidis</i>	Elser and Huntoon, 1909 (36)
	<i>M. intracellularis</i>	Gordon, 1921 (38)
	<i>Neisseria intracellularis</i>	
<i>N. cinerea</i>	<i>Micrococcus cinereus</i>	Von Lingelsheim, 1906 (97)
	<i>M. catarrhalis</i> (type 2) ^a	Elser and Huntoon, 1909 (36)
		Gordon, 1921 (38)
	<i>Neisseria pharyngis</i>	Wilson and Smith, 1928 (104)
	<i>Neisseria cinerea</i>	Wilson and Wilkinson, 1983 (103)
		Murray and Branham, 1939 (74)
<i>N. sicca</i>	<i>Diplococcus pharyngis siccus</i>	Von Lingelsheim, 1906 (97)
		Elser and Huntoon, 1909 (36)
		Gordon, 1921 (38)
	<i>Neisseria pharyngis</i>	Wilson and Smith, 1928 (104)
	<i>N. sicca</i>	Wilson and Wilkinson, 1983 (103)
		Murray and Branham, 1939 (74)
<i>N. subflava</i>	<i>Diplococcus pharyngis flavus</i> group III	Von Lingelsheim, 1906 (97)
	Chromogenic group III	Elser and Huntoon, 1909 (36)
	Chromogenic group 3	Gordon, 1921 (38)
	Strain F ^b	Wilson, 1928 (105)
	<i>Neisseria pharyngis</i>	Wilson and Smith, 1928 (104)
		Wilson and Wilkinson, 1983 (103)
	<i>N. subflava</i>	Murray and Branham, 1939 (74)
	<i>N. subflava</i>	Reyn, 1974 (80)
	<i>N. subflava</i> (biovar <i>subflava</i>)	Vedros, 1984 (95)
<i>N. flava</i>	<i>Diplococcus pharyngis flavus</i> group I	Von Lingelsheim, 1906 (97)
	Chromogenic group I	Elser and Huntoon, 1909 (36)
	Chromogenic group 4	Gordon, 1921 (38)
	Strain F ^b	Wilson, 1928 (105)
	<i>Neisseria pharyngis</i>	Wilson and Smith, 1928 (104)
	<i>N. flava</i>	Murray and Branham, 1939 (74)
	<i>N. subflava</i>	Reyn, 1974 (80)
	<i>N. subflava</i>	Wilson and Wilkinson, 1983 (103)
	<i>N. subflava</i> (biovar <i>flava</i>)	Vedros, 1984 (95)
<i>N. perflava</i>	<i>Diplococcus pharyngis flavus</i> group II	Von Lingelsheim, 1906 (97)
	Chromogenic group II	Elser and Huntoon, 1909 (36)
	Chromogenic group 5	Gordon, 1921 (38)
	Strains A, B, C, and E ^c	Wilson, 1928 (105)
	<i>Neisseria pharyngis</i>	Wilson and Smith, 1928 (104)
	<i>N. perflava</i>	Murray and Branham, 1939 (74)
	<i>N. subflava</i>	Reyn, 1974 (80)
	<i>N. subflava</i>	Wilson and Wilkinson, 1983 (103)
	<i>N. subflava</i> (biovar <i>perflava</i>)	Vedros, 1984 (95)
<i>N. mucosa</i>	<i>Diplococcus mucosus</i>	Von Lingelsheim, 1906 (97)
	<i>Neisseria mucosa</i>	Reyn, 1974 (95)
	<i>N. mucosa</i>	Wilson and Wilkinson, 1983 (103)
<i>B. catarrhalis</i>	<i>Micrococcus catarrhalis</i>	Von Lingelsheim, 1906 (97)
	<i>M. catarrhalis</i> (type 1)	Elser and Huntoon, 1909 (36)
	<i>Neisseria pharyngis</i>	Wilson and Smith, 1928 (104)
	<i>N. catarrhalis</i>	Wilson, 1928 (105)
	<i>Branhamella catarrhalis</i>	Reyn, 1974 (80)
	<i>Moraxella</i> (B.) <i>catarrhalis</i>	Bøvre, 1984 (15)

^a *N. cinerea* isolates were identified as *B. catarrhalis* subtypes on the basis of their distinct colonial morphology.

^b Strain F was not tested for acid production from fructose.

^c Strains A, B, C, and E conform to *N. perflava* based on their patterns of acid production. It must be remembered that *N. mucosa* was not recognized and may also correspond to some or all of these strains.

isolated on nonselective medium, some strains identified as *N. meningitidis* may have been *N. subflava* biovar *subflava* (Table 2).

Saccharolytic *Neisseria* spp. In the early 1920s, the saccharolytic commensal *Neisseria* spp. were called by different names (Table 3). Von Lingelsheim described *Micrococcus pharyngis siccus* (*N. sicca*), *M. pharyngis cinereus* (*N. cinerea*), *Diplococcus pharyngis flavus* groups I (*N. flava*), II (*N. perflava*), and III (*N. subflava*), and *D. mucosus* (*N. mucosa*); all species except *N. mucosa* were characterized

by their patterns of acid production from glucose, maltose, and sucrose, but not from fructose (97).

Elser and Huntoon (36) described *M. pharyngis siccus*, *D. mucosus*, and three groups of saccharolytic organisms analogous to the *N. subflava* biovars which they called the chromogenic groups I (*N. flava*), II (*N. perflava*), and III (*N. subflava*). These organisms were classified according to their patterns of acid production from carbohydrates. Because the nitrate reduction test was not used to distinguish strains of *N. mucosa* from *N. perflava* (5), strains of *N. mucosa* may

have been grouped with *N. perflava*; this would be supported by the fact that Elser and Huntoon identified at least two serogroups within the chromogenic group II (*N. perflava*). These serologic studies also showed that isolates of the chromogenic groups I (*N. flava*) and III (*N. subflava*) were identical but distinct from those of the chromogenic group II (*N. perflava*).

Gordon (38) characterized gram-negative diplococci from the oro- and nasopharynxes of persons with colds and influenza and healthy persons by their patterns of acid production from many carbohydrates including glucose, maltose, sucrose, fructose, and lactose. Some isolates produced acid reactions within 24 h that remained unchanged after incubation for 7 days, other isolates produced acid reactions within 24 h which became alkaline after 7 days, and still others were negative after 24 h but produced acid within 7 days. These reactions were reproducible.

Gordon (38) identified *M. pharyngis siccus* and six chromogenic organisms but did not identify *D. mucosus*. Of the six chromogenic groups numbered 1 to 6, groups 3, 4, and 5 correspond to *N. subflava*, *N. flava*, and *N. perflava*, respectively, according to their growth characteristics. The chromogenic groups 1, 2, and 6 do not appear to correspond to *Neisseria* spp., although Gordon thought that groups 2 and 3 corresponded to the groups I (*N. flava*) and III (*N. subflava*) that Elser and Huntoon had found to be serologically identical (36). The isolates belonging to group 2 produced acid from fructose but not from maltose, which is inconsistent for *N. flava*.

Wilson (105) characterized six strains, named A through F, that were representative of strains of saccharolytic *Neisseria* spp. isolated on blood agar or Fildes pepsinized sheep blood agar. The strains were characterized by their colony morphology and acid production from carbohydrates and were tested weekly during a 3-month period. Most strains gave reproducible patterns of acid production from glucose and maltose in 2 to 3 days, and variable acid production was observed from fructose and sucrose in 4 to 6 days. In contrast to previous investigators (36, 38), Wilson was unable to identify isolates based on the descriptions of the colonial morphology and acid production and suggested that, with the exception of *N. meningitidis* and *B. catarrhalis*, all previously recognized species should be subspecies within a single species.

Retrospective interpretation of these data is speculative but, if we assume that strains that gave variable acid reactions in sucrose-containing media were actually sucrose positive, strains A to F can be assigned to modern *Neisseria* spp. Based on the characteristic wrinkled colonies and acid production from glucose, maltose, fructose, and sucrose, strain D was *N. sicca*. Strains A, B, C, and E were either *N. perflava* or *N. mucosa*, although strain A may have been *N. sicca*. Strain F was either *N. flava* or *N. subflava*; acid production from fructose was not determined. No distinction between *N. flava* and *N. subflava* could be made in this study because only one strain representing this colony type was characterized.

Wilson and Smith (104) also recognized the discrepancies between the colonial morphology descriptions and the patterns of acid production for *B. catarrhalis* and the chromogenic species. They determined patterns of acid production from glucose, maltose, and sucrose by 50 isolates in serum peptone water broth and on ascitic agar medium; fructose was not tested. Twenty-five isolates gave the same reactions in both media. Of the 25 isolates that gave different reactions in the two media, acid was produced from glucose more

frequently in the serum peptone water medium, whereas acid was produced more frequently from sucrose on the ascitic agar medium; acid was produced from maltose almost equally in both media. The reproducibility of acid production from an individual carbohydrate was not determined. Wilson and Smith also observed differences in the colonial morphology of individual strains on different media as well as changes in morphology that occurred with prolonged incubation, which suggested that colonial morphology should not be a major criterion for classification. Because of the variability of the cultural and biochemical characteristics of the gram-negative cocci, these investigators concluded that "instead of dividing them up into a number of so-called species—*catarrhalis*, *flavus*, *cinereus*, *mucosus*, *siccus*—they should be grouped under the broad term *Neisseria pharyngis* . . .".

Asaccharolytic *Neisseria* spp. Two species, *Micrococcus catarrhalis* (*B. catarrhalis*) and *M. cinereus* (*N. cinerea*), were described in detail by von Lingelsheim in 1906 (97). Elser and Huntoon (36) did not identify *M. cinereus* but described two types of *M. catarrhalis* among strains they characterized according to colonial morphology. Strains in the first group were *B. catarrhalis*, whereas strains in type 2 appear to have been *N. cinerea*.

Gordon (38) did not identify *M. cinereus* among his isolates but divided strains of *M. catarrhalis* into four groups on the basis of their growth characteristics. Strains in the largest subgroup correspond to *B. catarrhalis*. Isolates belonging to the third subgroup produced small, translucent, flat, grey, smooth, glistening colonies that resemble *N. cinerea*. These isolates were also difficult to maintain in subculture, a characteristic common to some *N. cinerea* and *N. gonorrhoeae* isolates (unpublished observations). It is difficult to identify retrospectively isolates belonging to the second and fourth subgroups; strains belonging to the fourth subgroup produced pinpoint colonies that were difficult to maintain on blood agar and may not have been neisserias.

In 1934, Huntoon (50) described an organism, *N. pseudo-catarrhalis*, whose description was consistent with that of *N. cinerea* (*N. cinereus*) (97). Strains of *N. cinerea* were not recognized and correctly identified again until 1962 in Germany (10) and in 1984 in the United States (63).

Modern Studies

In the studies described above, several problems were introduced and perpetuated in the classification and nomenclature of the *Neisseria* spp. and *B. catarrhalis*. These were due to the use of inappropriate media for the detection of acid production from carbohydrates, the inappropriate use of colonial morphology for the classification of species, and the lack of important differential tests including the oxidase reaction, nitrate reduction, and the production of polysaccharide from sucrose. These problems may be summarized as (i) the failure to distinguish *N. cinerea* from *B. catarrhalis* and *N. gonorrhoeae*; (ii) the failure to differentiate between isolates belonging to the species *N. perflava*, *N. flava*, and *N. subflava*; (iii) the failure to identify isolates of *N. lactamica* or *N. mucosa*; and (iv) the failure to differentiate between *N. meningitidis* and *N. polysaccharea*.

Although investigators in the early 1900s classified *Neisseria* spp. by cell morphology and arrangement, colony morphology, and acid production from carbohydrates (36, 38), it was recognized that the classification of the species could not be based on either cell or colonial morphology but more reliably on patterns of acid production from carbohy-

drates. By 1928, however, the use of patterns of acid production from carbohydrates was also questioned (104, 105). A review of these publications shows that strains were tested in media that were rich in peptone or serum, contained litmus or Andrade indicator, and often were adjusted to pH 7.8. We know now that these media are unsuitable for detecting acid production from organisms that produce acid from carbohydrates by oxidation (1, 48). Thus, the media used by early investigators were not suitable for detecting acid production by *Neisseria* spp., and in retrospect, it is not surprising that they were unable to clearly differentiate between the species and biovars that may now be readily identified if appropriate media are used.

In 1954, Hugh and Leifson (48) showed that bacterial species produced acid from carbohydrates by either fermentation or oxidation and that oxidative species produced less acid from carbohydrates than fermentative species. An oxidation-fermentation medium was formulated to detect acid production by oxidative species by reducing the peptone concentration of the medium relative to the carbohydrate concentration.

Pathogenic *Neisseria* spp. Although *Neisseria* spp. have been described as facultative anaerobes, there do not appear to be any publications that verify this fact prior to the 1980s. It is possible that this statement has persisted in taxonomic literature from the years when the anaerobic *Veillonella* spp. were included in the family *Neisseriaceae* (19, 74, 75). Although Berger initially found that strains of *N. gonorrhoeae* and *N. meningitidis* were inhibited by, and could not reduce, 0.1% (wt/vol) nitrite (5), he subsequently found that some strains of *N. gonorrhoeae* could reduce 0.001% (wt/vol) nitrite (7). Strains of *N. gonorrhoeae* belonging to diverse serogroups can reduce 0.001% (wt/vol) nitrite within 24 h and 0.01% (wt/vol) within 48 h (56). We found, moreover, that strains of *N. gonorrhoeae* could grow under anaerobic conditions by nitrite respiration (57). This explained the previous observations of Short et al. (88) that *N. gonorrhoeae* strains could be grown initially but not in subculture under anaerobic conditions. Subsequent studies have shown that all of the human *Neisseria* spp. with the possible exception of *N. meningitidis* can grow under anaerobic conditions by nitrite respiration (unpublished observations). Few strains of *N. meningitidis* can reduce 0.01% (wt/vol) nitrite, and they vary in their ability to reduce 0.001% (wt/vol) nitrite (F. E. Ashton, F. Collins, J. A. Ryan, and B. B. Diena, Abstr. Fifth Int. Pathogenic *Neisseria* Conf. 1986, abstr. no. I-2).

Saccharolytic *Neisseria* spp. In 1960, Berger (1) showed that *Neisseria* spp. produced acid from carbohydrates by oxidation whereas *Gemella haemolysans* produced acid by fermentation. He devised a modified oxidation-fermentation medium containing 1% serum and phenol red indicator to detect acid production by *Neisseria* spp. Berger and co-workers (2, 9) found that strains of *N. perflava*, *N. sicca*, *N. flava*, and *N. subflava* consistently produced acid from the disaccharides maltose and sucrose but variably from the monosaccharides glucose and fructose. Furthermore, the final pH of the medium was lower in media inoculated with *N. perflava* and *N. sicca* strains than in those inoculated with *N. flava* or *N. subflava* strains (2). They were unable to obtain reproducible acid production from fructose by strains of *N. flava* and concluded that these entities were variants of the same species which would be named *N. subflava* (9). It should be noted that, in their studies, Berger et al. (2, 9) determined acid production on a nutrient peptone agar medium containing 1% carbohydrates and litmus indicator,

not the modified oxidation-fermentation medium (2) in which acid may have been detected more sensitively. Berger also showed that strains of *N. flava* and *N. subflava* produce ammonia from peptone (6); the release of ammonia into the medium may have neutralized acid produced from carbohydrates (58, 59). The distinction between *N. perflava* and the *N. flava*-*N. subflava* species was further supported by the observation that *N. perflava* strains produced polysaccharide from sucrose whereas *N. flava*-*N. subflava* strains did not (3). Berger et al. (2, 9) also examined the serological relatedness among *N. sicca*, *N. perflava*, *N. flava*, and *N. subflava* and confirmed the observation of Elser and Huntoon (36) that *N. flava* and *N. subflava* were serologically identical but distinct from *N. perflava*. Berger et al. (2, 9) also confirmed that, although *N. sicca* and *N. perflava* were biochemically identical, they were serologically distinct. Berger and Brunhoeber (9) concluded that *N. flava* and *N. subflava* were variants of the same species but distinctly different from *N. perflava* and *N. sicca*.

We confirmed Berger's observations that a modified oxidation-fermentation medium (58) and a selective isolation medium for commensal *Neisseria* spp. (59). We developed the modified oxidation-fermentation medium to avoid difficulties encountered with cysteine-Trypticase agar medium. Although cysteine-Trypticase agar medium deeps have been inoculated by pipetting dense suspensions of strains onto the surface of the medium and stabbing to inoculate growth into the medium, acid reactions obtained with this method are often very weak and difficult to interpret. We overcame this problem by inoculating media heavily by using swabs and were able to obtain unequivocal acid reactions for all species (58). We have used modified oxidation-fermentation medium, however, because the acid reactions are more easily interpreted than those obtained in cysteine-Trypticase agar medium (59). In contrast to Berger's studies, we were consistently able to differentiate between strains of *N. flava* and *N. subflava* in fructose-containing modified oxidation-fermentation medium (58), but we concur with Berger and Brunhoeber (9) that these species are probably variants of the same species. We have continued to determine the ability of isolates to produce acid from fructose (59) and were intrigued by the fact that strains of *N. subflava* (fructose negative) were rarely isolated whereas strains of *N. flava* (fructose positive) were isolated frequently.

Elser and Huntoon (36) observed serological heterogeneity among *N. perflava* isolates, which was probably due to the inclusion of *N. mucosa* isolates in the chromogenic group II. It is interesting that Berger et al. (2, 9) did not observe any serological heterogeneity among *N. perflava* isolates that might have indicated the existence of *N. mucosa* strains. Berger (3) introduced a test to detect polysaccharide production from sucrose for the classification of *Neisseria* spp. Strains of *N. perflava*, *N. sicca*, and *N. mucosa* produce polysaccharide from sucrose, whereas strains of *N. flava* and *N. subflava* do not (3). Again, *N. mucosa* strains, which produce polysaccharide from sucrose, were not detected. *N. mucosa* was not recognized and redescribed until 1959 (96).

Two nomenclatures have been used for the saccharolytic *Neisseria* spp. In the United States, the saccharolytic organisms were maintained as separate species, *N. sicca*, *N. perflava*, *N. flava*, and *N. subflava*, until 1974 (19, 80). In the United Kingdom, the saccharolytic species *N. subflava*, *N. flava*, and *N. perflava* have been combined in a single species, *N. subflava*, which was previously named *N. pharyngis* (103). *N. mucosa* and *N. sicca* have also been listed as

separate species, and *N. cinerea* has been listed as a species of uncertain status (103). The name *N. pharyngis* is still frequently used in English publications (51).

Asaccharolytic *Neisseria* spp. Berger also studied the asaccharolytic *Neisseria* spp. Berger and Wulf (11) observed two distinct colonial morphologic types among isolates identified as *N. catarrhalis*; these were designated group I and group II (10). Berger et al. (4, 10) characterized these isolates as *N. catarrhalis* by nitrate reduction and serologic studies. Strains belonging to *N. catarrhalis* group I reduced nitrate, whereas group II strains were nitrate negative. The groups were also serologically distinct (4). Berger and Paepcke (10) concluded that *N. catarrhalis* group I strains conformed to the description of *N. catarrhalis* and those belonging to group II belonged to *N. cinerea*.

Apart from the description of *N. pseudocatarrhalis* in 1934 (50), *N. cinerea* strains were not reported in English language publications until 1984, when we isolated a strain on Martin-Lewis medium from the cervix of a patient with arthritis (63). *N. cinerea* strains are colistin susceptible (6, 63) but occasionally have been isolated on gonococcal selective media (34, 63).

"New" Species

***N. flavescens*.** *N. flavescens* was isolated from an outbreak of epidemic meningitis in Chicago (20) and may not have been isolated since then. Although this species has been reported elsewhere (78, 89, 98), insufficient differential tests were performed to identify the isolates conclusively; they may have been *N. cinerea* (63). It is possible that this species was a hybrid organism that had characteristics of both the pathogenic and the commensal species. Similar to the saccharolytic species, *N. flavescens* strains are pigmented and colistin susceptible and produce polysaccharide from sucrose (6) but are genetically more related to the pathogenic species (39, 44).

***N. mucosa*.** As noted earlier in the discussion of the saccharolytic *Neisseria* spp., *D. mucosus* (*N. mucosa*) was described in 1906 (97) but was not recognized again until 1959, when it was redescribed by Véron et al. (96). Strains of *N. mucosa* are distinguished from those of *N. perflava* and *N. sicca* by their ability to reduce nitrate (Table 2).

***N. lactamica*.** *N. lactamica* was described by Hollis et al. (46) in 1969 and by Berger (8) as *N. meningococcoides*. *N. lactamica* strains are colistin resistant, grow on gonococcal selective medium, and are differentiated from *N. meningitidis* by their ability to produce β -galactosidase that cleaves lactose to glucose from which acid is produced (Table 2). Strains of *N. lactamica* had not been described in studies previous to 1969 (11, 36, 38, 104, 105) probably because the reported strains were isolated from adults and not children.

***N. elongata*.** *N. elongata* was described in 1970 by Bøvre and Holton (16). Strains of *N. elongata* are rod shaped and elongate into filaments when exposed to sublethal concentrations of penicillin (71). Strains of *N. elongata* are normal flora of the oropharynx and have been isolated from cases of pharyngitis (95). Although rod shaped, *N. elongata* is considered to be a member of the genus *Neisseria* because of its genetic affinity to the true neisserias, its similarity to them based on cellular lipid and carbohydrate composition, and the characteristics of its glycolytic enzymes (95). Two subspecies, *N. elongata* subsp. *elongata* and *N. elongata* subsp. *glycolytica*, have been described; these are differentiated on the basis of their ability to produce acid from glucose (95).

***N. polysaccharea*.** *N. polysaccharea* was described in 1983 (82) and is characterized by its ability to produce acid from glucose and maltose and polysaccharide from sucrose (Table 2). Strains of this species were previously identified as nontypable strains of *N. meningitidis* (13). Strains of *N. polysaccharea* also may have been identified previously as *N. subflava* if their ability to produce polysaccharide from sucrose was not determined (3, 39).

***N. gonorrhoeae* subsp. *kochii*.** Recently, neisserial strains that exhibited characteristics of both *N. gonorrhoeae* and *N. meningitidis* were isolated from patients with conjunctivitis in rural Egypt (69). Isolates resembled *N. gonorrhoeae* because they produced acid only from glucose and did not produce γ -glutamylaminopeptidase. They did not, however, react with gonococcal protein I-specific monoclonal antibodies and, unlike *N. meningitidis*, required cystine/cysteine for growth on auxotyping medium. The "Egyptian" isolates produced large, pigmented colonies similar to those of *N. meningitidis* strains and gave a positive reaction in a temperature-sensitive transformation assay for *N. gonorrhoeae*, although at a lower frequency than did gonococcal strains (69). For practical purposes, these strains have been identified as *N. gonorrhoeae* subsp. *kochii* because they are thought to have been the organism described by Koch in 1883 (64). Clinical isolates of this species would be identified as *N. gonorrhoeae* by biochemical tests but may not be identified by serological tests alone. To our knowledge, this subspecies has not been isolated in the United States, although strains of this species were isolated from men with urethritis in Alexandria, Egypt (unpublished data).

NEISSERIA AND RELATED SPECIES: PATHOGENS OR SAPROPHYTES?

Of the *Neisseria* and related species, only *N. gonorrhoeae* strains are always pathogenic. Strains of this species infect mucosal surfaces of the cervix, urethra, rectum, and oro- and nasopharynx, causing symptomatic or asymptomatic infections (71, 73). Gonococcal infections of the oro- and nasopharynx and the rectum may be asymptomatic more frequently than those of the urogenital sites. However, strains of *N. gonorrhoeae* with patterns of requirements that include arginine, hypoxanthine, and uracil or proline, arginine (citrulline), and uracil have been associated with asymptomatic infections of urogenital sites (22, 28).

Strains of *N. meningitidis* may also be pathogens, being associated with epidemic meningitis in many geographic areas (71, 73). The pathogenicity of *N. meningitidis* is generally associated with specific serogroups and serotypes. Of a total of 13 serogroups of meningococci, encapsulated strains belonging to the serogroups A, B, C, and W-135 have been most frequently associated with epidemics; group A strains have been associated with most epidemics, whereas group B, C, and W-135 strains have caused sporadic epidemics (73). Meningococcal strains may be carried asymptotically in the oro- and nasopharynx (12, 37, 59) and have been isolated from urogenital sites in men and women (73). Between 3 and 30% of healthy persons may be asymptomatic carriers of meningococci in nonepidemic geographic areas. During epidemics in military recruits, although $\geq 95\%$ of recruits may be asymptomatic carriers of the epidemic strain, only 1% develop systemic disease.

B. catarrhalis strains were thought to be normal flora of the oro- and nasopharynx. Recently, however, this species has been recognized as a pathogen that causes pneumonia (91), systemic disease (32), sinusitis (21), otitis media (29),

respiratory infections (68), and ophthalmia neonatorum (100). This species is not frequently isolated from the oropharynxes of healthy adults (11, 59, 60) and either may be normal flora of respiratory sites other than the oro- or nasopharynx or may colonize certain individuals in a carrier state similar to that of the meningococcus.

Many commensal *Neisseria* spp. have been sporadically isolated from disseminated sites, blood, and cerebrospinal fluid (73), but no correlation has been established between any species and syndrome that would warrant its designation as a pathogen. Commensal *Neisseria* spp. appear to be opportunistic pathogens (24, 31, 42); some infections attributed to these species have occurred in persons who may be predisposed to infections due to deficient immune systems (87).

HABITAT AND PREVALENCE OF *NEISSERIA* SPP.

The prevalence of gonorrhea will not be discussed in this review. *N. meningitidis* strains are carried as normal flora in the oro- and nasopharynx of adults (76, 84, 101, 102) and children (12, 37). The prevalence of *N. meningitidis* carriage varies geographically and may occur more frequently in adults with gonorrhea (76, 84, 101, 102) and in homosexual men (53, 106). The carriage rate of *N. meningitidis* in children is also usually low, being $\leq 1\%$ during the first 4 years of life and increasing thereafter (37).

The carriage rate of *N. lactamica* is generally higher than that of *N. meningitidis* in children (12). The *N. lactamica* carriage rate increased in children from approximately 4% at 3 months to a peak of 21% in children 18 to 24 months, declining thereafter to 2% by age 14 to 17 years (37). It has been estimated that 59% of children have been colonized by *N. lactamica* at least once by the age of 4 years (37). Unlike *N. gonorrhoeae* and *N. meningitidis*, *N. lactamica* has not been implicated as a primary pathogen, although its role as an opportunistic pathogen has been described (65).

The commensal *Neisseria* and the related species, *B. catarrhalis* and *K. denitrificans*, are normal inhabitants of the oro- or nasopharynx or both (11, 36, 38, 46, 59, 97, 104, 105) and are occasionally isolated from other sites (24, 31, 32). Commensal *Neisseria* spp. rarely grow on media selective for the gonococcus (55, 59). Thus, the oropharyngeal carriage rate of the colistin-susceptible commensal species must be determined on a colistin-free medium.

It is impossible to determine the carriage rate of commensal *Neisseria* spp. in early studies (36, 38, 104, 105) because of the uncertainty of the identifications. Most studies of the prevalence of *Neisseria* spp. and *B. catarrhalis* were performed with nonselective media (11, 36, 38, 104, 105), which neither inhibited the growth of other bacterial species nor permitted differentiation between groups of *Neisseria* and related species. Thus, it is probable that determination of the carriage rates of different species grown on these media underestimated their true prevalence because of overgrowth by either non-neisserial species or the sucrose-positive *Neisseria* spp. Some studies have been performed with selective differential media that selected for commensal *Neisseria* spp. and *B. catarrhalis* and further differentiated between the asaccharolytic species (4) or between several different groups of species (59, 60).

In a study by Berger and Wulf (11), the carriage rates of commensal *Neisseria* spp. in adults were as follows: *N. sicca*, 45%; *N. perflava*, 40%; *N. subflava*-*N. flava*, 11%; and "*N. catarrhalis*," 3%. It must be remembered that *N. mucosa* was not recognized by these authors (11); thus, it is

not possible to determine whether it was present or how its presence would affect the stated carriage rates for *N. sicca* and *N. perflava*. Using a selective medium, Berger (4) found that asaccharolytic strains accounted for 15% of all neisserial isolates but did not give the relative carriage rates of *N. cinerea* and *B. catarrhalis* (10).

Recently, we studied the prevalence and persistence of *Neisseria* and related species in the oropharynx of adults (59). We found that adults had one of two general patterns of colonization. Some adults were colonized heavily by several strains of the sucrose-positive species, *N. mucosa* and *N. perflava*-*N. sicca*, whereas others were colonized sparsely by several *Neisseria* spp. These patterns of colonization were generally persistent, although they were occasionally disrupted; a person usually colonized heavily by sucrose-positive strains would have the alternative pattern of colonization by many species which was replaced by the original pattern of colonization within a short period of time. The most dramatic finding was that, whereas most persons were colonized by two or three species, 5% were colonized by four or five species (Fig. 1). These observations were not unique to Seattle, Wash., where this study was performed. A subsequent survey of 35 patients in DeKalb County, Georgia (60), showed that the *Neisseria* spp. colonized adults in patterns and prevalence similar to what was observed previously. The prevalence of some species may vary geographically, however. Strains of colistin-resistant *N. subflava* biovar *perflava* have been isolated repeatedly in Chicago (54, 55) and infrequently in DeKalb County (60) but not in Seattle (59). In contrast, *K. denitrificans* strains were isolated from 16% of patients in DeKalb County (60) but not in Chicago, Ill., or Seattle (54, 55, 59).

IDENTIFICATION OF *NEISSERIA* AND RELATED SPECIES

Bacteriology

The accurate identification of *Neisseria* spp. must be made with tests that differentiate between *Neisseria* related species. The *Neisseria* spp. are gram negative and oxidase positive (95). With the exception of *N. elongata*, the *Neisseria* spp. and *B. catarrhalis* are diplococci, whereas *K. denitrificans* strains are coccobacilli that may occasionally appear to be diplococci in Gram-stained smears. The bacillary shape of *K. denitrificans* strains can be demonstrated by a penicillin disk test (71). Subinhibitory concentrations of penicillin inhibit cell wall synthesis, and bacilli elongate to filamentous cells which are easily distinguished from true cocci. In addition to their morphologic similarity to the gonococcus, *K. denitrificans* strains must be included in schema to identify *Neisseria* spp. because they also grow on gonococcal selective media and produce acid from glucose (45, 46, 95).

Strains of *N. meningitidis* (71), *N. lactamica* (71), *N. cinerea* (63), *N. polysaccharea* (81), and *K. denitrificans* (46) grow as translucent, nonpigmented colonies that closely resemble the gonococcus on isolation media. Of these species, all except *N. cinerea* usually grow on gonococcal selective media (71, 73, 82) (Table 2). Occasionally, however, strains of *N. cinerea* have grown on gonococcal selective media, despite the fact that they are colistin susceptible, and have been misidentified as *N. gonorrhoeae* (34, 63). Although strains of most commensal species, *N. subflava* biovars *subflava*, *flava*, and *perflava*, *N. sicca*, *N. mucosa*, and *B. catarrhalis*, rarely grow on gonococcal

FIG. 1. Frequency of isolation of *Neisseria* spp. and *B. catarrhalis* from 202 adults in Seattle, Wash. Shaded boxes represent the isolation of the corresponding species or combination of species from the number of patients indicated. The patterns of colonization are arranged in order of increasing complexity from one to five species.

isolation media because they are colistin susceptible, some strains of *N. subflava* biovar *perflava* and *B. catarrhalis* are colistin resistant and will grow on selective media (33, 54, 55). Colonies of these species, however, are pigmented or opaque and should be distinguished from *N. gonorrhoeae* on isolation media or growth media, although the pigmentation is more easily determined by harvesting growth on a cotton applicator or smearing it on filter paper with a loop. Strains of most *Neisseria* spp. are also easily distinguished from *N. gonorrhoeae* by their ability to produce acid from maltose and other carbohydrates (71, 73). The species that are most difficult to distinguish from *N. gonorrhoeae* by biochemical characteristics are *N. cinerea*, *B. catarrhalis*, and *K. denitrificans*. These species may be differentiated from each other by the characteristics listed in Table 4.

When specimens are taken from sites that are usually sterile, for example, conjunctivae, blood, and cerebrospinal fluid, they are usually cultured on nonselective media on which all *Neisseria* spp. may grow if present in the specimen. Similarly, *Neisseria* spp., *B. catarrhalis*, and *K. denitrificans* may also be isolated when pharyngeal specimens are cultured on blood or chocolate agar.

Selection of Tests

Many types of tests are available for the identification of *Neisseria* and related species. Tests range from the traditional biochemical tests that must be incubated for 24 to 48 h before results can be obtained to tests for the rapid identification of *Neisseria* spp. that can provide an identification within 4 h after inoculation. The traditional tests have the advantage that they provide a more detailed characterization of isolates but have the major disadvantage that their use may delay the identification of an isolate unnecessarily. In contrast, some rapid tests may not always provide an accurate identification because they give limited information about an isolate. Many of the rapid tests have been specifically designed to confirm gram-negative, oxidase-positive isolates that were presumptively identified on selective media as *N. gonorrhoeae*. Other rapid tests include several that can be used to identify isolates to the species level. Thus, the microbiologist must decide what he/she needs to know about the isolate which, in turn, will determine the choice of both the isolation medium and the tests used to identify the isolate.

Patients at high risk for gonorrhea may attend sexually transmitted disease clinics specifically for the diagnosis of sexually transmitted diseases. The laboratory diagnosis of gonorrhea in patients attending sexually transmitted disease clinics may be based on either a "presumptive" or a

TABLE 4. Biochemical characteristics that differentiate between *Neisseria* and related species that may be isolated on gonococcal selective media and misidentified as *N. gonorrhoeae* with rapid procedures that detect acid production from carbohydrates

Species	Acid from:		Superoxol	Hydroxyprolyl-aminopeptidase	γ-Glutamyl-aminopeptidase	Nitrate reduction	Deoxyribo-nuclease	Colistin susceptibility ^a
	Glucose	Maltose						
<i>N. gonorrhoeae</i>	+	—	+	+	—	—	—	R
<i>N. meningitidis</i> ^b	+	—	—	NA ^c	+	—	—	R
<i>N. cinerea</i>	[+] ^d	—	—	+	—	—	—	S
<i>B. catarrhalis</i>	—	—	—	—	—	{+} ^e	+	[R] ^f
<i>K. denitrificans</i>	+	—	—	+	—	+	—	R

^a R, Resistant; S, susceptible.

^b Maltose-negative strains.

^c NA, Not applicable when γ-glutamylaminopeptidase is produced.

^d [+], Some strains produce weak acid reactions which are not delayed and are not glucose positive in all test systems.

^e {+}, Reaction weak or delayed, cannot be performed reliably as a rapid test (unpublished observations).

^f [R], Some strains are colistin resistant and grow on selective media.

confirmed diagnosis of *N. gonorrhoeae*. A presumptive laboratory diagnosis of *N. gonorrhoeae* based on the observation of intracellular gram-negative diplococci in polymorphonuclear leukocytes in urethral exudate, or the growth of a gram-negative, oxidase-positive diplococcus on a selective medium for *N. gonorrhoeae*, may suffice to confirm a diagnosis of gonorrhea in symptomatic men with urethritis because the Gram stain has a positive predictive value >95% (73). The laboratory identification of *N. gonorrhoeae* in cervical, rectal, and pharyngeal specimens must be made by isolating and identifying *N. gonorrhoeae* from specimens (73).

Specimens from high-risk patients and low-risk patients with a clinical diagnosis of gonorrhea are plated directly on selective media that enhance the isolation of the pathogenic *Neisseria* spp. However, strains of the nonpathogenic species *N. lactamica* and *K. denitrificans* and some strains of *N. subflava* biovar *perflava* and *B. catarrhalis* are colistin resistant and also grow on this medium (33, 54, 55). It should be noted at the same time, however, that some strains of *N. gonorrhoeae* may not be isolated on selective media if they are vancomycin susceptible (71); chocolate agar or media containing lower concentrations of vancomycin must be used to isolate these strains.

In contrast, when low-risk patients (for example, children) are diagnosed with throat or conjunctival infections in hospital clinics, private doctors' offices, and emergency rooms, specimens are cultured on a nonselective medium such as blood or chocolate agar. All *Neisseria* and related species can grow on these media. The isolate must be identified to the species level by using an appropriate procedure.

When it is anticipated that a pathogenic *Neisseria* species is present in a specimen from a low-risk patient, for example, in cases of suspected sexual abuse or meningococcal meningitis, the specimen should be cultured on a selective medium or a colistin susceptibility test should be performed to ascertain whether the organism is more likely to be a pathogenic or a nonpathogenic species. However, because of the implications of a diagnosis of gonorrhea in a low-risk patient, an isolate must be identified to the species level to avoid misidentifying a nonpathogenic species as *N. gonorrhoeae*; presumptive criteria cannot be used to identify *N. gonorrhoeae* in low-risk patients (100).

Traditional Tests

In a reference laboratory, *Neisseria* and related species are characterized by their colonial morphology on gonococcal selective media and biochemical tests that are shown in Table 2. It may not be necessary to use all of these tests to identify these species, but all should be used to confirm the identity of an isolate from a systemic infection which is a member of a species that is not typically pathogenic; a limited number of these tests can be used to identify most species. The procedures for performing these tests have been described previously (58, 71). Traditional tests must be inoculated from pure cultures of the isolate and frequently must be incubated for 48 h before the results can be obtained and the organism identified. Although strains from many anatomic sites may be isolated within 24 to 48 h, it may take several days to isolate strains from rectal specimens contaminated by *Proteus* spp. Thus, 48 to 72 h may elapse before the isolate is identified.

With respect to the *Neisseria* and related species, the choice of isolation media and confirmation tests also depends on whether specimens are being obtained to confirm a

clinical diagnosis of gonorrhea or to identify a gram-negative, oxidase-positive diplococcus obtained from a site that is usually sterile. In the first instance, the decision will be further influenced by the patient population from which the specimen will be obtained, that is, whether the patients are at high or low risk for gonorrhea.

Rapid Tests for Identification of *Neisseria* and Related Species

Rapid methods for the identification of *Neisseria* and related species have been developed. The methods currently in frequent use include tests to detect acid production from carbohydrates, chromogenic tests to detect specific enzymes, and serologic tests (71, 73). Recently, deoxyribonucleic acid (DNA) probe tests have also been developed (93). Their applications to *N. gonorrhoeae* detection will be described later.

Acid production tests. Tests that detect acid production from carbohydrates usually consist of glucose, maltose, lactose, and sucrose suspended in buffers and dehydrated for storage. Several products are available for the rapid determination of acid production from carbohydrates. These include the Minitek (BBL Microbiology Systems, Cockeysville, Md.), Quadferm + (Analytab Products, Inc., Plainview, N.Y.), RIM-N (American Micro Scan, Campbell, Calif.), *Neisseria*-Stat (Richardson Scientific, Dallas, Tex.), and *Neisseria*-Kwik (Micro-Biologics, St. Cloud, Minn.) tests. In these procedures, dense suspensions or loops full of organisms are plated into tubes or wells containing the carbohydrates. The tests are incubated according to the directions of the manufacturer, and patterns of acid production can usually be read in 2 to 4 h. Because the tests to detect acid production must be inoculated from a pure culture, a strain may not be identified until 24 h after isolation.

Some *Neisseria* spp. can be identified by their patterns of acid production alone, i.e., without supplemental tests. Other species (for example, *N. perflava*, *N. sicca*, and *N. mucosa*) produce acid from the same carbohydrates. *N. mucosa* strains can be distinguished from *N. perflava* and *N. sicca* by their ability to reduce nitrate. Differentiation between *N. perflava* and *N. sicca* is more difficult. Historically, these species have been differentiated on the basis of colony morphology. Strains of *N. sicca* generally produce nonpigmented colonies that adhere to the agar surface and become wrinkled on prolonged incubation, whereas strains of *N. perflava* generally produce pigmented colonies that are smooth and easily emulsified. These characteristics may not be exclusive to each species, however, but probably will suffice for most until more discriminatory differential characteristics are identified.

Because a test for acid production from fructose generally is not included in commercial rapid test systems, no differentiation is made among the biovars of *N. subflava*, *N. subflava*, *N. flava*, and *N. perflava*. Furthermore, strains of *N. flava* and *N. subflava* as well as strains of *N. polysaccharaea* resemble *N. meningitidis* in acid production tests. Thus, without further examination, strains of these species may be misidentified as *N. meningitidis* (13). Strains of *N. flava* and *N. subflava* should be distinguishable because their colonies are opaque and pigmented. In addition, strains of *N. flava* and *N. subflava* are colistin susceptible and have not been reported to be isolated on gonococcal selective media. When isolated on nonselective medium, colistin susceptibility should be determined with a colistin disk

TABLE 5. Tests for differentiating between *Neisseria* and related species that might be routinely isolated on gonococcal selective medium and misidentified as *N. gonorrhoeae* or *N. meningitidis* with rapid procedures to detect acid production from carbohydrates^a

Acid from:	Species	Additional differential tests
Maltose	<i>N. meningitidis</i>	
	<i>N. polysaccharea</i>	Produces polysaccharide from sucrose
	<i>N. subflava</i> (biovar <i>perflava</i>)	Produces acid from sucrose and polysaccharide from sucrose
Glucose	<i>N. gonorrhoeae</i>	Superoxol positive, colistin resistant
	<i>K. denitrificans</i>	Reduces nitrate; superoxol negative, colistin resistant
	[<i>N. cinerea</i>]	[Superoxol negative, colistin susceptible]
None	[<i>N. gonorrhoeae</i>]	[Superoxol positive, colistin resistant]
	<i>N. cinerea</i>	Superoxol negative, colistin susceptible
	<i>N. flavescens</i>	Superoxol negative, colistin susceptible; produces polysaccharide from sucrose
	<i>B. catarrhalis</i>	Superoxol negative; produces deoxyribonucleases; reduces nitrate

^a Brackets indicate atypical reactions in the primary test.

susceptibility test or by determining the ability of the isolate to grow on gonococcal selective media. Strains of *N. polysaccharea* can be distinguished by determining their ability to produce polysaccharide from sucrose (39).

Some problems may be encountered when rapid tests are used to determine acid production from carbohydrates by *N. gonorrhoeae*. Acid reactions by some strains of *N. gonorrhoeae* and *N. meningitidis* may not be easily interpreted in some tests (99). Some strains of *N. gonorrhoeae*, including those that belong to the AHU auxotype, are weak producers of acid from glucose and appear to be glucose negative (72). In contrast, some strains of *N. cinerea* traditionally considered to be glucose negative (58, 95) have given positive glucose reactions in some rapid tests (18, 34, 63). Studies have shown that strains of *N. cinerea* do produce carbon dioxide from glucose (17). It is possible that these strains produce acid from glucose but rapidly overoxidize it to carbon dioxide; the weak reaction in glucose may be due to the accumulation of a small amount of acid or to acidification caused by the formation of carbonic acid. Irrespective of the cause of this reaction, adequate controls must be used to ensure accurate interpretation of acid production tests from glucose. Additional tests such as the superoxol (30% H₂O₂) test or colistin susceptibility test should be performed to confirm the identification of either *N. gonorrhoeae* or *N. cinerea* when the acid production reactions are equivocal (Table 5).

As noted earlier, strains of *K. denitrificans* also grow on gonococcal selective media, and if they appear to be diplococcal in shape, they may be misidentified as *N. gonorrhoeae* because they also produce acid from glucose (47). Superoxol-negative *K. denitrificans* strains may be distinguished from superoxol-positive *N. gonorrhoeae* strains also by their ability to reduce nitrate (73).

Enzyme substrate tests. Rapid identification procedures for *N. gonorrhoeae*, *N. meningitidis*, *N. lactamica*, and *B.*

catarrhalis have been developed based on the studies of D'Amato et al. (26). Gram-negative, oxidase-positive diplococcal isolates are inoculated into chromogenic substrates that, based on the color change observed, indicate the production of β -D-galactosidase (*o*-nitrophenyl- β -D-galactopyranoside test), γ -glutamylaminopeptidase, or hydroxyprolylaminopeptidase by strains of *N. lactamica*, *N. meningitidis*, and *N. gonorrhoeae*, respectively; strains of *B. catarrhalis* do not produce these enzymes and are presumptively identified if none of the enzymes are produced by the test isolates. Loopfuls of the growth or dense suspensions of isolates are inoculated into tubes (Gonochek II; E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) or onto moistened filter papers containing the substrates (Identicult-*Neisseria*, IDN; Scott Laboratories, Fiskeville, R.I.). The *o*-nitrophenyl- β -D-galactopyranoside test, which identifies *N. lactamica* strains, is easily interpreted. The γ -glutamylaminopeptidase test may be easily interpreted in the Gonochek II test which contains a substrate that turns from colorless to yellow when the enzyme is present. In the Identicult-*Neisseria* test, however, the substrate for the γ -glutamylaminopeptidase test changes from red to purple, a color change that may be subtle and, if interpreted incorrectly, could result in misidentification of strains of *N. meningitidis* and other *Neisseria* spp. as *N. gonorrhoeae* (W. O. Schalla, J. S. Lewis, J. S. Knapp, and J. W. Biddle, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, C267, p. 367). The enzyme substrate tests have been invaluable for differentiating *N. lactamica* from *N. gonorrhoeae* by the *o*-nitrophenyl- β -D-galactopyranoside test and the maltose-negative *N. meningitidis* strains from *N. gonorrhoeae* strains by the γ -glutamylaminopeptidase test (85).

Products that incorporate enzyme substrate tests alone must be used according to the limitations of the manufacturer; that is, only strains isolated on selective media should be confirmed in these tests. It should be noted that some nonpathogenic *Neisseria* spp. (29, 30) also produce hydroxyprolylaminopeptidase and may produce γ -glutamylaminopeptidase, which may result in their being misidentified as *N. gonorrhoeae* or *N. meningitidis* (Table 6). Strains of *N. cinerea* and *K. denitrificans* and colistin-resistant strains of *N. perflava* that grow on gonococcal selective medium, however, produce hydroxyprolylaminopeptidase (30, 34, 54; unpublished data) and will be misidentified as *N. gonorrhoeae* if not confirmed with other procedures. Thus, when strains of these species are isolated on gonococcal selective medium, they may be misidentified as *N. gonorrhoeae* or *N. meningitidis*.

Products such as the Rapid N/H System (Innovative Diagnostic Systems, Inc., Decatur, Ga.), Vitek *Neisseria-Haemophilus* Identification card (Vitek Systems, Inc., Hazelwood, Mo.), and the HNID panel (American Micro Scan, Sacramento, Calif.) combine enzyme substrate tests with other biochemical tests that provide additional characterization of the isolate. The combination of biochemical and enzyme substrate tests has provided accurate identification of not only *Neisseria* spp. but also *Haemophilus* and other species (52) and may permit accurate identification of strains otherwise inaccurately identified with enzyme substrates alone. These products may also be used to identify strains isolated on nonselective media.

Serologic tests for laboratory identification of *N. gonorrhoeae*. Serologic tests for the identification of *N. gonorrhoeae* strains include a direct test to detect gonococcal antigens in specimens (Gonozyne; Abbott Laboratories,

TABLE 6. Enzymes produced by *Neisseria* and related species that may be isolated on gonococcal selective medium and misidentified as *N. gonorrhoeae* or *N. meningitidis*

Enzyme produced	Species	Additional differential characteristics
Hydroxyprolylaminopeptidase	<i>N. gonorrhoeae</i>	Superoxol positive, colistin resistant
	<i>K. denitrificans</i>	Reduces nitrate; superoxol negative, colistin resistant
	<i>N. cinerea</i>	Superoxol negative, colistin susceptible
	<i>N. subflava</i> (biovar <i>perflava</i>)	Produces acid from sucrose and polysaccharide from sucrose
	<i>N. flavescens</i>	Superoxol negative, colistin susceptible; produces polysaccharide from sucrose
	<i>N. polysaccharea</i>	Produces acid from glucose and maltose; produces polysaccharide from sucrose
γ -Glutamylaminopeptidase	<i>N. meningitidis</i>	Produces acid from glucose and maltose; superoxol negative; colistin resistant
No reaction	<i>B. catarrhalis</i>	Superoxol negative; produces deoxyribonuclease; reduces nitrate

North Chicago, Ill.) and coagglutination and fluorescent-antibody (FA) tests for culture confirmation.

The Gonozyne test was developed to directly detect gonococcal antigens in urethral and cervical exudate. Specimens are immersed in a diluent containing plastic beads that absorb gonococci and their antigens. The antigens bound to the bead are labeled with anti-gonococcal antibody conjugated to horseradish peroxidase which, in turn, produces a yellow color when incubated with the substrate. No color develops if gonococci are not present in the specimen. The test is specific and sensitive for gonococci in urethral specimens from men but is less sensitive than cervical culture in women (86). The test may provide a method for processing specimens from patients in geographic areas distant from the clinical laboratory; however, strains of *N. lactamica* and *N. cinerea* have given false-positive reactions. Thus, this test is presumptive and should be limited to detecting *N. gonorrhoeae* in specimens from high-risk patients. A presumptive identification of *N. gonorrhoeae* made by using this procedure should be identified by culture procedures if the results are to be used for medicolegal purposes.

Coagglutination and FA tests use monoclonal antibodies to detect *N. gonorrhoeae* strains in primary or pure cultures. These products generally contain a cocktail of gonococcal protein IA- and IB-specific monoclonal antibodies. Protein I is the major gonococcal outer membrane protein and is expressed by gonococcal strains as either protein IA or IB (but not both); these proteins have different structures and patterns of antigenicity (62). Thus, protein I-specific monoclonal antibody reagents must contain several different antibodies to react with all gonococci. Because the serologic tests can be performed with colonies from the primary isolation plate and do not require the isolation of a pure culture, an isolate may be identified 24 h earlier than is possible with the rapid carbohydrate or enzyme-substrate tests, which must be inoculated with a suspension prepared from a pure culture. The GonoGen (New Horizons Diagnostics, Cockeysville, Md.), the Phadebact Monoclonal GC OMNI reagent (Pharmacia, Rahway, N.J.), and the Meritec-GC reagent (Meridian Laboratories, Cincinnati, Ohio) are coagglutination tests for identifying *N. gonorrhoeae*, whereas the SYVA Microtrak *Neisseria gonorrhoeae* Culture Confirmation Test (SYVA, Palo Alto, Calif.) is an FA reagent that uses monoclonal antibodies.

The GonoGen test is performed on glass slides and viewed in oblique light against a black background. A dense suspension (McFarland no. 3) of the isolate is heated in a boiling-water bath for 10 min, and, after being cooled to room

temperature, a drop of the suspension is spread in marked wells on a glass slide with a negative control and a test reagent. The reaction is interpreted after the antibody-antigen mixture has been rotated for 2 min. Positive and negative control tests should be run with each set of unknown tests. The reaction mixture in the control well should show no agglutination; the results for the test suspension are invalid if agglutination is observed in the control well. If the control well shows no reaction, a positive reaction in the test well confirms that the isolate is *N. gonorrhoeae*.

The Phadebact Monoclonal OMNI test replaced the Phadebact Gonococcus Test, which used polyvalent antibodies, and is performed in a similar manner. Organisms are suspended in 0.7% saline to an optical density equivalent to a 0.5 McFarland standard and heated for 5 min in a boiling-water bath, after which a drop of the cooled suspension is reacted for 1 min with a drop of a control and a test reagent in marked areas on a white card. The agglutinin formed in a positive reaction is blue due to the incorporation of methylene blue in the reagent. The Meritec-GC test is also performed on a white card; red dye is incorporated into the reagents for visualization of the reaction. This test uses a bacterial suspension (optical density = McFarland no. 3) that is boiled for 10 min and reacted with test and control monoclonal antibody reagents. A positive reaction is recorded if a reaction occurs within 1 min with the test reagent but not with the control reagent.

The SYVA monoclonal FA test is performed by preparing a very thin smear from five colonies presumptively identified as gonococci with 5 μ l of distilled or deionized water. The smear is air dried, heat fixed, and immediately stained with 30 μ l of the reagent. The slide is then incubated at 37°C for 15 min, after which excess reagent is removed from the slide without disturbing the smear; the slide is rinsed for 5 to 10 s with a gentle stream of distilled or deionized water, air dried, and sealed under a cover slip with mounting fluid. The slide is viewed under an oil immersion objective of a fluorescent microscope. A positive reaction is recorded if the smear contains fluorescent, apple-green, kidney-shaped diplococci characteristic of *N. gonorrhoeae*. Cells of nongonococcal *Neisseria* spp. should be visible, but will not stain. Comparisons should be made between positive and negative control slides when the reactions of unidentified strains are interpreted.

An FA reagent that uses polyclonal antibodies (Difco Laboratories, Detroit, Mich.) is also available for the identification of *N. gonorrhoeae* strains. This product should be used only for the presumptive identification of *N. gonor-*

rhoeae because not all strains react with the reagent (77, 94) and cross-reactions of the reagent with other *Neisseria* spp. have been reported (40).

Monoclonal antibody diagnostic reagents have generally been highly specific and sensitive for *N. gonorrhoeae* when used to identify isolates from high-risk patients in primary cultures on gonococcal selective media (23, 30, 66, 67, 70, 99) (D. L. Elliman, W. M. Janda, D. Celig, K. L. Ristow, and R. Shone, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, C273, p. 368; J. S. Lewis, J. W. Biddle, M. E. Shepherd, and J. S. Knapp, Abstr. Seventh Int. Meet. Int. Soc. Sex. Transm. Dis. 1987, p. 90). Some problems, however, have been documented with all reagents. Strains of *N. meningitidis*, *N. lactamica*, *N. cinerea*, and *K. denitrificans* have cross-reacted with the GonoGen and Phadebact OMNI reagents (30, 70) (S. S. Barth, C. Tatsch, and S. J. Gibson, Abstr. Annu. Meet. Am. Soc. Microbiol., 1987, C270, p. 368; W. M. Janda, J. M. Stevens, and L. M. Wilcoski, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, C31, p. 337; A. C. Kuritza, C. Chapis, P. Gallo, and S. C. Edberg, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, C33, p. 337; unpublished observations). *N. gonorrhoeae* isolates belonging to the serovar IA-4 isolates failed to react with the GonoGen reagent (70). Because the distribution and frequency of IA-4 isolates may vary geographically and temporally, it is impossible to anticipate where and when these problem isolates may be encountered or to determine their prevalence.

Difficulties have been encountered with the interpretation of the Phadebact OMNI reagent test. After cross-reactions with nongonococcal isolates were observed, it was recommended that suspensions be prepared to an optical density equivalent to a 0.5 McFarland standard and that the saline in which the suspensions were prepared be pH 7.0 to 7.5. Carlson et al. (23) found that saline at pH 7.4 was optimal for the preparation of suspensions. It was further recommended that reactions of $\pm++$ be interpreted as equivocal and that the identity of organisms giving this reactions be confirmed by other tests. We have noted that some *N. gonorrhoeae* isolates give equivocal reactions under these test conditions (unpublished observations). At the time of writing, the guidelines for the interpretation of this test have not been clarified.

Nongonococcal *Neisseria* spp. have not reacted with the Microtrak FA reagent (30, 66, 99). Occasionally, however, strains of *N. gonorrhoeae* have not reacted in this test (Lewis et al., Abstr. Seventh Int. Meet. Int. Soc. Sex. Transm. Dis. 1987). Nonspecific Fc binding of the FA reagent to other bacterial species, including some strains of *Staphylococcus aureus*, has been noted. As a result, laboratorians have been notified that they should test only gram-negative, oxidase-positive organisms isolated from gonococcal selective media with the Microtrak reagent. Similarly, it is possible that cross-reactions between the coagglutination reagents and nongonococcal isolates may also result from Fc binding of the reagent to other organisms.

New Technologies for Identification of *N. gonorrhoeae*

Nucleic acid probes. Recently, DNA probes for the identification of *N. gonorrhoeae* strains have been developed. The ORTHOProbe (Ortho Diagnostics, Carpinteria, Calif.) detects chromosomal sequences in gonococcal strains with a biotinylated DNA probe (Y. A. Jean Louis and R. J. Rice, Program Abstr. 27th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 721, 1987; Kuritza et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1988). This test was highly

specific and highly sensitive when colonies from 24-h cultures were tested; it was less sensitive, however, when older cultures were tested. Nongonococcal isolates may be identified as *N. gonorrhoeae* if medium components are introduced into the test.

Chemiluminescent tests with DNA probes corresponding to gonococcal ribosomal ribonucleic acid or single-stranded oligonucleotides of analyte DNA have been developed to detect gonococci (M. E. Harper, J. S. Lewis, K. H. Mayer, S. M. Opal, J. J. Hogan, C. L. Milliman, V. Jonas, R. S. Bhatt, D. L. Kacian, and the Neisseria Development Team, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, C35, p. 337.; M. E. Harper, C. Gonzales, M. S. You, C. V. Gegg, D. Kranig-Brown, Y. Y. Yang, R. A. Respass, and P. R. Roeder, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, C36, p. 338.; J. Kolberg, D. Besemer, M. Stempien, and M. Urdea, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, C38, p. 338).

Few evaluations have been performed with the DNA probe tests; thus, problems associated with the tests, such as false-positive and -negative reactions with *N. gonorrhoeae* and other *Neisseria* and related species, have not been encountered. If problems are encountered with probe tests, it may be anticipated that there will not be correlations between these and problems that have been encountered with other technologies currently used. The DNA probe tests have been evaluated as culture confirmation tests but may offer a rapid direct test for *N. gonorrhoeae* if they can detect gonococci in specimens.

Interpretation of Results

As outlined above, problems have been encountered with all tests for the identification of *N. gonorrhoeae* strains. Few problems have been encountered when nongonococcal *Neisseria* spp. are identified (30) because these isolates generally give unequivocal results in biochemical tests. Evaluations of new products, however, have generally been conducted in high-risk patient populations and have frequently included only the pathogenic and related species, *N. gonorrhoeae*, *N. meningitidis*, *N. lactamica*, and *B. catarrhalis*, and a few strains of commensal *Neisseria* spp. Unfortunately, test strains of the sucrose-positive *N. subflava* biovar *perflava* evaluated in these studies are easily distinguished from other species; strains of the sucrose-negative biovars have rarely been evaluated (49). Until recently, strains of *N. cinerea* have not been routinely included in evaluations, and their importance has been challenged because they are infrequently isolated on gonococcal selective medium. The specificities and sensitivities of procedures evaluated in high-risk patient populations are valid only for these populations and cannot be extrapolated to low-risk populations in which nongonococcal *Neisseria* spp. may be encountered less frequently. Laboratorians must keep this in mind when selecting and interpreting the results of rapid tests for the identification of strains isolated from unusual specimens or on nonselective media.

Incorrect identification of nongonococcal isolates as *N. gonorrhoeae* have resulted from the misuse of products. Laboratories must follow the manufacturers' instructions carefully and observe the limitations recommended for products. It is important that testing of isolates be limited to gram-negative, oxidase-positive diplococci, and some products should be used only to identify strains isolated on gonococcal selective media and not those isolated on nonselective media.

Test results must be interpreted with most caution when the strain is an oropharyngeal isolate because this is the normal habitat for commensal *Neisseria* spp. (59). Strains of *N. lactamica* may be isolated more frequently from children than from adults (12, 37, 59) and may be misidentified as *N. gonorrhoeae* if inappropriate tests are used exclusively for their identification (34, 54, 100). Similarly, strains of *N. meningitidis* may also be normal flora in the oropharynges of adults and children (12, 37, 59). *N. cinerea* has been isolated frequently from the oropharynx of both adults and children (59; unpublished data), and, occasionally, strains have cross-reacted weakly with gonococcal monoclonal antibody reagents (30). In instances that may have medicolegal implications (that is, when a presumptive diagnosis of gonorrhea is made in a child), it is important that the isolate be purified and the identification confirmed by using several tests based on different principles, for example, an acid production and a rapid enzyme or serologic test, before the isolate is confirmed as *N. gonorrhoeae*. The strain should also be stored for further study or sent to a reference laboratory for confirmation (100).

It is important that the growth characteristics of the organisms be considered when the results of serological diagnostic tests are interpreted. When the cultural characteristics and the serologic test results are not consistent with an identification of *N. gonorrhoeae*, the isolate should also be tested in procedures that use different diagnostic principles, for example, acid production and serologic or enzyme substrate tests.

Mules, Horses, or Donkeys

In recent years, several unusual strains of gram-negative, oxidase-positive diplococci have been isolated that are obviously neisserias but cannot be clearly identified as a recognized species (43, 51, 69). The strains isolated by these investigators exhibited characteristics of *N. gonorrhoeae* and *N. meningitidis* and in some cases could not be unequivocally assigned to either species (43, 73). The strains isolated by Hodge et al. (43) have been rarely isolated. In contrast, the strains isolated from conjunctivitis in rural Egypt (69) are prevalent in this geographic area and were assigned the subspecific epithet *kochii*.

Although *N. flavescens* has been recognized as a species since 1939 (74) and reported by other investigators (78, 89, 98), the only confirmed isolates of this species are those isolated by Sara Branham (20); others are thought now to have been strains of *N. cinerea* (63). *N. flavescens* is clearly phenotypically different from other *Neisseria* spp., but its relationship to other species is not clear. In some respects, *N. flavescens* is an asaccharolytic member of the saccharolytic group (6); it is a pigmented, polysaccharide-producing species more closely related to the *N. gonorrhoeae* group (35, 44).

Thus, occasionally unusual strains may be isolated and it will be easy in some instances (20) to distinguish them from other *Neisseria* spp. However, it will be impossible to unequivocally identify strains such as those isolated by Mazloun et al. (69) and Hodge et al. (43) because they are clearly hybrids between species that are themselves closely related genetically (39, 44). Although it is easy to understand the frustration expressed by Ehret and Judson (35), hybrid strains are mules and cannot be turned into horses or donkeys! These strains must be extensively characterized and should probably be reported to the clinician as "unusual *N. gonorrhoeae*/*N. meningitidis*" or "hybrid *N. gonor-*

rhoeae/*N. meningitidis*," unless certain confirmatory tests are given more importance than others in determining the identification of the strain for clinical purposes. It should be clearly understood, however, that any such prioritization may have no taxonomic status.

SUMMARY

The pathogenic *Neisseria* spp., *N. gonorrhoeae* and *N. meningitidis*, have been studied extensively and rapid identification procedures have been designed to distinguish these species from the commensal *Neisseria* and related species that are normal flora of the oro- and nasopharynx. The commensal *Neisseria* spp. have been largely ignored except for isolated studies. It is important that we know about these species, however, because not only may some be misidentified as pathogenic species if identified with inappropriate procedures, but also they may occasionally be isolated from unusual sites and must be correctly identified to the species level for clinical purposes.

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