

Branhamella catarrhalis: an Organism Gaining Respect as a Pathogen

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INTRODUCTION	293
PATHOGENIC POTENTIAL OF <i>B. CATARRHALIS</i>	294
Critique of Sampling Procedures	294
Importance of Adequate Descriptions of Isolates	295
Bacteremia, Endocarditis, and Meningitis	295
Otitis Media, Sinusitis, and Keratitis	295
Pseudogonococcal Conjunctivitis and Urogenital Infections.....	297
Lower Respiratory Tract Infections.....	297
Analogy between <i>B. catarrhalis</i> and <i>N. meningitidis</i> Infections	298
Healthy Carriers of <i>B. catarrhalis</i>	298
Isolation rates.....	298
Selective culture media for <i>B. catarrhalis</i>	299
Virulence.....	299
Immunity	300
IDENTIFICATION AND BIOCHEMICAL CHARACTERISTICS OF <i>B. CATARRHALIS</i>	301
Differential Tests.....	301
Action on carbohydrates	301
Reduction of nitrate and nitrite	301
Hydrolysis of DNA	301
Hydrolysis of tributyrin and other esterase substrates	302
Hydrolysis of aminopeptidase substrates.....	302
Response to superoxol (30% H ₂ O ₂)	302
Other differential tests.....	302
Stages in the Identification Process.....	303
Strain Differentiation: Typing	303
Restriction endonuclease analysis of DNA	303
OMP and LOS analyses.....	303
SUSCEPTIBILITY TO ANTIBACTERIAL AGENTS	303
Non-β-Lactam Agents	303
Unequal Susceptibility of β-Lactamase-Negative Strains to β-Lactam Agents	304
β-Lactamase-Positive Strains	305
Detection methods	305
Prevalence	305
Problems in determination of resistance to β-lactam antibiotics.....	305
Genetic features	307
Possible influence in mixed infections: indirect pathogenicity	308
Characteristics of the β-Lactamases	308
STRUCTURE AND COMPOSITION OF <i>B. CATARRHALIS</i>	309
Capsule and Pili.....	309
Envelope	309
DNA.....	311
WHAT'S IN A NAME?	311
CONCLUSIONS AND PROSPECTS	312
ACKNOWLEDGMENTS	312
LITERATURE CITED	312

INTRODUCTION

Branhamella catarrhalis received little respectful attention until recently. Strains isolated from clinical material were often disregarded because of the repeated, confident assertion that the organism is nonpathogenic and commonly

present in the normal pharynx (117, 209, 232, 316). Consequently, medical technologists usually elected not to identify organisms referred to as nongonococcal, nonmeningococcal neisseriae or normal flora (58, 221, 248, 262, 268). In a discussion during a symposium on *B. catarrhalis* held in 1985, C. D. Bluestone [Drugs 31(Suppl. 3):38, 1986] stated his impression that, in non-university hospitals in the United States, "the organism is still frequently called *Neisseria catarrhalis* and is not considered pathogenic." On the other

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TABLE 1. Chronology of differential biochemical tests developed for *Neisseria* spp. and *B. catarrhalis*

Test	Date	Authors (reference)
Acid production from carbohydrates in		
Rich basal medium	1905	Dunn and Gordon (108)
Low-concn peptone medium	1983	Knapp and Holmes (178)
Buffered salts solution	1970	Catlin (61)
Polysaccharide synthesis from sucrose	1948	Hehre and Hamilton (142)
	1961	Berger (20)
Nitrate and nitrite reduction	1959	Véron et al. (308)
	1961	Berger (22)
Butyrate esterase action on		
Tributyrim		
Reduced opacity in agar	1962	Berger and Paepcke (25); Pike et al. (236)
Acid production in fluid	1981	Riou et al. (254)
4-Methylumbelliferyl butyrate	1988	Vanechoutte et al. (301)
Indoxyl butyrate	1989	Dealler et al. (88)
Deoxyribonucleate hydrolysis	1964	Catlin and Cunningham (69)
	1977	Riou and Guibourdenche (256)
γ -Glutamyl- β -naphthylamide hydrolysis	1967	Mannheim and Bürger (199)
	1978	D'Amato et al. (84)
<i>o</i> -Nitrophenyl- β -D-galactopyranoside hydrolysis	1968	Corbett and Catlin (80)
Superoxol (30% H ₂ O ₂) decomposition	1982	Saginur et al. (266)

hand, a developing appreciation of the pathogenic potential of *B. catarrhalis* and concern over the increasing prevalence of β -lactamase-producing (β la⁺) strains in clinically significant infections are reflected in numerous recent reports. These are summarized in several valuable reviews (e.g., see references 140, 158, 305, and 321). The 1989 symposium, "*Branhamella catarrhalis*: a Microbiological and Clinical Update," illustrates the rising interest in this organism.

Originally described in German as *Mikrokokkus catarrhalis* by R. Pfeiffer (cited by Frosch and Kolle [125]) and then in English as *Micrococcus catarrhalis* (e.g., see reference 108), the species was subsequently classified in the genus *Neisseria* (26, 41, 149). In 1970, it was transferred to the new genus *Branhamella* (60), named for Sara E. Branham. Her studies (described in a biographical sketch by Pittman [239]) greatly contributed to knowledge of the neisseriae. *B. catarrhalis* was accepted in the 1974 edition of *Bergey's Manual* (245), but was reduced to a subgenus within the genus *Moraxella* in the 1984 edition of *Bergey's Manual* (37). My reasons for retaining the name *Branhamella catarrhalis* will be discussed in a later section. In describing the numerous studies of this organism, however, I shall use the name that was given to the bacteria in each report.

B. catarrhalis has been regarded as one of the commonest inhabitants of the pharynx (195). A current textbook (324) estimates its presence as 12% in the nose and nasopharynx and 10 to 97% in the oropharynx. Early studies had found "*Micrococcus catarrhalis*" in 33% of nasal cultures (9), 18% of nasopharyngeal cultures (112), and 46% of nose and throat cultures from healthy persons and 45% of subjects with the common cold (130). "*M. catarrhalis*" was recognized as a gram-negative diplococcus, capable of growth at 22°C, which displays certain well-described colony characteristics and has no action on "fermentable substances" (9, 108, 112).

Significant advances in our understanding of *B. catarrhalis* awaited the development of additional tests (Table 1). These enabled Berger (23, 25) to show that the common, nonsaccharolytic, gram-negative cocci comprised two distinct species, *Neisseria cinerea* and "*N. catarrhalis*." Described in German and later in French (256), *N. cinerea* usually was overlooked until 1984, when it was further characterized by Knapp et al. (180). Two studies of bacteria isolated from throat cultures on semiselective media showed that the

ratios of *N. cinerea*/*B. catarrhalis* were 26:1 (23) and 6:1 (179). Accurate species identification together with the application of methods for typing strains and determining their virulence are needed in further studies of carrier and case isolates.

The intent of this review is to provide a critical evaluation of *B. catarrhalis* and its relation to disease, susceptibility to antibiotics, basic biological and differential features, and areas of fundamental research in need of further investigation.

PATHOGENIC POTENTIAL OF *B. CATARRHALIS*

Virulence is not an exclusive characteristic of the bacterium, but involves bacterium-host interactions. The process of infection starts with bacterial attachment to mucosal cells and may proceed through several distinguishable steps, possibly ending in dissemination (204, 285). The outcome depends on numerous variables, such as the number and characteristics of bacteria and nonspecific and specific immune defenses. Immunodeficiency states and certain chronic debilitating diseases are predisposing factors. My evaluation of the pathogenic potential of *B. catarrhalis* considers the methods used for collecting and processing specimens and bacterial identification as well as clinical observations.

Critique of Sampling Procedures

The early view that *B. catarrhalis* has no significance in bronchopulmonary infections is due in part to a failure to appreciate that expectorated sputum is contaminated with indigenous pharyngeal bacteria. Transtracheal puncture was introduced as a procedure for collecting uncontaminated material from the lower respiratory tract (127, 158, 224, 270), but it causes patient discomfort. Therefore, Geckler et al. (127) and Aitken and Thornley (5) compared transtracheal aspirates with good-quality sputum specimens plated quantitatively and found good agreement between the results. It is essential to prepare and Gram stain a smear of the expectorated sputum and observe the numbers of host cells and bacteria. Good-quality specimens contain at least 25 leukocytes and no more than 10 squamous epithelial cells per

low-power ($\times 100$ magnification) field (269). The presence of more than 25 squamous epithelial cells indicates that a specimen is unsatisfactory since these cells are derived from oropharyngeal mucous membranes (73, 127, 241). The sputum is liquefied with buffered *N*-acetyl-L-cysteine (28, 211), dithiothreitol (5), or Sputasol (Oxoid Ltd., London, England) (200, 279) and mixed to permit a uniform sampling. For research purposes, many workers then prepare serial dilutions for plating on agar medium since they attach etiological significance to *B. catarrhalis* when its numbers of CFU are $>10^7$ /ml (28, 93, 211, 218, 267) or 10^8 /ml (5, 200, 277).

Middle-ear fluid (MEF) obtained by tympanocentesis has been the preferred specimen for research on otitis media because the tympanic cavity is normally sterile, but this invasive procedure is not done routinely in pediatric practice (272, 274). Appropriate nasopharyngeal cultures (but not throat cultures) in which *B. catarrhalis* constitutes the majority of colonies were considered by Kamme et al. (168) and Schwartz et al. (272) to be of some help in predicting the middle-ear pathogen in acute otitis media. For maxillary sinus infections, on the other hand, the results of nasopharyngeal cultures from children (309) or nasal cultures from adults (46, 163) were of little predictive value; the reliability compared with sinus aspirates was 20% for *B. catarrhalis* (163).

Importance of Adequate Descriptions of Isolates

Chapters in the 1974 editions of three widely used manuals (62, 82, 245) for the first time listed nitrate reduction as a characteristic of *B. catarrhalis*. This test and others developed more recently are needed to differentiate *B. catarrhalis* from other species of gram-negative cocci, such as *N. cinerea*, that do not form acid from carbohydrates.

The characteristics of the isolates from the first five cases of systemic disease listed in Table 2 were compatible with "*N. catarrhalis*" as it was known, but did not include nitrate reduction. For these strains and for others reported in later papers that did not specify the differential tests performed, or at least cite a pertinent reference, I have noted that a particular characteristic was not stated. Such strains are referred to in this review as presumptively identified *B. catarrhalis* since the descriptions were insufficient to establish that the identifications satisfy the criteria currently accepted for determining the species.

This critical approach probably underestimates the percentage of strains adequately characterized in the laboratory, but focuses attention on the necessity that published reports provide sufficient detail to confirm the identification.

Bacteremia, Endocarditis, and Meningitis

At least 21 cases of meningitis (cited in references 77, 113, 126, and 287) and 1 case of endocarditis (76) which occurred before 1965 were caused by nonsaccharolytic, gram-negative diplococci resembling "*N. catarrhalis*." Confirmed *B. catarrhalis* strains were isolated from patients with bacteremia (13, 50, 72, 98, 140, 197, 218, 268) and endocarditis (300), and presumptively identified strains were recovered from other patients with systemic disease (Table 2). Six of the isolates were said to be penicillin resistant (13, 242, 268) and seven produced β -lactamase (11, 72, 75, 98, 138, 140, 306). Identical characteristics of the isolates from two or more relevant specimens established their role in the diseases afflicting at least 17 of these patients. Suppurative arthritis in a 3-month-

old girl which affected two joints, knee and hip, was caused by a penicillin-susceptible strain (confirmed by nitrate reduction and DNase production) (151); dissemination of the bacteria through the bloodstream probably occurred, although a blood culture was sterile. Similarly, a hematogenous route of infection was presumed for a case of acute purulent pericarditis in a 65-year-old man (183). A case of *B. catarrhalis* septicemia with disseminated intravascular coagulation leading to death in 1983 was cited by McLeod et al. (205).

Petechial rash, ecchymoses, or purpura fulminans (indicated by P in Table 2), occurred in nine children. These cutaneous manifestations are characteristic of meningococcal septicemia (illustrated in Youmans et al. [324]). Two children with *B. catarrhalis* bacteremia, including one who developed purpura, did not appear to be seriously ill and their bacteremia was unsuspected (13).

Immunodeficiency/suppression or neutropenia was conductive to many of these infections (11, 31, 50, 52, 72, 75, 98, 144, 268). Septicemia in an infant with acquired immunodeficiency syndrome was caused by *B. catarrhalis* (presumptive identification) (320). Other predisposing conditions mentioned were neurosurgery (113), gynecological surgery (105), inguinal hernia surgery (218), otolaryngological surgery (219), aortic prosthesis and dental extractions (242), hemodialysis (300), chronic obstructive pulmonary disease (140, 287), and mouth-to-mouth resuscitation and type A influenza (306). No predisposing condition was evident in seven children (13, 31, 75, 77, 118, 232) or two adults (138, 197).

Otitis Media, Sinusitis, and Keratitis

"*Neisseria* sp." (not identified) was considered a sufficient identification of strains isolated from MEF from 15 children in a study published in 1979 (272) and from 18 children in a 1982 report (129). By 1985, however, a textbook (324) could state that *B. catarrhalis* is "convincingly associated" with acute suppurative otitis media and sinusitis.

Evidence for this comes from bacteriological studies of MEF obtained by needle aspiration through the tympanic membrane. Many cultures yielded "*N. catarrhalis*" (78, 135, 141, 166, 168) and *B. catarrhalis* (30, 184, 188, 189, 194, 265, 274, 290, 305, 310). The organism also was isolated from aspirates obtained by puncture of the maxillary sinus (30, 46, 163, 225, 309).

The identity of *B. catarrhalis* was confirmed by nitrate reduction or DNase production by the strains reported in eight of the foregoing studies (46, 163, 166, 168, 188, 225, 274, 305), but the other identifications were presumptive. The middle ear and sinuses normally are sterile. Cultures of the external auditory canal did not contain *B. catarrhalis* (141, 166, 168, 184, 274), thereby excluding this site as a likely source of MEF specimen contamination (189). External auditory canal cultures from 4 of 72 children were reported as "*Neisseria* sp." (43); these isolates which had not been identified further were erroneously listed as *B. catarrhalis* in a later review (44).

Additional evidence of the pathogenic role of *B. catarrhalis* in many of these infections was the observation of gram-negative diplococci occurring intracellularly in polymorphonuclear leukocytes in MEF. Also, immunoglobulin A (IgA) class antibodies specific for *B. catarrhalis* were detected in MEF and in sera from some children with otitis media (189).

Otitis media is the most frequent diagnosis made by physicians who provide health care for children (30, 274).

TABLE 2. *B. catarrhalis* strains (presumptive or confirmed) isolated from patients with systemic disease

Yr ^a	Ref- erence	Patients		Features of infection ^b	Positive specimen (no.) ^c	Characteristics ^d				
		Age	No.			GN diplo- cocci	Oxidase positive	Nonsac- charo- lytic	NO ₃ reduction to nitrite	DNase pro- duced
1965	232	2.5 yr	1	Bacteremia, meningitis, P	Blood, CSF, throat	Yes	Yes	Yes	NS	NS
1967	77	14 mo	1	Meningitis, P	CSF, throat	Yes	Yes	Yes	NS	NS
1969	118	6 mo	1	Septicemia, meningitis, P	Blood, CSF, bone marrow	Yes	Yes	Yes	NS	NS
1970	113	36 yr	1	Meningitis, purulent rhinorrhea	CSF, nasal pus	Yes	Yes	Yes	NS	NS
1974	52	8 yr	1	Septicemia, polyarthropathy, P	Blood, skin lesion	Yes	Yes	Yes	NS	NS
1976	242	69 yr	1	Endocarditis	Blood (7)	NS	NS	NS	NS	NS
1976	105	45 yr	1	Endocarditis	Blood (6)	NS	NS	NS	NS	NS
1980	218	71 yr	1	Pneumonia	Blood	Yes	Yes	Yes	Yes	NS
1980	98	19 mo	1	Bacteremia, P	Blood (6)	Yes	Yes	Yes	Yes	NS
1980	287	64 yr	1	Bacteremic pneumonia	Blood (2), ETS	Yes	NS	NS	NS	NS
1983	50	3 yr	1	Septicemia, P	Blood, CSF, PF, lung ^e	NS	NS	Yes	Yes	Yes
1983	197	19 yr	1	Bacteremic pneumonia	Blood	Yes	Yes	Yes	Yes	NS
1983	11	3.5-4 yr	3	Bacteremia	Blood	NS	NS	NS	NS	NS
1983	144	24 yr	1	Septicemia, sinusitis, subcutaneous nodules	Blood (3)	NS	NS	NS	NS	NS
1976-1985 ^f	268	31 mo	1	Septicemia, P	Blood	Yes	Yes	Yes	Yes	NS
1976-1985 ^f	268	15, 39, 41 yr	3	Septicemia	Blood	Yes	Yes	Yes	Yes	NS
1985	300	62 yr	1	Endocarditis	Blood (2)	Yes	Yes	Yes	Yes	Yes
1985	13	11 mo	1	Bacteremia, tonsillar exudate, P	Blood	Yes	Yes	Yes	Yes	Yes
1985	13	4 yr	1	Bacteremia, sinusitis	Blood	Yes	Yes	Yes	Yes	Yes
1985	72	69 yr	1	Bacteremia	Blood	Yes	Yes	Yes	Yes	NS ^g
1985	306	2.5 mo	1	Bacteremia	Blood	NS	NS	NS	NS	NS
1987	140	69 yr	1	Bacteremic pneumonia	Blood, ETS	Yes	Yes	Yes	Yes	Yes
1977-1988 ^f	31	1-36 mo	5	Bacteremia	Blood	NS	NS	NS	NS	NS
1988	138	40 yr	1	Septicemia	Blood (3)	NS	NS	NS	NS	NS
1988	219	5 yr	1	Bacteremia, meningitis	Blood, CSF	Yes	NS	NS	NS	NS
1989	75	4 yr	1	Pneumonia, otitis media, P	Blood	NS	NS	NS	NS	NS
1989	75	7 yr	1	Tracheitis, bacteremia	Blood, ETS	NS	NS	NS	NS	NS

^a Date of isolation given in text or estimated from the date manuscript was submitted or published.

^b P, Petechiae and/or purpura or ecchymoses.

^c Number of blood cultures yielding bacterial growth is given in parentheses. CSF, Cerebrospinal fluid; ETS, endotracheal secretion; PF, pleural fluid.

^d GN diplococci, Gram-negative diplococci observed; NS, not stated.

^e Specimens from all sites yielded mixed cultures of *B. catarrhalis*, the predominant organism, and *H. influenzae*.

^f Patient(s) identified by reviewing records for the period indicated.

^g Tributyrin hydrolysis was positive (a test substituted for DNase production).

Middle-ear infections are usually caused by microorganisms that originate in the nasopharynx, traverse the Eustachian tube, and invade the tympanic cavity. Recent studies of isolates from MEF show that *B. catarrhalis* is the third most common species, behind *Streptococcus pneumoniae* and *Haemophilus influenzae* (30, 189, 194, 265, 305, 310).

In 1967, Coffey et al. (78) emphasized that "*N. catarrhalis*" shares with pneumococci and *H. influenzae* the ability to invade the tympanic cavity. This insight was based on finding pure cultures in MEF from 49 (7%) of 698 children (mean age, 1 year 10 months) studied during a 2-year period ending in June 1966. Mixed cultures were isolated in three other cases. The otitis media was bilateral in 35% of the 49 children. Smears of 23 (47%) of the MEF specimens revealed gram-negative diplococci, either extracellular or intracellular in polymorphs. Recent observations indicate that the size of the population in MEF exceeds 10⁴ CFU/ml when bacteria can be seen in a smear (310). Stenfors and Räsänen (290) developed a method of quantitative microscopy and demonstrated that the bacterial content was 2.6 × 10⁷/ml (median value) in MEF characterized as mucopurulent, but only 2.0 × 10⁵/ml in "sticky glue"-type effusion.

Prior to 1980 the reported frequency of *B. catarrhalis* in cultures of MEF obtained from children with acute otitis media was <10% (78, 135, 166, 168, 189, 274, 305). Unac-

countably, a sharp increase in the percentage was found in a study conducted by Shurin et al. (274) in Cleveland, Ohio, between October 1980 and February 1982. Strains, confirmed by nitrate reduction and DNase activity, were isolated in pure culture from MEF obtained from 20 (20%) of 98 children. In a 2-year extension of this study (305), *B. catarrhalis* was identified in MEF from 15% of children with otitis media and was more common in fall and winter (20%) than in spring and summer (11%; *P* < 0.05). The frequencies of the organism in positive MEF cultures examined in other American studies after 1981 were 14% (310), 16% (30), and 19% (184).

On the other hand, in a Finnish study of acute otitis media, Ruuskanen et al. (265) presumptively identified *B. catarrhalis* in 7% of 3,332 MEF cultures examined over a 6-year period, 1980 to 1985. This was 12.5% of all MEF cultures that showed bacterial growth. Coexisting viral infection, especially with respiratory syncytial virus, was detected in children with otitis media (265). A Swedish study of acute otitis media revealed pure cultures of *B. catarrhalis* in 9% of MEF cultures from 75 children; 43% of the accompanying nasopharyngeal specimens from these children contained presumptive *B. catarrhalis* (194).

The cause of keratitis reported in 11 patients since 1980 was attributed to *B. catarrhalis* (196).

Pseudomonococcal Conjunctivitis and Urogenital Infections

A case of conjunctivitis in a 5-day-old girl was described in 1987 by Romberger et al. (260). A strain isolated from the purulent eye discharge was reported as *N. gonorrhoeae* resistant to penicillin. The diagnosis of gonorrhea was reported to the public health department and the parents were subjected to intramuscular injections of cefotaxime. This strain subsequently was identified as *B. catarrhalis* by a state laboratory and the Centers for Disease Control in Atlanta, Ga. The stressful implications of the erroneous identification can be imagined.

Historically, ophthalmia neonatorum was attributed to *N. gonorrhoeae*, but the identification often was based solely on the characteristics of bacteria seen in Gram-stained preparations of conjunctival exudate (248, 284, 297). *B. catarrhalis* (confirmed by nitrate reduction) as a cause of ophthalmia neonatorum was first reported by Spark et al. (284) in 1979 in an infant with fever of 3-week duration and a conjunctivitis that was partially suppressed by short-term parenteral antibiotic therapy. Righter and Nicol (248) described another case of conjunctivitis in an infant who received only ineffective topical therapy for months because cultures of purulent discharge from both eyes were reported as growing normal flora, not *N. gonorrhoeae* or *N. meningitidis*. Thirteen additional cases of conjunctivitis in infants were reportedly caused by *B. catarrhalis* (cited in references 260, 297, and 317). Furthermore, a confirmed strain was isolated from conjunctival discharge obtained from a previously healthy, 58-year-old woman with acute bilateral conjunctivitis (172).

Confirmed *B. catarrhalis* strains were isolated from urethral exudates from four men with urethritis (96, 278) and from urine specimens collected 5 days apart from a woman with chronic pyelonephritis (153). The organism was the only pathogen isolated from a urinary tract infection in a 13-year-old girl (2). Strains provisionally identified as "*N. catarrhalis*" were recovered from four anal specimens cultured on selective medium (29). Johnson (158) cited additional cases of urogenital infections associated with "*N. catarrhalis*" and pointed out that their pathogenic role remained speculative because of the possibility of infection with *Chlamydia trachomatis* or *Ureaplasma urealyticum* that would not have been detected by conventional bacteriology.

Lower Respiratory Tract Infections

Pneumonia, acute exacerbations of chronic bronchitis, tracheitis, and empyema are caused by *B. catarrhalis*. The isolates were regarded as primarily opportunistic in many cases. The factors predisposing to infection include underlying disease of the bronchial tree or lung parenchyma, especially chronic obstructive pulmonary disease, and an immunodeficiency state due to chemotherapeutic agents or abnormalities involving the cellular or humoral immune systems (58, 134, 140, 277). Thus, *B. catarrhalis* pneumonia was reported in patients with multiple myeloma (91, 209), chronic lymphocytic leukemia (287), or quantitative abnormalities of serum immunoglobulins (171, 192). Advanced age appears to be a factor, possibly due to the significant reductions of IgG and IgM seen after age 60 years (51). Viral damage to respiratory tract epithelium, for example, by respiratory syncytial virus infection (6, 319), may promote invasion by *B. catarrhalis*. A high risk of aspiration from the oropharynx has been identified as a predisposing factor in some patients (259, 277).

B. catarrhalis pneumonia may present a clinical picture similar to other bacterial pneumonias, but generally is a milder illness except in the immunocompromised patient (321). An indication of its relative mildness is found in reports that most blood cultures are negative (58, 85, 118, 209, 221, 241, 259, 313).

The earlier reluctance to accept a pathogenic role for *B. catarrhalis* has diminished in view of evidence of its presence in pure cultures in sites that normally are sterile. Thus, it has been isolated from pericardiocentesis fluid aspirated from a patient with acute pericarditis (183). Pleural effusion specimens obtained by thoracentesis from each of two patients with empyema yielded *B. catarrhalis* on two occasions (83, 313). The organism was isolated from transtracheal or endotracheal aspirates from 47 patients with pneumonia or acute bronchopulmonary infections (5, 73, 99, 171, 173, 192, 209, 224, 270, 279, 287, 316). Sections of lung tissue from two patients who died with untreated pneumonia revealed intracellular and extracellular gram-negative diplococci (209, 277), and *B. catarrhalis* was isolated in pure culture from postmortem lung secretions (277). Furthermore, elevated antibody titers were detected in convalescent sera from patients with *B. catarrhalis* bronchopulmonary infections (28, 71, 197).

A sputum culture usually yields clinically significant results if the specimen is of good quality and is inoculated by using quantitative methods (described above). Nevertheless, mixed cultures of *B. catarrhalis* with *H. influenzae* or pneumococci or both are isolated from substantial numbers of sputum specimens (73, 200, 241, 277). Some authors questioned the role of *B. catarrhalis* as a respiratory pathogen in cases of mixed infection with acknowledged pathogens (134, 259). Others omitted such cases from consideration (207, 279). As expected, sputa from patients with aspiration pneumonia often yielded mixed cultures typical of oropharyngeal flora (259).

Seeking further evidence of the clinical significance of *B. catarrhalis* in bronchopulmonary infection, Yuen et al. (328) conducted a case control study of 26 patients ("cases") whose expectorated sputum yielded pure cultures of confirmed *B. catarrhalis* and patients ("controls") whose sputum cultures contained only commensal *Neisseria* spp. (DNase negative). Proportionately more cases than controls ($P < 0.05$) showed three or more clinical features of bronchopulmonary infection (cough with increased purulence of the sputum, fever of $>37.4^{\circ}\text{C}$, leukocytosis, chest X-ray signs, and crepitations and/or dullness of the chest). Furthermore, intracellular gram-negative diplococci were observed in 13 of the 26 sputum specimens containing *B. catarrhalis* and none were detected in the control sputa.

Lower respiratory tract infections caused by *B. catarrhalis* in children tend to differ from those in adults by the absence of underlying respiratory disease (200). Thus, *B. catarrhalis* bronchopneumonia developed in three children who were intubated and on mechanical ventilation following elective surgery or trauma (173) and also was reported in a 15-day-old, prematurely born infant whose lungs had appeared normal 5 days earlier (228). On the other hand, preexisting lung disease was recognized in five children, 6 weeks to 6 months of age, who suffered from *B. catarrhalis* pneumonia. All had been born prematurely, entered a hospital in Phoenix, Ariz., during winter months, and experienced precipitous clinical deterioration requiring emergency intubation and mechanical ventilation (19).

Tracheitis caused by *B. catarrhalis* developed in a previously healthy 11-month-old girl (137) and also in a 4-year-old

girl following local injury to the trachea by a cuffed endotracheal tube (114). Epithelial damage by respiratory syncytial virus infection was considered a predisposing factor in the development of *B. catarrhalis* tracheitis in a 9-year-old boy (319) and a 6-month-old boy (6).

A nonhealing, thoracic, surgical wound infection was described by Gray et al. (132) in a 59-year-old woman who had undergone an open lung biopsy procedure. *B. catarrhalis* was isolated from deep thoracotomy wound specimens as well as from tracheal secretions and sputum, and smears of each revealed numerous polymorphonuclear leukocytes and intracellular and extracellular gram-negative diplococci.

The peak incidence of *B. catarrhalis* respiratory infections occurs in winter months. This was found south of the equator in Western Australia (93) and New Zealand (279), as well as in Denmark (269), The Netherlands (85, 86), England (200), Scotland (57), Japan (203), and the United States (241).

Davies and Maesen (86) described a remarkable 10-year study at the Regional Public Health Laboratory in Heerlen, The Netherlands. Approximately 1,700 positive cultures from respiratory tract specimens were obtained per year. The frequency of *B. catarrhalis* strains, which in 1977 was 5%, steadily increased until in 1982 it reached 20% and by 1986 it accounted for 26% of the respiratory tract pathogens. This inexplicable rise occurred in a relatively constant antibiotic climate and was not merely the result of increased awareness in the laboratory.

Person-to-person transmission of *B. catarrhalis* was thought responsible for some hospital-acquired infections. Pulmonary infections in three premature infants were traced to an attending nurse (139). Acute bronchitis caused by a β la⁺ strain developed in a previously healthy, 22-year-old nurse who worked in a chest ward (207). Calder et al. (57) undertook an environmental study of chest wards in which clusters of cases were found adjacent to index patients. Nasopharyngeal carriage of *B. catarrhalis* was identified in 7% of staff members and 8% of patients. Six *B. catarrhalis* cultures were isolated from 117 settle plates that were exposed for 6 to 8 h in a duty room frequented by carriers and in a chest ward. Also, experiments showed that the organism survived for at least 3 weeks in sputum (57). This circumstantial evidence of environmental spread illustrated the need for a means of determining strain relatedness.

The development of restriction endonuclease typing (See subsection, "Strain Differentiation") made it possible to trace the source of an outbreak of respiratory illness due to β la⁺ *B. catarrhalis* that occurred in an intermediate care unit. Patterson et al. (230) analyzed isolates from three staff members and ten patients, four with pneumonia, four with bronchitis, and two who were asymptomatic (hospitalized for nonrespiratory conditions). The typing results indicated that the isolates from five patients (including one asymptomatic) and two staff probably had a common origin.

Analogy between *B. catarrhalis* and *N. meningitidis* Infections

B. catarrhalis resembles *N. meningitidis* in many respects in spite of their very significant genotypic differences. Their natural habitat is believed to be human beings exclusively, and the nasopharynx is the site of initial mucosal colonization (37, 145, 204, 212, 307). Presumably this is due to the distribution of specific host cell receptors (16). Both organisms often are carried asymptotically (212, 292). The carriage rates are lower than formerly believed, however,

owing to the recognition of distinctions between *B. catarrhalis* and *N. cinerea* and the differentiation of nontypable meningococci from *N. lactamica* and *N. polysaccharea* (177, 258). *N. meningitidis* may be more pathogenic and less dependent than *B. catarrhalis* on predisposing conditions for the manifestation of disease. Nevertheless, both possess capsules and endotoxin which may facilitate later stages of infection.

Systemic infections with both organisms may be accompanied by petechial rashes, purpura, or ecchymoses. Such lesions were found in nine patients (Table 2), all of whom were ≤ 8 years of age, and in a 64-year-old man with pneumonia from whom *B. catarrhalis* was isolated in pure culture from a transtracheal aspirate (316). Disease caused by either organism may display a rapid onset of symptoms (118, 205) or be the cause of an unsuspected bacteremia in young children who present with fever and upper respiratory infections (13).

An unknown number of infections with *B. catarrhalis* have been attributed to *N. meningitidis* (52, 72, 77, 192, 205, 232). This error would result from the failure to use adequate differential tests because of the belief that gram-negative diplococci seen in purulent spinal fluid or good-quality sputum must be the established pathogen.

The term commensal, which refers to the nondetrimental coexistence of organisms, has frequently been applied to *B. catarrhalis* but not to *N. meningitidis*. Characterizing one organism as a commensal (205, 324) or a nonpathogen (117) and another as a pathogen carries unwarranted implications of qualitative differences. Actually, the distinction between their potential pathogenicity appears to be primarily quantitative.

Healthy Carriers of *B. catarrhalis*

Isolation rates. Various culture media containing selective agents, together with differential tests sufficient to confirm the identity of *B. catarrhalis*, were used in investigations of carrier rates (Table 3). Also, in two of these studies the prevalence of *N. cinerea* was determined as 14.5% (23) and 28.2% (177) compared with <5% for *B. catarrhalis*. Nasopharyngeal colonization of healthy children in Cleveland, Ohio, was 46% in fall and winter, but only 9% ($P < 0.005$) in spring and summer, overall 18% (305). Higher percentages of *B. catarrhalis* were isolated from children younger than 24 months than from older children (Table 3).

A corresponding age relationship was observed by Arkwright (9) in 1907. "*Micrococcus catarrhalis*" was isolated "from a much larger proportion of infants under one year than of older children or of adults: the last furnished proportionately the fewest strains" (9). Further evidence came from three studies in the 1980s of nasopharyngeal specimens taken in fall or winter months from Swedish children who showed no symptoms of respiratory infections. *B. catarrhalis* was isolated from 45% of children 2 years or younger, but from only 17% aged 6 to 7 years (194) and, similarly, from 36% of children 2 years or younger, but from only 13% who were 6 to 9 years old (48). Mølsted et al. (210) studied 191 children aged 1 to 7 (mean, 4.5) years who attended day-care nurseries and isolated *B. catarrhalis* from 58%. Selective media were not used in these investigations, and some of the strains possibly were *N. cinerea* since not all identifications were confirmed. In contrast to results with children, *B. catarrhalis* was recovered from only 3% of blood agar cultures of posterior nasal specimens from 286 healthy men (mean age, 20 years) who were military service recruits in Finland (163).

TABLE 3. Isolation of confirmed *B. catarrhalis* strains on semiselective and selective media

Author(s)	Yr	Place	Subjects (no. in study)	Culture site	<i>B. catarrhalis</i> (%)
Berger (21)	1961	Hamburg	Adults (172)	Throat ^a	0.6
Knapp (177)	1982–1983	Seattle	Adults (202)	Oropharynx ^b	4.5
Calder et al. (57)	1984	Edinburgh	Adults (148) ^c	Nasopharynx	7.4
DiGiovanni et al. (93)	1985	Perth ^d	Adults (123)	Nasopharynx	2.4
Van Hare et al. (305)	1982–1983	Cleveland	Children <24 mo (37)	Nasopharynx	24.0
			Children ≥24 mo (14)	Nasopharynx	0
Vaneechoutte et al. (302)	1988	Ghent	Children 3–12 yr (178)	Saliva	48.9
			Children 3–12 yr (75)	Throat	30.7

^a Carriage of "*N. catarrhalis*" was reported by Berger (21) as 15% (26 of 172 subjects); however, after *N. cinerea* was defined in a later study (25), retests of the 26 strains identified 25 (14.5%) as *N. cinerea* and 1 (0.6%) as "*N. catarrhalis*" (23).

^b *N. cinerea* was identified in 28.2% of the cultures.

^c Hospital staff and patients whose illness was not attributed to *B. catarrhalis* infection. All other study groups were made up of subjects without respiratory symptoms.

^d Western Australia.

Selective culture media for *B. catarrhalis*. Selective media designed to improve the detection of gonococci and meningococci are inhibitory for many *B. catarrhalis* strains principally due to the action of colistin (73, 81, 177, 325). Thus, only 12 strains were isolated from 1,204 throat cultures on a medium containing colistin (6 µg/ml) and other selective agents (325). However, a good recovery of *B. catarrhalis* and inhibition of various other species are obtained on semiselective media containing vancomycin and trimethoprim, each at final concentrations of 3 µg/ml (81, 179, 305) or higher (93, 283). Other semiselective media contained 25 µg of ristocetin per ml (21) or 10 U of bacitracin per ml (207). Some investigators added maltose (21), sucrose (179), or DNA (283) to facilitate differentiation of colonies. Corkill and Makin (81) initially added DNA to their semiselective medium, but found that the zone representing DNase activity around single colonies of *B. catarrhalis* was not always easy to detect. Vaneechoutte et al. (302) improved the selective action of a sheep blood agar containing vancomycin (10 µg/ml), trimethoprim (5 µg/ml), and amphotericin B (2 µg/ml) by adding sodium acetazolamide (10 µg/ml). Incubation of this medium in air greatly reduced the growth of *Neisseria* species due to inhibition of the activity of carbonic anhydrase, an enzyme typically produced by *Neisseria* species, but not by *B. catarrhalis* (24, 37, 302, 307). The enhanced selectivity of the medium was considered responsible for the high rates of *B. catarrhalis* isolation (Table 3) since rates were far lower on semiselective media or on blood agar (302).

Virulence

Features associated with virulence in other bacteria are also present in *B. catarrhalis*. Pili may facilitate adherence to mucosal surfaces. An outer coat analogous to a capsule and the tendency of cells to form clusters may reduce the effective actions of host defenses. The spontaneous release of outer membrane blebs provides a mechanism for the dispersal of endotoxin into peripheral tissues (285).

Studies of the virulence of clinical isolates are hampered by the low virulence of *B. catarrhalis* for laboratory animals (113, 125, 130). Intraperitoneal inoculation of mice with enormous doses proved lethal, but the bacteria were not found in the heart blood, and death was thought to be due to the "toxic effect of the bacterial protein" (130).

Animal models of otitis media secondary to bacterial infection make use of chinchillas and gerbils. Middle-ear inoculations of *B. catarrhalis* resulted in the development of effusions. Nevertheless, viable bacteria were not recovered

after 24 h (95, 106), although elevated levels of neutral phosphatase and hexosaminidase were found in 4-day samples of chinchilla effusions (95). Killed *B. catarrhalis* cultures also produced effusions (106), suggesting the involvement of endotoxin in inflammatory changes seen in the middle ear (89). Experiments in which five *B. catarrhalis* isolates were added to specimens of mucoid effusion material aspirated from middle ears of young children showed that viability was retained for 18 to 36 days (291). However, conditions affecting bacterial viability in MEF from living chinchillas and in MEF following experimental inoculation are hardly comparable.

The mechanisms enabling *B. catarrhalis* to colonize the human nasopharyngeal mucous membrane are virtually unknown, but must involve adherence. Nonadherent bacteria are bathed by secretions containing antibacterial enzymes and products of local immune systems and, moreover, are likely to be swept away by the flushing effects of mucus and ciliary beating (195). Studies of adherence have demonstrated specific interactions between adhesins on bacterial surfaces and receptors on mucosal surfaces (16). The adhesins of gonococci and meningococci include pili and heat-modifiable outer membrane proteins, for example, gonococcal protein II (285, 292).

Investigators at Nagasaki University described interesting research on *B. catarrhalis* adherence. The text of one paper (249) is in Japanese, but the abstract, technique, and the labels and legends for figures are in English. A second study (203) is described in English. For the adherence assay, squamous epithelial cells scraped from the oropharynx were washed twice in phosphate-buffered saline to remove loosely attached bacteria and then suspended in phosphate-buffered saline containing Ca²⁺ and Mg²⁺ to give 10⁵ human cells per ml. Bacteria from overnight cultures in Mueller-Hinton broth were washed, and a suspension was adjusted to an optical density of 0.1 at 620 nm. "These suspensions contained from 1–6 × 10⁷ CFU/ml" (249). The attachment mixture contained equal volumes (249) or a ratio of 1 part cells to 100 bacteria (203). After incubation for 30 min in a shaking 37°C water bath, this mixture was washed four times to eliminate nonadherent bacteria and then collected on a cover glass and Gram stained. Counts were made at ×1,000 magnification of the number of attached bacteria per cell for 50 consecutive epithelial cells. The *B. catarrhalis* strains (identity confirmed by nitrate reduction) were recent isolates from respiratory infections.

Rikitomi et al. (249) studied 60 samples of oropharyngeal cells obtained from 25 patients with chronic bronchopulmo-

nary infections. *B. catarrhalis* adhered in greater numbers to these cells than to cells from healthy persons ($P < 0.001$); the mean number of bacteria per cell ranged between seven and less than one per oropharyngeal cell from patients compared with less than one per cell from healthy adult controls. In contrast, adherence to cells from patients and control subjects was low and approximately equal for control bacteria characterized as *Neisseria* sp.

Mbaki et al. (203) investigated the seasonal frequency of *B. catarrhalis* infections in experiments with oropharyngeal cells from 48 patients who suffered from various types of chronic bronchopulmonary disease involving *B. catarrhalis* and other bacteria. The adherence of *B. catarrhalis*, in terms of mean number of bacteria per oropharyngeal cell, was highest in winter (3.11 ± 1.30) and lowest in summer (0.40 ± 0.25), with intermediate values in spring (1.07 ± 0.45) and autumn (1.84 ± 1.30). The value was 0.70 ± 0.16 for adherence to oropharyngeal cells obtained in December (winter) from nine infection-free control subjects. Mbaki et al. (203) considered that high adherence levels in winter were related to low environmental temperatures. However, low humidity in heated rooms may be more relevant since some oropharyngeal cell samples were from hospitalized patients.

A provocative observation by Rikitomi et al. (249) was the possibly greater adherence to oropharyngeal cells of β la⁺ cells (two strains tested) than β -lactamase-negative cells (one strain). Unfortunately, the technical difficulty in adjusting suspensions of these *B. catarrhalis* cells to contain equal numbers of CFU per milliliter raises doubts about the validity of comparisons between strains when data are expressed as mean bacteria per cell. In view of the possibly significant differences between numbers of CFU per milliliter in the initial attachment mixtures, it would have been preferable to express results in terms of (mean number of bacteria per human cell)/(number of bacterial CFU per milliliter).

Studies of the activities of *B. catarrhalis* in human cell cultures are needed to elucidate the complex process of infection. Adherence is required, but is not sufficient for cytotoxicity and invasion (292). *N. lactamica* cells, for example, adhere irreversibly to human cells but are not internalized (273). Upon introduction into human nasopharyngeal organ cultures, pilated meningococci attach to microvilli of nonciliated cells and in 6 to 12 h become trapped and pulled into membrane-bound vesicles in a manner resembling phagocytosis (204). Nearby ciliated epithelial cells, to which meningococci do not attach, soon lose ciliary activity and undergo sloughing believed due to the action of lipopolysaccharide and peptidoglycan monomers released by the cocci (204, 292).

B. catarrhalis was shown to produce histamine during growth in broth supplemented with histidine (1 mg/ml). Devalia et al. (90) speculated that this ability could contribute to the pathogenicity of such strains in the lower respiratory tract.

A mechanism for iron acquisition is a requirement for bacterial growth and, therefore, for pathogenicity (285). Iron-binding proteins present at mucosal surfaces (lactoferrin) and in blood serum (transferrin) of the human host have high association constants for iron resulting in insufficient free iron for bacterial growth. Schryvers and Lee (271) showed that *B. catarrhalis* cells possess membrane-associated proteins that bind human lactoferrin and human transferrin, thereby enabling the bacteria to utilize these sources of iron for growth. On the other hand, lactoferrin and transferrin from nonhuman species apparently are not

bound, which may contribute to the specificity of *B. catarrhalis* for human beings.

Some clinical isolates are resistant to killing by normal human serum and others are susceptible (46, 71, 282). The molecular basis of this complement-dependent bactericidal action in *B. catarrhalis* has not been investigated, but is presumably multifactorial as in *N. gonorrhoeae* (246).

Prellner (243) demonstrated that *B. catarrhalis* can bind the complement component C1q with high efficiency without the participation of antibodies. She speculated that, in patients with acute otitis media, C1q binding might contribute to abnormal C1 complexes leading to impaired complement activation and defective opsonization of bacteria.

IgA1 protease was not produced by any of 45 confirmed strains of *B. catarrhalis* (208, 305). Koomey and Falkow (182) showed that this is due to the absence of the IgA1 protease-encoding gene in *B. catarrhalis* rather than to the presence of a silent or defective allele. IgA1 protease has been considered a virulence factor since it specifically cleaves and inactivates human IgA1, but Sparling (285) has questioned its clinical importance. Furthermore, an IgA1 protease-deficient *N. gonorrhoeae* mutant is unimpaired in its attachment to and invasion of human mucosal cells in organ culture (79).

Immunity

Circumstantial evidence of the importance of immunoglobulins as mediators of defense against *B. catarrhalis* is found in the strong clinical association discussed earlier between various immunodeficiency states and systemic disease or bronchopulmonary infections. Furthermore, the greater susceptibility of older individuals may be due in part to the significant decline of serum IgG and IgM concentrations by the sixth decade of life (51).

The development of antibodies reactive with *B. catarrhalis* has been investigated in patients suffering from maxillary sinusitis (46), acute bronchopulmonary infections (28, 71, 197), and acute otitis media (189). The serological methods involved immunodiffusion (46), complement fixation (46), an enzyme-linked immunoassay (189, 197), a bactericidal assay (71), and an immunofluorescent-antibody test (28). Modest increases of antibody titers between acute and convalescent sera occurred in most, but not all, patients. Antibodies were also detected in control sera from substantial numbers of healthy persons (28, 46, 189), possibly due to immunogenic stimulation by *B. catarrhalis* present in the pharyngeal normal flora (189).

Leinonen et al. (189) found that IgA class antibody directed against *B. catarrhalis* developed in the sera of eight of nine children, 2 to 6 years old, who suffered from acute otitis media caused by *B. catarrhalis*. IgA antibodies were not detected in control sera from children with otitis media caused by pneumococci and *H. influenzae*. Although half of these control sera contained IgG reactive with *B. catarrhalis*, the titers did not increase during convalescence. Middle-ear aspirates from which *B. catarrhalis* was cultured contained IgA and IgG antibodies directed against the pathogen. The presence and titers of both immunoglobulins depended on the age of the child. Using the same enzyme-linked immunoassay, Malkamäki et al. (197) detected high and rising titers of IgG and IgA antibodies in serum samples from a 19-year-old man with bacteremic pneumonia caused by *B. catarrhalis*.

B. catarrhalis is recognized as a T-cell-independent mitogen which stimulates DNA synthesis in human B lympho-

TABLE 4. Differential biochemical reactions of *B. catarrhalis* and possibly confusing *Neisseria* spp.^a

Species	Acid formation from:				Polysaccharide from sucrose	Reduction of nitrate	Hydrolysis of:		
	Glucose	Maltose	Lactose	Sucrose			DNA	Tributyryn	γ -GNA ^b
<i>B. catarrhalis</i>	0	0	0	0	0	+	+	+	0
<i>N. cinerea</i>	0	0	0	0	0	0	0	0	0
<i>N. flavescens</i>	0	0	0	0	+	0	0	0	0
<i>N. gonorrhoeae</i>	+	0	0	0	0	0	0	0	0
<i>N. meningitidis</i>	+	+	0	0	0	0	0	0	+
<i>N. caviae</i> ^c	0	0	0	0	0	+	+	+	0

^a +, Most strains (>90%) give positive reactions; 0, negative reactions in tests with adequate controls.

^b γ -GNA, *N*- γ -Glutamyl- β -naphthylamide.

^c This organism, designated *Neisseria caviae* (257, 307) or *Moraxella (Branhamella) caviae* (37), is isolated from guinea pigs; if isolated from human sources, it might be called *B. catarrhalis* in spite of differences in DNA base composition, DNA-DNA hybridization reactions (174), genetic transformation frequencies (148), serological (24) and biochemical (15, 74, 257, 264, 298) characteristics, and major cellular fatty acids (298).

cytes. This B-cell activation is related to the binding of bacteria to IgD present on the B cell. Forsgren et al. (120) offered the suggestion that B-cell binding may alter the immune response of the host and confer a selective advantage upon *B. catarrhalis*.

IDENTIFICATION AND BIOCHEMICAL CHARACTERISTICS OF *B. CATARRHALIS*

The appearance of bacteria in a properly Gram-stained preparation should be evaluated carefully. *B. catarrhalis* is gram negative, but has a tendency to resist decolorization (9, 23, 60, 245, 257). This interaction with the crystal violet dye may be responsible for the inhibition of growth on crystal violet medium found for *B. catarrhalis* but not *N. cinerea* (4).

The bacteria characterized in Table 4 are oxidase-positive, nonmotile, gram-negative cocci commonly arranged in pairs with their abutting sides flattened. Division occurs in two planes at right angles to one another, thereby preventing chain formation which occurs when cell division is restricted to one plane (15). The potentially confusing coccobacillary cells of *Moraxella* and *Acinetobacter* species elongate into snakelike cells when division is blocked by sublethal concentrations of penicillins or certain other antibacterial agents. Since elongation does not occur with *B. catarrhalis* or *Neisseria* species, this is an easy way to distinguish them from nonsaccharolytic, short paired rods (63).

B. catarrhalis colonies on blood agar are nonhemolytic, circular, grayish white, opaque, convex, and $\cong 2$ mm in diameter after 48 h. Colonies when pushed tend to remain intact and slide across the agar like a hockey puck on ice [P. A. Shurin, *Drugs* 31(Suppl. 3):38-39, 1986].

Differential Tests

Action on carbohydrates. *B. catarrhalis* does not produce acid from any of 9 (69) or 10 (112) carbohydrates. Negative results are observed uniformly with various methods (9, 61, 178) including the rapid carbohydrate degradation tests designated RIM-N (94, 155, 185), Gonobio-Test (94), and API QuadFERM⁺ (109, 156). Minitek test reactions were correct in some instances (94), but not all (233).

Aberrant reactions in carbohydrate tests more often confuse the identification of *Neisseria* species. False-positive reactions in rapid glucose utilization tests were recorded for some strains of *N. cinerea* (40, 94, 177) which led to their presumptive identification as *N. gonorrhoeae* in some cases (317). Rare meningococcal isolates are maltose negative (293). Glucose-negative reactions have been observed with

meningococci and gonococci (73, 82, 109, 177, 178, 245). Such strains often, but not always, give positive reactions after a few subcultures on a favorable medium (212) or in a test with 10% (wt/vol) glucose, which facilitates transport of the sugar (62).

N. flavescens possesses an amylosucrase that builds sucrose into a polysaccharide which reacts with iodine (20, 61, 142). This reaction differentiates *N. flavescens* from *N. cinerea* (Table 4) and *N. polysaccharea* from *N. meningitidis* (177, 258).

Reduction of nitrate and nitrite. *B. catarrhalis* reduces NO₃ to NO₂ and beyond (22, 24, 37, 69, 73, 99, 256). The absence of both NO₃ and NO₂ is confirmed by the negative reaction that persists after addition of the NO₂ test reagents followed by zinc dust, which reduces NO₃ nonenzymatically. Berger (23) found that 0.03 to 0.01% (wt/vol) NO₃, rather than the customary 0.1%, provides a more sensitive test useful for revealing weak NO₃ reduction. The test for NO₃ reduction, when modified for use as a rapid method, was found difficult to interpret (315) or unreliable (177).

NO₂ is denitrified by all species given in Table 4 provided that growth occurs. Because of NO₂ toxicity, the customary 0.1% (wt/vol) concentration should be reduced for tests of *B. catarrhalis*, *N. cinerea*, *N. gonorrhoeae*, and *N. meningitidis*. Seven *B. catarrhalis* strains were able to grow in the presence of 0.005% (wt/vol) KNO₂, but not 0.03%; the type strain ATCC 25238 (Ne 11) tolerated 0.01% (69). Denitrification of NO₂ by *B. catarrhalis* occurs without the formation of gas (24, 25, 69), whereas nitrogen gas is produced by *N. cinerea* and *N. flavescens* (24, 25, 307). The test for gas production requires a large volume of broth (e.g., 25 ml) when NO₂ is used at 0.005 or 0.01% so that enough gas will accumulate in the inverted collection vial.

Hydrolysis of DNA. The original finding of DNase production by *B. catarrhalis* (69) was confirmed by subsequent investigators who also demonstrated the differential value of the characteristic (4, 73, 81, 226, 256). The customary DNase test medium contains 0.2% (wt/vol) DNA. Various reagents detect the zone of hydrolysis that develops around an area of dense bacterial growth. The Frazier reagent used in three studies (69, 73, 256) contains mercuric chloride, 15 g dissolved in 100 ml of distilled water, plus 20 ml of concentrated hydrochloric acid. Alternatively, a solution of 0.1 N HCl was used (4, 81) or toluidine blue, 0.1% (wt/vol) (226).

The time of incubation can significantly affect the DNase test results. For example, one *B. catarrhalis* strain examined by Corkill and Makin (81) produced no detectable DNase activity after 24 h, whereas an 18-mm-diameter zone was found with a duplicate culture incubated for 48 h. In my

experience, the type strains *N. cinerea* ATCC 14685, *N. flavescens* ATCC 13120, and *N. gonorrhoeae* ATCC 19424 produced 1+ zones of DNase detected by the addition of the Frazier reagent after incubation of cultures for 4 days; 2+ zones were found with *B. catarrhalis* ATCC 25238 and 4+ zones were found with *Staphylococcus aureus* (unpublished observations). For definitive results, control cultures of *B. catarrhalis* and a typically negative *Neisseria* species should be incubated in parallel with test cultures to enable direct comparisons of DNase zone diameters.

Hydrolysis of tributyrin and other esterase substrates. Tributyrin hydrolysis is a valuable differential characteristic (73, 154, 201, 247, 250, 257). The original test was relatively unsatisfactory, however, because the hydrolysis of 1% (vol/vol) tributyrin incorporated in an agar medium (25) produced only a partial reduction of opacity that was difficult to see (256). A micromethod with 0.1% (vol/vol) tributyrin in agar revealed hydrolysis by *Neisseria* species as well as *B. catarrhalis* (238). Recent methods take advantage of the 10-fold-greater amount of butyric acid liberated from tributyrin by an esterase of *B. catarrhalis* than by enzymes of *Neisseria* species likely to be isolated from human sources (254). In one method a disk containing tributyrin and phenol red indicator is added to a dense bacterial suspension prepared in buffer solution. A positive reaction is seen as the change from red to yellow (acid pH). Disk tests usually give positive results (154, 201, 257), but negative reactions were reported for 2 of 31 isolates (109) and 1 of 28 (247) *B. catarrhalis* strains.

4-Methylumbelliferyl butyrate and indoxyl butyrate are used in place of tributyrin in other tests for esterase. Release of the 4-methylumbelliferone moiety by hydrolysis is detected by its fluorescence under UV light (301). Hydrolysis of indoxyl butyrate releases indoxyl, which spontaneously forms indigo blue (88).

Hydrolysis of aminopeptidase substrates. *N*- γ -Glutamyl- β -naphthylamide and L-hydroxyproline- β -naphthylamide are substrates used in rapid tests of γ -glutamylaminopeptidase and hydroxyprolylaminopeptidase, respectively. Mannheim and Bürger (199) first demonstrated that *N*- γ -glutamyl- β -naphthylamide was hydrolyzed by *N. meningitidis* but not by *B. catarrhalis*. This was confirmed by other investigators (84, 147, 255), who also reported negative reactions for *N. gonorrhoeae*. A purified arylamidase from "*N. catarrhalis*" which lacked activity on *N*- γ -glutamyl- β -naphthylamide had strong activity for alanine β -naphthylamine and several other amino acid β -naphthylamines (18). The production of hydroxyprolylaminopeptidase by *N. cinerea*, but not by *B. catarrhalis*, is of possible differential value (147, 177). This enzyme was produced by all *N. gonorrhoeae* strains examined (84, 147, 150) and by various percentages of *N. meningitidis* (84, 147, 177).

Response to superoxol (30% H₂O₂). All species listed in Table 4 produce catalase detected with 3% H₂O₂. The use of 30% H₂O₂ was recommended as a differential test (266). A positive superoxol test is seen as brisk bubbling immediately after dropping 30% H₂O₂ onto a young (\leq 24-h) culture. Weak bubbling or a reaction delayed for 3 s or longer is considered negative. Positive reactions were obtained with 99.9% of *N. gonorrhoeae* isolates (8, 266, 326). Most *N. meningitidis* strains gave negative reactions, but 98% of group A meningococci were positive (8). The superoxol test appears to lack differential value for *B. catarrhalis* since both negative and positive reactions have been reported (4, 8, 177, 326).

Other differential tests. Serological tests involving classic

agglutination methods were unsuccessful due to the notorious spontaneous agglutination of *B. catarrhalis* in 0.85% NaCl solution (9, 24, 112, 130, 143, 245). Precipitin tests were used to distinguish *B. catarrhalis* from *N. cinerea* (24, 25). An immunodiffusion technique showed that one *B. catarrhalis* strain (ATCC 8176) was serologically unrelated to *N. cinerea* or to strains of eight other *Neisseria* species (263). Eliasson (110) investigated a trypsin-sensitive, heat-stable P antigen located on the surface of *B. catarrhalis*. He suggested that an antibody specific for P antigen might serve as a reagent for the identification of *B. catarrhalis*.

A DNA probe of chromosomal origin was developed and found to be 100% specific for *B. catarrhalis* (D. Beaulieu and P. H. Roy, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, D249, p. 124). Such a probe will reduce the time required for identification since it can be used on primary cultures with only a short pretreatment to release DNA.

Growth at 22°C and growth on nutrient agar have long been considered typical of *B. catarrhalis* (4, 25, 82, 108, 117, 212, 245). Unfortunately, the components of nutrient agar are poorly defined and nutritionally variable (62). Additional variables (often unstated) are the inoculum density, which should be low enough to minimize spurious reactions due to carry-over of nutrients in cells grown on an adequate medium, time of incubation, and atmospheric humidity. A dry incubation atmosphere deters growth. *B. catarrhalis* ATCC 25238 did not grow on nutrient agar (Difco Laboratories) during incubation in an atmosphere of humidified air at either 22 or 36°C for 6 days, although sparse growth occurred on blood agar at 22°C. *N. cinerea* ATCC 14685 failed to grow on either nutrient or blood agar at 22°C, but moderate growth occurred on nutrient agar at 36°C (unpublished findings). The failure of 11 clinical isolates of *B. catarrhalis* to grow on nutrient agar incubated at 22°C led Doern and Morse (99) to recommend that this test not be included among the criteria for identification.

Some *B. catarrhalis* strains are able to grow on modified Thayer-Martin agar and other colistin-containing selective media (29, 94, 99, 109, 177, 257, 325). Thus, contrary to earlier findings (212), growth inhibition on these media is not a reliable differential characteristic.

Nutritional requirements were investigated by Riou and Guibourdenche (257). They found that all of 178 strains of *B. catarrhalis* required arginine for growth in Catlin's chemically defined medium (64). On the other hand, *N. cinerea* required cysteine, proline, and arginine (180, 257) and also histidine and biotin (unpublished findings). Juni et al. (164) independently showed the arginine requirement in a simpler defined medium and found that most strains could utilize ornithine in place of arginine. An attempt to demonstrate multiple nutritional biotypes used ion-exchange chromatography to detect the use and production of amino acids by *B. catarrhalis* grown in nutrient broth (27). The utilization of various carbon sources and additional physiological reactions were reported (15, 73).

Weiner and Penha (314) recently reported the value of Bacto TB Hydrolysis Reagent (Difco) as a means of detecting Tween 80 hydrolysis by *B. catarrhalis*. Negative results were obtained with 152 strains of *Neisseria* species and 41 strains of *Moraxella* species. On the other hand, lipase production detected in complex medium supplemented with 0.01% (wt/vol) Tween 80 was reported as positive for *Moraxella lacunata*, *M. liquefaciens*, *M. osloensis*, and *Acinetobacter* strains, as well as for *B. catarrhalis* (15). Although a potentially useful differential test, Tween 80 hydrolysis needs further evaluation.

Stages in the Identification Process

Various commercial enzyme substrate kits for rapid tests have been described (94, 150, 155, 177, 212, 233, 315). Unfortunately, some rapid tests yield only negative reactions for *B. catarrhalis* or, worse, false identifications (40, 94, 109, 233). Rapid tests may be justified provided that the results are rapidly communicated and the physicians fully recognize the possible provisional nature of a bacterial identification. In a survey described by Stanek (289), physicians indicated their willingness to sacrifice some degree of accuracy in return for the prompt delivery of test results. However, it is unacceptable to give a genus and species name to an isolate solely on the basis of negative reactions. A second stage in the identification process is needed.

The identity of *B. catarrhalis* is best confirmed by positive reactions in three differential tests (Table 4) since none of them is 100% sensitive or specific. Before undertaking these tests, it is important to verify the microscopic appearance and purity of the culture. The coccobacillary form of several *Moraxella* species that reduce NO₃ (37) must be recognized (63). The possible difficulties associated with the use of inocula from a primary culture are well known, but a further example is instructive. A mixed culture obtained from a transtracheal aspirate contained *B. catarrhalis* which provided growth factors that enabled *H. influenzae*, the undetected strain, to grow and form acid in a glucose-containing medium. Accordingly, the diplococcus was initially identified as *N. gonorrhoeae* in three different laboratories (53).

The identification of atypical strains is a third stage. This may require methods used primarily in research. An organism isolated from blood cultures gave aberrant results in four tests, but was identified as an atypical strain of *B. catarrhalis* on the basis of cell wall fatty acid and lipopolysaccharide analyses (322). DNA base composition was determined in an effort to identify oxidase-positive, nonsaccharolytic, gram-negative cocci isolated from urogenital cultures (240). The guanine-plus-cytosine content (69 mol%) proved that the isolates were not related to *Branhamella* or *Neisseria* species. Isolates of interest like these should be preserved for possible use in further studies (64).

Survey specimens were sent to "qualified reference laboratories" as part of the proficiency testing program of the College of American Pathologists. *B. catarrhalis* was correctly identified by 74% of 685 laboratories in 1983 and 82% of 688 laboratories in 1985 (162). This represents 125 failures to recognize *B. catarrhalis* in 1985. (Greater respect is needed!)

Strain Differentiation: Typing

The profiles of electrophoretic mobilities of soluble proteins are of potential value for identifying and differentiating *B. catarrhalis* strains (122). Following their separation by electrophoresis through gels, proteins were stained nonspecifically with amido black (122, 123) or were visualized specifically with substrates for 12 metabolic enzymes (74) or with five substrates specific for esterases (235). Analysis of the profiles of 80 *B. catarrhalis* strains enabled Picard et al. (235) to identify 34 esterase zymotypes and trace the spread of strains between patients with nosocomial bronchopulmonary infections.

Restriction endonuclease analysis of DNA. In restriction endonuclease typing, DNA obtained by lysis of whole cells is purified and digested with a restriction endonuclease, an enzyme that recognizes and attacks a specific DNA base

sequence. This target sequence is relatively uncommon in the DNA of any given strain and is likely to differ for DNAs of unrelated strains. Agarose gel electrophoresis separates the DNA fragments produced by cleavage, and their pattern of bands is revealed by reaction with ethidium bromide.

Patterson et al. (230) used three restriction enzymes (*Pst*I, *Hind*III, and *Hae*III) to analyze isolates from a nosocomial outbreak of *B. catarrhalis* respiratory infections. The DNA fragment patterns were similar for strains isolated from five patients and two staff members, indicating the clonal origin of these seven isolates; different patterns were displayed by four other isolates. The same investigators (229) later examined 14 β la⁺ *B. catarrhalis* strains collected over a 16-month period from other patients in the same hospital. Twelve distinct DNA fragment patterns were identified, 10 in epidemiologically unrelated strains. Identical fragment patterns were displayed by isolates from two patients located on the same ward for over 2 months.

Dickinson et al. (92) used *Pst*I and *Cla*I restriction endonucleases in a study of *B. catarrhalis* isolates from the nasopharynx and MEF of seven children who experienced one or two episodes of otitis media. Strains from six of these children were unique in their DNA fragment patterns. However, in seven of eight episodes, the pair of isolates from the pharynx and middle ear had identical DNA fragment patterns, which suggested that the populations had a common origin and that these pharyngeal isolates were virulent.

A method is needed for classifying the DNA fragment patterns and identifying the variable sequences. This will facilitate the consolidation of findings from different studies and evaluation of nucleotide sequence diversity.

OMP and LOS analyses. Eight major outer membrane proteins (OMPs) of *B. catarrhalis* were characterized by Bartos and Murphy (14). The patterns of electrophoretic mobilities were similar for each of 50 strains of diverse origins. Further investigation (216) may show whether the antigenic determinants of these OMPs have the diversity required for serotyping in the way meningococci and gonococci are typed (124). In a preliminary study of lipooligosaccharides (LOS), Murphy (215, 216) detected antigenic differences by use of an immunoblot assay. Vaneechoutte et al. (303) developed an inhibition enzyme-linked immunosorbent assay to determine LOS antigenic differences. Three LOS types were distinguished which represented 61, 29, and 5% of 302 strains tested. No relation was discerned between the LOS type and the clinical or geographical origin of the strains.

SUSCEPTIBILITY TO ANTIBACTERIAL AGENTS

Susceptibility implies that the antibacterial agent gains access to a cellular target in a concentration sufficient to suppress a particular vital function. Antibiotics penetrate wild-type gram-negative bacteria by two routes. Outer membrane porin channels are permeable to hydrophilic solutes of relatively low molecular weight (<600 in enteric bacteria) (223). Tetracyclines, chloramphenicol, and most β -lactam agents diffuse through porin channels, although passage is impaired by bulky side chains and hydrophobicity (222, 223). Diffusion through the phospholipid bilayer regions of the outer membrane is the principal means of entry of rifampin, erythromycin, vancomycin, fatty acids, and various other hydrophobic compounds (213, 223).

Non- β -Lactam Agents

The high susceptibility to erythromycin and rifampin typical of *B. catarrhalis* is related to the considerable hydro-

phobicity of its cell surface (131). Rifampin MICs are 0.007 to 0.25 $\mu\text{g/ml}$ (49, 103). The range of erythromycin MICs, which was 0.02 to 0.3 $\mu\text{g/ml}$ in 1970 (166), was nearly the same in some recent studies (7, 107, 259); in two others (103, 119), the range extended to 1.0 $\mu\text{g/ml}$. A few erythromycin-resistant strains (MICs not stated) were reported (93, 221, 277, 313). Differences of technique, such as medium pH and incubation atmosphere (47, 107), can account for minor differences between erythromycin test results. However, genuine resistance indicated by MICs in the range 4 to 16 $\mu\text{g/ml}$ was recorded for a few *B. catarrhalis* strains isolated in Japan (218), The Netherlands (296), Sweden (165, 225), and the United States (49).

Gram-negative bacteria can gain increased resistance to erythromycin by at least two mechanisms: alteration of a protein in the 50S ribosomal subunit (324) and reduced permeability of the outer membrane which provides a protective barrier against various antibiotics (191, 222). The characteristics of two erythromycin-resistant *B. catarrhalis* strains described by Kallings (165) (her strains 1 and 7) suggest to me that their mechanisms of resistance differed. Strain 1 displayed low MICs (micrograms per milliliter) of penicillin V (≤ 0.125), ampicillin (≤ 0.064), cefaclor (≤ 0.25), doxycycline (≤ 0.125), and trimethoprim (TMP)-sulfamethoxazole (SMX; 0.5), but the MIC of erythromycin was 4 $\mu\text{g/ml}$. These characteristics are compatible with the idea that strain 1 carries a mutation that specifically affects the erythromycin target. Strain 7, which is also β -lactamase negative, displays high MICs (micrograms per milliliter) of penicillin V (> 8), ampicillin (0.5), cefaclor (4), doxycycline (0.5), and TMP-SMX (16), in addition to erythromycin (> 8). Such multiple resistances suggest that the strain may carry one or more mutations that result in reduced permeability. Unaccountably, these levels of resistance of strain 7 are similar to those of strain 2 which produces β -lactamase. In this connection, it is important to recognize that β -lactamase production is a trait that may be superimposed upon genetically atypical strains.

Differences in susceptibility to fatty acids and lipids also indicate outer membrane permeability differences. Free fatty acids naturally present in culture media are inhibitory to the growth of many *N. gonorrhoeae* strains, and this toxicity is neutralized by starch (64, 213). Arko and Odugbemi (8) examined seven *B. catarrhalis* strains and observed that the growth of three was suppressed on an area of a starch-containing medium that had been exposed to alpha-amylase. They suggested that digestion of the starch eliminated its detoxifying action. It would be interesting to determine the response to fatty acids in a more controlled study of additional strains.

Tetracycline resistance (MIC, 16 $\mu\text{g/ml}$) was detected in 1988 in two *B. catarrhalis* isolates from Texans with chronic lung disease (49). Previously, the highest MICs were ≤ 0.5 $\mu\text{g/ml}$ reported in three surveys (7, 251, 259) and 1.0 $\mu\text{g/ml}$ in another (103). For the related drug doxycycline, MICs ranged between ≤ 0.125 and 4 $\mu\text{g/ml}$ in Sweden (165, 225), but were ≤ 1 $\mu\text{g/ml}$ in Spain (119) and 0.06 to 0.25 $\mu\text{g/ml}$ in Australia (251).

Chloramphenicol MICs fell within the range of 0.25 to 1.0 $\mu\text{g/ml}$ for isolates in the United States (7, 103, 259) and Spain (119), but were as high as 4 $\mu\text{g/ml}$ in Sweden (165).

TMP MICs have ranged between 4 and > 64 $\mu\text{g/ml}$ (3, 10, 99, 252, 294, 302) and SMX MICs were reported to be 2 to 32 $\mu\text{g/ml}$ (252). The target of TMP is dihydrofolate reductase, and in *B. catarrhalis* the affinity of TMP for enzyme is weak (10). The TMP/SMX combination is more effective than

either agent alone (3, 252) due to the inhibitory actions of TMP and SMX on different steps in the vital pathway of folate biosynthesis (reviewed in reference 67). Most strains are susceptible (103, 121, 193) and display TMP/SMX MICs within the range 0.02/0.4 to 1.6/30 $\mu\text{g/ml}$ (7, 49, 259, 299). Rare strains were considered resistant (86, 93, 313) and MICs of 4.0/76 $\mu\text{g/ml}$ were reported (299). Since TMP is less active than SMX, the optimum ratio for most *B. catarrhalis* strains is not 1:20, the ratio for a commercially available TMP/SMX combination, but is closer to 2:1 or 1:1 (252) or 1:4 (165).

Vancomycin can be used in selective media for *B. catarrhalis* because the typical range of MICs is 32 to 128 $\mu\text{g/ml}$ (99, 302). Reports from one group (7, 140), however, gave the lower limit as a MIC of < 0.5 $\mu\text{g/ml}$. This suggests that some isolates may represent hypersusceptibility mutants, like those of *N. gonorrhoeae* (181), which would fail to grow on vancomycin-containing selective media.

Colistin susceptibility was considered typical of *B. catarrhalis* in the 1970s (99). Although many recent isolates are susceptible (73, 283), others are sufficiently resistant to enable them to grow on selective medium containing colistin at 7.5 $\mu\text{g/ml}$ (81, 99, 109). A correlation between increased resistance to colistin and β -lactamase production was observed (81, 99), but was not confirmed in another study (3).

The inhibitory concentrations of 11 heavy-metal ions for 56 strains of *B. catarrhalis* were determined by an agar dilution method. Riley and Taylor (253) found that only 10% of the strains were inhibited by 0.1 M molybdenum, whereas 100% were inhibited by 0.0001 M cadmium ions. The strains were not equally susceptible to a given metal ion, but all strains were inhibited by 0.001 M each silver and mercury, 0.01 M cobalt, and 0.1 M each arsenate, chromium, iron, lead, manganese, and nickel ions. Resistance to multiple metal ions which characterized many strains was unrelated to β -lactamase production.

Unequal Susceptibility of β -Lactamase-Negative Strains to β -Lactam Agents

As with equality (some individuals are more equal than others), so it is also true of susceptibility that some strains are more susceptible than others. Thus, Kamme (166) found 60-fold differences between ampicillin MICs (range, 0.005 to 0.3 $\mu\text{g/ml}$) and penicillin G MICs (range, 0.01 to 0.6 $\mu\text{g/ml}$) for confirmed "*N. catarrhalis*" strains isolated in 1969 from children with otitis media and purulent rhinitis. More recent studies of strains lacking β -lactamase found ampicillin MICs (micrograms per milliliter) as high as 0.5 (165), 0.78 (267), 1.0 (225), and > 4 (85), and Davies et al. (87) recorded a cefuroxime MIC of 16 for one isolate in a study that established the range as 0.25 to 1 $\mu\text{g/ml}$ for 17 other isolates from patients with bronchitis.

The targets of β -lactam antibiotics are penicillin-binding proteins that catalyze the final stages of peptidoglycan biosynthesis (286). Mutations that reduce the affinity of binding proteins for penicillins lead to increased resistance. Other mutations that affect the outer membrane may increase resistance to penicillin by reducing permeability (191, 222). Although the levels of resistance may not be dramatically increased by these structural modifications, they should not be disregarded. The chromosomally mediated resistance in *N. gonorrhoeae* is a clinically important form of penicillin resistance in β -lactamase-negative strains (286). Therefore, when analyzing results of *B. catarrhalis* susceptibility tests, it is important to separate β -lactamase-negative

strains from those producing β -lactamase in view of their differing mechanisms of resistance. Furthermore, potentially significant genetic differences between strains may be concealed by MICs considered for clinical purposes to indicate susceptibility.

This is illustrated in the interesting study by Sweeney et al. (299) of 10 *B. catarrhalis* isolates from patients with pneumonia (referred to as P strains) and 15 isolates that colonized healthy adults (C strains). Two P and three C strains were β -lactamase negative. The MICs of six penicillins and seven cephalosporins, determined by a standard broth macrodilution method, showed that the five strains were susceptible according to the accepted interpretative breakpoints. Combining the results for the β -lactamase-negative and β la⁺ strains led to the conclusion that the susceptibilities of the 10 P strains did not differ significantly from those of the 15 C isolates (299).

On the other hand, if the data for the β -lactamase-negative strains are considered separately, the MICs (expressed as geometric means) reveal susceptibility differences of potential genetic significance. For example, the penicillin G MICs for the P and C isolates were 0.06 and 2.52 μ g/ml, respectively, a 42-fold difference. For both ampicillin and piperacillin, the MICs were 0.06 μ g/ml for the P isolates and 1.59 μ g/ml for C isolates, a 26-fold difference. The best example is the 53-fold difference between the MICs of mezlocillin, 0.06 μ g/ml for P and 3.17 μ g/ml for C isolates. Investigations of many more β -lactamase-negative strains are needed as a basis for definitive conclusions. However, real differences between the geometric mean MICs might indicate differences between the outer membrane compositions of P and C isolates that could be of potential medical importance, for example, by affecting the adhesions responsible for adherence to host mucosal surfaces (249, 292). It should be noted that Soto-Hernandez et al. (282) reported ampicillin MICs of 0.008 μ g/ml for six β -lactamase-negative isolates: three from patients who met the criteria for bronchopulmonary disease and three from patients who did not meet these criteria (so-called colonizing strains). However, the strains colonizing patients may differ from C strains isolated from healthy adults.

β -Lactamase-Positive Strains

Detection methods. Biological methods detect β -lactamase by its reduction of the antibacterial effectiveness of a penicillin or cephalosporin for a susceptible indicator strain. Biochemical methods detect enzymatic cleavage of the β -lactam ring (101). Nitrocefin and PADAC are cephalosporins that undergo color changes from yellow to red and purple to yellow, respectively, in positive tests for β -lactamase. An acidometric method detects the acid pH that develops when penicillin is hydrolyzed to penicilloic acid. A positive reaction in the iodometric test with penicillin is seen as a color change of the starch-iodine indicator from blue to colorless when iodine is bound by the β -lactam cleavage product.

The results obtained with these methods are not equally reliable (101, 156, 234). For example, one *B. catarrhalis* strain that produces a low level of β -lactamase was sent to laboratories as part of the 1983 proficiency survey by the College of American Pathologists. Only the nitrocefin assay gave an acceptable percentage (96%) of positive results. The percentages detected in other tests were as follows: acidometric, 78%; PADAC, 69%; iodometric, 64% (162). The superior performance of the nitrocefin test is related to the

higher rate of hydrolysis of nitrocefin compared with that of penicillin by *B. catarrhalis*.

Prevalence. Strains isolated before 1970 were susceptible to penicillin (12, 15, 33, 69, 78, 245) and did not produce β -lactamase (4). β -Lactamase also was not detected in 46 *B. catarrhalis* strains isolated between 1970 and 1976 in France (234), 18 isolates from patients in the United States between 1952 and 1975 (311), or 5 from Japanese patients in 1976 to 1979 (218).

Two strains isolated in 1976 (Table 5) produced an enzyme identified as the BRO-1 β -lactamase (311). In Sweden (198), England (231), France (56), and Belgium (224), β la⁺ strains were detected in 1977. Interestingly, in southern Netherlands, which lies next to Belgium, a β la⁺ strain was not isolated until February 1980 in spite of a "constant search" (86), but the frequency rose to over 60% by 1985 to 1986. β -Lactamase was detected in 46% of 250 isolates from Swedish outpatients in 1983 (121). In Nagasaki, Japan, the β la⁺ strains reached 50% in 1980 and 1981, 61% in 1982, and 74% in 1983 (218). In Beijing, China, 25 (83%) of 30 isolates were reported in 1988 (329), and in Western Australia the percentage of *B. catarrhalis* β la⁺ isolates from bronchopulmonary infections doubled between 1982 and 1985 (93). Furthermore, although the prevalence of *B. catarrhalis* in acute otitis media of Finnish children remained approximately 7%, the β la⁺ isolates increased from 30 to 71% between 1980 and 1985 (265). A national collaborative study of *B. catarrhalis* strains submitted by 15 U.S. medical centers found that 84% of 378 respiratory isolates were β la⁺ in 1987 to 1988 (J. H. Jorgensen, L. A. Maher, A. W. Howell, J. S. Redding, and G. V. Doern, Progr. Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1286, 1989).

The rising frequency of β la⁺ strains has been attributed to the selective action of β -lactam agents used for treatment. However, community-acquired β la⁺ strains are the sources of some bronchopulmonary infections (86, 200, 207, 229) and most cases of acute otitis media. Furthermore, two recent studies show that β la⁺ *B. catarrhalis* strains are carried by healthy children. β -Lactamase was detected in 78% of 94 isolates from Belgian school children (302) and in 57 (54%) of 105 isolates from day-care children in Sweden (210). Thirty-one of these Swedish β la⁺ carriers had not received antibiotics during the previous 6 months.

Problems in determination of resistance to β -lactam antibiotics. The formation of bacterial clusters is a source of technical difficulty in quantitative susceptibility tests. For example, aberrant killing curves were obtained in a study of the activity of a new cephalosporin against 10 strains of β la⁺ *B. catarrhalis*. A massive decline of CFU per milliliter occurred during 6 h of incubation, but this was followed after 24 and 48 h by "regrowth," i.e., an increase of CFU to reach the number attained by the drug-free control culture (327). One possible explanation for regrowth (66) is that vortex mixing of the broth deposited clusters of cells on the glass above the fluid level, out of reach of the cephalosporin. Subsequent vortexing reintroduced viable cells into the broth in which the effective cephalosporin concentration was reduced by β -lactamase.

The results of susceptibility tests represent the outcome of complex interactions among (i) the cell, its physiological state, relative permeability, and penicillin-binding proteins; (ii) the concentration and physicochemical characteristics of the β -lactamase; and (iii) the particular antibiotic.

The outer membrane of *B. catarrhalis* is not an efficient permeability barrier (116). However, the barrier function

TABLE 5. Prevalence of β -lactamase-producing (β la⁺) strains confirmed as *B. catarrhalis*

Reference	Place	Yr	Source of cultures ^a	Detection method ^b	No. of strains		
					Total	β la ⁺	%
311	Arizona	1976	Sputum	Ncef	NS ^c	1	
311	Puerto Rico	1976	Conjunctiva	Ncef	NS	1	
198	Sweden	1977	Nasopharynx, children	Ncef, iodo	53	2	3.8
224	Belgium	1977	TA, BPI	Ncef	15	1	6.7
294	The Netherlands	1980	Sputum, BPI	Ncef or biol	27	2	7.4
		1981			50	19	38.0
		1982			42	20	47.6
85	The Netherlands	1981	Sputum, BPI	Ncef	307	66	21.5
		1983-1984			737	355	48.2
86	The Netherlands	1985-1986	Sputum, BPI	Ncef	NS	NS	63-70
206	Scotland	1981-1982	Sputum, BPI	Ncef	96	42	43.8
		1983			116	68	58.6
		1984			44	34	77.3
269	Denmark	1986	TA, sputum, BPI	Biol or Ncef	106	52	49.1
234	France	1981-1985	Sputum, pharynx	Ncef, iodo, acid	134	NS	56.8
277	New Zealand	1980-1982	Sputum, BPI	Acid, Ncef	99	38	38.4
279	New Zealand	1983	Sputum, BPI	Acid	NS	2	2.7
		1984			NS	13	18.6
		1985			22	11	50.0
313	Australia	1984	Sputum, BPI	Ncef	13	7	53.8
267	Japan	1983-1984	Sputum, BPI	Ncef, acid	47	29	61.7
160	California	1978-1979	Sputum, BPI	Ncef	7	5	71.4
274	Ohio	1980-1982	MEF, children	Biol	26	20	76.9
305	Ohio	1982-1984	MEF, children	Ncef	61	46	75.4
			Throat, children		63	46	73.0
7	Tennessee	1982-1983 ^d	TA, sputum, BPI	Ncef	53	46	86.8
283	Tennessee	1983-1986	Sputum	Ncef	31	24	77.4
241	Texas	1983-1985	TA, sputum, BPI	Ncef	220	158	71.8
119	Spain	1986-1987 ^d	TA, sputum, BPI	Iodo	61	47	77.0
302	Belgium	1987	BPI	Ncef	83	NS	69.8
		1988	Oral, school children		94	73	77.7

^a TA, Transtracheal or endotracheal aspirates; BPI, bronchopulmonary infections; MEF, middle-ear fluid.

^b Ncef, Nitrocef; iodo, iodometric; biol, biological; acid, acidometric.

^c NS, Not stated.

^d Date of isolation given in related paper or estimated from the date manuscript was submitted.

may be increased by mutation, and the ensuing reduced permeability will limit entry of antibiotics into the periplasmic space and lead to increased resistance. A second barrier is provided by β -lactamase which amplifies the resistance to β -lactam agents (222). The result is the maintenance of a periplasmic concentration low enough to permit the continuing action of peptidoglycan-synthesizing enzymes in spite of a potentially inhibitory β -lactam concentration in the external environment.

Susceptibility test results are affected by the physiological state of the inoculum and the composition of the antibiotic-containing medium. These factors may partly explain the differences shown in Table 6 between the ranges of benzylpenicillin MICs in tests with media containing or lacking blood which were recorded by Doern and colleagues (100, 103) and Dunkin et al. (107). The use of media supplemented with cysteine resulted in reduced activity of amoxicillin/clavulanic acid (281).

Inoculum density is another factor that significantly affects the MIC determined for β la⁺ strains (102, 111, 160, 184, 231). Differences between the number of cells spot inoculated on agar media may account to some extent for differences between the results reported by Doern and Tubert (103) (Table 6) and Yokota et al. (323) (Table 7) for antibiotics that are readily hydrolyzed. A better correspondence is seen in the values reported by these authors for MICs of cefotaxime and other antibiotics that are comparatively resistant to inactivation by β -lactamase. The inoculum effect

in broth (2-ml final volume) dilution tests was most conspicuous with relatively susceptible β la⁺ strains tested at high densities (102). For example, in tests of five strains with ampicillin, inocula of 10⁴ CFU/ml gave MICs of 0.025 μ g/ml, whereas with 10⁷ CFU/ml the MICs ranged between 0.4 and 3.2 μ g/ml.

B. catarrhalis strains characterized by an ampicillin MIC of \leq 0.25 μ g/ml were considered susceptible (162) or, more recently, MICs ranging between 0.125 and 0.5 μ g/ml were defined as moderately susceptible (97). A peculiarity of β la⁺ strains is that some appear to be susceptible. Ampicillin MICs of 0.03 (103), 0.06 (101), $<$ 0.125 (7), and \leq 0.125 (85, 184, 259, 267) μ g/ml were reported for β la⁺ strains. Significantly, two strains with ampicillin MICs of 0.064 μ g/ml hydrolyzed nitrocef in at only 60% of the mean value found for 13 strains with ampicillin MICs of \geq 0.25 μ g/ml (295). Two other aberrant *B. catarrhalis* strains were β -lactamase negative by the nitrocef in disk test, but were recognized as β la⁺ when cell extracts were tested with nitrocef in solution (193). Both strains produced a β -lactamase identified as the 1908 type (now designated BRO-2; see below) and were more susceptible to six β -lactam antibiotics than strains that produced β -lactamases of the Ravasio (BRO-1) type.

The idea (193) that cellular production of BRO-2 activity might be less than that of BRO-1 was confirmed by Wallace et al. (311). The mean activity of BRO-1 β -lactamase in cell-free extracts from 11 clinical strains was 149.1 \pm 56.5 (standard deviation) nmol of nitrocef in per min per mg of

TABLE 6. MICs of β -lactam antibiotics reported for β -lactamase-positive (β la⁺) *B. catarrhalis*

Authors (reference)	No. of strains	Test medium ^a	CFU in inoculum	Antibiotic ^b	MIC (μ g/ml)	
					Range	Geometric mean
Doern et al. (100)	11	M-H agar with 5% heated blood	10 ⁴ /spot	Pen	1.25–4.0	10.1
				Ampicillin	0.32–5.0	1.5
Doern and Tubert (103)	58	M-H agar	10 ⁴ /spot	Pen	0.015–4.0	0.74
				Ampicillin	0.03–4.0	0.89
				Carbenicillin	0.25–16	2.0
				Ticarcillin	0.5–4.0	1.4
				Piperacillin	0.125–1.0	0.25
				Cephalothin	1.0–8.0	4.1
				Cefuroxime	0.5–4.0	1.5
				Cefotaxime	0.06–2.0	0.38
				Cefoxitin	0.125–1.0	0.23
				Amox/Clav (2:1)	0.007–0.25	0.06
				Dunkin et al. (107)	35	Agar with 4% lysed blood
Philippon et al. (234)	49	Agar	3 × 10 ³ –5 × 10 ³ /spot	Pen	2.0–32	9.78
				Amox	0.5–32	2.86
				Clav	8–128	30.27
				Sulbac	1–32	5.38
				Amox/Clav ^e	0.12–0.5	0.14
				Amox/Sulbac ^e	0.12–0.5	0.15

^a M-H, Mueller-Hinton.

^b Pen, Benzylpenicillin; Amox, amoxicillin; Clav, clavulanate; Sulbac, sulbactam.

^c Bacteria were incubated in Mueller-Hinton broth for several hours, thereby generating log-phase cells for inoculation.

^d NS, Not stated.

^e Fixed concentration of inhibitor, 5 μ g/ml.

protein, with a range of 60.4 to 217.4. The mean value for BRO-2 extracted from nine isolates was 65.5 \pm 43.1 nmol of nitrocefin per min per mg of protein (range, 29.0 to 167.7; but only one extract had activity in excess of 100).

The production of β -lactamase does not necessarily translate into resistance in the host (97). Infections by some β la⁺ *B. catarrhalis* strains have been treated successfully with benzylpenicillin, ampicillin, or amoxicillin (98, 160, 200). On the other hand, failures in the treatment of children with acute otitis media occurred in some cases of *B. catarrhalis* infection with >10⁴ CFU/ml (310). Johnsson and Brorson (161) found significant differences among the relative rates of hydrolysis of ampicillin, cefaclor, and cefuroxime by intact β la⁺ cells tested at pH 6 and 8. This might affect the outcome of treatment of an infection of the maxillary sinus or middle ear where the pH may be nonphysiological (47). In view of the uncertainties regarding the number of bacteria, the concentrations of β -lactamases and antibiotic, and the environmental conditions at the site of infection, it is expedient in the clinical situation to consider that a β la⁺ strain is likely to be unaffected by β -lactam agents that can be cleaved by the β -lactamase.

Genetic features. The production of β -lactamase is apparently constitutive in *B. catarrhalis* (56, 115, 276, 295). The precautions needed to confirm the absence of induction are given by Bush and Sykes (55).

The genetic determinant of β -lactamase, *bla*, is thought to be located on the chromosome. This conclusion was originally based on the inability of investigators to visualize plasmid DNA in lysates of various β la⁺ strains (56, 100, 160, 229, 230, 237, 294, 304). These negative findings may have been due to preparative difficulties (17) and the low percentage of plasmid-carrying strains (17, 311). Recently, bands of extrachromosomal DNA have been detected in *B. catarrhalis* (17, 167, 170, 311) and also in several *Moraxella* species (167, 311).

It is important to note, however, that many β la⁺ *B. catarrhalis* strains lack plasmids and strains carrying plas-

mids may not produce β -lactamase (17, 167, 311). As yet, the title of the 1986 communication, "Plasmid-Mediated β -Lactamase in *Branhamella catarrhalis*" (167), is not justified.

A 12.2-kilobase plasmid, designated pLQ510, was isolated from the β la⁺ strain E22 (17). Radiolabeled, purified pLQ510 DNA, which was used as a molecular probe to screen 17 *B. catarrhalis* strains, gave a positive signal with only three: strain E22, a second known plasmid carrier, and E7. The β la⁺ strain E7 was found in repeated tests to lack plasmid DNA. Homology was detected between the pLQ510 probe and chromosomal DNA of strain E7, but no homology was found with 11 other β la⁺ strains. Beaulieu et al. (17) concluded that the homology did not come from the *bla* gene.

The *H. influenzae* gene that encodes β -lactamase is plasmid associated, but in the majority of β la⁺ strains the plasmid genome is integrated into the chromosome. A possible correlation between integration and virulence was suggested by the observation that ampicillin-resistant *H. influenzae* strains carrying extrachromosomal *bla* displayed reduced virulence in the infant rat model compared with resistant strains carrying integrated *bla* sequences (214).

Kamme and co-workers in Sweden reported successful intraspecific (169) and intergeneric transfer of β -lactamase production by conjugation (170). The *bla* genes of *B. catarrhalis* and *M. nonliquefaciens* donor strains were transferred to a *B. catarrhalis* recipient strain that was β -lactamase negative and resistant to rifampin and novobiocin. Recombinants were selected on a medium containing benzylpenicillin and rifampin (167). An unusual feature of β la⁺ *B. catarrhalis* donor strains was their loss of ability to transfer *bla* unless they were subcultured on penicillin-containing medium immediately after primary isolation (170). Wallace et al. (311) confirmed the transfer of *bla* from donor strains of *B. catarrhalis* and *M. nonliquefaciens* to the same two *B. catarrhalis* recipient strains that had been used by Kamme et al. (167).

The addition of 100 μ g of DNase I to the filter membranes that hold the mating populations and the absence of inten-

tionally added DNA were considered sufficient to identify the mechanism of transfer as conjugation. I suggest the alternative possibility, transformation. Transformation of streptomycin resistance is known to occur between *B. catarrhalis* and strains originally designated *M. nonliquefaciens* (33, 59) but more recently assigned to the new species *M. osloensis* (15, 37).

Three testable considerations support my argument that the mechanism of the reported *bla* transfers could be transformation. (i) DNase activity may be lost due to proteolysis and diffusion from the filter membrane into the underlying medium. A fraction of the cells crowded on the membrane may remain competent for transformation which can occur before the end of the overnight incubation period allowed for mating and phenotypic expression. (ii) DNA released from cells spontaneously can elicit transformation. I (65) showed that the cell-to-cell transfer of *N. gonorrhoeae* chromosomal genes occurred during cocultivation of a competent recipient strain with a donor strain that lacked a conjugative plasmid; DNA was not added experimentally, but DNase abolished this transfer. Dorward et al. (104) found that a vehicle for DNA transfer was naturally elaborated membrane vesicles (blebs). Such blebs, which are also produced by *B. catarrhalis* during growth (143, 159, 217), may provide a natural mechanism for transfer of transforming DNA between cells. (iii) The tendency of donor cells to transfer DNA may be enhanced by growth on penicillin-containing medium. β -Lactam antibiotics are known to disorganize the wall structure of gram-negative bacteria, thereby increasing permeability (191) and, possibly, the tendency for the formation of membrane blebs.

Is the worldwide increase of β la⁺ *B. catarrhalis* since the late 1970s due to the dissemination of one highly communicable progenitor strain? Apparently it is not. Differences between β la⁺ strains were demonstrated by restriction endonuclease typing (229, 230). Patterson et al. (229) identified 12 distinct *Hae*III digestion patterns of chromosomal DNA among a group of β la⁺ strains.

How, then, did *B. catarrhalis* gain the ability to produce β -lactamase when historically the species was penicillin susceptible? Mutations of a preexisting, functional *bla* gene can produce minor changes of the enzyme product. However, interbacterial transfer must be responsible for the recent, sudden appearance of an efficient β -lactamase. The large increase in β la⁺ strains does not imply that large numbers of *bla* gene transfers occurred. Continued multiplication of the recipients of occasional transfers, especially in the presence of β -lactam agents, the genetic stability of *bla* in its chromosomal location, and person-to-person spread within the community and between regions by international travel can account for the high prevalence of β la⁺ strains.

Possible influence in mixed infections: indirect pathogenicity. In localized, mixed infections with a penicillin-susceptible pathogen and a β la⁺ commensal, the β -lactamase is thought to protect the pathogen by inactivating a penicillin used for therapy. This effect has been referred to as indirect pathogenicity (44, 85, 165, 205, 312). Wardle (312) found suggestive evidence of protection in a few patients with bronchopulmonary infections who showed no clinical or radiological improvement during treatment with ampicillin or amoxicillin. Penicillin-susceptible strains of pneumococci or *H. influenzae* together with β la⁺ *B. catarrhalis* were identified in sputum specimens and β -lactamase was detected, but the antibacterial activity expected of the penicillin was absent. Subsequently, favorable therapeutic re-

sponses were obtained with antibiotics to which each of the organisms was susceptible.

A mouse model was used by Renneberg and Walder (244) to study the survival of a penicillin-susceptible pneumococcal strain mixed with either of two *B. catarrhalis* strains, one β la⁺ and the other (control) β -lactamase negative. Threads impregnated with bacteria were implanted subcutaneously on the backs of mice, which were then treated with ampicillin by periodic injection into the hind leg. Threads were removed after 8 h and immersed in phosphate-buffered saline, which was immediately used for assays of CFU and ampicillin concentrations. The β -lactamase produced during the mixed infection greatly reduced the antibacterial effect of the ampicillin.

The probability of ampicillin failure in mixed infections depends in part on the characteristics and the magnitude of the populations and the β -lactamase. *B. catarrhalis* β -lactamases are membrane associated and released only in minimal amounts (100, 115, 116). Thus, many β la⁺ strains may not release enough β -lactamase to protect other bacteria in a localized site.

Characteristics of the β -Lactamases

A recent classification placed the Ravasio and BRO-1 β -lactamases of *B. catarrhalis* in group 2c (54). Enzymes in 2c hydrolyze carbenicillin at least 75% as fast as benzylpenicillin and hydrolyze cloxacillin and many cephalosporins at relatively low rates; the isoelectric point (pI) typically falls below neutrality. Investigators have characterized β -lactamases of strains isolated from patients in England (231, 276), France (56, 234), Belgium (115), The Netherlands (294, 295), Sweden (111), the United States (220, 305, 311), and Japan (323). These enzymes are distinct from the plasmid genome-encoded TEM-1 β -lactamase found in *N. gonorrhoeae*, *H. influenzae*, and *Escherichia coli* (56, 111, 115, 234, 294, 311).

A large proportion of the β -lactamase remains associated with the cell debris obtained by ultrasonication of *B. catarrhalis* cells (111, 115). However, the presence of 0.5% Triton X-100 during ultrasonication results in >90% solubilization of enzyme (116). This indicates that this β -lactamase is strongly membrane associated.

Different methods used for enzyme extraction yield different amounts of β -lactamase (275) and other cellular proteins. Crude extracts are known to contain substances that affect enzyme activity either by interacting with the enzyme itself or by interfering with its reaction with substrate compounds (276). The use of crude extracts with weak enzyme activity in many of the studies may account in part for the diversity of substrate profiles and pIs reported and for the inference by some investigators that there are a number of distinct types of *B. catarrhalis* β -lactamase, perhaps as many as five (294) or six (295). On the other hand, with the exception of strain 939 described by Stobberingh et al. (294), Kamme et al. (167) found no evidence that *B. catarrhalis* possesses more than the one type of β -lactamase which they designated BRO-1. More recently, Wallace et al. (311) classified the Ravasio-type β -lactamase as BRO-1 and the related 1908 type as BRO-2.

Isoelectric focusing separates proteins by aligning them as sharp bands at their isoelectric points in a pH gradient produced electrophoretically. The β -lactamase bands are detected with nitrocefin overlays (275). Multiple, "satellite" bands often appear which are believed to represent biologically active molecules with altered net charges resulting from the loss and/or modification of specific amino acid

TABLE 7. β -Lactam antibiotics and the β -lactamase of *B. catarrhalis* NNBR-8303^a

Substrate	V_{max}^b	K_m (μ M)	MIC (μ g/ml) ^c
Benzylpenicillin	100	75	100
Ampicillin	66	11	50
Carbenicillin	77	3.7	50
Ticarcillin	51	26	25
Piperacillin	85	29	100
Cloxacillin	6	2.2	50
Cephaloridine	4	1.5	12.5
Cephalothin	8	2.9	25
Cefuroxime	18	155	0.78
Cefotaxime	8	147	0.20
Cefoxitin	1	7.4	0.20

^a The β -lactamase was purified by column chromatography on DEAE-Sephadex. (Data taken from Yokota et al. [323] with permission of the publisher.)

^b Maximum rate of hydrolysis of β -lactam substrate relative to that of benzylpenicillin, which is assigned a value of 100.

^c Determined in tests of one loopful (approximately 5 μ l) of an overnight broth culture on sensitivity disk agar-N containing twofold dilutions of antibiotic; inoculum estimated as 10⁸ CFU/ml.

residues, possibly by enzymatic action (276). The β -lactamase in a crude extract (parent enzyme) of *B. catarrhalis* 2001E focused as four major bands, A, B, C, and D, with pI values of 7.2, 6.4, 6.0, and 5.6, respectively (276). The activity of band A was not sufficient to permit further analyses, but B, C, and D were isolated and found to have similar substrate profiles. These differed somewhat, however, from the profile displayed by the less purified parent enzyme. Gel filtration of the parent enzyme gave a single peak of activity, corresponding to a molecular weight of 41,000. Molecular weights estimated for bands B, C, and D were 41,000, 41,000, and 36,500, respectively.

Kinetic constants of the β -lactamase purified from strain NNBR-8303 are given in Table 7. The data also show the correlation between the antibacterial activity (MIC) of the various β -lactam substrates and their resistance to cleavage by the β -lactamase. Not shown are diffusibility differences between the antibiotics which may also affect the MIC (222). The enzyme efficiently inactivates ampicillin as shown by the high rate of hydrolysis (V_{max} of 66) and the fairly high affinity for this substrate (K_m of 11 μ M). With cefotaxime as substrate, on the other hand, both the maximum rate of hydrolysis (V_{max} of 8) and the substrate affinity (K_m of 147 μ M) are low, indicating the relative inability of the enzyme to attack cefotaxime. This is consistent with the low MIC of cefotaxime (0.2 μ g/ml) compared with that of ampicillin (50 μ g/ml). This purified β -lactamase had a pI of 5.4 and an approximate molecular weight of 33,000 (323).

M. nonliquefaciens strains isolated in Sweden (111, 170) and the United States (311) also produce β -lactamase, a trait that appears to have been acquired in the recent past. The β -lactamases from *M. nonliquefaciens* and *B. catarrhalis* resemble each other (111). Wallace et al. (311) identified isoelectric focusing patterns characteristic of BRO-1 and BRO-2 in both organisms and found that relatively similar substrate profiles were displayed by the β -lactamases from both organisms. Benzylpenicillin, ampicillin, and methicillin were hydrolyzed at approximately equal rates. The hydrolysis rates for cefaclor and nitrocefin were higher and that for cephaloridine was much lower than that for benzylpenicillin.

Clavulanic acid and sulbactam are examples of active-site-directed, irreversible inhibitors called suicide inhibitors (55). Low concentrations of these agents inactivate *B. catarrhalis*

β -lactamases (115, 234, 294, 323), including both BRO-1 and BRO-2 (116). Since suicide inhibitors protect amoxicillin from the hydrolytic action of β -lactamase, the MIC of amoxicillin plus clavulanate is lower than the MIC of either agent alone (Table 6).

The important work of sequencing the gene encoding the BRO-1 β -lactamase is under way (D. Beaulieu, L. Piche, and P. H. Roy, Progr. Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1120, 1989). Comparisons of this sequence with those of genes encoding BRO-2 and other possibly different β -lactamases will show the extent of their divergence.

STRUCTURE AND COMPOSITION OF *B. CATARRHALIS*

Capsule and Pili

B. catarrhalis, like *N. gonorrhoeae* (227), produces capsules visible by electron microscopy as a fibrillar coat. The fuzzy appearance is seen in an ultrathin section of *B. catarrhalis* stained with magnesium uranyl acetate and lead citrate (illustrated in plate 10.2, Fig. 1, of the 1974 edition of *Bergey's Manual* [245]). Hellio et al. (143) used ruthenium red staining to show the polysaccharide composition of this fibrillar coat, which is seen here (Fig. 1c and d) as spicule-like material (sp) projecting from the outer membrane. The aggregated (autoagglutinated) cells appear to be held by their spicules.

Pili, also called fimbriae, are filamentous appendages that extend from the outer membrane and serve as adhesins (16). Pili were not observed in electron micrographs of *B. catarrhalis* ATCC 25238^T (143), ATCC 8176 (318), or ATCC 8193 (318). Preliminary observations of four clinical isolates revealed piliation (215). Wistreich and Baker (318) reported in 1971 that a strain identified as "*N. catarrhalis*" was piliated and agglutinated erythrocytes from rabbit and mouse, but not human (O, Rh₀+), sources. Scanning electron microscopy revealed adhesion of these cells and their pili to agglutinated mouse erythrocytes. Soto-Hernandez et al. (282) observed 4+ hemagglutination of human group O Rh⁺ erythrocytes by 16 *B. catarrhalis* clinical isolates. Further work is needed to determine whether this disagreement concerning erythrocytes is due to structural differences of pili or of human erythrocyte receptors.

Hemagglutination was not observed with strain ATCC 25238 (282) as expected from its lack of pili (143). A feature of strain ATCC 25238 (earlier designated Ne 11) which is valuable for genetic studies is its high frequency of intraspecific transformation (33, 68). The absence of piliation is interesting in view of the association between piliation (indicated by distinctive colony types [39, 64]) and transformability of *N. gonorrhoeae* (65, 181) and *M. nonliquefaciens* (39).

Envelope

The envelope or cell wall (Fig. 1a and b) resembles that of *N. gonorrhoeae* (227). Two trilaminar structures (cm and om) can be seen between which lie a periplasmic space and the dense peptidoglycan-containing layer (pg). The cytoplasmic membrane (cm), which is the innermost layer, carries penicillin-binding proteins (PBPs) at its outer face (286). PBPs 1, 2, and 3 of *B. catarrhalis* have molecular weights of 77,000, 68,000, and 39,000, respectively (128). PBP 3, like *E. coli* PBP 5/6, possesses a weak β -lactamase activity.

Proteins, LOS, and phospholipids are found in the outer

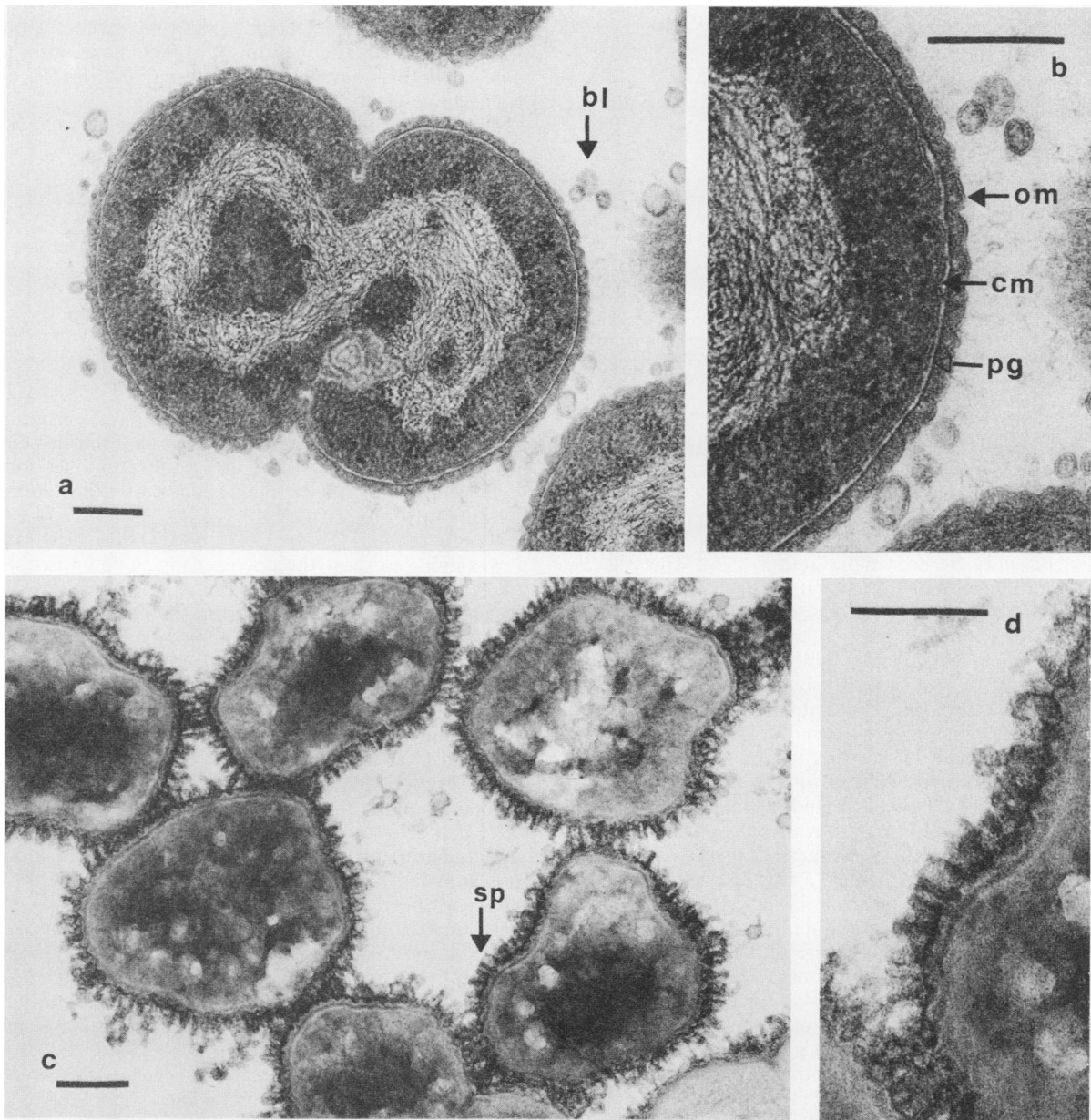


FIG. 1. Ultrastructure of *B. catarrhalis* ATCC 25238^T seen in thin sections. (a and b) Fixation with OsO₄; (c and d) ruthenium red staining. Abbreviations: bl, blebs; om, outer membrane; cm, cytoplasmic membrane; pg, peptidoglycan-containing layer; sp, spicule-like material. Bars, 0.2 μ m. (Reprinted from *Annales de l'Institut Pasteur/Microbiology* [143] with permission of the publisher.)

membrane. It should be noted that LOS is a variety of lipopolysaccharide that lacks the long, surface-exposed polysaccharide constituting the O antigen of smooth enteric bacteria (133, 159, 303). The absence of O antigen increases outer membrane permeability, resulting in enhanced susceptibility to erythromycin, rifampin, and other hydrophobic agents (222, 223).

Outer membrane vesicles or blebs are released spontaneously during growth (159, 217, 264). Their membranous appearance is evident in Fig. 1 (bl) and in the electron micrograph of outer membrane vesicles that Murphy and Loeb (217) obtained from the supernatant of a *B. catarrhalis* broth culture. Such preparations contain LOS and have a protein-binding pattern similar to that of outer membranes prepared by other methods (159, 217, 264).

N. gonorrhoeae blebs are highly toxic for embryonated eggs (227). In the early literature, blebs were referred to as free endotoxin (227, 264) and, indeed, they function as a packaging system for the distribution of endotoxin. The toxicity is due to the biological activity of lipid A, the glycolipid component of LOS. Lipid A is anchored in the outer membrane and is covalently bound to core oligosaccharides (133). The compositions of the *B. catarrhalis* "lipopolysaccharide" and its lipid A and core oligosaccharides were reported (1, 159).

Eight major OMPs (OMP-A to OMP-H) whose approximate molecular weights ranged between 98,000 and 21,000 were identified in *B. catarrhalis* (14, 216, 217). OMP-F has a molecular weight of 43,000 that is not altered by 100°C

TABLE 8. DNA base compositions

Species	G+C content (mol%) determined by:					
	Chromatography (68,69) ^a	Buoyant density in CsCl (38)	<i>T_m</i> (thermal denaturation midpoint)			
			202 ^b	148	261	258
<i>B. catarrhalis</i>						
Strain ATCC 25238 ^{Tc}	40.8 ^d	41.0	41	41.3	41.0	
Other strains ^e	40.0–43.0	41.0–42.0		41.6		
<i>N. cinerea</i>		49.0		50.9		50.5
<i>N. flavescens</i>	50.2 ^d	46.5–47.5	49		49.6	50.1
<i>N. gonorrhoeae</i>				53.3		53.2
<i>N. meningitidis</i>	51.3 ^d		51.5	52.0	52.8	53.2
<i>N. caviae</i>		44.5		47.3	46.9	

^a Numbers in parentheses are reference numbers.

^b Reference number.

^c Type strain of the species, originally designated Ne 11.

^d G+C content was derived from the A+T/G+C ratio.

^e Excludes "*N. catarrhalis*" NCTC 4103, which deviates enough to suggest that the species designation may be incorrect (15, 33, 59, 69).

treatment, in contrast to OMP-C, -D, -E, -G, and -H which are heat modifiable (217).

Porins are proteins that typically exist as trimers forming channels through the outer membrane for passive diffusion of small hydrophilic nutrients and antibiotics (223). OMP 2 of *N. meningitidis* is a porin characterized by a molecular weight of 40,000 to 42,000 (124). In view of the relationship between response to hydrophilic antibiotics and porin function, it will be of interest to investigate the *B. catarrhalis* porin(s). I speculate that OMP-F may be a porin.

The fatty acid profiles of members of the family *Neisseriaceae* have been determined in various laboratories (45, 147, 157, 187, 190, 298), but caution is needed in comparing the results. The fatty acid content is greatly affected by the culture medium components and incubation temperature (157, 187). For *B. catarrhalis* cells grown on supplemented GC medium base agar, 20% of the total fatty acids were recorded as 17:1 acid (where the number left of the colon indicates the number of carbon atoms and that on the right is the number of double bonds). However, 17:1 acid was not detected in cells grown on Trypticase soy agar (187). Various authors (147, 157, 187, 298) agree that the 16:0, 16:1, and 18:1 acids are abundant in *B. catarrhalis* and several species from animals, including *N. caviae* (this species is described in Table 4, footnote c). Nevertheless, the fatty acid profiles differ. By determining the double-bond positions (Δ) in 18:1 acids, Sugimoto et al. (298) distinguished *B. catarrhalis* (Δ 11 18:1, 64% of total fatty acids) from *N. caviae* (Δ 9 18:1, 42%).

DNA

The guanine-plus-cytosine (G+C) contents of DNA preparations from *B. catarrhalis* and possibly confusing *Neisseria* species are given in Table 8. The findings for a given species were in agreement, except for several values (especially for *N. flavescens*) determined by the CsCl gradient centrifugation method. Also, divergent G+C values determined by a chromatographic method were reported (186) for 11 strains of *N. caviae* (range, 47.7 to 50.4 mol%; not included in Table 8).

Differences between the G+C contents of *B. catarrhalis* and *N. meningitidis* DNA, for example, indicate base sequence differences sufficient to explain the absence of DNA-mediated transformation of streptomycin resistance between transformable strains of these species (68), their low degree

of DNA relatedness shown by DNA-DNA hybridization (174), and their lack of stable DNA duplexes (176). The "*N. catarrhalis*" genome contains approximately 2.3×10^6 nucleotide pairs (175). They must encode several thousand gene products. In view of the DNA differences, it is not surprising that a two-dimensional polyacrylamide gel electrophoresis system which separated 200 polypeptides from cellular extracts revealed only 9% similarity between the locations of polypeptide spots on electrophoretograms of a *B. catarrhalis* strain and a reference *N. gonorrhoeae* strain (152).

WHAT'S IN A NAME?

A scientific bacterial name should be unambiguous, recognizable in all languages, and stable (42, 280). "One of the annoying problems facing the practicing physician is the constant reclassification and changing names of microorganisms" (324). Although aware of the inconvenience of the name change, investigators working before 1970 recognized that substantial differences between "*N. catarrhalis*" and the "true neisseriae" mandated their separation into different genera (1, 15, 33, 68, 146, 174, 190). This separation was validated by subsequent studies (147, 152, 159, 261, 288).

Ideally, a genus is composed of species that are relatively similar phenotypically and show 50 to 65% DNA relatedness determined under conditions optimal for DNA reassociation (42). The "true neisseriae" associated with human hosts include *N. gonorrhoeae*, *N. meningitidis*, and the less pathogenic *N. flavescens*, *N. cinerea*, *N. sicca*, *N. subflava*, *N. mucosa*, and *N. polysaccharea* (177, 258, 307). Their levels of DNA relatedness are appropriate for membership in a single genus. On the other hand, relations between these organisms and *B. catarrhalis* are minimal or absent as determined by (i) genetic transformation of streptomycin resistance (33, 68, 148); (ii) DNA base composition (Table 8); (iii) DNA homologies determined by several techniques (136, 148, 174, 176); and (iv) rRNA binding to DNA (261).

In spite of the widespread agreement that it is inappropriate to include "*N. catarrhalis*" in the genus *Neisseria*, the name of the genus to which the species should be transferred is controversial. Two names have been proposed: *Branhamella catarrhalis* (Catlin in 1970 [60]) and *Moraxella (Branhamella) catarrhalis* (Bøvre in 1979 [35]). The latter is an emended version of the name *Moraxella catarrhalis*

which was suggested in 1968 (146) in consideration of the genetic relatedness demonstrated independently by Bøvre (32, 34) and by Catlin (59, 70). The extensive work of the Oslo group and their rationale for including bacterial rods and cocci in the genus *Moraxella* are summarized in two reviews (39, 145). Bøvre found it expedient to divide the genus *Moraxella* into the subgenus *Branhamella* (to contain coccal organisms) and the subgenus *Moraxella* (to contain rod-shaped organisms). This is an awkward arrangement, however, since subgenus names are now rarely used (280), *Moraxella (Branhamella) catarrhalis* is unnecessarily long, and deletion of the subgenus name leaves the possibly confusing *M. catarrhalis*. The name *Branhamella catarrhalis* served to distinguish the organism from the so-called nonpathogenic neisseriae and focused attention on their differing potentials for pathogenicity. The proposed association with moraxellae, most of which possess only limited pathogenicity (39, 145), would relegate *B. catarrhalis* to an unfamiliar conglomerate of cocci and rods.

The differences revealed by DNA-rRNA hybridization are so great that Rossau et al. (261) recommended the removal of *Branhamella* and *Moraxella* from the family *Neisseriaceae* in which they were classified (36, 39, 146). The genus *Neisseria* is now placed in the beta group of the new class *Proteobacteria* (a taxonomic category higher than the family), whereas *Branhamella* and *Moraxella* are in the gamma group (288). The genetic relatedness between *Branhamella* and *Moraxella* is genuine, but it can be acknowledged adequately, I believe, by assigning these genera to a new family, *Branhamaceae*.

CONCLUSIONS AND PROSPECTS

B. catarrhalis has finally gained respect as a pathogen thanks to the many recent reports of its causal role in a variety of infections. Much has been learned about its structural, biochemical, and differential characteristics, responses to antibacterial agents, and β -lactamases. Future studies must use strains precisely identified by standard methods. Research is needed to enhance the molecular and genetic respectability of the organism. Initial strategies may use transformation to develop isogenic strains carrying auxotrophic and antibacterial resistance markers. The possible cell-to-cell transmission of chromosomal loci mediated by DNA associated with blebs should be investigated. Knowledge of the genes and their organization in *B. catarrhalis*, *N. caviae*, and *M. nonliquefaciens* is needed. This will provide a sounder basis for judgment concerning the relatedness and evolution of these organisms, permit comparisons with the genetic markers of *N. gonorrhoeae*, and aid the identification of gene products.

A multifactorial problem centers around the genetic loci encoding *bla*, their origin, mode of transfer, features of chromosomal integration, and phenotypic effects, in addition to β -lactamase production, possibly associated with their acquisition. The rapid, worldwide increase in the percentage of β la⁺ strains is remarkable. In some regions this has been accompanied by rising numbers of *B. catarrhalis* infections. The increasing incidence of bronchopulmonary infections may be due in part to the greater use of immunosuppressive agents. However, this could not account for the documented increases of childhood otitis media caused by *B. catarrhalis*. Possibly the success of β la⁺ strains is associated with an improved mechanism of adherence to mucosal membranes, especially of children, or some other virulence-associated property. Further investigations of this problem as well as

the virulence of case and carrier strains and the molecular basis of pathogenesis await the development of organ cultures or animal models. The recent logarithmic increase of publications illustrates the increased awareness of *B. catarrhalis* and growth of interest in the multitude of significant questions now apparent.

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