

## *Branhamella (Neisseria) catarrhalis*: Criteria for Laboratory Identification

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Eleven clinically significant isolates of *Branhamella catarrhalis* grew well on modified Thayer-Martin medium and produced beta-lactamase, but did not grow on nutrient agar at 22°C. Minimum inhibitory concentrations of vancomycin, colistin, and trimethoprim were found to be higher than the concentrations of these antibiotics in modified Thayer-Martin medium. The criteria necessary for laboratory identification of *B. catarrhalis* are discussed.

*Branhamella (Neisseria) catarrhalis* has long been considered a harmless upper respiratory tract commensal of humans (5). Its recent recognition as a human pathogen, particularly as a cause of significant lower respiratory tract disease in patients with antecedent-compromised pulmonary function (7-10; M. A. Johnson, W. L. Drew, and K. Montgomery, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, Abstr. no. C158, p. 336), underscores the need for prompt and reliable isolation and identification of *B. catarrhalis* by the clinical microbiology laboratory. Criteria that have been used in distinguishing *B. catarrhalis* include colonial and Gram stain morphology; lack of pigmentation; oxidase production; failure to produce acid from glucose, maltose, sucrose, lactose, and fructose; inability to synthesize polysaccharide from sucrose (2); growth at 22°C on nutrient agar (3); reduction of nitrate and nitrite (11); and absence of growth on modified Thayer-Martin (MTM) medium (7, 12). We recently analyzed 11 clinically significant isolates of *B. catarrhalis* with regard to these and other parameters. A 12th strain, obtained from the American Type Culture Collection (ATCC), was included for comparison.

All 12 strains were oxidase-producing, gram-negative diplococci which grew well on 5.0% sheep blood agar incubated at 37°C in a humidified atmosphere containing 10% CO<sub>2</sub>. Discrete, smooth, glistening, whitish-gray colonies (1- to 3-mm diameter) were observed after 18 h of incubation. All strains reduced nitrate and nitrite; failed to produce acid from glucose, maltose, and sucrose; and did not synthesize polysaccharide from sucrose. Slide agglutination and counterimmunoelectrophoresis analyses for *Neisseria meningitidis* serogroups A to D and

X to Z were uniformly negative, as was direct fluorescent-antibody typing for *Neisseria gonorrhoeae*.

Unexpectedly however, and in contradistinction to the ATCC control strain, none of the 11 clinical isolates of *B. catarrhalis* grew on nutrient agar incubated at 22°C. In addition, all clinical isolates grew well on MTM medium incubated at 37°C in humidified 10% CO<sub>2</sub>, whereas the ATCC control strain did not. Smooth, regular grayish colonies (2- to 4-mm diameter) were observed after 18 h of incubation.

Growth on MTM medium was of interest insofar as the definitive description of *B. catarrhalis* (11) stated that this organism was inhibited by polymyxin B and similar antibiotics, i.e., colistin, which is incorporated into MTM medium, and since failure to grow on this medium is thought to be a property of *B. catarrhalis*. For this reason, the minimum inhibitory concentrations (MICs) of the antibacterial agents incorporated into MTM medium (i.e., vancomycin, colistin, and trimethoprim) were determined for all 12 strains of *B. catarrhalis*. The results are listed in Table 1.

The concentrations of vancomycin, colistin, and trimethoprim in MTM medium were 3.0, 7.5, and 5.0 µg/ml, respectively. All 11 clinical isolates of *B. catarrhalis* uniformly demonstrated MICs significantly higher than the concentrations of vancomycin, colistin, and trimethoprim in MTM medium, thus explaining their ability to grow on this medium. The failure of the ATCC strain to grow on MTM medium was apparently due to its sensitivity to colistin (MIC = 1.25 µg/ml). This value is significantly lower than the concentration of colistin (7.5 µg/ml) in MTM medium.

All 11 clinical isolates were also found to produce β-lactamase when examined by the iodimetric overlay method of Baldwin et al. (1). The

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TABLE 1. *Vancomycin, colistin, and trimethoprim MICs for B. catarrhalis*<sup>a</sup>

Strain	Source of isolate	$\beta$ -Lactamase production <sup>b</sup>	MIC ( $\mu$ g/ml)		
			Vancomycin	Colistin	Trimethoprim
Clinical isolates					
324 <sup>c</sup>	Pharynx	+	40	20	20
2233 <sup>d</sup>	Eye	+	>40	40	>40
4120 <sup>d</sup>	TTA <sup>e</sup>	+	>40	40	>40
NRL-A1649 <sup>f</sup>	TTA	+	>40	80	>40
318 <sup>d</sup>	Blood	+	>40	20	40
NRL-1262 <sup>f</sup>	TTA	+	>40	40	20
NRL-1650 <sup>f</sup>	TTA	+	>40	40	>40
634 <sup>c</sup>	Cervix	+	10	>80	20
NRL-1656 <sup>f</sup>	TTA	+	>40	80	>40
NRL-1246 <sup>f</sup>	TTA	+	>40	40	>40
W786 <sup>c</sup>	Peritoneal exudate	+	>40	40	>40
Control strain					
ATCC 8176		-	20	1.25	20

<sup>a</sup> MICs were determined by agar dilution with chocolate Mueller-Hinton agar and an inoculum size of  $10^8$  organisms per spot; plates were incubated at 37°C in humidified 10% CO<sub>2</sub> for 20 h.

<sup>b</sup>  $\beta$ -Lactamase production determined as described by Baldwin et al. (1).

<sup>c</sup> Isolates obtained from R. Jones, Kaiser Permanente Hospitals, Portland, Ore.

<sup>d</sup> Isolates recovered at the University of Oregon Health Sciences Center, Portland, Ore.

<sup>e</sup> TTA, Transtracheal aspirate.

<sup>f</sup> Isolates obtained from P. Piot, University of Washington, Seattle, Wash.

ATCC strain did not produce  $\beta$ -lactamase (Table 1). These results are consistent with the reports of others (4, 6, 8-10) that clinically significant isolates of *B. catarrhalis* often produce  $\beta$ -lactamase. The apparent strict correlation among  $\beta$ -lactamase production, resistance to colistin, and virulence observed in this study is presently being investigated in more detail.

The observations presented in this report emphasize the need to reexamine the microbiological characters used to identify *B. catarrhalis*. Growth at 22°C on nutrient agar and failure to grow on MTM medium should not be considered identification criteria of *B. catarrhalis*. To examine the latter criterion, ca.  $10^5$  colony-forming units of *B. catarrhalis* strains 324, 634, W786, and ATCC 8176 were plated on MTM medium. After 48 h of incubation,  $3 \times 10^3$  colony-forming units were observed for strains 324, 634, and W786; no colonies were observed with strain ATCC 8176. Therefore, the failure to detect growth on MTM with strain ATCC 8176 was not due to insufficient inocula. Until more extensive studies have been conducted, we suggest that the following minimal criteria be employed in the routine laboratory identification of *B. catarrhalis*: gram-negative diplococci; oxidase production; growth on 5.0% sheep blood agar incubated at 37°C in humidified 10% CO<sub>2</sub>; lack of pigmentation; failure to produce acid from glucose, maltose, and sucrose; and reduction of nitrate and nitrite.

The importance of thorough microbiological characterization of isolates resembling *B. catarrhalis* is underscored by its seeming emergence as a human pathogen. Furthermore, it is obviously necessary to distinguish this organism from *N. gonorrhoeae* and *N. meningitidis* since *B. catarrhalis* is clearly found in sites indigenous to these bacteria and possesses growth properties previously thought to be characteristic only of the "pathogenic *Neisseria*," i.e., failure to grow at 22°C on nutrient agar and growth on MTM medium.

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