Antimicrobial Susceptibility of *Branhamella catarrhalis* Isolates from Bronchopulmonary Infections

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Fifty-four clinical isolates of *Branhamella catarrhalis* from patients with bronchopulmonary infections were studied. The MICs for 50 and 90% of the isolates and the geometric mean MICs were determined for 11 antimicrobial agents. All the strains were resistant to trimethoprim but were susceptible to clavulanate-potentiated amoxicillin (Augmentin; Beecham Research Laboratories, London), chloramphenicol, co-trimoxazole, erythromycin, cefotaxime, and cefuroxime. Beta-lactamase-negative strains were uniformly susceptible to penicillin and ampicillin.

Branhamella catarrhalis is gaining increasing recognition as a respiratory pathogen. An increase in the incidence of *B. catarrhalis* infections has been reported by several centers (10, 15). More disturbing has been the alarming rate at which the proportion of β -lactamase-producing strains has increased (3, 4, 10). A notable rise in the rate of isolation of clinically significant *B. catarrhalis* strains causing infective exacerbations in patients with chronic pulmonary disease was reported for the winter months of 1982 to 1983 in Edinburgh; of these strains, 51% produced β -lactamase (10). Of considerable concern was the fact that 40% of those patients who had received ampicillin did not respond and thus required hospitalization. Of 81 patients, 53% acquired their infections in the hospital. As few studies are available regarding the antimicrobial susceptibility of *B. catarrhalis* (2, 6), we report our findings for clinical isolates obtained from patients with bronchopulmonary infections.

A total of 54 clinical isolates of *B. catarrhalis* were studied (35 β -lactamase positive and 19 β -lactamase negative). Seven *B. catarrhalis* strains (NCTC 3622, 3623, 3625, 4103, 11015, 11016, and 11020) were obtained from the National Collection of Type Cultures, Central Public Health Laboratory, London, England. As no β -lactamase-positive *B. catarrhalis* strains were available from the National Collection of Type Cultures, the reference strains listed above were included for comparison with our β -lactamase-negative clinical isolates. In addition, a β -lactamase-positive *Staphylococcus aureus* strain (B1555), obtained from Beecham Research Laboratories, London, and a β -lactamase-negative *S. aureus* strain (NCTC 6571) were included. The *B. catarrhalis* strains were identified by rapid carbohydrate utilization tests (18). All the isolates were tested for DNase activity (16) and β -lactamase production (14). To investigate the previously suggested markers of virulence (5, 9, 13, 15), we studied growth on nutrient agar at 22 and 37°C and on modified Thayer-Martin and New York City media by using an inoculum containing ca. 10^4 to 10^6 CFU. Agar dilution MICs were determined by the method described by Ericsson and Sherris (8).

The following 11 antibiotics were used: penicillin, ampicillin, Augmentin, erythromycin, chloramphenicol, cefuroxime, cefotaxime, trimethoprim, co-trimoxazole (trimethoprim-sulfamethoxazole) sulfamethoxazole, and colistin. Tetracycline was not tested because of local antibiotics policy. The drug concentrations were made by using a

commercially available preparation (ADATAB; Mast Laboratories, Liverpool, United Kingdom); concentrations were based on previously described MICs (2, 5). The antibiotics were incorporated in Diagnostic Susceptibility Test agar (DM215; Mast Laboratories) containing 7% lysed horse blood in accordance with the manufacturer's directions. Control plates with no antibiotics were made with each batch. As the determination of MICs by this method is less inoculum dependent (8) than by other methods, an overnight culture in brain heart infusion broth was diluted 1:50 and applied to the surface of the plate with a multipoint inoculator (MI400; Mast Laboratories); 2 μ l of inoculum was delivered. This dilution was found to be satisfactory in a pilot study. The inoculum was estimated to contain ca. 10^4 CFU per spot. All the plates were incubated in air at 37°C for 18 h. The MIC was defined as the lowest concentration of a given antibiotic that inhibited visible surface growth.

The MICs of the antibiotics used are shown in Table 1, and those for the β -lactamase-positive and β -lactamase-negative *S. aureus* are included for comparison. Whereas all the *B. catarrhalis* strains were resistant to trimethoprim, the MIC of colistin was uniformly ≤ 2 μ g/ml (except for one NCTC strain for which the MIC was 4 μ g/ml). All the strains were susceptible to Augmentin. All our isolates produced DNase and grew at 22 and 37°C on nutrient agar but failed to grow on New York City and modified Thayer-Martin media after overnight incubation. On the basis of the MIC susceptibility correlates derived from achievable serum concentrations (1), all the β -lactamase-producing *B. catarrhalis* strains were resistant to penicillin, ampicillin, trimethoprim, and colistin but susceptible to the remaining antibiotics. The β -lactamase-negative strains, including all NCTC strains, were resistant to trimethoprim but susceptible to the other antibiotics. Analysis of the individual and geometric mean MICs showed that erythromycin, Augmentin, and the newer cephalosporins were the most effective antibiotics against all the strains, but they conferred no advantage over penicillin or ampicillin against the β -lactamase-negative strains.

Although the natural history, course, and mechanism of the disease process produced by *B. catarrhalis* are not known, it is interesting that the mortality and morbidity caused by this organism are being reported more often (10, 13, 17). Its unique ability to produce an important enzyme, DNase (16), may be responsible for the inflammation of mucus membranes (9). Initially, strains producing β -lactamase were thought to confer increased virulence (9, 13, 15). Doern and Morse (5) suggested that colistin resistance or

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TABLE 1. MICs (micrograms per milliliter)^a for *B. catarrhalis*

Antibiotic	β -Lactamase-positive strains (n = 35)			MIC for standard <i>S. aureus</i> ^b	β -Lactamase-negative strains (n = 26)			MIC for standard <i>S. aureus</i> ^c
	Geometric mean MIC	MIC ₅₀	MIC ₉₀		Geometric mean MIC	MIC ₅₀	MIC ₉₀	
Penicillin	4.97	4.0	8.0	>32.0	0.03	0.01	0.01	0.01
Ampicillin	1.48	1.0	2.0	>16.0	0.01	0.005	0.03	0.125
Augmentin	0.05	0.06	0.125	>1.0	0.01	0.005	0.005	0.125
Erythromycin	0.07	0.03	0.125	0.25	0.13	0.03	0.125	0.125
Chloramphenicol	0.51	0.5	0.5	4.0	0.48	0.5	0.5	4.0
Cefuroxime	0.67	0.5	2.0	1.0	0.22	0.125	0.25	1.0
Cefotaxime	0.17	0.125	0.5	2.0	0.03	0.01	0.06	2.0
Trimethoprim	17.82	16.00	32.00	>4.0	20.00	16.00	32.00	>4.0
Sulfamethoxazole	5.68	2.0	16.0	>16.00	3.54	2.0	4.0	16.00
Co-trimoxazole	3.9	2.0	8.00	8.00	2.03	2.0	4.0	2.0
Colistin	1.15	1.0	2.0	>32.00	1.22	1.0	2.0	>32.00

^a Determined on Diagnostic Susceptibility Test agar. MIC₅₀, MIC for 50% of the isolates; MIC₉₀, MIC for 90% of the isolates.

^b *S. aureus* B1555 (β -lactamase positive).

^c *S. aureus* NCTC 6571 (β -lactamase negative).

failure to grow at 22°C was an additional index. Our results obtained from a larger number of isolates causing bronchopulmonary infections did not agree with these findings.

Clinically significant *B. catarrhalis* strains are no longer uncommon, particularly in the respiratory tract, and over half of these isolates are capable of producing β -lactamase (2, 3, 10). Furthermore, the presence of β -lactamase-producing organisms in sputum has been blamed for the neutralization of β -lactam antibiotics and for the prolongation of respiratory illness (3, 12, 17). This, together with the rapid increase in the number of β -lactamase-positive strains, has raised the question regarding the choice of antibiotics in acute chest infections (3, 7, 10). As life-threatening infections are not generally caused by *B. catarrhalis*, it is sufficient to treat most patients with oral antimicrobial drugs. All *B. catarrhalis* strains are uniformly resistant to trimethoprim, so the use of this drug in the treatment of bronchopulmonary infections requires careful consideration. Many workers (6, 15) have concluded that the β -lactamase of this organism is chromosomally associated. As the plasmids for the *Enterobacteriaceae* carrying genes coded for β -lactamase production can be transferred to *B. catarrhalis* (11), it would be interesting to find out whether an increase in such plasmids in the respiratory tract is responsible for the increase in β -lactamase-producing strains.

Finally, because *B. catarrhalis* can be found at sites indigenous to pathogenic *Neisseria* spp. and shares some physiological and biochemical characteristics with them, it is important that a thorough microbiological screening system be established for detection and identification of *B. catarrhalis*.

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