ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1986, p. 774–776
0066-4804/86/110774-03$02.00/0

Vol. 30, No. 5

Disk Diffusion Susceptibility of *Branhamella catarrhalis* and Relationship of β-Lactam Zone Size to β-Lactamase Production

ILEICE LUMAN,[1] REBECCA W. WILSON,[1] RICHARD J. WALLACE, JR.,[2] AND DONALD R. NASH[2]*

Departments of Pathology[1] and Microbiology,[2] The University of Texas Health Center at Tyler, Tyler, Texas 75710

Received 14 April 1986/Accepted 29 July 1986

We tested 231 isolates of *Branhamella catarrhalis* for β-lactamase production and drug susceptibility by the National Committee for Clinical Laboratory Standards disk diffusion method. The nitrocephin disk (Cefinase) identified β-lactamase in 98% of the enzyme-producing strains, and a zone diameter of inhibition of ≤29 mm for penicillin correctly predicted the presence of β-lactamase in 99% of the isolates. No resistance to erythromycin, tetracycline, trimethoprim-sulfamethoxazole, or amoxicillin-clavulanic acid was observed.

Branhamella catarrhalis has received increasing attention as a potential pathogen of the upper and lower respiratory tract (4). Otitis media, sinusitis, bronchitis, and bronchopneumonia (the latter two primarily in patients with underlying lung disease) appear to be the major clinical diseases (9, 17, 18, 21). In a recent study with Gram stain-directed cultures, *B. catarrhalis* was recovered from 5% of all acceptable sputum samples from patients with chronic lung disease over a 30-month period, and 11.5% of the samples contained a recognized pathogen; this latter incidence approached 20% in the winter months (16).

Of major concern therapeutically has been the recognition that *B. catarrhalis* produces β-lactamase. The first known report of β-lactamase in this species appeared in 1977 (10). Since that time β-lactamase production has spread, and many isolates now produce the enzyme (2, 14, 20). The β-lactamase is cell bound and appears to be more difficult to detect than TEM-1 in *Haemophilus influenzae* and *Neisseriae gonorrhoeae*. Isoelectric focusing of crude β-lactamase preparations has revealed two distinct enzyme patterns, referred to as Ravasio and 1908 (7). Prior studies have indicated that the Ravasio enzyme variety is the most common in the United States, being present in 90% of β-lactamase-positive *B. catarrhalis* strains (11).

All isolates tested to date have been susceptible to erythromycin, tetracycline, amoxicillin-clavulanic acid, cefoxitin, and the broad-spectrum cephalosporins by agar dilution techniques (1, 2, 6, 20). Little information is available on disk diffusion, including the acceptability of Mueller-Hinton agar (MHA) and the zone diameters for β-lactams which would identify β-lactamase-producing strains.

(An abstract of this work was presented at the Annual Meeting of the American Society for Microbiology, 25 March 1986, in Washington, D.C. [Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, abstr. A72, p. 13].)

Isolates of *B. catarrhalis* recovered from sputum samples submitted to the clinical laboratory of a chest hospital between February 1983 and October 1985 were evaluated. *B. catarrhalis* was identified by growth of nonpigmented colonies on 5% sheep blood agar and chocolate agar, a characteristic Gram stain, absence of acid production on glucose, maltose, lactose, and sucrose, and production of DNase (4, 5). β-Lactamase testing was performed with whole cells and a nitrocephin disk (Cefinase; BBL Microbiology Systems, Cockeysville, Md.) and by the addition of extracts from acetone-treated cells (11) to a solution of nitrocephin (15).

Disk diffusion susceptibility testing was done at the time of isolation with MHA by the procedure recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (12). Isolates which failed to grow adequately were retested under 5% CO_2 with chocolatized MHA. The majority of isolates were frozen at −70°C and regrown at a later date for the β-lactamase testing of cell extracts and for isoelectric focusing (IEF).

Crude extracts from acetone-treated cells containing β-lactamase were submitted to IEF on polyacrylamide gels with LKB instruments. The details of this procedure and IEF results for many of the current isolates have been presented elsewhere (11).

A total of 231 isolates were identified during the study period. With the Cefinase disk, 171 isolates (74%) were β-lactamase positive. Of the 231 isolates, 146 were also tested for β-lactamase production by using cell extracts. Two isolates which were β-lactamase negative by the disk test were positive with the acetone-treated cell extracts. Both isolates which gave an initial negative β-lactamase result had the 1908-type enzyme.

Twenty-six isolates (11%) failed to grow adequately on MHA in room air and had to be restudied in 5% CO_2 on chocolatized MHA. The other 89% produced good growth on overnight incubation on MHA. By disk diffusion, no isolates were identified which were resistant to erythromycin, moxalactam, amoxicillin-clavulanic acid, chloramphenicol, cefotaxime, trimethoprim-sulfamethoxazole, or sulfisoxazole. There was no difference in mean zone size for any of these drugs, except cefotaxime, related to whether the isolates produced β-lactamase. The mean and range of the zone diameters are listed in Table 1.

Zone diameters of inhibition for penicillin G and their relationship to β-lactamase production are shown in Fig. 1. All β-lactamase-positive isolates had inhibitory zones 27 mm in diameter or less, including isolates identified as having either the 1908 or Ravasio β-lactamase pattern on IEF. Among the β-lactamase-negative isolates, all but one had inhibitory zones 29 mm in diameter or greater. (Based on zones of inhibition to all β-lactams, this isolate is suspected of being a β-lactamase-producing strain, but unfortunately was unavailable for repeat testing.) The ≥29-mm diameter of inhibition used to define the susceptibility of *Staphylococcus aureus* to penicillin G correctly predicted the β-lactamase activity in 230 of the 231 isolates (99.6%).

* Corresponding author.

TABLE 1. Comparison of the mean zone diameters of inhibition of β-lactamase-positive and β-lactamase-negative isolates of *B. catarrhalis* against 11 antimicrobial agents

Antimicrobial agent	β-Lactamase positive strains			β-Lactamase negative strains		
	No. tested	Mean zone (mm) ± SD (range)	% Resistant strains[a]	No. tested	Mean zone (mm) ± SD (range)	% Resistant strains
Chloramphenicol	171	36.4 ± 3.6 (28–50)	0	60	36.1 ± 2.8 (28–42)	0
Tetracycline	171	29.2 ± 4.1 (21–45)	0	60	28.8 ± 2.3 (24–34)	0
Erythromycin	171	31.1 ± 4.2 (19–48)	0	60	32.0 ± 4.2 (19–42)	0
TMP-SMX[b]	152	26.8 ± 4.5 (14–48)	0	53	27.1 ± 3.1 (21–36)	0
Sulfisoxazole	171	39.8 ± 7.0 (21–60)	0	60	39.7 ± 6.4 (21–50)	0
Moxalactam	148	42.9 ± 5.3 (22–66)	0	53	42.3 ± 6.4 (20–54)	0
Cefotaxime	171	33.1 ± 5.0 (22–46)	0	60	37.7 ± 3.9 (29–46)	0
Cephalothin	171	19.6 ± 2.5 (13–27)	0.6	60	36.7 ± 4.1 (22–46)	0
Penicillin G	171	12.0 ± 5.4 (6–27)	100	60	36.0 ± 4.1 (25–46)	1.7
Ampicillin	171	17.6 ± 4.8 (6–31)	97.1	60	40.6 ± 4.3 (26–50)	1.7
Methicillin	171	8.7 ± 3.9 (6–28)	62.0	60	24.1 ± 4.7 (14–34)	0

[a] Resistance was defined by current NCCLS standards, except that a zone diameter of inhibition of ≤29 mm was used to define resistance to penicillin and ampicillin.

[b] Isolates requiring chocolate agar were not included for TMP-SMX (trimethoprim-sulfamethoxazole).

Comparable results were obtained with ampicillin (Fig. 2). The β-lactamase-positive isolates all had zone diameters of inhibition of ≤31 mm, with only 5 of 171 (2.9%) having zone diameters of inhibition of ≥29 mm. Two of the five isolates with zone diameters of inhibition of ≥29 mm had the 1908 enzyme pattern, two were of the Ravasio type, and one was unavailable for IEF testing. Isolates with the 1908 β-lactamase pattern had mean zone diameters of inhibition of almost 29 mm, compared with 17 mm for isolates with the Ravasio β-lactamase pattern. Among the 60 β-lactamase-negative isolates, all but one had zone diameters of inhibition of ≥29 mm. This was the same isolate that had a small zone diameter of inhibition when tested against penicillin G. The ≥29 mm zone diameter of inhibition used to define susceptibility of *S. aureus* to ampicillin correctly predicted the β-lactamase activity in 225 of 231 isolates (97.4%).

Less accurate predictions of the presence of β-lactamase could be made by using methicillin zone diameters of inhibition. With the NCCLS criterion of ≥14 mm to define susceptibility and ≤9 mm for resistance, 106 of 171 β-lactamase-positive isolates were resistant, while all 60 β-lactamase-negative isolates were susceptible. Thus, this zone diameter of inhibition correctly predicted the presence of β-lactamase in only 61.3% of the isolates. However, the two IEF patterns gave markedly different results. Eight of 10 isolates (80%) with the 1908 enzyme pattern were susceptible to methicillin, compared with only 5 of 86 isolates (5.8%) with the Ravasio pattern.

The zone diameters of inhibition obtained with cephalothin showed a bimodal distribution of isolates similar to that observed with methicillin, penicillin, and ampicillin. Only one isolate (0.4%) was resistant, while 137 of 171 (80.1%) of β-lactamase-positive isolates, as well as all of the β-lactamase-negative isolates, fell into the susceptible category by the current NCCLS criterion (≥18 mm). With a cutoff of ≥29 mm for susceptibility, as was used for penicillin, 100% of the β-lactamase-positive isolates and 96.7% of the β-lactamase-negative isolates would be correctly identified for the presence or absence of the enzyme.

The results in Table 2 compare the mean zone diameters of inhibition obtained for different β-lactams between isolates demonstrating the 1908 and Ravasio β-lactamase patterns. Zone diameters of inhibition for 1908-type isolates were significantly larger than those for Ravasio-type isolates for all β-lactams tested, with the largest differences noted between the penicillins and cefotaxime.

The ≤29-mm zone diameter of inhibition used to define resistance of *S. aureus* to penicillin G correctly identified all 171 of the β-lactamase-producing isolates of *B. catarrhalis* in the current study. Virtually identical results were obtained by Oberhofer and Towle, who tested 79 isolates of *B. catarrhalis* by standard disk diffusion and compared the zone diameters of inhibition obtained with penicillin with the results of β-lactamase testing by the penicillin-starch paper strip method (14). In their study, all 32 β-lactamase-positive isolates were identified as resistant using the zone diameter of inhibition of ≤29 mm, while 44 of 47 β-lactamase-negative isolates (94%) were correctly identified as susceptible. In both studies, the 20-mm zone diameter of inhibition normally used for identifying TEM-1 β-lactamase-producing strains of *H. influenzae* and *N. gonorrhoeae* (3, 12) missed almost one-third of the β-lactamase-producing isolates of *B. ca-*

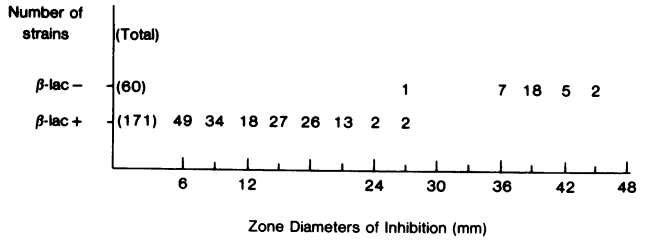

FIG. 1. Relationship between β-lactamase production in *B. catarrhalis* and zone diameters of inhibition with penicillin G.

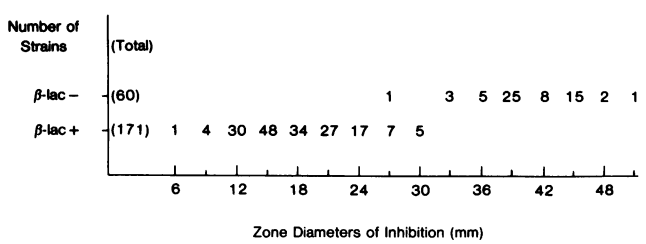

FIG. 2. Relationship between β-lactamase production and zone diameters of inhibition with ampicillin.

TABLE 2. Mean zone diameters of inhibition to various β-lactams of β-lactamase-producing strains of *B. catarrhalis* with the Ravasio or 1908 enzyme pattern

β-Lactam	1908 pattern		Ravasio pattern		t^a	P^a
	No.	Mean zone (mm) ± SD	No.	Mean zone (mm) ± SD		
Penicillin	9	22.7 ± 2.5	86	11.6 ± 4.8	11.4	0.0001
Ampicillin	9	28.6 ± 2.2	85	17.4 ± 4.4	12.6	0.0001
Methicillin	9	16.6 ± 4.5	85	8.5 ± 4.6	11.4	0.0001
Cephalothin	9	23.4 ± 2.5	85	19.6 ± 2.4	4.4	0.0015
Moxalactam	9	48.0 ± 3.4	84	42.7 ± 5.6	3.9	0.0023
Cefotaxime	9	41.0 ± 2.7	84	32.5 ± 4.6	8.2	0.0001

[a] Analysis of variance for unbalanced designs.

tarrhalis. Thus, we would recommend the use of a zone diameter of inhibition of ≤29 mm to penicillin as indicative of the presence of β-lactamase in *B. catarrhalis* strains.

The accuracy of the various methods used to detect the β-lactamases of *B. catarrhalis* has not been assessed in detail. A recent College of American Pathologists survey of almost 700 microbiology laboratories for a single strain of β-lactamase-positive *B. catarrhalis* showed nitrocephin to be 96% accurate in detecting the enzyme, compared with less than 80% for other approved methods (8). Thus, detection methods other than nitrocefin should not be used until their accuracy has been studied.

Previous reports of MIC determinations for *B. catarrhalis* have shown all isolates to be susceptible to erythromycin, tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole, moxalactam, and cefotaxime (1, 2, 4, 6, 19, 20). Although we did not perform MIC determinations, the current disk susceptibility data for a larger number of isolates support the previous data that showed no strains resistant to these agents in the United States at present. Slevin et al. (18) reported that 3 of 96 isolates and 10 of 95 isolates of *B. catarrhalis* were not susceptible to erythromycin and trimethoprim-sulfamethoxazole, respectively. Details of the susceptibility method were not provided, however.

Previous susceptibility studies of ampicillin and penicillin for *B. catarrhalis* have shown a 90% MIC (MIC_{90}) for β-lactamase-producing strains of 1.0 to 5.0 μg/ml, values which are within the susceptible to moderately susceptible category of the NCCLS (12). Numerous investigators have reported treatment failures with ampicillin or amoxicillin for β-lactamase-producing strains of *B. catarrhalis* (13, 18, 22). On this basis, we believe the criteria for susceptibility or resistance of *B. catarrhalis* to penicillin or ampicillin should currently be based on the presence or absence of β-lactamase.

Differences observed between isolates producing the 1908 and Ravasio β-lactamase patterns indicate that the latter are less susceptible to the β-lactams, particularly the penicillins. Preliminary studies comparing the two types suggest that the apparent functional differences in the two enzyme varieties are related to a quantitatively lower concentration of enzyme produced by the 1908-type isolates.

LITERATURE CITED

1. **Ahmad, F., D. T. McLeod, M. J. Croughan, and M. A. Calder.** 1984. Antimicrobial susceptibility of *Branhamella catarrhalis* isolates from bronchopulmonary infections. Antimicrob. Agents Chemother. **26**:424–425.
2. **Alvarez, S., M. Jones, S. Holtsclaw-Berk, J. Guarderas, and S. L. Berk.** 1985. In vitro susceptibilities and β-lactamase production of 53 clinical isolates of *Branhamella catarrhalis*. Antimicrob. Agents Chemother. **27**:646–647.
3. **Biddle, J. W., J. M. Swenson, and C. Thornsberry.** 1978. Disc agar diffusion antimicrobial susceptibility tests with β-lactamase producing *Neisseria gonorrhoeae*. J. Antibiot. **31**:352–358.
4. **Doern, G. V.** 1986. *Branhamella catarrhalis*—an emerging human pathogen. Diag. Microbiol. **4**:191–201.
5. **Doern, G. V., and S. A. Morse.** 1980. *Branhamella* (*Neisseriae*) *catarrhalis*: criteria for laboratory identification. J. Clin. Microbiol. **11**:193–195.
6. **Doern, G. V., K. G. Siebers, L. M. Hallick, and S. A. Morse.** 1980. Antibiotic susceptibility of β-lactamase-producing strains of *Branhamella* (*Neisseria*) *catarrhalis*. Antimicrob. Agents Chemother. **17**:24–29.
7. **Farmer, T., and C. Reading.** 1982. β-Lactamases of *Branhamella catarrhalis* and their inhibition by clavulanic acid. Antimicrob. Agents Chemother. **21**:506–508.
8. **Jones, R. N., and H. M. Sommers.** 1986. Identification and antimicrobial susceptibility testing of *Branhamella catarrhalis* in United States laboratories, 1983–1985. Drugs **31**(Suppl. 3):34–37.
9. **Louie, M. H., E. L. Gabay, G. E. Mathisen, and S. M. Finegold.** 1983. *Branhamella catarrhalis* pneumonia. West. J. Med. **138**:47–49.
10. **Malmvall, B.-E., J. E. Brorsson, and J. Johnsson.** 1977. In vitro sensitivity to penicillin V and β-lactamase production of *Branhamella catarrhalis*. J. Antimicrob. Chemother. **3**:374–375.
11. **Nash, D. R., R. J. Wallace, Jr., V. A. Steingrube, and P. A. Shurin.** 1986. Isoelectric focusing of beta-lactamases from sputum and middle ear isolates of *Branhamella catarrhalis* recovered in the United States. Drugs **31**(Suppl. 3):47–53.
12. **National Committee for Clinical Laboratory Standards.** 1979. Performance standards for antimicrobial disk susceptibility tests. Approved standard ASM-2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
13. **Ninane, G., J. Joly, M. Kraytman, and P. Piot.** 1978. Bronchopulmonary infection due to β-lactamase-producing *Branhamella catarrhalis* treated with amoxycillin/clavulanic acid. Lancet **i**:257.
14. **Oberhofer, T. R., and D. W. Towle.** 1982. Evaluation of the rapid penicillinase paper strip test for detection of beta-lactamase. J. Clin. Microbiol. **15**:196–199.
15. **O'Callaghan, C. H., A. Morris, S. M. Kirby, and A. H. Shingler.** 1972. Novel method for detection of β-lactamases by using a chromogenic cephalosporin substrate. Antimicrob. Agents Chemother. **1**:283–288.
16. **Pollard, J. A., R. J. Wallace, Jr., D. R. Nash, J. I. Luman, and R. W. Wilson.** 1986. Incidence of *Branhamella catarrhalis* in the sputa of patients with chronic lung disease. Drugs **31**(Suppl. 3):102–107.
17. **Shurin, P. A., C. D. Marchant, C. H. Kim, G. F. Van Hare, C. E. Johnson, M. A. Tutihasi, and L. J. Knapp.** 1983. Emergence of beta-lactamase-producing strains of *Branhamella catarrhalis* as important agents of acute otitis media. Pediatr. Infect. Dis. **2**:34–38.
18. **Slevin, N. J., J. Aitken, and P. E. Thornley.** 1984. Clinical and microbiological features of *Branhamella catarrhalis* bronchopulmonary infections. Lancet **i**:782–783.
19. **Stobberingh, E. E., B. I. Davies, and C. P. A. van Boven.** 1984. *Branhamella catarrhalis*: antibiotic sensitivities and β-lactamases. J. Antimicrob. Chemother. **13**:55–64.
20. **Sweeney, K. G., A. Verghese, and C. A. Needham.** 1985. In vitro susceptibilities of isolates from patients with *Branhamella catarrhalis* pneumonia compared with those of colonizing strains. Antimicrob. Agents Chemother. **27**:499–502.
21. **Wald, E. R., G. J. Milmoe, A. Bowen, J. Ledesma-Medina, N. Salamon, and C. D. Bluestone.** 1981. Acute maxillary sinusitis in children. N. Engl. J. Med. **304**:749–754.
22. **Wardle, J. K., R. Freeman, and H. R. Ingham.** 1982. *Branhamella catarrhalis*. Lancet **i**:1244.