

Predicting Susceptibility of *Streptococcus pneumoniae* to Ceftriaxone and Cefotaxime by Cefuroxime and Ceftizoxime Disk Diffusion Testing

NATALIE WILLIAMS-BOUYER,* ANTONIO HERNANDEZ, AND BARBARA S. REISNER

Department of Pathology, University of Texas Medical Branch, Galveston, Texas 77555-0740

Received 18 March 1999/Returned for modification 20 May 1999/Accepted 26 July 1999

In this study, disk diffusion testing with ceftizoxime and cefuroxime was evaluated for use in predicting the susceptibility of *Streptococcus pneumoniae* to ceftriaxone and cefotaxime. Of the 194 isolates included in this study, 138 were susceptible, 34 were intermediate, and 22 were resistant to cefotaxime by MIC testing; 138 isolates were susceptible, 35 were intermediate, and 21 were resistant to ceftriaxone by MIC testing. A zone of inhibition around the cefuroxime disk of ≥ 32 mm correctly categorized 101 of 138 isolates as susceptible to cefotaxime and ceftriaxone. A zone of inhibition around the ceftizoxime disk of ≥ 26 mm correctly categorized 111 of 138 isolates as susceptible to cefotaxime and 114 of 138 as susceptible to ceftriaxone. We conclude that disk diffusion can separate *S. pneumoniae* isolates susceptible to ceftriaxone and cefotaxime from those that are not susceptible. Isolates not falling into the susceptible category by disk diffusion require additional testing to determine the MIC.

Over the past several years, resistance of *Streptococcus pneumoniae* to penicillin has been increasing. Currently at our institution, 20% of *S. pneumoniae* isolates are resistant to penicillin and 20% are intermediate. Although the majority of penicillin-resistant isolates remain susceptible to the broad-spectrum cephalosporins, in vitro resistance and clinical failures with these agents have occurred (3, 4, 9). Presently, our pneumococcal cefotaxime resistance rate is 5%, with 2.5% of the strains intermediate. Therefore, testing of susceptibility of *S. pneumoniae* to the broad-spectrum cephalosporins is becoming more important and should be performed routinely for isolates causing serious infections (8).

The use of disk diffusion to screen for extended-spectrum cephalosporin resistance in pneumococci has been studied (1, 2, 5, 6, 10), and interpretive criteria for susceptibility testing with 30- μ g cefotaxime and ceftriaxone disks have been described (2, 6); however, susceptibility testing with these disks is not recommended due to an excessive number of minor interpretive discrepancies between disk diffusion and MIC test results. Because of this problem, the use of disk diffusion testing with less potent cephalosporins (e.g., ceftizoxime, cefuroxime, and ceftazidime) to predict susceptibility to extended-spectrum cephalosporins has been proposed (2).

In this study, we evaluated the use of disk diffusion testing with 30- μ g ceftizoxime and cefuroxime disks to predict the susceptibilities of 194 *S. pneumoniae* isolates to cefotaxime and ceftriaxone. Isolates were either obtained from fresh culture ($n = 167$) or received from clinical stock cultures from other medical centers (University of Iowa [$n = 11$], Duke University [$n = 6$], and University of Alabama at Birmingham [$n = 10$]) to ensure adequate numbers of resistant strains. Table 1 summarizes the penicillin and cephalosporin resistance patterns of these isolates. Sources included lung lavage fluid, sputa, wounds, eyes, ears, noses, and sterile body fluids (blood and peritoneal, pleural, and joint fluids). Isolates were suspended

in defibrinated sheep blood and frozen at -70°C prior to testing.

Frozen isolates were subcultured twice and incubated at 35°C in 5% CO_2 for 18 to 24 h prior to susceptibility testing. The MIC was determined with PASCO Supplemental MIC frozen panels with sheep blood supplement (Difco Laboratories, Detroit, Mich.). The antibiotic concentrations tested were 0.12 to 16 $\mu\text{g/ml}$ for cefotaxime and 0.25 to 8 $\mu\text{g/ml}$ for ceftriaxone. Following an overnight incubation, study organisms were taken directly from blood agar plates and suspended in tryptic soy broth. A nephelometer (A-Just turbidity meter; Abbott Laboratories, Chicago, Ill.) was used to adjust bacterial cell suspensions to a turbidity equal to a 1.0 McFarland standard ($\sim 10^8$ CFU/ml). An aliquot of this standardized suspension was added to the blood supplement to yield approximately 10^6 CFU/ml. MIC trays were inoculated with these suspensions (within 10 min of turbidity adjustment) and incubated at 35°C for 18 to 24 h in a non- CO_2 incubator. Interpretation of the MIC of each drug was made according to National Committee for Clinical Laboratory Standards guidelines (8) (susceptible, ≤ 0.5 $\mu\text{g/ml}$; intermediate, 1.0 $\mu\text{g/ml}$; and resistant, ≥ 2.0 $\mu\text{g/ml}$). For quality control, *S. pneumoniae* 49619 was tested with each batch. Disk diffusion testing was performed according to National Committee for Clinical Laboratory Standards guidelines with Mueller-Hinton agar with 5% sheep blood (BBL, Cockeysville, Md.) (7). Plates were incubated at 35°C for 18 to 24 h in CO_2 . For quality control, *Escherichia coli* 25922 (cefuroxime disk zone, 20 to 26 mm) on Mueller-Hinton II plates (BBL) and *S. pneumoniae* 49619 (ceftizoxime disk zone, 28 to 34 mm) on Mueller-Hinton agar with 5% sheep blood were included with each batch to verify the potency of the antibiotic disks.

The zones of inhibition around the surrogate disks were correlated with the MICs of ceftriaxone and cefotaxime to determine the minimum zone of inhibition that would separate susceptible from nonsusceptible isolates (Fig. 1 and 2). The zone of inhibition around the cefuroxime disk that identified the greatest number of cefotaxime- and ceftriaxone-susceptible isolates without incorrectly classifying any resistant or intermediate isolates was 32 mm. Of the 194 isolates tested in this

* Corresponding author. Mailing address: Department of Pathology, University of Texas Medical Branch, 301 University Blvd., Galveston, TX 77555-0740. Phone: (409) 747-1424. Fax: (409) 772-5683. E-mail: nmwillia@utmb.edu.

TABLE 1. *S. pneumoniae* isolate penicillin and extended-spectrum cephalosporin resistance patterns

Penicillin interpretive category (MIC)	No. of isolates with indicated phenotype					
	Cefotaxime (MIC)			Ceftriaxone (MIC)		
	Susceptible (≤ 0.5 $\mu\text{g/ml}$)	Intermediate (1.0 $\mu\text{g/ml}$)	Resistant (≥ 2.0 $\mu\text{g/ml}$)	Susceptible (≤ 0.5 $\mu\text{g/ml}$)	Intermediate (1.0 $\mu\text{g/ml}$)	Resistant (≥ 2.0 $\mu\text{g/ml}$)
Susceptible (≤ 0.06 $\mu\text{g/ml}$)	75	0	0	75	0	0
Intermediate (0.12–1.0 $\mu\text{g/ml}$)	44	8	0	45	7	0
Resistant (≥ 2.0 $\mu\text{g/ml}$)	19	26	22	18	28	21

study, 101 of 138 (73.2%) cefotaxime-susceptible isolates and 101 of 138 (73.2%) ceftriaxone-susceptible isolates were accurately predicted by using this zone of inhibition as a cutoff. All intermediate and resistant isolates had a cefuroxime zone of inhibition of ≤ 31 mm. The use of a ceftizoxime zone of 26 mm or greater accurately predicted 114 of 138 (82.6%) cefotaxime-susceptible isolates and 111 of 138 (80.4%) ceftriaxone-susceptible isolates. All intermediate and resistant isolates had a ceftizoxime zone of inhibition of ≤ 25 mm.

Our findings were very similar to those reported by Fried-

land et al. (5), who tested only 23 penicillin-resistant *S. pneumoniae* isolates and found cefuroxime and ceftizoxime zones of ≥ 31 and ≥ 26 mm, respectively, to most accurately identify isolates susceptible to the broad-spectrum cephalosporins. Our cefuroxime results differed from but our ceftizoxime results agreed with those reported by Barry and Fuchs (2), who found a cefuroxime zone of ≥ 28 mm and a ceftizoxime zone of ≥ 26 mm to be the best predictors. This study was comparable in scope to our study and included 52 penicillin-intermediate and 67 penicillin-resistant strains.

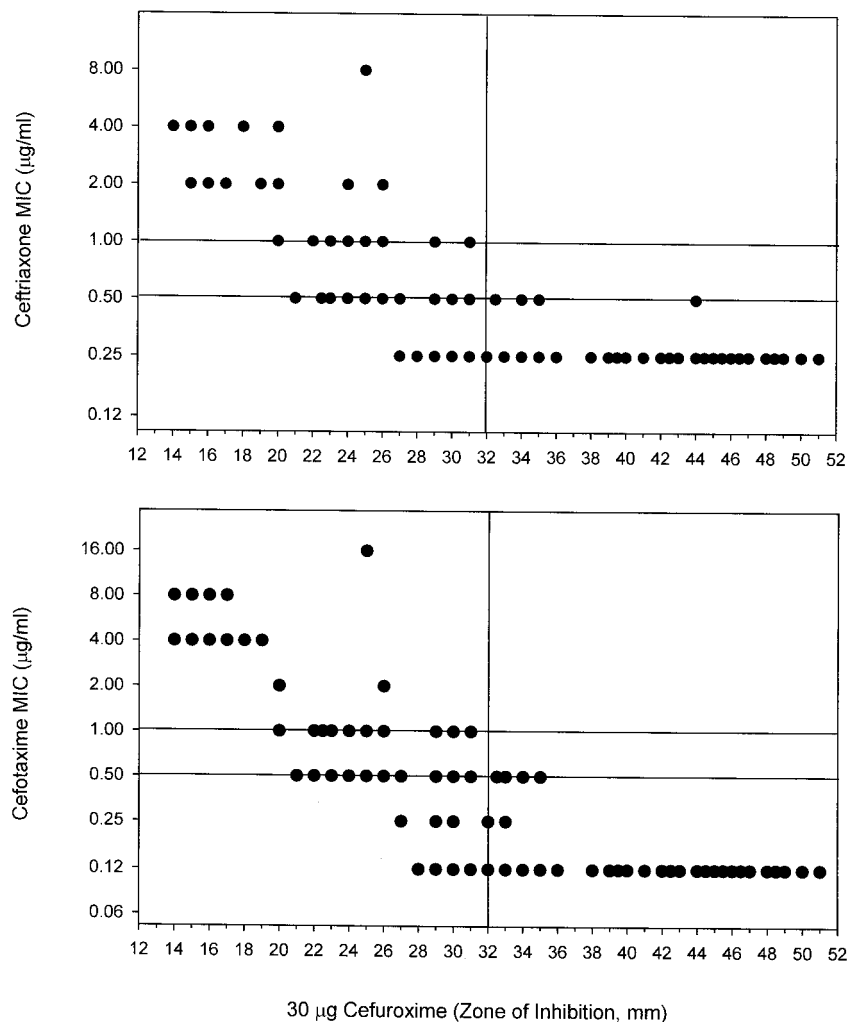


FIG. 1. Scattergrams comparing zones of inhibition around cefuroxime disks with broth microdilution MICs of cefotaxime and ceftriaxone (some dots represent more than one isolate for which the MICs and zones of inhibition are the same).

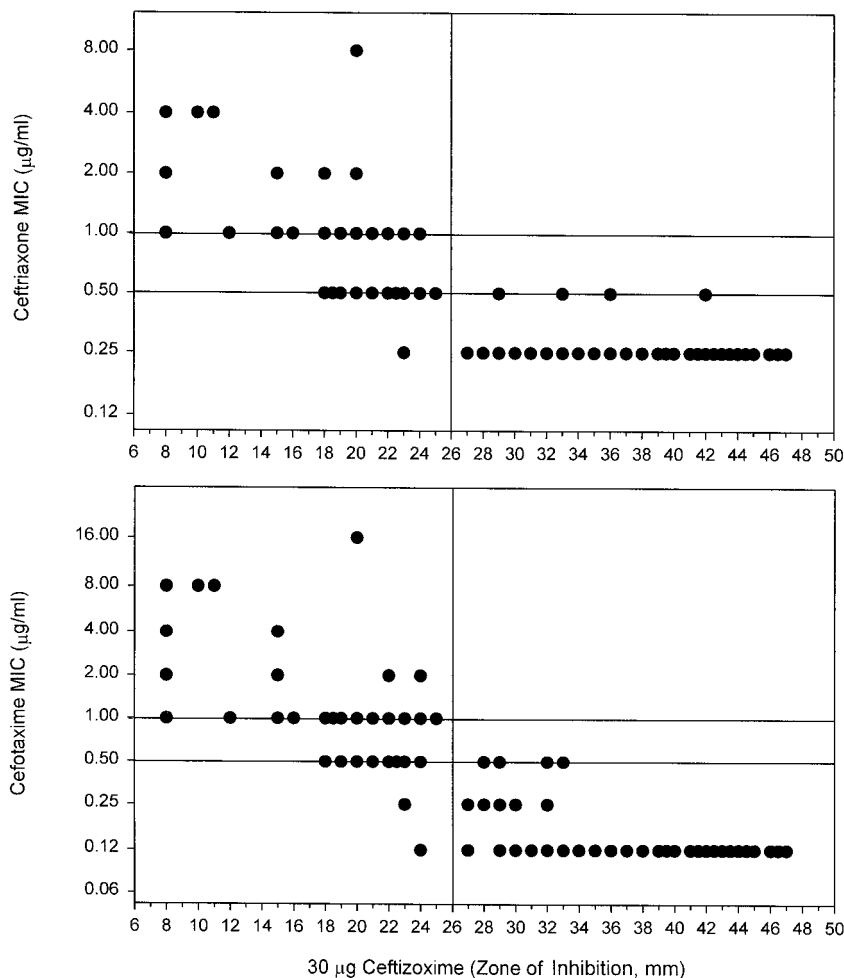


FIG. 2. Scattergrams comparing zones of inhibition around ceftizoxime disks with broth microdilution MICs of cefotaxime and ceftriaxone (some dots represent more than one isolate for which the MICs and zones of inhibition are the same).

Based on the results of our study, we believe that disk diffusion testing can be used to predict susceptibility of *S. pneumoniae* to the broad-spectrum cephalosporins. We recommend the use of a ceftizoxime disk rather than a cefuroxime disk, because the former identified more susceptible isolates in our study and because its zone size, 26 mm, has been consistently found in three studies to be a reliable breakpoint for susceptible strains. This recommendation is also supported by the findings of Friedland et al. (5), who observed that a ceftizoxime disk provided the clearest means of distinguishing strains for which ceftriaxone and cefotaxime MICs were ≥ 1.0 µg/ml.

We propose that further studies be performed to more firmly establish the most accurate zone size for predicting susceptible isolates. Given variabilities in test media (i.e., Mueller-Hinton agar with 5% sheep blood), it would be worthwhile to conduct an interlaboratory study in which several commercial media (from BBL and Remel, etc.) and several lot numbers from each manufacturer are used to determine reproducibility of the disk diffusion method and to establish the performance characteristics of the primary media used in laboratories today. Such a study would generate peer-reviewed data, thereby helping establish standards for testing *S. pneumoniae* for resistance to extended-spectrum cephalosporins by this test method.

Currently, our laboratory uses disk diffusion to test *S. pneumoniae* isolates from respiratory tract specimens for sensitivities to oxacillin (reported as penicillin), erythromycin, clindamycin, trimethoprim-sulfamethoxazole, and vancomycin. For non-penicillin-susceptible isolates, our laboratory subsequently uses the E-test to determine the MIC of penicillin. Additionally, for these isolates, physicians at our institution have requested that the broad-spectrum cephalosporins be examined by the E-test as well. Use of the ceftizoxime disk during initial testing would provide a more cost-effective method to identify isolates that are susceptible to broad-spectrum cephalosporins. Of the isolates included in our study, approximately half of those that were intermediate or resistant to penicillin were susceptible to cefotaxime or ceftriaxone (Table 1). For these isolates, the screening method described above would reduce the need for further MIC testing, except for those isolates with zones of < 26 mm. Because this approach delays the reporting of test results, we suggest that isolates causing serious infections (i.e., from blood and cerebrospinal fluid) be tested directly by a MIC method.

We thank Difco Laboratories for supplying PASCO Supplemental MIC frozen panels and sheep blood supplement, and we thank Lizzie Harrell (Duke University), Ronald Jones (University of Iowa), and

David Briles (University of Alabama at Birmingham) for providing *S. pneumoniae* clinical isolates.

REFERENCES

1. **Barry, A., S. Brown, and W. Novick.** 1995. Criteria for testing the susceptibility of *Streptococcus pneumoniae* to cefotaxime and its desacetyl metabolite using 1 μ g or 30 μ g cefotaxime disks. *Eur. J. Clin. Microbiol. Infect. Dis.* **14**:724–726.
2. **Barry, A. L., and P. C. Fuchs.** 1996. Surrogate disks for predicting cefotaxime and ceftriaxone susceptibilities of *Streptococcus pneumoniae*. *J. Clin. Microbiol.* **34**:2609–2612.
3. **Bradley, J., and J. Connor.** 1991. Ceftriaxone failure in meningitis caused by *Streptococcus pneumoniae* with reduced susceptibility to beta-lactam antibiotics. *Pediatr. Infect. Dis. J.* **10**:871–873.
4. **Friedland, I., S. Shelton, M. Paris, S. Rinderknecht, S. Ehrett, K. Krisher, and G. McCracken, Jr.** 1993. Dilemmas in diagnosis and management of cephalosporin-resistant *Streptococcus pneumoniae* meningitis. *Pediatr. Infect. Dis. J.* **12**:196–200.
5. **Friedland, I. R., S. Shelton, and G. H. McCracken, Jr.** 1993. Screening for cephalosporin-resistant *Streptococcus pneumoniae* with the Kirby-Bauer disk susceptibility test. *J. Clin. Microbiol.* **31**:1619–1621.
6. **Jorgensen, J. H., J. M. Swenson, F. C. Tenover, M. J. Ferraro, J. A. Hindler, and P. R. Murray.** 1994. Development of interpretive criteria and quality control limits for broth microdilution and disk diffusion antimicrobial susceptibility testing of *Streptococcus pneumoniae*. *J. Clin. Microbiol.* **32**:2448–2459.
7. **National Committee for Clinical Laboratory Standards.** 1997. Performance standards for antimicrobial disk susceptibility tests for bacteria that grow aerobically: approved standard M2-A6, 6th ed. National Committee for Clinical Laboratory Standards, Wayne, Pa.
8. **National Committee for Clinical Laboratory Standards.** 1999. Performance standards for antimicrobial susceptibility testing: document M100-S9. National Committee for Clinical Laboratory Standards, Wayne, Pa.
9. **Sloas, M., F. Barrett, J. Chesney, B. English, B. Hill, F. Tenover, and R. Leggiadro.** 1992. Cephalosporin treatment failure in penicillin- and cephalosporin-resistant *Streptococcus pneumoniae* meningitis. *Pediatr. Infect. Dis. J.* **11**:662–666.
10. **Tenover, F., J. Swenson, and L. McDougal.** 1992. Screening for extended-spectrum cephalosporin resistance in pneumococci. *Lancet* **340**:1420.