

Screening for Cephalosporin-Resistant *Streptococcus pneumoniae* with the Kirby-Bauer Disk Susceptibility Test

IAN R. FRIEDLAND,* SHARON SHELTON, AND GEORGE H. McCracken, JR.

Department of Pediatrics, University of Texas Southwestern Medical Center, Dallas, Texas 75235-9063

Received 4 January 1993/Accepted 22 February 1993

Kirby-Bauer disk susceptibility tests with five standard cephalosporin disks were performed on 23 penicillin-resistant *Streptococcus pneumoniae* isolates for which ceftriaxone MICs were 0.125 to 4 µg/ml. Cefuroxime disk inhibition zone diameters distinguished clearly isolates for which ceftriaxone MICs were ≥2 µg/ml from more susceptible strains, whereas cephalothin, ceftizoxime, cefotaxime, and ceftriaxone disks distinguished these isolates less clearly than the cefuroxime disk did.

Patients with pneumococcal meningitis who fail therapy with an extended-spectrum cephalosporin have been reported recently (2, 3, 6). For the causative strains in these patients, cefotaxime or ceftriaxone MICs were found to be ≥2 µg/ml. Because an extended-spectrum cephalosporin is most frequently used as empiric treatment for childhood meningitis, the early detection of strains with decreased susceptibilities to cephalosporins is vital. We performed Kirby-Bauer disk susceptibility tests with standard cephalosporin disks on 23 penicillin-resistant pneumococcal strains, including 5 strains for which ceftriaxone MICs were ≥2 µg/ml; 4 of the strains were isolated from children with meningitis who failed cefotaxime or ceftriaxone therapy (3, 6).

Colonies from overnight growth on blood agar plates were suspended in Mueller-Hinton broth, and the turbidity was adjusted to match that of a 0.5 McFarland standard. The suspension was then streaked onto Mueller-Hinton blood agar plates, cefotaxime, ceftriaxone, cefuroxime, ceftizoxime, and cephalothin disks (all 30 µg; BBL Sensi-Disc; Becton Dickinson Microbiology Systems, Cockeysville, Md.) were placed on each plate, and zones of growth inhibition were measured after 20 h of incubation at 35°C in room air (1). MICs were determined by broth microdilution in Mueller-Hinton broth supplemented with 3% lysed horse blood with inocula of approximately 5×10^5 CFU/ml. MBCs were determined by plating 10 µl from clear microtiter wells onto blood agar and incubating the agar plates for 20 h in 5% CO₂ at 35°C (4).

All disk tests and MIC and MBC measurements were done in triplicate. Different lots of medium were used for each of the three measurements, but the same disk lot was used throughout the study. Median MICs and MBCs and all three disk zone diameters for each strain are reported. The MICs of penicillin (log₂) correlated with the MICs (log₂) of cefuroxime ($r = 0.58$, $P = 0.004$), ceftriaxone ($r = 0.55$, $P = 0.006$), cefotaxime ($r = 0.52$, $P = 0.011$), and, to a lesser extent, ceftizoxime ($r = 0.46$, $P = 0.027$). Because of the selective inclusion of strains for which cephalosporin MICs are high, the correlation between penicillin and cephalosporin MICs was not as great as that observed when consecutive strains isolated in our hospital laboratory were analyzed (for example, for penicillin versus cefotaxime MICs, $r = 0.93$ and $P < 0.001$) (3).

Fifteen strains were relatively resistant to penicillin (MICs, 0.1 to 1.0 µg/ml) and eight strains were highly resistant to penicillin (MICs, ≥2 µg/ml). For 8 strains ceftriaxone MICs were ≤0.5 µg/ml, for 10 strains ceftriaxone MICs were 1 µg/ml, for 1 strain the ceftriaxone MIC was 2 µg/ml, and for 4 strains ceftriaxone MICs were ≥4 µg/ml. Cefotaxime MICs were within one twofold dilution of the ceftriaxone MICs. The ceftriaxone MICs and MBCs for 19 strains were the same, and the MBCs for the other four strains were twofold greater than the corresponding MICs. The distribution of inhibition zone diameters obtained with each of the disks is shown in Fig. 1A to D. Some inhibition zones (with each of the disks) had indistinct borders or had a zone of light growth within a larger inhibition zone. In such instances, the inhibition zone diameters were measured from the innermost ring of colonies. The inhibition zone diameters plotted against cefotaxime MICs were similar to the results obtained with inhibition zone diameters plotted against ceftriaxone MICs (data not shown). The ranges of disk inhibition zone sizes were greater with the cefuroxime, ceftizoxime, and cephalothin disks than with the extended-spectrum cephalosporin disks, and the variation between zone sizes measured on each of three occasions was lowest for the cefuroxime disk. The mean square errors of the three measures were 2.74, 3.33, 4.41, 5.86, and 7.10 for the cefuroxime, ceftriaxone, cefotaxime, ceftizoxime, and cephalothin disks, respectively (one-way analysis of variance for each disk). An inhibition zone diameter cutoff of 20 mm with the cefuroxime disk distinguished strains for which cefotaxime or ceftriaxone MICs were ≤1 µg/ml from those for which MICs were ≥2 µg/ml. The corresponding cutoff for the cefotaxime and ceftriaxone disks was 30 mm, although there was overlap of MICs for strains at this measurement. Three strains for which ceftriaxone MICs were ≥4 µg/ml had no inhibition zones with the ceftizoxime disk, but two other strains for which ceftriaxone MICs were 4 and 2 µg/ml sometimes had zone diameters of ≥15 mm. With the cephalothin disk, strains for which ceftriaxone MICs were different could not be distinguished. With all disks there was overlap of inhibition zone sizes of strains for which ceftriaxone MICs were 1 µg/ml and those for which ceftriaxone MICs were <1 µg/ml, but a ceftizoxime disk diameter of 25 mm separated these strains on all but three occasions. All strains for which the ceftriaxone MIC was ≥1 µg/ml had cefuroxime disk inhibition zone diameters of ≤30 mm on at least two of the three measurements.

Only a relatively small number of cephalosporin-resistant

* Corresponding author.

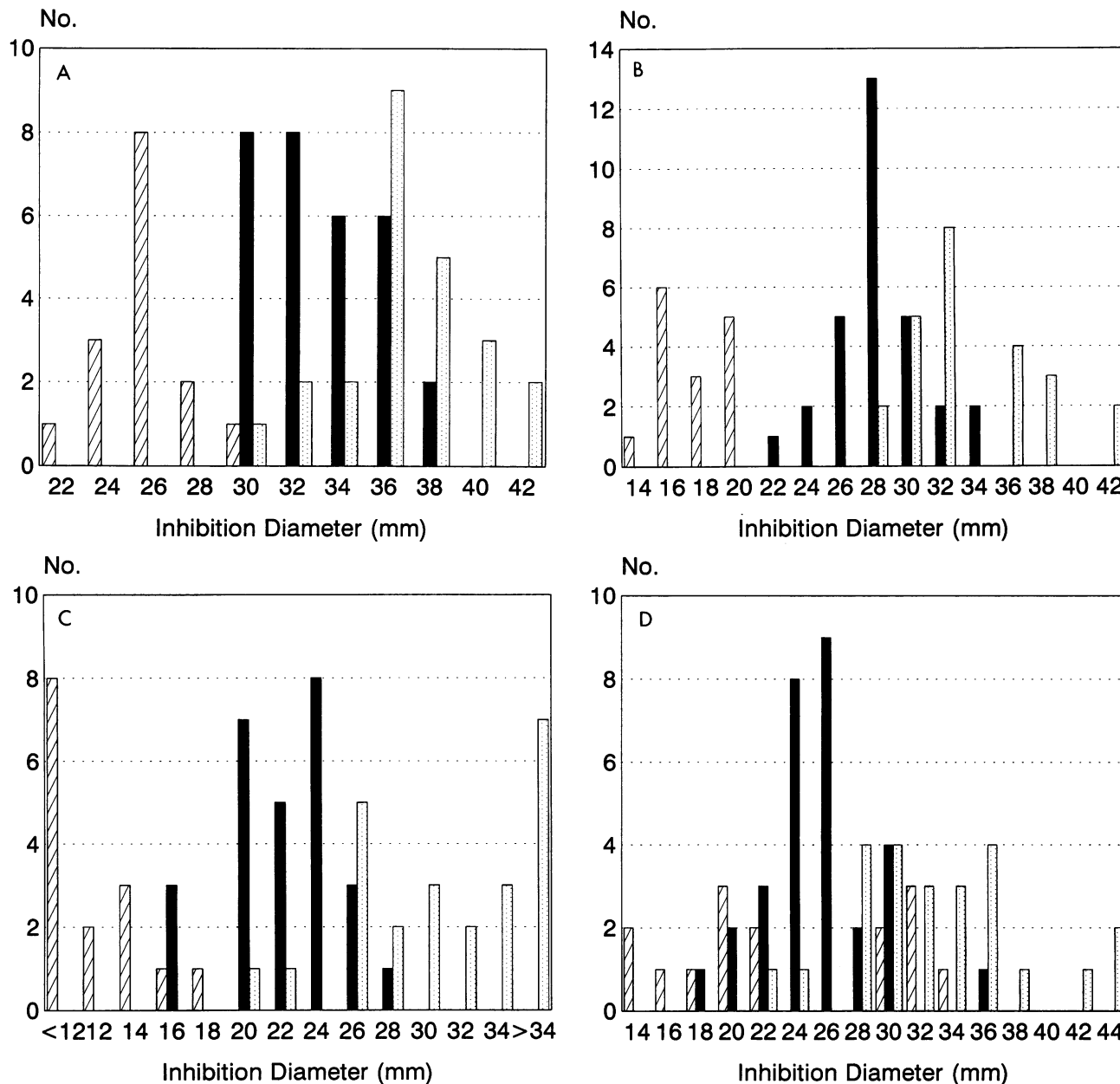


FIG. 1. Kirby-Bauer disk susceptibility testing of 23 penicillin-resistant pneumococcal strains grouped according to their ceftriaxone MICs (▨, ≥2 µg/ml; ■, 1 µg/ml; □, <1 µg/ml). The distributions of inhibition zone diameters obtained with ceftriaxone (A), cefuroxime (B), ceftizoxime (C), and cephalothin (D) disks are shown. Inhibition zone diameters were measured on three separate occasions, and all three values are included. Odd numbered values are grouped with the next even-numbered value.

pneumococcal strains have been isolated in the United States and are available for testing. As more cephalosporin-resistant strains become available, the sensitivities and specificities of various screening methodologies can be compared more accurately. The E test is simple to perform and has been shown to be an accurate screening tool for measuring antibiotic susceptibility (5). It is, however, expensive for routine use in testing all pneumococcal isolates, and cheaper methodologies such as the conventional Kirby-Bauer disk test deserve thorough evaluation.

Our data are based on results with small numbers of organisms, but they suggest that Kirby-Bauer susceptibility

testing with a cefuroxime or a ceftizoxime disk provides a clearer distinction of strains than does susceptibility testing with extended-spectrum cephalosporin disks. This is analogous to the better discrimination of penicillin-resistant and -susceptible strains obtained with an oxacillin disk compared with that obtained with a penicillin disk. The cefuroxime disk clearly distinguished strains for which MICs were ≥2 µg/ml from those for which MICs were <2 µg/ml, and the ceftizoxime disk provided the clearest means of distinguishing strains for which MICs were ≥1 µg/ml from more susceptible strains. The fact that the ceftizoxime disk may be useful in detecting cephalosporin-resistant strains has been

reported recently (7), but it is not clear from that brief report how many strains for which MICs were 2 or 1 $\mu\text{g/ml}$ were included. The cephalothin disk does not appear to be useful in distinguishing resistant from susceptible strains. It should be noted that antibiotic disk diffusion tests were done in room air according to current recommendations (1), and it is possible that slightly different results would be observed if these tests were conducted with added CO_2 .

There is no consensus on the definition of resistance to the extended-spectrum cephalosporins specific for *Streptococcus pneumoniae*. Because patients with meningitis caused by strains for which MICs are 2 $\mu\text{g/ml}$ have failed cefotaxime or ceftriaxone therapy, we believe that the definition of cephalosporin resistance should, as a minimum, include strains for which MICs are 2 $\mu\text{g/ml}$. These strains appear to be easily distinguished from more susceptible strains by standard cefuroxime disk testing. This cutoff may not be adequate, however, as illustrated by a child who we recently managed. The child had pneumococcal meningitis caused by a strain for which the ceftriaxone MIC was 1 $\mu\text{g/ml}$. The cerebrospinal fluid of this child failed to become sterilized after 48 h of ceftriaxone therapy (unpublished data). It is important to be able to detect strains for which ceftriaxone MICs are 1 $\mu\text{g/ml}$. Such strains appear to be most readily distinguished from more susceptible strains with a ceftizoxime disk with a zone diameter cutoff of 25 mm. A cefuroxime disk zone diameter cutoff of 30 mm may be useful for detecting such strains, although a number of more susceptible strains will be considered resistant if this cutoff is used. At this time, conventional MIC determinations or possibly the E test is required to confirm ceftriaxone MICs.

We recently recommended that initial empiric management of patients with pneumococcal meningitis should include vancomycin and cefotaxime or ceftriaxone (3). We suggest that pneumococcal strains, particularly those isolated from patients with meningitis, should be screened not only with an oxacillin disk but also with a cefuroxime or ceftizoxime disk. Oxacillin-resistant isolates (zone diameter,

≤ 19 mm) for which cefuroxime disk inhibition zone diameters are 21 to 30 mm should be regarded as possibly cephalosporin resistant, and those for which inhibition zone diameters are ≤ 20 mm should be regarded as definitely resistant pending MIC determinations. The corresponding zone diameters for the ceftizoxime disk (≤ 25 and ≤ 18 mm, respectively) would identify resistant strains, but these breakpoints would sometimes include more susceptible strains.

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