Development of Interpretable Criteria and Quality Control Limits for Broth Microdilution and Disk Diffusion Antimicrobial Susceptibility Testing of Streptococcus pneumoniae

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A five-center collaborative study was undertaken to develop quality control and specific interpretive criteria for susceptibility testing of Streptococcus pneumoniae against 12 antimicrobial agents. MICs were determined for 248 pneumococcal clinical isolates (with an emphasis on resistant strains) by the National Committee for Clinical Laboratory Standards (NCCLS)-recommended broth microdilution procedure incorporating lyzed horse blood-supplemented Mueller-Hinton broth. NCCLS disk diffusion testing was also performed for each isolate by using Mueller-Hinton sheep blood agar incubated in 5% CO2. Repetitive testing of S. pneumoniae ATCC 49619 with different sources and lots of media and disks allowed development of quality control ranges which encompassed approximately 95% of MIC and zone size values observed in the study. Good intra- and interlaboratory reproducibilities were seen with these testing methods and all of the drugs examined. On the basis of the results of this study, MIC interpretive criteria are proposed for 11 agents. Comparisons of MICs and disk diffusion zone sizes allowed disk diffusion zone size interpretive criteria to be proposed for five drugs and confirmed the use of the oxacillin disk test for prediction of penicillin susceptibility among pneumococci. Excessive numbers of minor-category interpretive errors precludes recommendation at this time of the disk diffusion method for testing of pneumococci against five of the drugs. Use of these proposed quality control and interpretive criteria should provide for reproducible test results and allow recognition of recently emerging resistance among pneumococcal clinical isolates.

Streptococcus pneumoniae clinical isolates resistant to penicillin or multiply resistant to several antimicrobial agent classes have been reported with increasing frequency in recent years throughout the world (5, 15, 24, 26, 29, 37). The prevalence of such strains has increased sharply in the United States since 1988 (4, 12, 19, 40, 44, 47). Reports of broad-spectrum cephalosporin resistance have made empirical therapeutic choices for serious infections such as meningitis very problematic (2, 10, 39). Because of the marked change in the susceptibility of pneumococci to commonly used antimicrobial agents, there is new interest in reliable methods for susceptibility testing of pneumococcal clinical isolates (9, 10, 20, 24, 38, 43). This five-center study has developed quality control and proposed interpretive criteria for the broth microdilution and agar disk diffusion tests for several conventional and recently developed antimicrobial agents against S. pneumoniae.

MATERIALS AND METHODS

Participant laboratories. Studies were conducted in five collaborating laboratories. They included Centers for Disease Control and Prevention (Atlanta, Ga.), Massachusetts General Hospital (Boston, Mass.), University of California, Los Angeles, Medical Center (Los Angeles, Calif.), Washington University and Barnes Hospital (St. Louis, Mo.), and University of Texas Health Science Center (San Antonio, Tex.).

Test organisms. Approximately 50 unique isolates of S. pneumoniae were tested in each of the five laboratories. They were either fresh clinical isolates, isolates referred to the laboratories for confirmation of antimicrobial resistance, or isolates selected from among the laboratories' stock culture collection to assure adequate numbers of resistant strains. These included 56 penicillin-resistant, 85 penicillin-intermediate, and at least 11 extended-spectrum cephalosporin-resistant strains.

Antimicrobial agents. Cefepime, cefotaxime, ceftriaxone, chloramphenicol, erythromycin, imipenem, meropenem, ofloxacin, penicillin (oxacillin for disk testing), tetracycline, trimethoprim-sulfamethoxazole, and vancomycin were the agents included in this study. Two different lots of susceptibility testing disks (one from Becton Dickinson Microbiology Systems, Cockeysville, Md., and one from Difco Laboratories, Detroit, Mich.) were utilized for each agent.

Quality control organisms. In order to ensure interlaboratory reproducibility, each laboratory tested a multiply resistant pneumococcal control strain and S. pneumoniae ATCC 49619.

Broth microdilution susceptibility tests. MICs were determined for each isolate with each drug by using cation-adjusted Mueller-Hinton broth supplemented with 3% lysed horse blood (MHLHB) in a microdilution format as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (34). A common lot of microdilution trays was provided to each laboratory, and a unique test lot of trays made with one of four distinct lots of Mueller-Hinton medium from one of three manufacturers (Becton Dickinson, Difco, or Unipath Oxoid [Ogdensburg, N.Y.]) was also tested in each
laboratory. Inocula were prepared by suspending growth obtained from sheep blood agar plates that had been incubated for 20 to 24 h in 5% CO₂. Colonies were suspended in 0.9% saline to obtain a suspension turbidity equivalent to a 0.5 McFarland standard. The suspension was further diluted within 15 min to provide a final inoculum density of 5 × 10⁸ CFU/ml in the wells of the microdilution trays. Colony counts were performed to ensure appropriate inoculum concentrations. Microdilution trays were incubated at 35°C in ambient air for 20 to 24 h prior to determination of MICs.

**Disc diffusion tests.** Disk diffusion tests were performed on each isolate and each drug according to standard NCCLS methods (33) using 150-mm-diameter Mueller-Hinton sheep blood agar (MHSBA) plates. Each laboratory was provided with a common lot of plates and one of two different lots of commercially prepared (Becton Dickinson or Remel) plates or laboratory-prepared plates with Oxoid Mueller-Hinton base. Plates were inoculated with an organism suspension equivalent to a 0.5 McFarland standard prepared in 0.9% saline as described above. Plates were incubated at 35°C in 5% CO₂ for 20 to 24 h prior to measurement of zone diameters.

**Testing protocol.** Each laboratory performed microdilution MICs on 50 clinical isolates from its own resources using the MHLHB test lot unique to that facility and disk diffusion tests using the MHSBA test lot for that laboratory. In addition, each laboratory performed 20 MIC determinations using its MHLHB test lot and five determinations with the MHLHB common lot on *S. pneumoniae* ATCC 49619 in order to develop MIC quality control limits for each drug (31). Ten separate determinations were performed on *S. pneumoniae* ATCC 49619 with the MHSBA common lot (with each of two disk lots), and 20 separate determinations were made with the MHSBA test lot unique to that laboratory (with each of two disk lots). Replicate testing was performed over a period of 5 or more days in order to appropriately assess intralaboratory and interlaboratory reproducibilities of MICs and zone diameters with each drug.

**Development of quality control ranges and interpretive criteria.** Data from all of the laboratories were analyzed to establish acceptable quality control limits for MICs and zone diameters for each drug according to the criteria advocated by the NCCLS (31). MICs and zone sizes for each drug were compared by scatter plots of the data generated by testing the clinical isolates in each laboratory. Proposed zone sizes were developed by use of the error-rate-bounded method (27).

**RESULTS**

Each of five laboratories tested two *S. pneumoniae* control strains and approximately 50 unique clinical isolates using the NCCLS reference MHLHB broth microdilution and MHSBA disk diffusion tests. Both control organisms and the clinical isolates grew reliably with all lots of media examined in the study. Repeat testing of *S. pneumoniae* ATCC 49619 yielded good intralaboratory and interlaboratory reproducibilities of MICs and zone sizes with the various agents tested in this study. Table 1 summarizes proposed MIC quality control limits on the basis of these tests. The proposed acceptable ranges include the observed modal MIC ± 1 log₂ dilution with all drugs, except the combination of trimethoprim-sulfamethoxazole, which demonstrated a dual mode of 0.25 and 0.5 μg/ml. Thus, the proposed MIC limits for that antimicrobial agent combination span 4 log₂ dilutions. The proposed MIC control limits encompassed more than 99% of the observed values for both the common lots of media and the test lots (Table 1).

Table 2 includes proposed disk diffusion zone diameter quality control limits derived from the replicate testing done in the five laboratories. The proposed zone diameter limits were first calculated on the basis of the medians and ranges of medians recorded with the medium test lots as suggested by Gavan et al. (11). In some cases, the ranges were expanded equally on each side of the calculated limits in order to encompass approximately 95% of the values recorded by the five laboratories. This allowed control zone diameter limits of 6 to 8 mm with most of the drugs. However, the chloramphenicol, ofloxacin, oxacillin, and tetracycline proposed zone diameter limits are somewhat narrower (5 mm) because of greater observed precision with those agents, and the proposed limits with imipenem are wider (12 mm) because of somewhat lesser precision observed with imipenem in this study (Table 2).

Scatter plots of MICs versus zone diameters are depicted in Fig. 1 for each drug. Interpretive criteria were first established for MICs of each drug (see Discussion); then, zone diameter interpretive criteria were proposed for each agent through use of the error-rate-bounded method (27) to select zone diameter breakpoints which would provide the fewest interpretive errors. Table 3 includes the proposed MIC and zone diameter interpretive criteria and a calculation of the interpretive errors which resulted from applying the proposed breakpoints to the data generated in this study. The correlation between MICs

### TABLE 1. Proposed MIC quality control limits for *S. pneumoniae* ATCC 49619 tested by broth microdilution using MHLHB

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Proposed MIC limits (μg/ml)</th>
<th>% Data included</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefepime</td>
<td>0.06–0.25</td>
<td>100</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>0.06–0.25</td>
<td>100</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0.03–0.12</td>
<td>100</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>2–8</td>
<td>100</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.03–0.12</td>
<td>100</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.03–0.12</td>
<td>100</td>
</tr>
<tr>
<td>Meropenem</td>
<td>0.06–0.25</td>
<td>100</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>1–4</td>
<td>100</td>
</tr>
<tr>
<td>Penicillin</td>
<td>0.25–1</td>
<td>100</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.12–0.5</td>
<td>99.3</td>
</tr>
<tr>
<td>Trim-sulfa</td>
<td>0.12–2.3–1–19</td>
<td>100</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.12–0.5</td>
<td>100</td>
</tr>
</tbody>
</table>

* Includes 22 values equal to or less than the lowest value tested (0.03 μg/ml) primarily with the common lot of trays.
* Includes 10 values equal to or less than the lowest value tested (0.12 μg/ml) primarily with the common lot of trays.

### TABLE 2. Proposed quality control zone size limits for *S. pneumoniae* ATCC 49619 tested on MHSBA

<table>
<thead>
<tr>
<th>Antimicrobial disk (μg)</th>
<th>Proposed zone size limits (mm)</th>
<th>% Data included</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefepime (30)</td>
<td>28–35</td>
<td>94.7</td>
</tr>
<tr>
<td>Cefotaxime (30)</td>
<td>30–35</td>
<td>96.6</td>
</tr>
<tr>
<td>Ceftriaxone (30)</td>
<td>30–35</td>
<td>96.4</td>
</tr>
<tr>
<td>Chloramphenicol (30)</td>
<td>23–27</td>
<td>96.4</td>
</tr>
<tr>
<td>Erythromycin (15)</td>
<td>25–30</td>
<td>96.4</td>
</tr>
<tr>
<td>Imipenem (10)</td>
<td>33–44</td>
<td>95.0</td>
</tr>
<tr>
<td>Meropenem (10)</td>
<td>28–35</td>
<td>96.4</td>
</tr>
<tr>
<td>Ofloxacin (5)</td>
<td>16–21</td>
<td>98.6</td>
</tr>
<tr>
<td>Oxacillin (1)</td>
<td>8–12</td>
<td>97.7</td>
</tr>
<tr>
<td>Tetracycline (30)</td>
<td>27–31</td>
<td>97.2</td>
</tr>
<tr>
<td>Trim-sulfa (1.25–23.75)</td>
<td>22–27</td>
<td>96.1</td>
</tr>
<tr>
<td>Vancomycin (30)</td>
<td>20–27</td>
<td>98.3</td>
</tr>
</tbody>
</table>

* Trim-sulfa, trimethoprim-sulfamethoxazole.
FIG. 1. Scattergrams representing MICs and zone diameters derived from testing 248 S. pneumoniae clinical isolates from five laboratories. Numbers of strains that are penicillin resistant are circled; numbers of strains intermediate to penicillin are boxed. Solid horizontal lines, proposed MIC interpretive criteria; solid vertical lines, corresponding proposed zone diameter interpretive criteria. Because of excessive interpretive errors, disk testing is not recommended with cefepime, cefotaxime, ceftriaxone, imipenem, or trimethoprim-sulfamethoxazole (see the text and Table 3). Sufficient clinical data are not yet available to propose interpretive criteria for meropenem.
FIG. 1—Continued.
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FIG. 1—Continued.
FIG. 1—Continued.
FIG. 1—Continued.
and zones was considered to be adequate to propose disk diffusion interpretive criteria with five of the drugs in the study (chloramphenicol, erythromycin, oxacillin, tetracycline, and vancomycin). Interpretive criteria for meropenem are not proposed at this time, because of the limited number of human clinical response data available. Because of excessive interpretive errors (mostly minor errors) with cefepime, cefotaxime, ceftriaxone, imipenem, and trimethoprim-sulfamethoxazole, disk testing is not recommended with those agents at the present time. Lastly, it is proposed that the interpretive breakpoints for penicillin MICs and oxacillin zone diameters remain unchanged from their previously suggested values (33, 34), despite 14% major interpretive errors with the oxacillin disk test (Table 3). The majority of errors occurred with strains whose penicillin MICs reflected borderline susceptibility to penicillin (MIC = 0.06 µg/ml) and which produced zone diameters of 13 to 17 mm.

DISCUSSION

The prevalence of S. pneumoniae clinical isolates resistant to primary therapeutic agents such as penicillin, chloramphenicol, trimethoprim-sulfamethoxazole, and tetracycline appears to have changed markedly in the last 2 to 5 years (4, 5, 24, 29, 44). In addition, reports of pneumococcal resistant to various extended- and broad-spectrum cephalosporins have occurred for the first time during this period (2, 6, 10, 39). These developments have placed greater emphasis on prompt and accurate recognition of resistant pneumococcal isolates by clinical microbiology laboratories.

The MIC procedure recommended by the NCCLS (34) for testing pneumococci includes the use of MHLHB. Lyzed horse blood-supplemented medium has been criticized as being tedious to prepare and not widely available from commercial sources. For those reasons Haemophilus test medium (HTM) was explored as an alternative to MHLHB (18, 20, 32); however, MICs tended to be somewhat lower in HTM than MICs in MHLHB, a finding which can affect the interpretation of results with some drugs (18). Perhaps more importantly, excessive growth failures and significantly lower MICs were seen with some contemporary lots of HTM used for pneumococcal testing in an early phase of the present study (data not shown). Because of these unexplained problems with some lots of HTM, we cannot recommend HTM at this time as an alternative to MHLHB for MIC testing of pneumococci.

The definitions of penicillin resistance in current use (34) are derived from the original breakpoints proposed by Hansman (13) and later endorsed by Jacobs et al. (16, 17). Thus, the definition of penicillin resistance used in this study was an MIC ≥ 2 µg/ml and MICs of 0.12 to 1 µg/ml were referred to as intermediate rather than "relatively resistant," for consistency with breakpoints proposed for the other agents studied. The oxacillin disk test has been found in prior studies to be reliable for predicting susceptibility to penicillin (24, 41). An acknowledged shortcoming of the oxacillin disk test is its inability to distinguish between strains that are penicillin resistant from those that are intermediately resistant to penicillin or those that demonstrate borderline susceptibility to penicillin (especially MICs of 0.06 µg/ml) (41, 48). However, the data from the present study reaffirm the concept that susceptible strains of pneumococci reliably produce zones of 20 mm or greater with the oxacillin disk screening test (Fig. 1). It is our view that it is important to define precisely the level of penicillin susceptibility of significant pneumococcal isolates (especially those causing meningitis) by determining an MIC. In areas with very low rates of penicillin resistance (e.g., less than 5%) or with isolates from patients who do not have meningitis, it may be considered cost-effective to screen isolates with the oxacillin disk test and to pursue MIC testing with penicillin and alternative agents only on isolates that have oxacillin zone sizes less than 20 mm. Current evidence indicates that high-dose penicillin (12 × 10⁶ to 24 × 10⁶ U/day given intravenously for adults) may be used to treat penicillin-intermediate pneumococcal infections outside the central nervous system (15, 23, 36, 38). However, clinical experience has shown that penicillin is not an adequate regimen for treatment of penicillin-resistant or -intermediate strains in patients who have meningitis (14, 15, 36, 45, 46).

Chloramphenicol resistance in pneumococci is most often due to production of the inactivating enzyme chloramphenicol acetyltransferase (19, 25). Chloramphenicol MICs for such strains are 8 µg/ml or greater (19). On the basis of the results of the present study, such strains can be accurately recognized by disk diffusion testing using the breakpoint indicated in Table 3. However, recent clinical experience in the use of chloramphenicol to treat meningitis due to penicillin-resistant pneumococcal strains has not been favorable (8). Thus, chlor-
amphotericin alone may not represent optimal therapy of pneumococcal meningitis due to penicillin-resistant strains, even when a chloramphenicol-susceptible strain is implicated.

Until recently, extended-spectrum cephalosporins have been used successfully for therapy of serious pneumococcal infections such as meningitis, including those due to penicillin-resistant or -intermediate strains (6, 22, 36, 46). Most pneumococcal strains have had cefotaxime or ceftriaxone MICs of ≤0.03 μg/ml (15, 23). However, pneumococcal strains with cefotaxime or ceftriaxone MICs of 2 to 32 μg/ml have been recognized recently in patients who have meningitis and who have failed treatment with either of those cephalosporins (2, 10, 39). Certain of these cephalosporin-resistant strains have shown intermediate susceptibility to penicillin (MICs of 0.25 to 0.5 μg/ml), while others have been penicillin resistant (2, 10). Cephalosporin resistance in these strains appears to be due to unique remodeling of the penicillin-binding proteins, which differs somewhat from the penicillin-binding-protein alterations that result in penicillin (but not cephalosporin) resistance (7, 30).

Clinical response to cefotaxime or ceftriaxone therapy has varied, with MICs of 0.5 to 1 μg/ml. In one study, seven of nine adult patients with meningitis responded favorably to cefotaxime therapy (200 to 350 mg/day) (45). It is not clear from that study if the patients with strains in the higher cephalosporin MIC range (0.5 to 1 μg/ml) responded less favorably than those for whose strains the MICs were lower (e.g., <0.03 μg/ml). However, a child with meningitis caused by a strain for which the MIC was 1 μg/ml failed therapy with low-dose (150 mg/kg of body weight) cefotaxime (1). A single patient described in a letter to the editor was said to fail therapy with cefotaxime for a strain with a cefotaxime MIC of 0.5 μg/ml (3).

The methodologies used to determine the cephalosporin MICs in some of the aforementioned studies have not been described in detail, making method- or medium-dependent differences impossible to distinguish. The aforementioned clinical responses combined with knowledge that cerebrospinal fluid levels of either cefotaxime or ceftriaxone are usually in the range of 4 to 4.5 μg/ml (10, 39) have led us to propose that pneumococcal isolates with cefotaxime or ceftriaxone MICs of 2 μg/ml or greater be regarded as resistant, at least with respect to patients with meningitis. On the basis of a review of the extant literature and unpublished clinical studies reviewed by the NCCLS Antimicrobial Susceptibility Testing Subcommittee, it is likely that strains for which MICs are ≤0.5 μg/ml will be responsive to therapy with high doses of either of these cephalosporins and, thus, should be categorized as susceptible. We propose that strains with cefotaxime or ceftriaxone MICs of 1 μg/ml be categorized as intermediate until more information becomes available to clarify the role of cephalosporin therapy for such strains, particularly for therapy of meningitis. It is likely that such strains would be responsive to cephalosporin therapy for infections outside the central nervous system. Because the susceptibility of pneumococci to cefotaxime and ceftriaxone appear to be almost identical to those features of cefotaxime and ceftriaxone (Fig. 1) (42) and because clinical response data reviewed by the NCCLS Antimicrobial Susceptibility Testing Subcommittee provided similar outcomes, we propose the same tentative interpretive criteria for cefepime (Table 3). Unfortunately, the disk diffusion test results accumulated in the present study did not correlate extremely well with MICs for these three cephalosporins. In particular, large numbers of minor errors compromised the diagnostic usefulness of the disk test for these agents (Table 3). Thus, we cannot recommend disk diffusion testing of pneumococcal isolates with cefepime, cefotaxime, or ceftriaxone at this time. The use of cefotaxime or ceftizoxime disk tests has been proposed to screen pneumococci for high-level (MICs ≥ 4 μg/ml) cephalosporin resistance if an MIC method is not readily available (9, 43). The present study did not evaluate use of a cefotaxime disk test for this purpose, but ceftizoxime zone diameter data generated during this study were not useful for accurate recognition of cephalosporin-resistant (MICs ≥ 2 μg/ml) strains (data not shown).

Much fewer data are available for review regarding the susceptibilities of the penicillin-, cefotaxime-, or ceftriaxone-resistant strains to other cephalosporins, including those administered by the oral route. Patients have developed meningitis while receiving therapy with cefaclor or cefprozil axetil for strains that ultimately were shown to be cephalosporin resistant in vitro (39). The MICs of cefaclor, cefprozil, loracarbef, and cefuroxime for pneumococcal strains that are cefotaxime or ceftriaxone resistant on the basis of the criteria indicated above are markedly elevated (data not shown), and the MICs of these four oral cephalosporins for penicillin-resistant or -intermediate strains are also elevated (data not shown). The NCCLS initially proposed application of the MIC interpretive criteria used for Haemophilus and nonfastidious bacteria for determination of pneumococcal susceptibility to those agents (34) on the basis of the reasoning that pneumococci and Haemophilus species often produce similar localized, non-life-threatening infections and that clinicians may expect to respond to a similar manner to oral beta-lactam therapy. However, it is our present opinion that the MIC interpretive criteria for those oral cephalosporins are too high for use with pneumococci. Evidence that penicillin-intermediate or -resistant pneumococci are clinically responsive to therapy with oral cephalosporins has not been provided. Additional data relevant to the activity of several oral beta-lactams against pneumococci are under evaluation and will be the subject of a future article and later recommendations by the NCCLS.

Imipenem has been suggested as a possible therapeutic alternative for the cephalosporin-resistant pneumococcal strains (45) and has been used successfully to treat one patient with a cefotaxime-resistant isolate (1). However, the cerebrospinal fluid penetration of imipenem may not be optimal for treatment of meningitis without causing the unwanted side effect of seizures (28). On the basis of the very limited data available, we tentatively propose that pneumococci with MICs of 0.12 μg/ml be categorized as susceptible and that strains with MICs of 1 μg/ml or greater be regarded as resistant (Table 3). Unfortunately, the disk diffusion test was found to correlate poorly with imipenem MICs (Fig. 1E) and cannot be recommended for testing of this agent.

The MIC interpretive criteria used for nonfastidious organisms (34) seem to characterize accurately levels of susceptibilities of pneumococci to erythromycin and ofloxacin (Fig. 1H and L, respectively, and Table 3). However, only criteria which define susceptibility to vancomycin (MIC ≤ 1 μg/ml; zone diameter ≥ 17 mm) can be proposed since vancomycin-resistant pneumococcal strains do not exist at this time (Fig. 1K and Table 3).

We propose adoption of the MIC interpretive criteria in current use for Haemophilus species (34) for tetracycline and trimethoprim-sulfamethoxazole since pneumococci and Haemophilus species often produce similar respiratory infections (e.g., otitis media and sinusitis) and pneumococcal strains might be expected to respond in a similar manner at those sites. Zone diameter interpretive criteria for tetracycline are proposed in Table 3. Numerous minor interpretive errors preclude us from recommending disk testing criteria for tri-
methoprim-sulfamethoxazole (Fig. 1J and Table 3). However, the majority of errors involved classifying strains that were intermediate by the MIC test as resistant on the basis of the disk diffusion method.

In summary, we have proposed MIC interpretive criteria for 11 antimicrobial agents against pneumococci using the NCCLS reference broth microdilution procedure. In addition, disk diffusion interpretive criteria specific for pneumococci are proposed for chloramphenicol, erythromycin, tetracycline, ofloxacin, and vancomycin. Because of excessive interpretive category errors, we do not recommend the disk diffusion test for cefepime, cefotaxime, ceftriaxone, imipenem, or trimethoprim-sulfamethoxazole. The data presented in this article have also served as the basis for the newest recommendations on testing of pneumococci by the NCCLS (35). It is our recommendation that as a minimum, all pneumococcal isolates from patients with meningitis be tested by an MIC method against penicillin and either cefotaxime or ceftriaxone as soon as isolated colonies are available. If resistance to those cephalosporins is detected, additional agents may need to be tested (e.g., vancomycin, chloramphenicol, and imipenem). For isolates from patients who do not have meningitis, some laboratories may wish to test additional agents initially while others may wish to test only for penicillin susceptibility for reasons of cost control. It can be assumed that pneumococcal isolates that are penicillin susceptible (as determined by the criteria described in this article) are also susceptible to the other beta-lactam antibiotics included in this study. It is also important to emphasize that the MIC and disk diffusion criteria proposed herein have been validated only for the procedures used in this study. The methods described in this article should provide laboratories with the information needed to assess accurately the level of susceptibility of contemporary pneumococcal clinical isolates.

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