Improved Medium for Antimicrobial Susceptibility Testing of
Haemophilus influenzae

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The need for complex growth media has complicated routine susceptibility testing of Haemophilus influenzae because of antagonism of certain antimicrobial agents by the medium or because of difficulties in interpretation of growth endpoints. Haemophilus test medium (HTM) is a simple, transparent medium for broth- or agar-based tests with H. influenzae. HTM incorporates Mueller-Hinton medium with additions of 15 μg of hematin per ml, 15 μg of NAD per ml, and 5 mg of yeast extract per ml as growth-promoting additives. Agar or broth microdilution MICs of 10 antimicrobial agents for a collection of 179 H. influenzae isolates determined by using HTM compared favorably with MICs determined by the conventional agar or broth dilution methods recommended by the National Committee for Clinical Laboratory Standards. Disk diffusion tests performed with HTM allowed accurate categorization of susceptible and resistant strains and were easier to interpret than tests performed with Mueller-Hinton chocolate agar. A particular advantage of HTM was the reliability of broth- or agar-based test results with trimethoprim-sulfamethoxazole. The results of this study suggest modification of current National Committee for Clinical Laboratory Standards MIC-interpretive criteria for H. influenzae with amoxicillin-clavulanate, chloramphenicol, and trimethoprim-sulfamethoxazole. Error rate-bounded analysis of MICs and disk diffusion zone sizes also suggest modified zone-interpretive criteria for ampicillin, amoxicillin-clavulanate, chloramphenicol, and tetracycline with HTM or conventional media. Interpretive zone sizes are newly proposed for cefaclor and rifampin disk diffusion tests.

Isolates of Haemophilus influenzae resistant to a variety of antimicrobial agents have been reported with increasing frequency in recent years (6, 12, 17–19). Whereas rapid tests for beta-lactamase (32) or chloramphenicol acetylmethylcase (1) provide useful information regarding two commonly used agents, reliable growth-based susceptibility tests are necessary to detect resistance to ampicillin by alternative mechanisms (24, 29) and to antimicrobial agents such as trimethoprim-sulfamethoxazole, tetracycline, erythromycin, rifampin, and cephalosporins (4, 5, 10, 17, 30, 33). The complex media needed to satisfy the exacting growth requirements (11) of H. influenzae have complicated the performance of antimicrobial agent susceptibility tests. The inclusion of animal blood or blood products often has made the resulting test media opaque (2, 22), making the interpretation of test results more difficult. Complex growth-promoting additives may result in variability of media performance from batch to batch or cause antagonism of certain antimicrobial agents, particularly trimethoprim-sulfamethoxazole (2, 6, 20).

This report describes the development of a medium formulation for susceptibility testing of H. influenzae which is simple and inexpensive to prepare, is transparent, and poses minimal risk of antagonism of antimicrobial agents.

MATERIALS AND METHODS

Test strains. A collection of 179 H. influenzae isolates of clinical origin from at least six different geographic locations was selected to include both susceptible and resistant strains for each of the study drugs.

Antimicrobial agents. Reagent grade powders of ampicillin, amoxicillin-clavulanate (2:1 ratio), cephalothin, cefamandole, cefaclor, chloramphenicol, erythromycin, rifampin, tetracycline, and trimethoprim-sulfamethoxazole (1:19 ratio) were obtained either from their respective manufacturers or from Sigma Chemical Co. (St. Louis, Mo.).

Beta-lactamase tests. All isolates were tested for beta-lactamase production by use of nitrocefin-impregnated paper disks (Cefnase; BBL Microbiology Systems, Cockeysville, Md.). Chloramphenicol acetylmethylase tests. Strains demonstrating apparent borderline susceptibility to chloramphenicol were tested by using a commercial-paper-disk method for the presence of chloramphenicol acetylmethylase (Remel, Lenexa, Kans.).

Test Media. (i) HTM. Haemophilus test medium (HTM) was prepared by using Mueller-Hinton broth or Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.) as the base to which 15 μg of bovine hematin (Sigma) per ml and 5 mg of yeast extract (Difco) per ml were added before autoclaving. The hematin was prepared as a stock solution by dissolving 50 mg of powder in 100 ml of 0.01 N NaOH with stirring and gentle heat to thoroughly dissolve the hematin. It was necessary to prepare fresh hematin each time the medium was made, since it was not stable as a concentrated stock solution. The hematin stock solution (30 ml) was added to each liter of medium to yield the final concentration of 15 μg/ml. After autoclaving, the final ingredient, 15 μg of β-NAD (Sigma) per ml, was added. This was accomplished by dissolving 50 mg of β-NAD in 10 ml of distilled water and then filter sterilizing by passage through a 0.22-μm-pore-size membrane filter. This stock solution (3 ml) was aseptically added to each liter of sterile, cooled medium. The β-NAD stock solution could be stored at 4°C for up to 1 month before use. For sulfonamide microdilution tests, 0.2 IU of thymidine phosphorylase (Burroughs Wellcome Co., Research Triangle Park, N.C.) per ml was aseptically added to the sterilized and cooled HTM broth. Initial studies with HTM and several of the antimicrobial agents used in this investigation did not reveal a significant effect of addition of

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divalent cations (Ca\(^{2+}\) and Mg\(^{2+}\)) to broth dilution tests. The completed HTM could be stored at 4°C for 3 months or longer without adverse effect.

(ii) Mueller-Hinton LHB. Mueller-Hinton broth (Difco) was supplemented with 5% lyzed horse blood (prepared in our laboratory) and 10 μg of β-NAD (Sigma) per ml as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (27), to produce Mueller-Hinton lyzed-horse-blood broth (LHB).

(iii) MH Choc. Mueller-Hinton agar (Difco) was supplemented with 1% hemoglobin (BBL) and 1% IsoVitaleX (BBL) for agar dilution tests as suggested in NCCLS M7-A (27). Chocolate Mueller-Hinton agar (MH Choc) plates (150 by 15 mm) for disk diffusion tests were obtained from BBL.

**Determination of growth kinetics in HTM.** Growth curves were performed with selected *H. influenzae* strains to determine the growth-promoting character of HTM. Initial inocula were prepared in Mueller-Hinton broth by suspending 24-h growth from enriched chocolate plates (BBL) to match the turbidity of the 0.5 McFarland standard. Inocula were further diluted and then added to 10 ml of HTM to achieve an inoculum density of approximately 10\(^3\) CFU/ml. Viable cell counts were determined initially by subculture of the HTM tubes to enriched chocolate agar plates and also after 4, 8, and 24 h of incubation at 35°C in ambient air.

**Susceptibility tests.** (i) Microdilution tests. Microdilution MIC tests were performed by using HTM or LHB containing twofold-concentration increments of antimicrobial agents dispensed in 100-μl amounts in plastic 96-well trays. Inocula were prepared by suspending 24-h growth from enriched chocolate agar plates (BBL) in Mueller-Hinton broth to visually equal the turbidity of the 0.5 McFarland standard. Inocula were further diluted and added to microdilution trays to achieve a final inoculum density of approximately 10\(^8\) CFU/ml. Trays were incubated for 16 to 20 h at 35°C in ambient air before interpretation of MIC endpoints.

(ii) Agar dilution tests. Agar dilution MICs were determined by using HTM or MH Choc agars incorporating twofold increments of the various antimicrobial agents. Initial inoculum preparation was done as described above. Final inocula consisted of 10\(^4\) CFU/μl spot applied to the surface of the plates with a Steers replicator. Plates were incubated for 16 to 20 h at 35°C either in ambient air or in 5% CO\(_2\) before MIC interpretation.

(iii) Disk diffusion tests. Disk diffusion tests were performed on either HTM or MH Choc agars according to the general guidelines of NCCLS M2-A3 (26). Inocula were prepared visually to match the turbidity of the 0.5 McFarland standard as described above. The following antimicrobial agent disks were used: ampicillin (10 μg), amoxicillin-clavulanate (20/10 μg), cephalothin (30 μg), cefamandole (30 μg), cefazolin (30 μg), chloramphenicol (30 μg), tetracycline (30 μg), trimethoprim-sulfamethoxazole (1.25/23.75 μg), rifampin (5 μg), and erythromycin (15 μg). Inoculated plates with antimicrobial disks applied to the surface were incubated for 16 to 18 h at 35°C either in ambient air or in 5% CO\(_2\) before measurement of zone sizes.

**RESULTS**

HTM is a newly formulated medium for susceptibility testing of *H. influenzae* by broth dilution, agar dilution, or disk diffusion methods. The HTM growth supplements cause the medium to appear slightly darker in color than unsupplemented Mueller-Hinton medium; however, its optical clarity allows measurement of disk diffusion zones from the back of the plate by using reflected or transmitted light as with unsupplemented Mueller-Hinton agar. Likewise, turbidity in broth dilution tests is readily determined by using either macro- or microdilution test methods.

The excellent growth-promoting properties of HTM were demonstrated by performance of kinetic growth studies of selected *H. influenzae* strains in HTM broth. Cell numbers increased rapidly during the initial 4 to 8 h of incubation and resulted in approximately 5 × 10\(^8\) CFU/ml by 24 h (Fig. 1). Likewise, ample growth lawns of *H. influenzae* were observed on HTM agar plates used for disk diffusion tests.

HTM broth microdilution or agar dilution MICs of the various drugs compared favorably with those determined by using either LHB or MH Choc agar (Table 1). Geometric mean and mode MICs were essentially equivalent by all methods with ampicillin, amoxicillin-clavulanate, cefamandole, and chloramphenicol. On the other hand, cefaclor microdilution MICs tended to be approximately one log\(_2\) dilution lower in HTM than MICs determined by using the other media or by HTM agar dilution. Cephalothin MICs were approximately two log\(_2\) dilutions lower by HTM microdilution testing than by other methods. Erythromycin MICs were one to two log\(_2\) dilutions higher when determined by either of the agar dilution methods than when performed by microdilution. Rifampin and tetracycline MICs tended to be one log\(_2\) dilution lower in HTM than in either of the conventional media. Lastly, trimethoprim-sulfamethoxazole MICs were generally two log\(_2\) dilutions higher in HTM broth than in LHB, a significant point with trimethoprim-sulfamethoxazole-resistant strains which demonstrated relatively low MICs in LHB.

Because we determined that some clinical isolates of *H. influenzae* (particularly those from respiratory sources) did not grow or produced very sparse growth in ambient air, we evaluated incubation of agar dilution MIC and disk tests in an atmosphere of 5% CO\(_2\), as well as in ambient air. Strains which grew in either atmosphere demonstrated close agreement between MICs (data not shown). However, disk diffusion zone sizes in CO\(_2\) tended to be slightly smaller (1 to 2 mm) on HTM or MH Choc with most drugs included in this...
TABLE 1. Correlation of broth or agar dilution MICs determined in HTM with those determined by using LHB or MH choc agar on 100 H. influenzae isolates

<table>
<thead>
<tr>
<th>Drug</th>
<th>HTM</th>
<th>LHB broth microdilution</th>
<th>MH Choc agar dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GM</td>
<td>Mode</td>
<td>GM</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>1.3</td>
<td>0.25</td>
<td>1.6</td>
</tr>
<tr>
<td>Amoxicillin-clavulanate</td>
<td>0.44</td>
<td>0.5</td>
<td>0.51</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>1.6</td>
<td>1</td>
<td>3.1</td>
</tr>
<tr>
<td>Cefamandole</td>
<td>0.35</td>
<td>0.5</td>
<td>0.51</td>
</tr>
<tr>
<td>Cefaclor</td>
<td>1.7</td>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>0.63</td>
<td>0.5</td>
<td>0.63</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.68</td>
<td>0.5</td>
<td>0.57</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>0.07</td>
<td>0.06</td>
<td>ND</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>0.35</td>
<td>0.25</td>
<td>0.28</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>1.6</td>
<td>2</td>
<td>5.3</td>
</tr>
</tbody>
</table>

* GM, Geometric mean.
ND, Not determined.

study, although zone sizes for erythromycin were often 2 to 3 mm smaller, and tetracycline zones were generally 2 to 3 mm larger when incubated in CO2.

Figure 2 depicts the scattergrams for all 10 antimicrobial agents, comparing broth microdilution MICs and disk diffusion zones (in CO2 incubation) for HTM and conventional media. Current NCCLS zone size interpretive criteria (26) are indicated on each graph with revised tentative zone size breakpoints proposed by us after a review of these data. Not depicted in Fig. 2, but listed in Table 2 are proposed changes in MIC-interpretive breakpoints for amoxicillin-clavulanate, chloramphenicol, erythromycin, and trimethoprim-sulfamethoxazole, as well as current NCCLS MIC-interpretive criteria (27) for all of the drugs. We propose revised MIC breakpoints to represent a better estimate of susceptibility and resistance in H. influenzae (see Discussion), whereas proposed changes in zone size-interpretive criteria stem from applying error rate-bounded analyses (25) to the data plots. Table 3 indicates the category errors encountered when current NCCLS and proposed MIC and zone criteria were applied to the data.

DISCUSSION

The increase in resistance of H. influenzae to ampicillin and other primary antimicrobial agents (6, 12, 17-19, 24) places new emphasis on reliable methods for susceptibility testing of this microorganism. The fastidious nature of H. influenzae has heretofore necessitated use of rather complex growth media for performance of susceptibility tests, often requiring use of blood or blood products (8, 22, 26, 27). Although several of these media provide luxuriant growth of H. influenzae, their complex constituents may antagonize certain antimicrobial agents (2, 6) and often complicate interpretation of test endpoints because of the opaque nature of media such as MH Choc (26, 27). Simply adding X and V factors (hemin and NAD) to Mueller-Hinton agar allows growth of some strains (3). However, in our experience, many strains do not grow well enough for reliable susceptibility testing with the addition only of those basic supplements to Mueller-Hinton base. Yeast autolysate has been shown to provide some important growth-stimulating factors when added to media for H. influenzae (28). Supplementation of Mueller-Hinton base with a commercial preparation of yeast autolysate plus hematin (Difco Supplement C) seemed to be a promising medium for susceptibility testing of H. influenzae (14, 15). However, at least one lot of Supplement C was inadvertently contaminated with microbial beta-lactamase, causing spurious ampicillin resistance (34). In addition, Supplement C is costly and significantly antagonizes trimethoprim-sulfamethoxazole (unpublished observation).

HTM appears to represent an improvement over previous medium formulations used for testing H. influenzae in that it is relatively simple to prepare and inexpensive, does not antagonize antimicrobials, and is transparent to facilitate readings of susceptibility tests. The relatively simple ingredients used in the preparation of HTM provide excellent batch-to-batch reproducibility of growth characteristics and susceptibility test results. The cost of the three HTM supplements (hematin, NAD, and yeast extract) was approximately 10% of the cost of either lysed horse blood plus NAD (for broth) or hemoglobin and IsoVitaleX (for agar) for supplementation of Mueller-Hinton medium. Addition of 0.1 to 0.2 IU of thymidine phosphorylase (5, 31) per ml to HTM broth markedly improved microdilution endpoints with trimethoprim-sulfamethoxazole. Even including the considerable expense of thymidine phosphorylase, the cost of HTM broth was less than one-half that of LHB. Thymidine phosphorylase was not found to be necessary for trimethoprim-sulfamethoxazole disk diffusion tests with HTM agar if yeast extract which contained relatively little thymidine was chosen for preparation of HTM agar.

MICs determined by using HTM broth or agar compared favorably with MICs determined by LHB or MH Choc agar dilution methods. In fact, trimethoprim-sulfamethoxazole MICs of strains resistant to that combination were somewhat higher in HTM than in LHB (data not shown), making categorization of resistant strains more obvious with HTM.

We recommend incubation of HTM agar tests in 5% CO2 on the basis that (i) it provides growth of virtually all isolates on initial testing and (ii) it does not adversely affect the accuracy of susceptibility tests. In our experience, many laboratory-adapted stock culture strains of H. influenzae and most fresh isolates from cerebrospinal fluid or blood grow adequately on HTM or MH Choc agar in ambient air. However, a significant proportion of fresh clinical isolates from other sources (perhaps 30% from respiratory, middle-ear effusions, and eyes) do not grow reliably without incubation in CO2. Although previous investigations have sug-
gested antagonism of erythromycin (21) or potentiation of tetracycline (7) by CO₂-related alterations of medium pH, or other differences in susceptibility when tests were incubated in CO₂ (16), we believe that these disadvantages are overshadowed by the striking growth promotion of CO₂ incubation with *H. influenzae*. Zone size-interpretive criteria suggested in Fig. 2 and Table 2 take into account the effect of CO₂ incubation of HTM or MH Choc disk diffusion tests.

We are not aware of compelling reasons to indicate that the susceptible MIC breakpoint for amoxicillin-clavulanate should be one dilution increment higher (on the basis of the amoxicillin component) than the breakpoint for ampicillin (9, 27). Indeed, three of four strains in this study which were beta-lactamase negative but ampicillin resistant (and thus not significantly affected by clavulanate) yielded amoxicillin-clavulanate MICs of 4 µg/ml (on the basis of amoxicillin). We therefore suggest that the appropriate susceptible MIC breakpoint for *H. influenzae* isolates with amoxicillin-clavulanate be ≤2 µg/ml, which is consistent with the recommended susceptible breakpoint for ampicillin (27).

Isolates included in this study for which chloramphenicol MICs were 8 or 16 µg/ml usually (13 of 15 isolates) produced detectable chloramphenicol acetyltransferase and thus should be classified as resistant. Therefore, we propose that a susceptible MIC breakpoint of 4 µg/ml be used for chloramphenicol tests with *H. influenzae* and that MICs of ≥8 µg/ml be termed resistant. We suggest that the MIC termed susceptible for optimal treatment of serious infections (as opposed to urinary infections) with trimethoprim-sulfamethoxazole (≤0.5 µg/ml [on the basis of the trimethoprim component]) indicated in footnote *m* of Table 2 in NCCLS M7-A (27) be applied to tests with *H. influenzae*. We further suggest that MICs of 1 to 2 µg/ml be termed moderately susceptible and that MICs of ≥4 µg/ml be regarded as resistant. Lastly, if the single susceptible erythromycin MIC breakpoint of ≤0.5 µg/ml proposed by Jones et al. (13) is used, the majority of *H. influenzae* strains will be categorized as resistant to erythromycin rather than as moderately susceptible, as suggested by NCCLS for strains for which MICs are 1 to 4 µg/ml (27).

Because of the revised MIC-interpretive criteria proposed above, and because further refinement of disk diffusion zone size-interpretive guidelines appear reasonable from our data, we propose new interpretive zone sizes for ampicillin, amoxicillin-clavulanate, chloramphenicol, and tetracycline for tests performed with either HTM or conventional MH Choc agar. The proposed zone sizes are listed in Table 2, and Table 3 depicts the effect of the proposed and previous zone breakpoints on categorization errors. In the majority of instances, the percentages of very major or major errors were reduced by application of the proposed zone sizes, e.g., ampicillin and tetracycline tests with HTM, and amoxicillin-clavulanate, chloramphenicol, and erythromycin with either medium.

Interpretive zone sizes for *H. influenzae* are initially proposed for cefaclor and rifampin. Guidelines for disk diffusion tests with these two antibiotics heretofore have not been published by NCCLS. Our proposed zone breakpoints for cefaclor differ by only 1 mm (Table 2) for the two media because of slightly larger zones on HTM. Likewise, pro-
FIG. 2—(continued)
Trimethoprim-Sulfamethoxazole

Tetracycline

Rifampin

Erythromycin

FIG. 2—(continued)
TABLE 2. Comparison of current NCCLS and proposed MIC breakpoints and disk diffusion zones

<table>
<thead>
<tr>
<th>Drug</th>
<th>MIC (µg/ml)*</th>
<th>MH Choc (NCCLS zones (mm)*</th>
<th>HTM zones (mm)**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>MS</td>
<td>R</td>
</tr>
<tr>
<td>Ampicillin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amoxicillin-clavulanate</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalothin</td>
<td>8</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td>Cefamandole</td>
<td>8</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td>Cefaclor</td>
<td>8</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>8</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.5</td>
<td>1-4</td>
<td>8</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>4</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rifampin</td>
<td>2</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

* S, Susceptible; MS, moderately susceptible; R, resistant.

** Proposed MIC breakpoints for rifampin are 1 mm larger on HTM than on MH Choc (Table 2).

The proposed susceptible breakpoint of ≥23 mm for erythromycin with *H. influenzae* suggested by Jones et al. (13) correlates only with the susceptible MIC breakpoint of ≥0.5 µg/ml. MIC-zone size plots of erythromycin values with either either medium combination resulted in an amalgam of similar values closely clustered near both MIC and zone breakpoints (Fig. 2). Thus, definition of a moderately susceptible-resistant breakpoint would prove difficult by any of the test methods.

We deemed trimethoprim-sulfamethoxazole zones uninter-

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TABLE 3. Percent category errors with current NCCLS and proposed interpretive criteria

<table>
<thead>
<tr>
<th>Drug</th>
<th>% Category error*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MH Choc (NCCLS M2-A3)</td>
</tr>
<tr>
<td></td>
<td>VM</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0</td>
</tr>
<tr>
<td>Amoxicillin-clavulanate</td>
<td>0</td>
</tr>
<tr>
<td>Susceptible MIC = 4a (NCCLS)</td>
<td>1.8</td>
</tr>
<tr>
<td>Susceptible MIC = 2 (proposed)</td>
<td>0</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>0</td>
</tr>
<tr>
<td>Cefamandole</td>
<td>0.9</td>
</tr>
<tr>
<td>Cefaclor</td>
<td>NA</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>0</td>
</tr>
<tr>
<td>Susceptible MIC = 4 (proposed)</td>
<td>1</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0</td>
</tr>
<tr>
<td>Resistant MIC = 8 (NCCLS)</td>
<td>17</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>0</td>
</tr>
<tr>
<td>Susceptible MIC = 2 (NCCLS)</td>
<td>NT</td>
</tr>
<tr>
<td>Susceptible MIC = 0.5 (proposed)</td>
<td>NT</td>
</tr>
<tr>
<td>Rifampin</td>
<td>NA</td>
</tr>
</tbody>
</table>

* Values in parentheses indicate that lack of corresponding zone-MIC intermediate categories introduces minor error. VM, Very major; M, major; m, minor; NA, not applicable; NC, no change from NCCLS; NT, not tested.

a MICs given in micrograms per milliliter.
pretable when using MH Choc, probably because of large amounts of thymidine contributed by the bovine hemoglo-
bin. However, zones on HTM were generally interpreted
without difficulty and allowed discrimination between sus-
ceptible and resistant strains. The interpretive zones sug-
gested by NCCLS (26) for use with fastidious organisms
appeared to fit well with the H. influenzae data derived in
this study (Tables 2 and 3).

Lastly, not all of our proposed zone-interpretive criteria
provided improvement in classifying the susceptibility of
strains in this study. However, the proposed breakpoints
appeared to better fit the distribution of data points, perhaps
providing for some margin of reading error. In particular, we
propose increasing the single zone breakpoints for ampicillin
and amoxicillin-clavulanate because the four beta-
lactamase-negative but ampicillin-resistant strains included
in this study showed zones very close to or exceeding the
NCCLS breakpoints (26). Zones with 10-μg ampicillin disks
were either 19 or 20 mm on HTM, and amoxicillin-
clavulanate zones ranged from 19 to 26 mm. We suggest that
our proposed C. P. increase in the interpretive zone sizes for
these two drugs might allow accurate categorization of such
strains without need for lower density (2-μg) disks as re-
cently proposed for ampicillin (23). It is important for all of
our proposed changes in interpretive MIC and zone criteria
to be subjected to additional testing experience and to be
reviewed by a consensus group such as NCCLS before ac-
ceptance.

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