Comparison of Etest to Broth Microdilution Method for Testing *Streptococcus pneumoniae* Susceptibility to Levofloxacin and Three Macrolides

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When the Etest was compared to broth microdilution for susceptibility testing of *Streptococcus pneumoniae*, levofloxacin, erythromycin, and penicillin results correlated for both methods; azithromycin and clarithromycin showed discrepancies of ≥2 dilutions for 95.8% and 31.5% of the isolates, respectively. Levofloxacin was active against 141 of 142 isolates (≥2.0 μg/ml), making it a potentially useful new fluoroquinolone.

Because of the development of penicillin and cephalosporin resistance in up to 24% of U.S. pneumococcal isolates (1, 3, 4, 9, 17), most clinical laboratories, including those in community hospitals, have begun routine testing of selected *Streptococcus pneumoniae* isolates. The need for alternate therapeutic agents, as well as a practical approach to determine susceptibility to different antimicrobial agents, is apparent. Levofloxacin, a new fluoroquinolone agent that is the *levo* isomer (or S isomer) of ofloxacin, has been reported to have improved activity against a variety of pathogens, including *S. pneumoniae*, compared with ofloxacin and ciprofloxacin (13, 18).

For community hospital laboratories, the Etest is frequently the method of choice, as it is simple to use, is applicable for single-isolate testing, and allows selection of individual drugs to be tested on the basis of local clinical practices. To avoid the dilemma of potential penicillin resistance, macrolides are increasingly used by clinicians. In this study, the Etest was compared to the broth microdilution (BMD) method for testing the susceptibility of recent clinical isolates of *S. pneumoniae* to three macrolides, penicillin, and levofloxacin. *Staphylococcus aureus* ATCC 29213, *S. pneumoniae* ATCC 49619 (intermediate penicillin resistance), and *S. pneumoniae* ATCC 49136 (penicillin susceptible) were used as control strains.

Levofloxacin, penicillin G, erythromycin, clarithromycin, and azithromycin standard powders were obtained from their respective manufacturers and were reconstituted in accordance with the manufacturer’s instructions for use on the same day to prepare microdilution plates, which were then stored at −70°C until use.

Microtiter trays containing Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) supplemented with cations and 2.5% lysed horse blood were inoculated with a final concentration of approximately 5 × 10⁵ CFU/ml (5 × 10⁵ CFU per well) and incubated at 35°C in ambient air for 20 to 24 h prior to determination of MICs as recommended by the National Committee for Clinical Laboratory Standards (10). Susceptibilities were interpreted on the basis of National Committee for Clinical Laboratory Standards-specified breakpoints (12). The interpretive breakpoint criteria for levofloxacin were based on preliminary information from the R. W. Johnson Pharmaceutical Institute (suscptibility: ≥2 μg/ml; intermediate resistance, 4 μg/ml; resistance: ≥8 μg/ml).

Drug MICs were determined with Etest strips (AB Biodisk, Piscataway, N.J.) containing levofloxacin, azithromycin, clarithromycin, erythromycin, and penicillin. Mueller-Hinton blood agar plates were inoculated and then incubated at 35°C in 5% CO₂ for 20 to 24 h as recommended by the manufacturer. The MIC was defined by the intersection of the growth ellipse with the manufacrurer’s instructions for use on the same day to prepare microdilution plates, which were then stored at −70°C until use.

Microtiter trays containing Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) supplemented with cations and 2.5% lysed horse blood were inoculated with a final concentration of approximately 5 × 10⁵ CFU/ml (5 × 10⁵ CFU per well) and incubated at 35°C in ambient air for 20 to 24 h prior to determination of MICs as recommended by the National Committee for Clinical Laboratory Standards (10). Susceptibilities were interpreted on the basis of National Committee for Clinical Laboratory Standards-specified breakpoints (12). The interpretive breakpoint criteria for levofloxacin were based on preliminary information from the R. W. Johnson Pharmaceutical Institute (suscptibility: ≥2 μg/ml; intermediate resistance, 4 μg/ml; resistance: ≥8 μg/ml).

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margin with the Etest strip by use of light reflected at an oblique angle to examine the plates.

Our BMD-determined MIC results (Table 1) indicated that the MIC of levofloxacin for 90% of the strains tested (MIC\textsubscript{90}) was 2.0 \(\mu\)g/ml, which compares favorably to those previously reported for ofloxacin (2.0 to 4.0 \(\mu\)g/ml) and ciprofloxacin (2.0 to 4.0 \(\mu\)g/ml); it was 2 dilutions higher than that of sparfloxacin (0.5 \(\mu\)g/ml) (15, 16). The Etest-determined MICs, obtained at the same time the BMD tests were performed, were within \(\pm 1\) dilution for all but one (99.7%) of the isolates tested (Table 2).

Resistance to the macrolides was notably higher among strains intermediately and fully resistant to penicillin (21% [4 of 19]) than among penicillin-susceptible strains (6.5% [8 of 123]). Etest-determined penicillin MICs were within \(\pm 1\) dilution for 97.9% of the isolates tested, and erythromycin MICs were within \(\pm 1\) dilution for 94.3% of the isolates. MICs for the remaining isolates were all within 2 dilutions of the BMD-determined MICs, except for one strain, for which the Etest-determined penicillin MIC was 3 dilutions lower. In contrast, for only 4.2% of the isolates tested were the Etest azithromycin MICs within \(\pm 1\) dilution of the BMD-determined azithromycin MICs. The remainder of Etest-determined azithromycin MICs were 2 or more dilutions greater than BMD-determined azithromycin MICs. Azithromycin MICs determined by the Etest and BMD showed the greatest discrepancy, with 18.5% major and 54.5% minor interpretive errors (Table 2). Similarly, for only 68.5% of the isolates tested were the Etest-determined clarithromycin MICs within \(\pm 1\) dilution of the BMD-determined MICs. However, although for 31% of the isolates tested there was a difference of 2 or more dilutions between the clarithromycin Etest- and BMD-determined MICs, there was negligible major or minor error for this antibiotic (using the calculations described by the National Committee for Clinical Laboratory Standards) (11).

Because the Etest blood agar plates were incubated in a \(\text{CO}_2\) environment, whereas BMD trays were incubated in ambient air in accordance with National Committee for Clinical Laboratory Standards guidelines (10), the discrepancy between MICs obtained by the two test systems may be due to the effects of \(\text{CO}_2\) on erythromycin and azithromycin, as has been proposed by us and others (2, 5, 14), and may relate to agar surface pH. The decrease in pH of the agar surface in the presence of \(\text{CO}_2\) may alter the antibiotics such that the Etest-determined MICs are consistently higher than the BMD-determined MICs of these antibiotics. At pH 8, un-ionized macrolides may cross the cytoplasmic membrane and easily bind the bacterial ribosomes. However, when the pH of the medium becomes acidic, these antibiotics ionize and may have greater difficulty crossing the cytoplasmic membrane (14). Because azithromycin has two basic groups, it is expected to be more sensitive to pH changes in the medium than is erythromycin. This is precisely what we observed: erythromycin MICs showed the least variation between the BMD and the Etest, whereas azithromycin MICs showed the greatest variation; clarithromycin MICs showed intermediate variation. Had the discrepant BMD- and Etest-determined MICs of azithromycin been due solely to poor growth of the \(S.\ pneumoniae\) isolates incubated without \(\text{CO}_2\), we would have expected to see similar discrepancies for all of the antimicrobial agents tested by these two methods. In fact, this was not the case. Only the macrolides showed a significant variation, i.e., \(\geq 2\) dilution difference between the BMD- and Etest-determined MICs (Table 2). These results indicate that caution should be used in interpreting Etest-determined macrolide MICs, as the MICs of some of these antibiotics were appreciably higher than those determined by the BMD method when the Etest plates were incubated in 5% \(\text{CO}_2\). Which test system is more clinically relevant remains to be determined.

Although previous studies utilized the agar dilution method for evaluation of susceptibility to levofloxacin (13, 18), neither the standard BMD method nor the Etest has been previously evaluated for levofloxacin versus \(S.\ pneumoniae\), despite its potential clinical utility. We found that the Etest provides a relatively simple and reliable method for determining MICs of levofloxacin, as well as penicillin and erythromycin, against \(S.\ pneumoniae\) isolates, as has also been reported by others (6–8). The Etest-determined MICs of these antibiotics showed excellent correlation with standard BMD-determined MICs. However, as Etest-determined MICs of the macrolides azithromycin and clarithromycin were appreciably higher than the BMD-determined MICs, this study suggests that the interpretive breakpoints for these antibiotics should be reevaluated for the Etest when determining MICs for \(S.\ pneumoniae\) incubated in the presence of 5% \(\text{CO}_2\).

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**REFERENCES**

