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Evaluation of In Vitro Spectra of Activity of Azithromycin, Clarithromycin, and Erythromycin Tested against Strains of *Neisseria gonorrhoeae* by Reference Agar Dilution, Disk Diffusion, and Etest Methods

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The macrolide-azilide susceptibility testing (agar dilution, disk diffusion, Etest) criteria for 105 *Neisseria gonorrhoeae* strains were evaluated. In addition, the potencies of azithromycin, clarithromycin, and erythromycin were studied. The most active macrolide-azilide agent was azithromycin (MIC at which 90% of the isolates are inhibited [MIC_{90}], 0.5 μg/ml) compared with clarithromycin (MIC_{90}, 1.5 to 2 μg/ml) and erythromycin (MIC_{90}, 2 to 4 μg/ml). The Etest (AB Biodisk, Solna, Sweden) was observed to produce MIC results very similar to those of the reference agar dilution test (GC agar base), with 100% of the results within 1 $\log_2$ dilution step of the reference MICs. The disk diffusion test zone diameters for all three drugs correlated at an acceptable level ($r = -0.81$ to -0.92) with the reference agar dilution MICs. Interpretive criteria for susceptibility were proposed for azithromycin at a MIC of ≤2 μg/ml and a disk diffusion test zone of ≥25 mm. No category for resistance was proposed because of the paucity of strains for which MICs were >2 μg/ml. These tentative criteria should be further validated by correlations with clinical trial data for gonococcal strains (as they emerge) that have azithromycin MICs above the proposed susceptible category range.

For the over 400,000 reported cases of gonorrhea in the United States each year, there are a wide variety of therapeutic options (2, 7), including the macrolides (erythromycin, clarithromycin), usually combined with rifampin, and the structurally related azilide azithromycin prescribed alone. Recent reports have documented the clinical efficacy of a single dose of azithromycin (1 or 2 g) for treatment of *Neisseria gonorrhoeae* (3, 4, 6, 11, 14) as well as another prevalent sexually transmitted disease caused by *Chlamydia trachomatis* (6, 11). Although the in vitro activities of these cited macrolide-azilides against several bacteria have been well established (1), a comprehensive comparison of the activities of the three agents against gonococci has not been conducted. Furthermore, a comparison of in vitro susceptibility methods with reference agar dilution (8), disk diffusion (9), and the alternative Etest (AB Biodisk, Solna, Sweden) method has not been reported. This study compares the results obtained from three test methods against these macrolide-azilides, studies the potencies of drugs in this class, and suggests (where possible) further interpretive criteria for susceptibility testing.

The following reagents and their sources were utilized: azithromycin (Pfizer Central Research, Groton, Conn.), clarithromycin (Abbott Pharmaceutical Products Division, Abbott Park, Ill.), and erythromycin and penicillin (Sigma Chemical Company, St. Louis, Mo.). The antimicrobial disks were purchased from Becton-Dickinson Microbiology Systems (Cockeysville, Md.) and Difco Laboratories (Detroit, Mich.); the Etest strips were manufactured by AB Biodisk. One hundred five strains of *N. gonorrhoeae* were evaluated. They consisted of recent clinical isolates from the U.S. Navy Environmental and Preventive Medicine Unit (San Diego, Calif.), the University of Iowa Hospitals and Clinics (Iowa City, Iowa), and other geographic locations worldwide. There were 25 strains of penicillinase-producing *N. gonorrhoeae*; 14 β-lactamase-negative, penicillin-resistant (MIC, ≥2 μg/ml) isolates; 58 β-lactamase-negative, moderately penicillin-susceptible (MIC, 0.12 to 1.0 μg/ml) strains; and 8 penicillin-susceptible (MIC, ≤0.06 μg/ml) strains (8). All 105 *N. gonorrhoeae* strains were evaluated according to National Committee for Clinical Laboratory Standards methods for disk diffusion and reference dilution (GC agar base) testing (8, 9). The Etest antimicrobial susceptibility testing was performed according to the manufacturer's guidelines found in the product package insert. All tests were performed in triplicate, and the average (mode or median) zone diameter and MIC were used for comparisons and for regression analyses by the method of least squares.

Table 1 lists the activities of four tested antimicrobial agents as found by two methods of determining MICs. Among the macrolide-azilide drugs, azithromycin was clearly the most active (MIC at which 90% of the isolates are inhibited [MIC_{90}], 0.5 μg/ml), and all MICs were ≤2 μg/ml, regardless of the method used. Clarithromycin was slightly more potent than erythromycin. No differences among the organisms were observed when they were categorized by geographic origin and by their penicillin or tetracycline resistance mechanisms (data not shown).

The MIC results of the reference agar dilution test and the Etest were very similar (Table 1), and the correlations (*r*) between the methods for each drug ranged from −0.88 (azithromycin) to −0.94 (erythromycin). Table 2 illustrates that all Etest MIC results were within 1 $\log_2$ dilution step of the reference MIC. A trend toward a slightly lower MIC result by the Etest was observed for all drugs examined.

Figure 1 presents the scattergrams comparing the reference agar dilution MIC results with the zones of inhibition around macrolide or azilide disks. Breakpoint criteria for susceptibility

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TABLE 1. Antigonococcal activities of penicillin and three macrolide-azilides[a]

Antimicrobial agent	Method	MIC (μg/ml)[b]		
		50%	90%	Range
Penicillin	Agar[c]	1	16	≤0.015–64
Azithromycin	Agar	0.25	0.5	0.06–2
	Etest	0.19	0.5	0.023–2
Clarithromycin	Agar	1	2	0.06–8
	Etest	0.5	1.5	0.032–8
Erythromycin	Agar	1	4	0.12–8
	Etest	0.75	2	0.047–6

[a] Activities of antimicrobial agents against 105 *N. gonorrhoeae* strains were tested by two dilution methods. Strains used included the following: penicillinase-producing *N. gonorrhoeae* (25 strains), β-lactamase negative penicillin resistant or intermediately resistant (72 strains), and organisms susceptible to penicillin for which MICs were ≤0.06 μg/ml (8 strains).

[b] Values under the boxheads 50% and 90% are MIC_{50}s and MIC_{90}s, respectively.

[c] Reference agar dilution (8).

have yet to be proposed for members of this antimicrobial class to predict the single-dose therapeutic success against uncomplicated gonorrheae (8, 9). However, an acceptable linear correlation between the results of both standardized test methods was achieved ($r = -0.81$ to -0.92). For all three drugs, the organism population distribution appeared unimodal over a range of 6 to 8 $\log_2$ dilution steps.

Single-drug, single-dose therapy with erythromycin has not been advised (7), and acceptable clinical cure rates (>95%) have only been reported for erythromycin (1 g) combined with 900 mg of rifampin (10). In the absence of a specific regimen for erythromycin and favorable clinical trial findings, no interpretive criteria can be offered (Fig. 1A). Similarly, clarithromycin has not been widely studied as a single-dose therapeutic agent directed against sexually transmitted pathogens, e.g., *N. gonorrhoeae* or *C. trachomatis* (Fig. 1B). Clarithromycin MICs for all gonococci tested, however, were at or below the susceptible breakpoint (2 μg/ml) used for most gram-positive pathogens (staphylococci) when treated by multiple-dose regimens (8). No criteria for gonococcal tests against clarithromycin are proposed, pending results from pivotal gonorrhea clinical trials.

For azithromycin (Fig. 1C), adequate in vitro and clinical results have been generated to recommend a breakpoint MIC (≤2 μg/ml) and a correlate zone diameter (≥25 mm). Only a category for susceptibility that includes all strains within that interpretation is tentatively proposed. Azithromycin MICs were 2 μg/ml for only two strains. Elevated MICs of erythromycin and clarithromycin (8 μg/ml) were shown for the same

TABLE 2. Comparison of macrolide-azilide MICs as determined for 105 *N. gonorrhoeae* strains by two dilution methods[a]

Antimicrobial agent	No. of strains for which Etest MIC/agar MIC ratio was follows:		
	0.5	1	2
Azithromycin	42	50	13
Clarithromycin	32	65	8
Erythromycin	52	53	0
Total	126	168	21

[a] For all macrolide-azilide agents tested, there were no Etest MIC/agar MIC ratios of ≤0.25 or ≥4 for any strains. All Etest MIC results were within 1 $\log_2$ dilution step of the reference MICs.

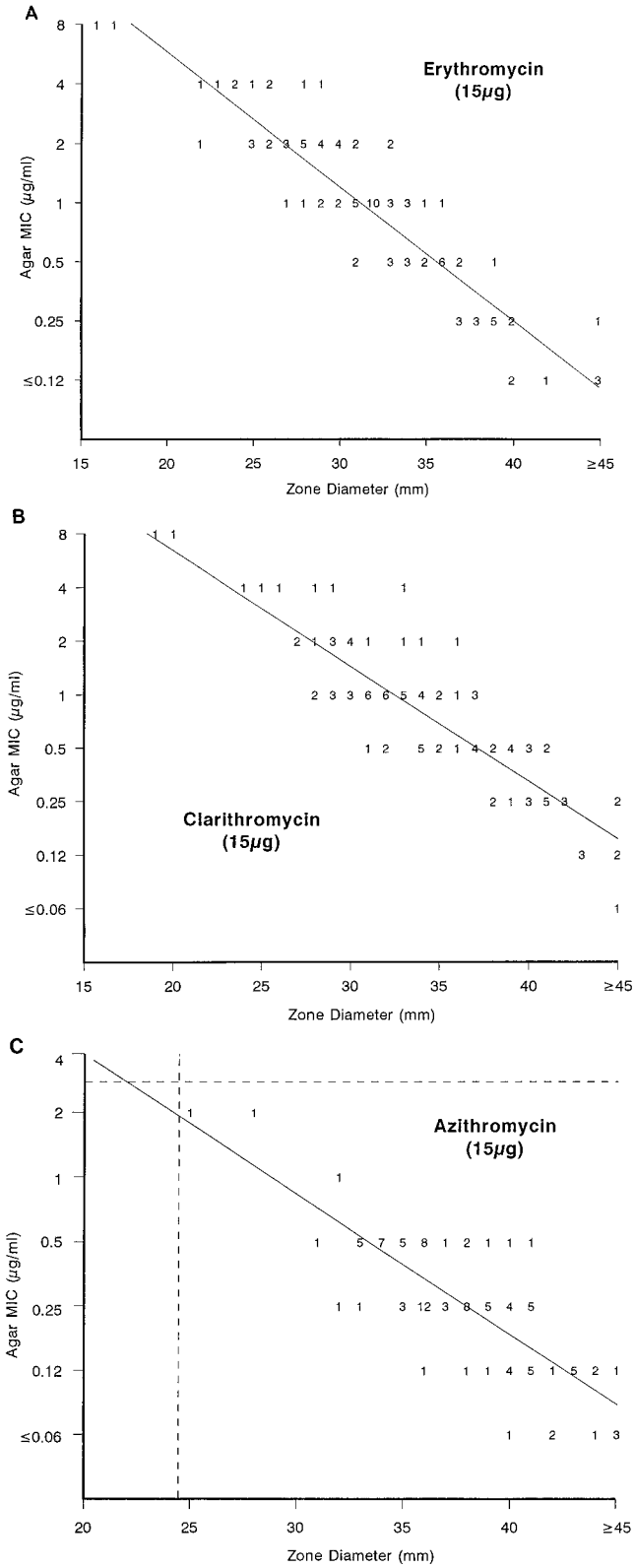

FIG. 1. Scattergrams comparing the erythromycin (A), clarithromycin (B), and azithromycin (C) reference agar dilution MICs with the zone of inhibition around 15-μg disks. The regression equations were $y = 16.3 - 0.23x$, $r = -0.92$, for erythromycin; $y = 15.9 - 0.21x$, $r = -0.88$, for clarithromycin; and $y = 15.1 - 0.21x$, $r = -0.81$, for azithromycin. The broken vertical and horizontal lines in panel C indicate the proposed interpretive criteria for susceptibility at a breakpoint MIC of ≤2 μg/ml and a zone of inhibition ≥25 mm in diameter.

two strains. The breakpoint MIC of azithromycin conforms to the guideline equation suggested by Moran and Levine (7), which asserts that a sustained concentration in serum should be maintained at fourfold greater than the MIC_{90} for approximately 10 h. The azithromycin MIC_{90} has been reported to be 0.25 to 0.5 μg/ml (this study; also references 1, 3, and 12). Other pharmacokinetic factors, such as drug concentrations in tissues and inflammatory cells, should also be considered when proposing a breakpoint for susceptibility. No interpretive test errors were found with these proposed interpretive criteria.

The azithromycin therapeutic success (1 or 2 g) for uncomplicated gonorrhea has consistently been between 95.2% (6, 14) and 99.2% (3). Cures of *C. trachomatis* infections have also been achieved by regimens in this cited dosing range (6, 11).

The Etest produced precise quantitative MICs over a wide range of dilutions for the studied macrolides and azithromycin. These data further validate this method as a simple method for testing pathogenic *Neisseria* species (5, 13). The disk diffusion test could also be utilized for the macrolides against gonococci but only when breakpoints validated by clinical trials become available. We believe that all three susceptibility testing procedures used for azithromycin when assessing gonococcal strains are accurate and clinically relevant.

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