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Disk Diffusion Susceptibility Testing of Two Macrolide Antimicrobial Agents: Revised Interpretive Criteria for Erythromycin and Preliminary Guidelines for Roxithromycin (RU 965)

RONALD N. JONES,[1]* ARTHUR L. BARRY,[1] PETER C. FUCHS,[1] AND CLYDE THORNSBERRY[2]

The Clinical Microbiology Institute, Tualatin, Oregon 97062,[1] and Centers for Disease Control, Atlanta, Georgia 30333[2]

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The 15-μg erythromycin disk was twice evaluated for interpretive accuracy against 417 and then 266 strains of gram-positive cocci, *Neisseria meningitidis*, and *Haemophilus influenzae* by using the criteria suggested by the National Committee for Clinical Laboratory Standards. These studies suggest a revision of streptococcal and *Staphylococcus* sp. interpretive guidelines to criteria (≥23 mm = susceptible, ≤13 mm = resistant) that are more compatible with in vivo erythromycin concentrations. It is also recommended that zone diameters be modified for *H. influenzae* (≥23 mm = susceptible, ≤22 = resistant) and that meningococci not be tested. A wide moderately susceptible category (1.0 to 4.0 μg/ml) would primarily include enterococcus strains and those organisms that would then respond only to parenteral administration of erythromycin. Roxithromycin (RU 965 or RU 28965), a new oxime ether erythromycin derivative, was also evaluated by investigator-prepared 15-μg disks and later with 30- and 60-μg commercial disks. Although roxithromycin was comparable to erythromycin in activity and regression line statistics, changes in the susceptible disk criteria were necessary because of superior roxithromycin serum concentrations and a longer serum half-life. Preliminary susceptible breakpoint criteria were ≥21 mm = susceptible, 10 to 20 mm = indeterminate, and ≤9 mm = resistant. By using the recommended interpretive criteria for both macrolides, >98% absolute agreement was obtained, therefore suggesting the application of a spectrum class concept.

Since erythromycin-like macrolide antimicrobials continue to be widely used, several similar drugs have been developed that have initially showed promise because of superior pharmacokinetics, increased antimicrobial potency, or both (2, 4, 11, 12, 14). Macrolides are principally applied to outpatient infections, to infections in penicillin-allergic patients, and to the treatment of *Mycoplasma* and *Chlamydia* infections and are the drugs of choice for legionellosis (3, 5, 13).

The recently described macrolide, roxithromycin (formerly RU 965 or RU 28965), has an antimicrobial activity and spectrum similar to those of erythromycin (1, 6). Its spectrum includes *Neisseria* spp., *Staphylococcus* spp., *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and other beta-hemolytic streptococci. It has only moderate activity against serogroup D streptococci and *Haemophilus influenzae* (1, 6). Another study demonstrated that roxithromycin was more active than erythromycin against eight species of *Legionella*, including 21 strains of *Legionella pneumophila* (7).

The National Committee for Clinical Laboratory Standards (NCCLS) Subcommittee for Disk Diffusion Tests has proposed a change in the interpretive criteria for reporting tests of penicillin and ampicillin against the streptococci (9). These proposals were developed to offer a greater predictive value for the in vitro test and may present the treating physician with more meaningful test information leading to the selection of the most appropriate dosing schedule, route of administration, or the need for combination chemotherapy. In the definitive NCCLS study (A. L. Barry and L. Thrupp, personal communications), data were also generated for modifying the interpretive guidelines of other drugs, such as erythromycin. In this report we evaluate the current erythromycin interpretive criteria and those suggested by the unpublished NCCLS study, using two separate collections of gram-positive and gram-negative organisms selected for variable susceptibility to the macrolides. In addition, we apply these new principles of macrolide susceptibility testing to tests with three different potencies of roxithromycin disks.

(These data were presented in part previously [R. N. Jones, Program Abstr. 14th Int. Conf. Chemother., abstr. no. WS11-12, 1985].)

MATERIALS AND METHODS

Antimicrobial agents. Roxithromycin was supplied by William J. Novick, Jr., of Hoechst-Roussel Pharmaceuticals, Inc., Somerville, N.J., and the comparison macrolide, erythromycin, was supplied by Abbott Laboratories, North Chicago, Ill. The drugs were diluted in Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) supplemented with calcium to 50 mg/liter and magnesium to 25 mg/liter as described in the NCCLS-approved standard, M7-A (10), and in previous investigations with this and other drugs (4, 6).

Bacterial strains. Recent isolates, typical of current clinical strains, were collected from the microbiology laboratories at the Cleveland Clinic Foundation, Cleveland, Ohio, St. Francis Hospital, Wichita, Kans., St. Vincent Hospital and Medical Center, Portland, Oreg., Northwestern Memorial Hospital, Chicago, Ill., and the Kaiser-Permanente Health Care Program Regional Laboratory, Clackamas, Oreg. These isolates were tested in two phases: 417 in phase I with in-laboratory-prepared roxithromycin disks and 266 in phase II with three concentrations of roxithromycin disks. The

* Corresponding author.

TABLE 1. Antimicrobial activity of roxithromycin and erythromycin against 433 bacterial isolates tested by NCCLS reference methods (9, 10)

Organism (no. tested)	Antimicrobial activity (μg/ml) of the following drug:			
	Roxithromycin		Erythromycin	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
S. aureus				
Penicillinase-positive (72)	0.5	1.0	0.25	0.5
Penicillinase-negative (26)	1.0	1.0	0.25	0.5
Methicillin-resistant (24)	>32	>32	>32	>32
Staphylococcus spp. (coagulase-negative)				
Penicillinase-positive (39)	>32	>32	>32	>32
Penicillinase-negative (12)	0.5	1.0	0.25	0.5
E. durans (3)	0.25		0.25	
E. faecalis (25)	8.0	>32	2.0	>32
E. faecium (16)	4.0	16	2.0	4.0
S. agalactiae (25)	0.25	0.25	0.12	0.12
S. bovis (10)	≤0.06	≤0.06	≤0.06	≤0.06
S. pneumoniae (25)	0.12	0.25	≤0.06	≤0.06
S. pyogenes (26)	0.25	>32	≤0.06	32
H. influenzae				
β-Lactamase-positive (50)	16	32	8.0	16
β-Lactamase-negative (45)	16	32	8.0	8.0
N. meningitidis (19)	0.5	1.0	1.0	2.0
L. monocytogenes (16)	0.5	2.0	0.25	1.0

phase I (417) isolates were distributed as follows: *Staphylococcus aureus*, penicillinase negative (26 strains); *S. aureus*, penicillinase positive (72 strains); *S. aureus*, methicillin resistant (24 strains); coagulase negative *Staphylococcus* spp. (51 strains); *Neisseria meningitidis* (19 strains); *Streptococcus agalactiae* (25 strains); *Streptococcus bovis* (10 strains); *S. pneumoniae* (25 strains); *S. pyogenes* (26 strains); *Enterococcus* (formerly *Streptococcus*) *durans* (3 strains); *Enterococcus faecalis* (25 strains); *Enterococcus faecium* (16 strains); *H. influenzae*, beta-lactamase negative (45 strains); and *H. influenzae*, beta-lactamase positive (50 strains). In phase II, the 266 strains were distributed as follows: *S. aureus* (70 strains, 20 methicillin resistant), coagulase-negative *Staphylococcus* spp. (30 strains, 10 methicillin resistant), *S. agalactiae* (20 strains), *S. bovis* (5 strains), *S. pneumoniae* (20 strains), *S. pyogenes* (20 strains), *E. durans* (3 strains), *E. faecalis* (20 strains), *E. faecium* (12 strains), *H. influenzae* (50 strains, 25 beta-lactamase positive), and *Listeria monocytogenes* (16 strains).

Antimicrobial susceptibility tests. Roxithromycin and erythromycin were compared against representative clinical isolates collected from at least six geographically separate institutions as described previously (4, 6). Broth microdilution test panels were prepared by Prepared Medical Laboratory, Tualatin, Oreg. The broth was supplemented with 2 to 3% Fildes peptic digest of horse blood for testing beta-hemolytic *Streptococcus* spp., *S. pneumoniae*, and *N. meningitidis*. For testing *H. influenzae*, the broth contained 3% lysed horse blood and 25 μg of NAD per ml. The final inoculum density was ca. 5×10^5 CFU/ml, and the MICs were recorded after 16 to 20 h of incubation at 35°C. These studies were performed in two laboratories with sufficient common quality control and stock strain controls to document comparability of reference testing procedures (10).

Disk agar diffusion tests were also performed by the current NCCLS standard procedure (9). Interpretive zone standards were selected by correlating zone diameters with MICs, using both the regression analysis method of least squares and a modified error-rate bounding method described by Metzler and DeHaan (8). The 15-μg roxithromycin disks were prepared by The Clinical Microbiology Institute, and the 30- and 60-μg disks used in phase II were prepared commercially by Difco. The 15-μg erythromycin disks for both phases were manufactured by BBL Microbiology Systems, Cockeysville, Md.

The results of the quality control organism determinations for erythromycin zone diameters and MICs were within the ranges recommended by the NCCLS (9, 10). The *S. aureus* ATCC 25923 median roxithromycin zones for the 15-, 30-, and 60-μg disks were 25 mm (range, 24 to 27 mm), 27 mm (range, 24 to 29 mm), and 29 mm (range, 27 to 31 mm), respectively. The roxithromycin modal MICs were 8.0 μg/ml (range, 8.0 to 16 μg/ml) for *E. faecalis* ATCC 29212 and 1.0 μg/ml (range, 0.5 to 2.0 μg/ml) for *S. aureus* ATCC 29213. All roxithromycin quality control data were based on 9 to 11 determinations.

RESULTS

Antimicrobial activity of roxithromycin and erythromycin. Table 1 shows the comparative activity results for 417 bacterial isolates tested against roxithromycin and erythromycin in phase I and of *L. monocytogenes* tested later in phase II. Roxithromycin had MICs for 50% of the strains (MIC_{50}) for the staphylococci and nonenterococcal streptococci (range, ≤0.06 to 0.5 μg/ml) that were approximately twice those of erythromycin (range, ≤0.06 to 0.25 μg/ml). Erythromycin was two- to fourfold more active against the 44 enterococci (*E. faecalis*, *E. faecium*, and *E. durans*). All strains having a resistant erythromycin MIC (≥32 μg/ml) also had similarly high roxithromycin MICs.

The *H. influenzae* isolates were only moderately susceptible to these macrolides. Only three strains had both roxithromycin MICs of ≤1.0 μg/ml and erythromycin MICs of ≤0.25 μg/ml. All other *Haemophilus* strains were very similar, with >80% of all MICs at 8.0 or 16 μg/ml for erythromycin and 16 or 32 μg/ml for roxithromycin. No differences were detected in the MIC results for β-lactamase-producing strains compared with enzyme-negative isolates. The 19 *N. meningitidis* strains were slightly more susceptible to roxithromycin (MIC_{50}, 0.5 μg/ml) than to erythromycin (MIC_{50}, 1.0 μg/ml).

The results for phase II comparative MICs were nearly identical to phase I results for all cited species (data not shown). *L. monocytogenes* was an additional tested organism in phase II for which a twofold greater activity was shown by erythromycin, but all macrolide MICs were ≤2.0 μg/ml.

Disk diffusion tests for erythromycin. The interpretive criteria found in the current NCCLS M2-A3 standard (9) indicate that an erythromycin MIC of ≤2.0 μg/ml is susceptible and correlates to a zone of inhibition of ≥18 mm. Conversely, the resistant criteria are ≥8.0 μg/ml and ≤13 mm. A total of 417 bacteria were tested with the 15-μg erythromycin disk in phase I. When testing all the strains listed in Table 1 and using the NCCLS interpretive criteria, an unacceptably high number of very major (16.3%) and minor (10.1%) errors were produced (Table 2). The false-susceptible (very major) errors were produced when testing the *H. influenzae* isolates, and the minor errors were contributed by the *H. influenzae* and meningococcal strains.

TABLE 2. Regression line statistics for the 15-μg erythromycin and various roxithromycin disk tests using the NCCLS disk diffusion method compared to the NCCLS reference broth microdilution method

Macrolide and organisms (no. tested)[a]	Regression interval (*n*)	*y* axis intercept ($\log_2$ [μg/ml] + 9)	Slope	Correlation coefficient (*r*)	% Interpretive errors using our criteria (% interpretive errors using NCCLS standards)[b]		
					Very major	Major	Minor
Erythromycin, phase I							
All organisms (417)	0.12–32 (295)	16.6	−0.31	0.81	0.0 (16.3)	0.0 (0.0)	26.6 (10.1)
Gram-positive (303)	0.12–32 (181)	12.6	−0.19	0.76	0.0 (0.0)	0.0 (0.0)	3.0 (5.9)
Haemophilus (95)	0.12–32 (95)	19.0	−0.38	0.71	0.0 (71.6)	0.0 (0.0)	0.0 (24.2)
Neisseria (19)	0.12–32 (19)	10.3	−0.05	0.15	NT (0.0)	NT (0.0)	NT (5.3)
Roxithromycin, phase I							
All organisms (415)	0.12–32 (335)	16.5	−0.31	0.81	0.0 (7.7)	0.0 (0.2)	23.4 (18.3)
Gram-positive (301)	0.12–32 (222)	13.2	−0.20	0.71	0.0 (0.0)	0.0 (0.0)	3.3 (5.6)
Haemophilus (95)	0.12–32 (95)	17.8	−0.31	0.71	0.0 (33.7)	0.0 (0.0)	90.5 (62.1)
Neisseria (19)	0.12–32 (19)	12.6	−0.15	0.35	0.0 (0.0)	0.0 (0.0)	5.3 (5.3)
Roxithromycin, phase II							
All organisms, 15 μg (266)	0.12–32 (212)	16.8	−0.33	0.87	0.0 (12.0)	0.0 (0.0)	29.7 (20.7)
All organisms, 30 μg (266)	0.12–32 (212)	17.6	−0.34	0.86			
All organisms, 60 μg (266)	0.12–32 (211)	17.8	−0.32	0.85			

[a] Phase I studies used 15-μg roxithromycin and erythromycin disks.

[b] Current NCCLS interpretive criteria (9, 10): ≥18 mm (≤2.0 μg/ml) = susceptible and ≤13 mm (≥8.0 μg/ml) = resistant. For roxithromycin, comparable breakpoints, conservatively adjusting for superior pharmacokinetics, would be ≥16 mm (≥4.0 μg/ml) = susceptible and ≤9 mm (≥16 μg/ml) = resistant. Very major, False-susceptible by disk test; major, false-resistant by the disk zone method; minor, moderately susceptible (intermediate or indeterminate) by one of the two susceptibility methods. NT, Not tested by this method.

With the 303 gram-positive cocci that were tested in phase I, 5.9% minor errors were found, all among the enterococci (Table 2, Fig. 1). The NCCLS is considering new interpretive criteria for the streptococci that would alter the susceptible zone breakpoint (≥18 mm) that divides the *Enterococcus* sp. population of zone diameters (Fig. 1). By applying these proposed criteria (Fig. 1, broken vertical line) the enterococci and nonenterococcal *Streptococcus* spp. were effectively separated into susceptible (MICs, ≤0.5 μg/ml) and moderately susceptible (MICs, 1.0 to 4.0 μg/ml) groups. Among this population of bacteria, the 173 *Staphylococcus* sp. strains were generally categorized as susceptible, with only 3 coagulase-negative *Staphylococcus* spp. (1.5% of the total) having moderately susceptible MICs. All other strains had a high grade of resistance (≥32 μg/ml) to erythromycin.

A few additional minor modifications of these proposed NCCLS erythromycin streptococcal criteria seem appropriate. (i) When testing *Staphylococcus* spp., the criteria for nonenterococcal *Streptococcus* spp. may be used. (ii) Use ≥23 mm and the MIC of ≤0.5 μg/ml as the only susceptible criteria for testing *H. influenzae*. This latter change would correctly identify the three susceptible strains in this study (Table 3). (iii) Meningococci should not be tested by this method because of high rates of minor error, e.g., susceptible zone diameters and only moderately susceptible MICs.

In the phase II erythromycin disk studies, only 266 organisms from species potentially considered within the spectrum of macrolides (see Materials and Methods) were analyzed. The regression equation was very similar to that in the previous study, $y = 16.8 - 0.33x$, $r = 0.86$. Again, *H. influenzae* and enterococci contributed most of the interpretive errors when the current NCCLS disk criteria were applied (9). These were 14 false-susceptible (5.3%) and 42 minor (15.8%) errors. By using the revised zone modifications described above, these discrepancies were reduced to only 1.1% minor errors.

Disk diffusion tests for roxithromycin. Because of the very similar potency and spectrum of the new oxime ether to those of erythromycin, comparable interpretive problems were encountered for its disk test (Table 2). Roxithromycin 15-μg disk regression line data showed nearly identical statistics to those of erythromycin. If the susceptible MIC breakpoint had been 1 $\log_2$ dilution higher (≤4.0 μg/ml) than that of erythromycin, the corresponding zones and error rates would have been nearly identical to those of erythromycin. These preliminary MIC and zone breakpoints can be easily justified by the superior roxithromycin pharmacokinetics (W. J. Novick, Jr., personal communication).

The roxithromycin disk testing of *H. influenzae* and meningococci also presented interpretive problems in phase I and were excluded from the regression line data shown in Fig. 2. When using roxithromycin MIC breakpoints 1 $\log_2$ dilution higher than those of erythromycin, the corresponding zones of inhibition were 2 to 3 mm smaller. By not using the proposed categorization changes, the roxithromycin-susceptible and -resistant interpretive criteria were ≤4.0 μg/ml (≥16 mm) and ≥16 μg/ml (≤9 mm), respectively. Table 2 indicates no major errors and 5.6% minor interpretive discrepancies when those criteria were applied. The wide moderately susceptible (indeterminate) category advocated for erythromycin could also be adapted for tests with roxithromycin. The resistant breakpoint remained unchanged, and the susceptible breakpoint was lowered to ≤1.0 μg/ml (≥21 mm). This minimized the minor error rate to only 3.3%. Streptococcal and *Staphylococcus* sp. MICs were also correctly categorized in a manner identical to that of erythromycin susceptibility tests.

H. influenzae strains were moderately susceptible by roxithromycin zone diameter criteria and generally resistant by MIC determinations (≥16 μg/ml). Because roxithromycin was more active than erythromycin against *N. meningitidis*, the disk test more correctly categorized these 19 strains as susceptible when either set of proposed interpretive criteria was used.

If enterococci and *H. influenzae* strains that are only moderately susceptible to erythromycin become susceptible

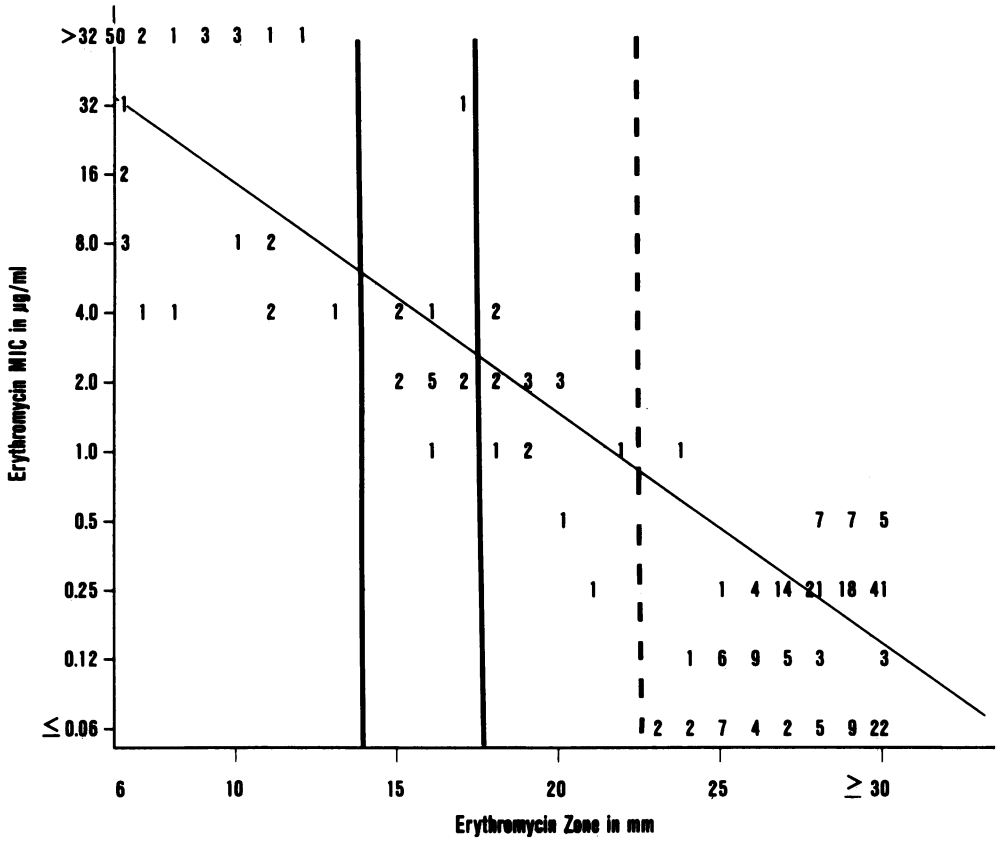

FIG. 1. Scattergram comparing erythromycin MICs and 15-µg disk zone diameters for 303 gram-positive cocci. Vertical solid lines, Current NCCLS breakpoints; vertical dashed line, suggested modification of the susceptible breakpoint. Number of cocci with specified MIC and zone diameter is plotted.

to roxithromycin by virtue of improved pharmacokinetics or favorable clinical trial data, then larger concentrations may be required in the test disks. In the phase II study, 15-, 30-, and 60-µg roxithromycin disks were tested. The statistics for these commercially prepared disks are found in Table 2. All very major errors continued to be among the *H. influenzae* strains, and minor errors were generally *H. influenzae* or enterococci. If these species were omitted from the regression statistics, the regression equation became $y = 16.9 - 0.33x$, $r = 0.92$. With the modified breakpoints of ≥21 mm (≤1.0 µg/ml) = susceptible and ≤9 mm (≥16 µg/ml) = resistant, the error rate was low, 8.5% minor discrepancies.

Comparative activity of roxithromycin and erythromycin against gram-positive cocci. Figure 3 shows the correlation of 311 (phase I) roxithromycin and erythromycin MICs. The regression line equation was $y = -1.47 + 1.06x$, and the correlation coefficient (r) was 0.98. The similar analyses for phase II isolates were $y = -1.73 + 1.07x$, $r = 0.99$. When using the current NCCLS erythromycin MIC interpretive breakpoints and similar breakpoints (1 dilution higher, Table 2) for roxithromycin, no major errors and only 5.1% minor errors were observed. These minor errors were among the enterococci that were intermediate or resistant to roxithromycin and intermediate or susceptible to erythromycin. Using corresponding zone diameters for each drug (Table 4), the minor error rate was 2.2% (data not shown), again without major interpretive discrepancies.

When the modified interpretive criteria were applied, the minor error rate was reduced to 1.9% for MIC comparisons and 1.5% for disk comparisons. The regression line equation for the zone diameter comparisons of roxithromycin and erythromycin 15-µg disks was $y = -1.30 + 1.03x$, with a correlation coefficient of 0.98. Therefore, the class-disk concept may be usable for these two macrolides.

DISCUSSION

Roxithromycin, a new oxime ether derivative of erythromycin, possesses an antimicrobial activity against most species comparable to that of erythromycin (1, 6). Generally, roxithromycin was twofold less active than erythromycin against gram-positive cocci and *H. influenzae* and had a twofold greater potency against *N. meningitidis* strains. These and our earlier data for *H. influenzae* (6) contrast dramatically with those reported by Barlam and Neu (1), who observed MICs for 90% of the strains (MIC_{90}) of ≤1.6 µg/ml for both macrolides. Results of previous studies also showed a significant antimicrobial activity against *Neisseria gonorrhoeae*, *Neisseria lactamica*, *Branhamella catarrhalis*, *Clostridium* spp., *Campylobacter* spp., and eight species of *Legionella* (1, 6, 7). Although the antimicrobial activity seemed modest, the strongest feature of roxithromycin was its pharmacokinetics after oral administration. Human pharmacokinetic trials with only 100- and 400-mg oral doses produced serum peaks of 6.0 and 15.0 µg/ml and

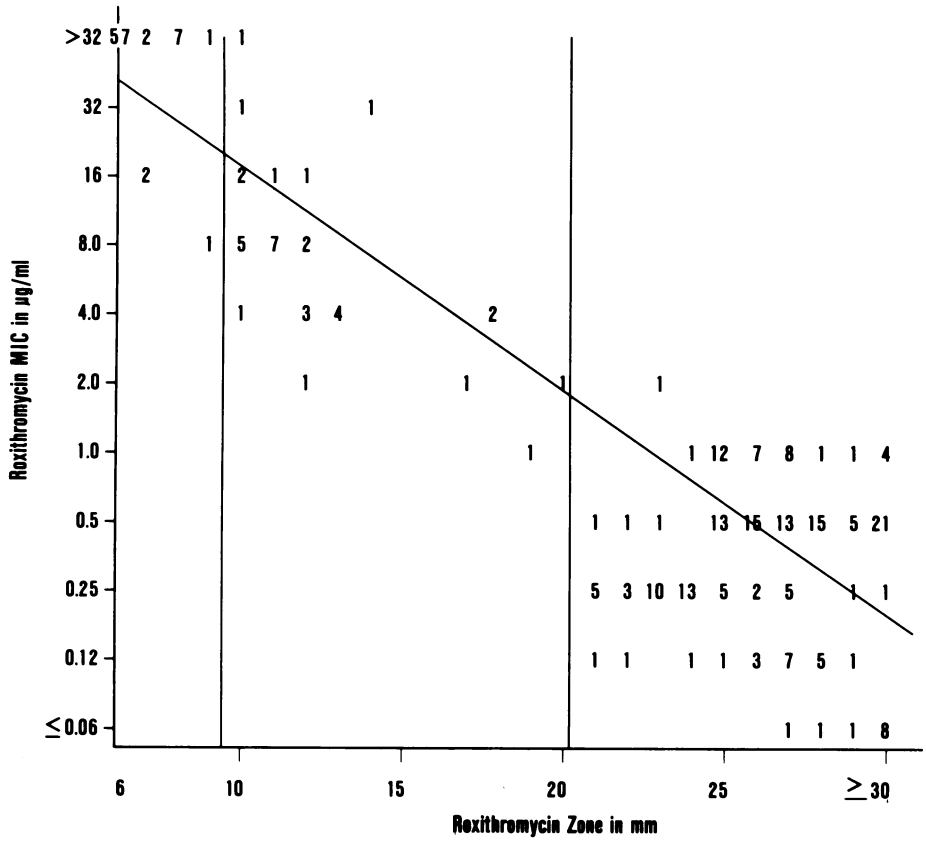

FIG. 2. Scattergram comparing roxithromycin MICs and 15-µg disk zones (phase I study) for 301 organisms, excluding *H. influenzae* and meningococci. Number of organisms with specified MIC and zone diameter is plotted.

trough concentrations of 4.0 and 4.5 µg/ml at 12 and 24 h, respectively. Infrequent gastrointestinal side effects have been reported after roxithromycin ingestion, and there has been no evidence of hepatic toxicity (W. J. Novick, Jr., personal communication).

Since the oral administration of erythromycin, even as esters, produces a low serum concentration, we believe that the current in vitro test standards for the 15-µg erythromycin disk require modification (9). Most studies with oral erythromycin preparations (5, 12) show peak concentrations of only 0.5 to 1.5 µg/ml, a level well below the current NCCLS susceptible breakpoint of ≤2.0 µg/ml (9, 10) and more compatible with the interpretive criteria determined by the NCCLS Streptococcal Collaborative Study (L. Thrupp and A. L. Barry, personal communications). This study established the regression line statistics for all clinically important antimicrobial agents using nearly all significant *Streptococcus* spp. These data for penicillin G and ampicillin were found in the most recent NCCLS standards (9, 10); the erythromycin statistics for both disk and dilution tests suggest a need for a lower susceptible MIC breakpoint, more comparable with serum levels after the usual oral doses.

TABLE 3. Distribution of erythromycin MICs and zone diameters for 95 *H. influenzae* strains tested by reference NCCLS methods (9, 10)

Erythromycin MIC (µg/ml)	Zone diam (mm)			
	6–13	14–17	18–22	>23
≥8.0	1	19	68	
4.0			4	
≤1.0				3

These revised erythromycin susceptibility criteria for the *Streptococcus* spp. appear to be similarly applicable to the interpretation of *Staphylococcus* sp. tests and, with minor modification, could be used for testing *H. influenzae* strains (Table 4). The MICs for all erythromycin-susceptible *Staphylococcus* spp. in this study were ≤0.5 µg/ml, and the zones of inhibition were ≥23 mm. The MICs and zones of inhibition for roxithromycin-susceptible *Staphylococcus* spp. were ≤1.0 µg/ml and ≥21 mm, respectively.

For these reasons, we present supporting data for revised interpretations of the 15-µg erythromycin disk test (9) and a preliminary set of guidelines for the 15-µg roxithromycin disk (Table 4). The roxithromycin human pharmacokinetics are sufficiently superior to those of erythromycin to consider the following in vitro test modifications; (i) susceptible breakpoints should be raised to concentrations at least equal to the mean drug concentrations achieved with clinical trial dose regimens (150 mg twice daily and 300 mg once a day); (ii) strains and species only susceptible to parenteral erythromycin (MICs, 1.0 to 4.0 µg/ml) may be susceptible to oral administration of roxithromycin (MICs, ≤8.0 µg/ml), negating the need for the moderately susceptible roxithromycin category; and (iii) preliminary 15-µg roxithromycin disk breakpoints may require modification to a higher disk content, e.g., 30 to 60 µg. The last two modifications must await

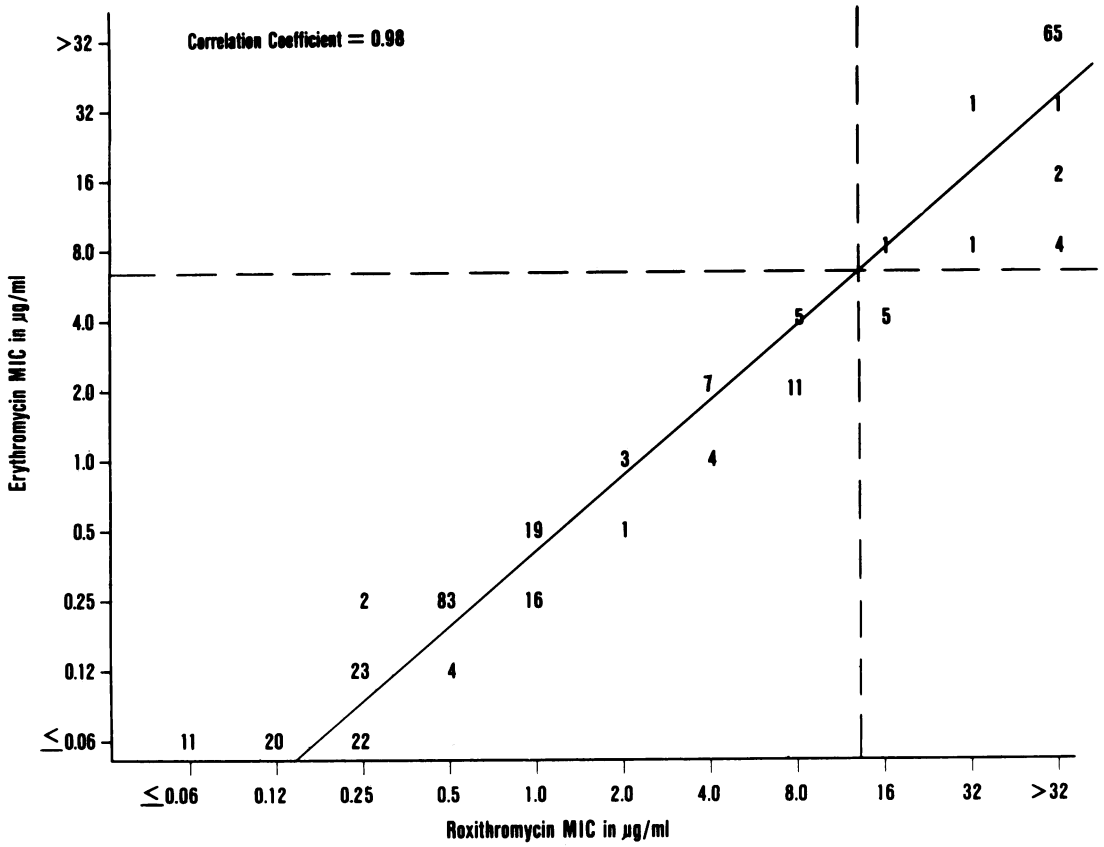

FIG. 3. Direct MIC comparisons of roxithromycin and erythromycin. Solid line, regression; broken lines, proposed (roxithromycin) or established (erythromycin) resistant MIC breakpoints. Number of organisms with specified roxithromycin and erythromycin MICs is plotted.

clinical trial results against these critical species or the development of a parenteral roxithromycin formulation. The organisms most affected by these interpretive criterion changes would be *H. influenzae* and enterococci. For the roxithromycin clinical trials, we recommend that strains for which MICs are 2.0 to 8.0 µg/ml and for which zones of inhibition are 10 to 20 mm, or both, be considered indeterminate but included for possible treatment. It is highly probable that parenteral erythromycin or combination therapy would be needed to achieve inhibitory tissue levels against these same species, the principle reason for the suggested modification of the current NCCLS interpretive criteria for erythromycin (9, 10).

TABLE 4. Proposed interpretive zone size criteria for erythromycin and roxithromycin

Macrolide (disk content) and organism tested	Zone size criteria (mm)			
	Susceptible	Moderately susceptible	Indeterminate	Resistant
Erythromycin (15 µg)				
Gram-positive cocci	≥23	14–22		≤13
Haemophilus sp.	≥23			≤22
Roxithromycin (15 µg), gram-positive cocci	≥21		10–20	≤9

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