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Tentative Interpretive Standards for Disk Susceptibility Tests with Moxalactam (LY127935)

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Moxalactam (LY127935; 6059-S) is a new beta-lactam antibiotic. We propose tentative zone standards for agar diffusion susceptibility tests with 30-μg disks. The final selection of minimal inhibitory concentration breakpoints for definition of resistant and susceptible categories must await clinical experience with this drug. Some of the clinical questions to be answered are defined. A moderately susceptible (intermediate) category is proposed for those strains with minimal inhibitory concentrations of 16 or 32 μg/ml (zones 15 to 22 mm in diameter). Strains with minimal inhibitory concentrations of ≥64 μg/ml are considered resistant, and those with minimal inhibitory concentrations of ≤8 μg/ml are considered susceptible. Tests with 30-μg disks did not satisfactorily separate strains with minimal inhibitory concentrations of 8 μg/ml from strains requiring <2 μg/ml for inhibition, because the regression line became parabolic at concentrations of 2 μg/ml and below. However, the disk tests were satisfactory for categorizing isolates into the above-described susceptible, moderately susceptible (intermediate), and resistant categories.

Moxalactam (LY127935; 6059-S) is a new beta-lactam antibacterial agent (a 1-oxa beta-lactam) with a broad spectrum of activity (3, 4, 4a, 8-10). The present report summarizes our efforts to establish tentative interpretive zone standards for the Bauer-Kirby disk technique for use during future clinical trials of this drug. Such standards may be subject to change once additional clinical experience is gained with this antibiotic. Some of the specific questions which need to be answered before establishing criteria for interpreting in vitro tests are outlined below.

MATERIALS AND METHODS

Tests with 463 selected bacterial isolates were performed at the Center for Disease Control, Atlanta, Ga., and at the University of California (Davis) Medical Center, Sacramento, Calif., as previously described (2, 5). Disk diffusion tests were performed with 30-μg cephalothin disks (BBL Microbiology Systems, Cockeysville, Md.) and 30-μg moxalactam disks (Eli Lilly & Co., Indianapolis, Ind.). The most recent procedure outlined by the National Committee for Clinical Laboratory Standards (7) was followed in both laboratories. A microdilution procedure was used to determine the minimal inhibitory concentrations (MICs) of moxalactam, cephalothin, cefamandole, and cefoxitin. Doubling dilutions of each antimicrobial agent ranged from 0.12 to 64 μg/ml. All dilutions were prepared in cation-supplemented Mueller-Hinton broth and the final inoculum contained about 10^5 colony-forming units per ml. MICs were recorded after 16 to 18 h at 35°C.

RESULTS

Definition of MIC categories. Before interpretive zone standards for the disk test are established, MIC breakpoints must be selected for defining resistant, intermediate, and susceptible categories. To accomplish this, the following three types of data should be considered: (i) the approximate blood levels that can be achieved during therapy with the dosage schedule normally used, (ii) the distribution of MICs among different species of pathogens likely to be treated with the antimicrobial agent, and (iii) clinical experience in treating different types of infections and types of microorganisms. At this time, there is little clinical experience in treating human infections with moxalactam, and a wide variety of dosage schedules are available; the more commonly utilized dosages and routes of administration have not been well established. However, there is now enough information available to support tentative breakpoints to be used during future clinical trials of this drug.

Blood levels. The results of some preliminary studies that were performed in human volunteers are summarized in Fig. 1 (data kindly provided by R. Kammer, Eli Lilly & Co.). By intravenous infusion of the drug over a 2-h period, very high peak serum levels can be

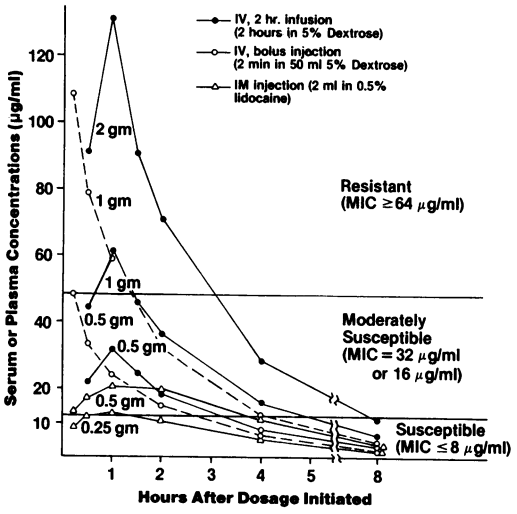

FIG. 1. *Human serum levels with varying dosages of moxalactam. Each value represents the mean blood level in four human volunteers (data generated by Shionogi Pharmaceutical Co. and provided by Eli Lilly & Co.). Approximate MIC breakpoints are superimposed as horizontal lines halfway between twofold dilution intervals (i.e., between 32 and 64 μg/ml [48 μg/ml] and between 8 and 16 μg/ml [12 μg/ml]). The midpoints were selected to compensate for the artifact imposed by testing serial twofold dilution intervals when determining MIC values. A fourth category (very susceptible) could be defined as an MIC of ≤2.0 μg/ml and included strains that should respond to low-dose intramuscular (IM) therapy. IV, Intravenous.*

achieved. With intravenous bolus injections, moderately high blood levels can be maintained for several hours. With intramuscular injections, somewhat lower blood levels can be obtained.

An organism is clearly resistant to a drug if the concentration required for inhibition (the MIC) exceeds the concentration that can be expected in the blood during therapy. With drugs such as moxalactam, dosages and routes of administration can be varied to achieve extremely high peak serum levels. There is no uniform agreement concerning the questions of what is the minimal ratio between blood levels and MICs or how long the blood level should exceed the MIC in order to reasonably expect therapeutic success. We arbitrarily elected to categorize microorganisms as resistant if the MIC was ≥64 μg/ml. With frequent intravenous injections of fairly large doses, it may be possible to successfully treat infections caused by resistant microorganisms, but this possibility remains to be demonstrated.

The susceptible category was arbitrarily defined to include microorganisms with MICs of ≤8 μg/ml. Serum levels in excess of 8 μg/ml can be maintained for 4 to 6 h with modest dosages. Another category of very susceptible microorganisms could be defined to include strains with MICs of ≤2.0 μg/ml; such microorganisms should respond to therapy with much less drug (i.e., with intramuscular injections).

A moderately susceptible (intermediate) category included microorganisms with MICs of 32 or 16 μg/ml. Such organisms are not properly categorized as resistant; i.e., they should respond to intravenous infusions of fairly large dosages (2 g or more every 6 or 8 h). On the other hand, moderately susceptible microorganisms are not properly classified in the susceptible category (MIC, ≤8 μg/ml). The therapeutic dosages differ for treating infections caused by moderately susceptible strains compared with infections caused by susceptible strains.

Because of the wide choice of therapeutic modalities with this relatively nontoxic drug, it seemed appropriate to utilize a broad intermediate or moderately susceptible category, based on available pharmacological information alone.

Distribution of MICs. The considerations outlined above were next viewed in the light of actual MIC determinations with recent clinical isolates. Figure 2 summarizes the results of a recent report by Jones et al. (4a), who studied 8,619 clinical isolates consecutively isolated in six different geographically separate medical centers. At least 95% of the *Enterobacteriaceae* and 86% of the *Bacteroides fragilis* isolates were inhibited by 2 μg/ml and were considered very susceptible. The resistant category (MIC, ≥64 μg/ml) included nearly all of the group D streptococci and many strains of *Pseudomonas aeruginosa* and *Acinetobacter* sp. Most *Staphylococcus aureus* strains, some strains of *P.*

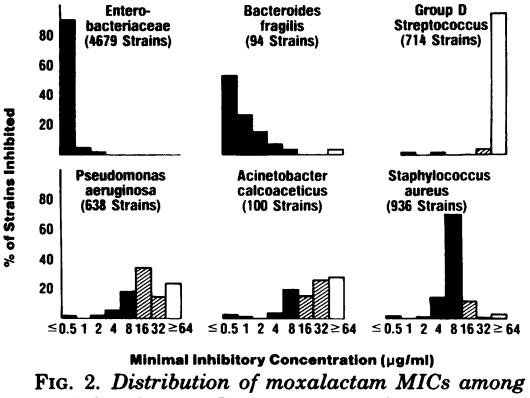

FIG. 2. *Distribution of moxalactam MICs among bacterial pathogens. Data were taken from a study of 8,619 clinical isolates (4a). Cross-hatched bars, moderately susceptible; solid bars, susceptible; open bars, resistant (see Fig. 1).*

aeruginosa, and some *Acinetobacter* spp. strains were considered to be susceptible but not very susceptible (i.e., MIC > 2 but ≤ 8 μg/ml). The moderately susceptible or intermediate category included only *P. aeruginosa*, *Acinetobacter*, and a few *S. aureus* isolates. Based on such distribution data alone, the susceptible category could be redefined to include strains with MICs of ≤2 μg/ml, leaving the intermediate category to contain strains with MICs of 4 to 32 μg/ml. Such a redefinition would move the majority of *P. aeruginosa* and *Acinetobacter* spp. isolates into the moderately susceptible category, but most *Enterobacteriaceae* would remain susceptible. However, such a shift in MIC breakpoints would change the interpretation of most staphylococci from susceptible to moderately susceptible. Whether that categorization is appropriate depends on the clinical experience in treating staphylococcal infections. Although a broad intermediate category of 4 to 32 μg/ml would clearly separate the bacterial populations, it may not be consistent with the pharmacological considerations outlined above. Consequently, we tentatively accepted an MIC breakpoint of ≤8 μg/ml for the susceptible category.

Clinical experience with moxalactam. The final decision of where the moxalactam MIC breakpoints are most appropriately located will depend largely upon clinical experience in treating various types of infections with this drug. Specifically, the following questions need to be answered. How do staphylococcal infections respond to therapy with different dosages of moxalactam? That is, are staphylococci susceptible or moderately susceptible to this drug? Do *P. aeruginosa* and *Acinetobacter* spp. infections respond to high-dose intravenous therapy? Do strains with MICs of ≥64 μg/ml respond at all, and are infections caused by strains with MICs of ≤8 μg/ml more likely to be cured clinically? Is moxalactam effective in treating anaerobic infections, especially when the *B. fragilis* group is involved? Are lower dosages appropriate for treating infections due to very susceptible (MIC, <2 μg/ml) enteric bacilli? Because of high urine levels, will *P. aeruginosa* urinary tract infections respond to low-dose therapy with moxalactam? Once that type of clinical experience has been gathered, the MIC breakpoints for categorizing microorganisms can be finalized. In the absence of such information, we tentatively decided to define the susceptible category as microorganisms with MICs of ≤8 μg/ml; our moderately susceptible (intermediate) category included those strains inhibited by 16 or 32 μg/ml, and all strains with MICs of ≥64 μg/ml were considered to be resistant. With these tentative definitions, we next examined the performance of 30-μg moxalactam disks.

Class concept. Before we proceeded with the development of a disk diffusion susceptibility testing procedure, it seemed appropriate to determine whether tests with an established cephalosporin could predict susceptibility or resistance to moxalactam. Table 1 summarizes data comparing the activity of moxalactam to the activities of cephalothin, cefamandole, and cefoxitin against the 463 strains included in this study. Microdilution tests with all four antimicrobial agents were categorized as being moderately susceptible or resistant (MIC, ≥16 μg/ml) and susceptible (MIC, ≤8 μg/ml). Tests with moxalactam were also categorized into groups with MICs of ≥64 and ≤32 μg/ml (resistant and susceptible or moderately susceptible). Table 1 lists the number of strains which were susceptible to one drug but not to another drug. Essentially all of the strains which were resistant to moxalactam were also resistant to the cephalosporins. However, a significant number of isolates were not susceptible to cephalothin, cefamandole, or cefoxitin but were susceptible (MIC, ≤8 μg/ml) to moxalactam. An even greater number of strains were moderately susceptible (MIC, 16 or 32 μg/ml) to moxalactam but were resistant to the other drugs. Consequently, we concluded that moxalactam represents a class of beta-lactam antimicrobial agents with a uniquely broad spectrum of activity. Tests with the established cephalosporins or cephamycins could not be expected to predict resistance to moxalactam.

Performance of 30-μg cephalothin disks. As a control measure, all 463 isolates in this study were tested with 30-μg cephalothin disks, as well as disks containing 30 μg of moxalactam. To express mathematically the relationship between MICs and zone diameters, a regression line was calculated by using the least-squares method. MIC correlates of ≤8 and ≥32 μg/ml were calculated for the current zone standards of ≥18 mm (susceptible) and ≤14 mm (resistant), respectively (Fig. 3). As observed previously (1), the disk test failed to categorize accurately the few strains with intermediate MICs or intermediate zone sizes. However, the majority of strains were clearly resistant or susceptible by both methods.

Because fairly high blood levels can be achieved with cephalothin, one could argue that the intermediate category should be expanded to include strains inhibited by 16 or 32 μg/ml, as we proposed for moxalactam. With that change in MIC breakpoints, 8 of 10 *Streptococcus faecalis* and only 5 enteric bacillus strains would be reclassified from the resistant category to the

intermediate category. Because of the distribution of MICs among the test strains included in this study, a change in MIC breakpoints would have little impact on the performance of the disk test.

Performance of 30-μg moxalactam disks. Figure 4 shows the correlation between MICs and zone diameters with moxalactam. The error rate-bounded method (6) applied to these data suggested zone size breakpoints of ≥23 mm for the susceptible category (MIC, ≤8 μg/ml) and ≤14 mm for the resistant category (MIC, ≥64 μg/ml). To further confirm these zone size breakpoints, a regression line was calculated by using all data with "on-scale" endpoints (Fig. 4, regression line A). The least-squares method appears to be inappropriate for analysis of these data because it assumes a straight line relationship and the curve becomes parabolic with the more susceptible strains. Based on the regression line alone, the zone size breakpoint of the susceptible category should be ≥20 mm, a breakpoint which is clearly inappropriate. To obtain a more realistic estimate of the correlation between zone sizes and MICs with microorganisms at or near the MIC breakpoints, the analyses were repeated, progressively eliminating zone data with lower MICs (Table 2). This was done in an effort to avoid the parabolic portion of the regression curve and to express the straight line portion which passes through the MIC breakpoints. The same type of analysis was applied to the data with cephalothin; elimination of the more susceptible MIC categories did not significantly influence the slopes or intercepts of the calculated regression formulas. With moxalac-

TABLE 1. *Cross-resistance analysis of MIC data with 463 microorganisms used to develop disk diffusion susceptibility testing interpretive critieria*

Antimicrobial agent	MIC (μg/ml)	% of strains resistant to an 8-μg/ml concn of[a]:			
		Moxalactam	Cefamandole	Cefoxitin	Cephalothin
Moxalactam	≤32		34	36	50
Moxalactam	≤8		18	20	34
Cefamandole	≤8	0.2		0	16
Cefoxitin	≤8	0.2	5		13
Cephalothin	≤8	0.2	0	0	

[a] Includes strains which were not susceptible (MIC, ≥16 μg/ml) to one drug but were susceptible (MIC, ≤8 or ≤32 μg/ml) to another.

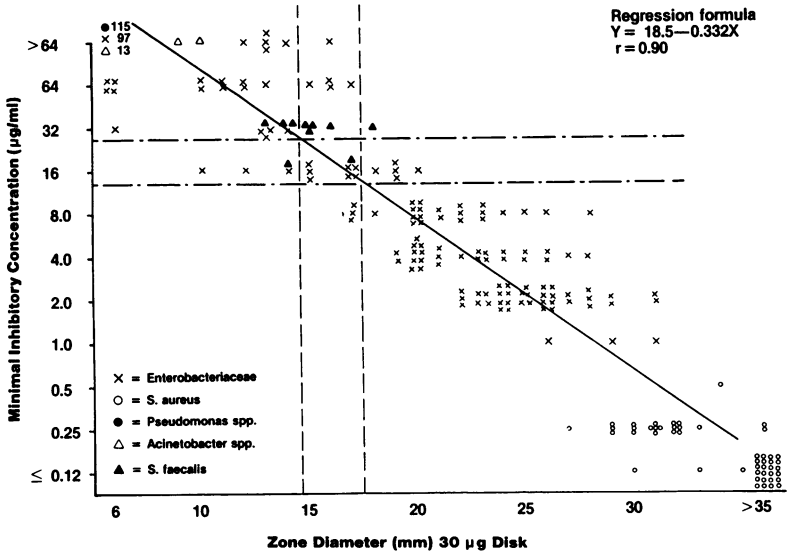

FIG. 3. *Correlation between microdilution MICs and zone diameters with 30-μg cephalothin disks. The regression line was calculated with all on-scale values. The intercept is expressed as $\log_2$ MIC + 9 (in micrograms per milliliter).*

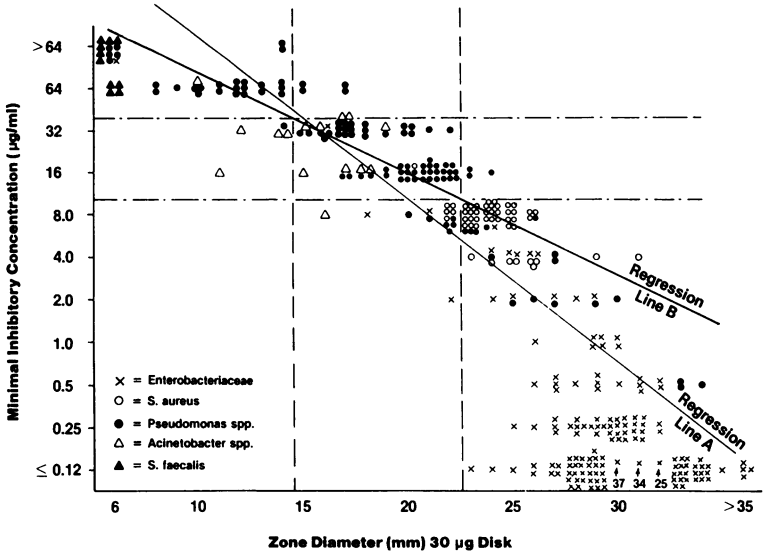

FIG. 4. *Correlation between MICs and zone diameters with 30-μg moxalactam disks. Regression line A was based on calculations with all on-scale endpoints. Regression line B was based on calculations with strains having MICs between 64 and 4.0 μg/ml (excluding strains with MICs of ≤2.0 μg/ml). See Table 2 for regression formulas.*

TABLE 2. *Regression analysis of moxalactam disk test data: effect of excluding data from susceptible strains to avoid the parabolic portion of the curve*

Range of MICs (μg/ml) included in calculations	Total no. of tests included	Regression formula		Correlation coefficient	MIC (μg/ml) correlate corresponding to a zone diam of:			
		Intercept (μg/ml)[a]	Slope		6 mm[b]	14 mm[c]	23 mm[d]	26 mm[e]
64 to 0.25 (not >64 or ≤0.12)	240	20.366	0.393	0.885	514.8	58.2	5.0	2.2
64 to 0.5 (not >64 or ≤0.25)	203	18.939	0.311	0.892	269.3	48.0	6.9	3.6
64 to 1.0 (not >64 or ≤0.5)	188	18.234	0.271	0.885	195.1	43.4	8.0	4.6
64 to 2.0 (not >64 or ≤1.0)	181	17.892	0.251	0.879	167.3	41.6	8.7	5.2
64 to 4.0 (not >64 or ≤2.0)	170	17.569	0.231	0.883	145.3	40.4	9.6	5.9

[a] Expressed as $\log_2$ MIC + 9.

[b] The maximal MIC that could be detected by a 30-μg disk, theoretically (i.e., a disk is 6.4 mm in diameter).

[c] The scattergram (Fig. 4) suggests that a 14-mm zone should correspond to an MIC of >32 but <64 μg/ml (48 μg/ml).

[d] The scattergram (Fig. 4) suggests that a 23-mm zone should correspond to an MIC of >8 but <16 μg/ml (12 μg/ml).

[e] The scattergram (Fig. 4) suggests that a 26-mm zone should correspond to an MIC of >2 but <4 μg/ml (3 μg/ml).

tam, a regression line was calculated for strains with MICs between 64 and 4.0 μg/ml (excluding strains with MICs of ≤2.0 and >64 μg/ml) (Fig. 4, regression line B). This regression line appears to better express the correlation between zone sizes and MICs at the MIC breakpoints. Interpretive zone size breakpoints of ≤14 and ≥23 mm correlate with MIC breakpoints of ≥64 and ≤8 μg/ml, respectively, confirming our conclusions drawn from the error rate-bounded method analysis. The resistant breakpoint would be the same for either regression line. Furthermore, if the MIC breakpoint for the susceptible category was reduced to ≤2.0 μg/ml, the zone size standard should be ≥26 mm (Fig. 4, regression line A). Because of the parabolic nature of the regression curve, the agar diffusion procedure cannot be expected to distinguish between categories accurately if MIC breakpoints of ≤2.0 μg/ml are used. The 30-μg disks appear

to be appropriate for categorizing strains with MIC breakpoints of ≥64 and ≤8 μg/ml and a moderately susceptible category of 16 or 32 μg/ml.

DISCUSSION

The considerations outlined in this report define the various types of data that are needed to establish MIC breakpoints and interpretive zone standards for the disk diffusion susceptibility test procedure for moxalactam. Final recommendations cannot be made until further clinical experience with chemotherapy against various types of infections is documented. If moxalactam proves to be ineffective in treating staphylococcal infections, in vitro tests with *S. aureus* would not be indicated. The accuracy of the disk test would be improved if tests with *S. aureus* were excluded from our data (Fig. 4). The disk test accurately separates the moderately susceptible pseudomonads and acinetobacters from the susceptible enteric bacilli. Experience in treating infections due to *P. aeruginosa* or *Acinetobacter* spp. will be needed to determine the relevance of such in vitro testing results. A sufficient number of moderately susceptible and resistant enteric bacilli were not available to assess the possibility of establishing zone standards that could be used only for tests with the *Enterobacteriaceae*. Enteric bacilli with zones in the moderately susceptible range should be tested further by a dilution procedure, if they are recovered from a serious infection that is to be treated with moxalactam. In the present series of tests, four enteric bacillus strains gave zones in the intermediate range; three were susceptible (MIC, ≤8 μg/ml), and one had an MIC of 32 μg/ml.

With the relatively nontoxic beta-lactam antimicrobial agents, a fairly broad range of dosage schedules can be utilized. Consequently, it is not possible to classify all microorganisms into resistant and susceptible categories. A third category (moderately susceptible) seems appropriate to describe those microorganisms which require greater dosages for therapy. A similar approach has already been recommended for tests with benzylpenicillin (7); i.e., a broad intermediate category has been defined to include the enterococci which are only moderately susceptible to penicillin. This approach might also be applied to tests with other beta-lactams.

In our series of tests, control data with 30-μg cephalothin disks demonstrated a linear relationship between the $\log_2$ MICs and zone diameters. However, with 30-μg moxalactam disks, the relationship was nonlinear; i.e., the curve became parabolic with the more susceptible enteric bacilli. An appropriate regression line was calculated by excluding data from the more susceptible strains and including only those data points which fell on the straight line portion of the regression curve. Because of such difficulties with regression analysis, the error rate-bounded method of Metzler and DeHaan (6) was also used to select an interpretive zone standard for defining the susceptible population. Also, because of the observed parabolic curve, tests with 30-μg disks should not be expected to be very accurate if MIC breakpoints below 2.0 μg/ml are used.

For use during future clinical trials with moxalactam, tentative zone standards of ≤14 and ≥23 mm are recommended for the reasons outlined above. Strains with zones 15 to 22 mm in diameter should be considered moderately susceptible (i.e., require a larger dosage). Additional pharmacological information and definition of the more commonly utilized dosage modalities may require modification of the MIC breakpoints and, consequently, the zone size standards.

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