Regression Curve Analysis of Cephalosporin Activity

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Regression lines were calculated for cephalothin, cephalaxin, and cephaloridine by relating zone diameters of inhibition to minimal inhibitory concentrations (MIC) obtained in Mueller-Hinton agar and in Trypticase Soy Agar. A regression line was calculated for cephaloglycin by obtaining MIC values in Trypticase Soy Agar at pH 6.6. Regression lines calculated from MIC values in Mueller-Hinton agar were practically superimposable on those based on MIC values in Trypticase Soy Agar. Organisms susceptible by disc testing to cephalothin were usually susceptible to cephalaxin and cephaloridine.

Regression curves demonstrating the relationship between zones of inhibition and minimal inhibitory concentrations (MIC) have been described by Sherris and co-workers (13) for cephalothin and by Wick (23) for cephalaxin. The purpose of the present study was threefold: (i) to determine regression curves for cephaloridine and cephaloglycin, (ii) to present the relative in vitro antibacterial activities of four cephalosporins, and (iii) to determine whether susceptibility data available by disc testing of one of the cephalosporins are applicable to the other cephalosporins.

MATERIALS AND METHODS

All bacteria studied were clinical isolates identified by the usual determinative procedures. Enterobacteriaceae were identified according to biochemical reactions and nomenclature of the taxonomic system of Ewing (4), as outlined in a previous report from this laboratory (20). Nonfermenting gram-negative bacilli were identified according to the method of King (7). Staphylococci were categorized by the tube coagulase test (Difco, Coagulase Plasma), and group D streptococci were defined with the bile-esculin test (16).

Disc susceptibility testing. Each bacterial strain was tested against 30-μg discs of cephalothin, cephaloridine, cephalaxin, and cephaloglycin. The disc test was performed as follows. Large (15 cm) petri dishes (Falcon Plastic, Los Angeles, Calif.) with Mueller-Hinton agar (BBL) at a depth of 5 to 6 mm were used within 4 days of preparation. A few colonies of the organism to be tested were inoculated into 3 ml of Mueller-Hinton broth (BBL) and incubated 2 to 4 hr. Turbidity was adjusted visually if necessary to a McFarland BaSO₄ standard prepared by adding 0.25 ml of BaCl₂ to 99.75 ml of 1% H₂SO₄ (0.36 n). Plates were inoculated by pipetting 2.5 ml of the broth onto the surface of the agar and distributing the inoculum evenly over the surface. The plates were then tipped for 3 to 5 min and the excess fluid was aspirated. Discs were applied and gently pressed against the agar with sterile forceps. Plates were incubated within 30 min at 35 to 37 C. After overnight incubation, the diameters of the zones of inhibition, including the diameter of the disc, were measured with a ruler on the undersurface of the petri dish.

Determination of MIC. MIC values were determined with an agar-dilution technique using the Steers, Foltz, and Graves inocula replicating apparatus (14). Each strain was tested against cephalothin, cephaloridine, and cephalaxin in Mueller-Hinton agar and Trypticase Soy Agar (BBL) at pH 7.4. Each strain was also tested against cephaloglycin in Trypticase Soy Agar at pH 6.6. Organisms to be tested were inoculated into 2 ml of Mueller-Hinton broth and incubated for 4 to 6 hr. Turbidity was adjusted visually to McFarland BaSO₄ standard 1 or 2 and transferred to wells complementary to the 36 prongs of the replicator. Since each prong delivers approximately 0.001 ml, final inoculum on the surface of the agar was in the order of 3 × 10⁶ to 6 × 10⁹ viable units. The MIC in micrograms per milliliter was read after 16 to 18 hr of incubation at 35 to 37 C as the concentration demonstrating no growth, a very fine barely visible growth, or a very few discrete colonies.

RESULTS

Regression lines and equations have been used to demonstrate the relationship between MIC values determined by the agar-dilution method and zone diameters obtained by the disc-diffusion method. (Regression curves for each antibiotic were calculated through the courtesy of Warren Wick at the Lilly Research Laboratories, Indianapolis, Ind.) As shown in Fig. 1–3, lines relating zone diameters to MIC values obtained in Mueller-Hinton agar are practically superimposable on those in which the MIC values were obtained in Trypticase Soy Agar. Since cephaloglycin MIC values (Fig. 4) were obtained at an acidic
pH, no comparisons were made between Trypticase Soy Agar and Mueller-Hinton agar.

The lowest MIC values and largest zone sizes were observed with the staphylococci as a group, with cephalothin and cephaloridine demonstrating the greatest activity against this group of organisms. Activity of each of the cephalosporins against Escherichia coli and Klebsiella species was similar to that of the others. All strains of Enterobacter species and Serratia marcescens were resistant to 50 μg of cephalothin, cephalaxin, and cephaloridine per ml; however, several strains of each of these two organisms were inhibited by 50 μg or less of cephaloglycin per ml. All strains of Pseudomonas aeruginosa and Herellea vaginicola were resistant to 50 μg of each of the four cephalosporins per ml. Inhibition of the group D streptococci varied with the cephalosporin and with the type of agar used.

![Fig. 1. Regression curve of cephalothin for zone diameters and MIC values. MIC values were determined in Mueller-Hinton agar in upper regression curve and in Trypticase Soy Agar in lower regression curve.](image)

![Fig. 2. Regression curve of cephalaxin for zone diameters and MIC values. MIC values were determined in Mueller-Hinton agar in upper regression curve and in Trypticase Soy Agar in lower regression curve.](image)

**DISCUSSION**

In Fig. 5, the regression lines for cephalaxin, cephalothin, and cephaloridine are compared to those obtained from data published by Sherris and associates (13) on cephalothin and Wick (23) on cephalaxin as well as from unpublished data kindly supplied by Wick. Line 1 in each case was derived from data obtained by Wick using the method described in a preliminary report of an international collaborative study sponsored by the World Health Organization. This method utilizes an inoculum yielding a dense but not completely confluent growth on the surface of the agar. In contrast, the method used in this study (line 3, Fig. 5) and that recommended by Bauer and associates [(1, 13) line 2, Fig. 5] utilize an inoculum which is dense and confluent and therefore heavier than that of the international collaborative study method. It is apparent that there is an inoculum effect which is most obvious with cephaloridine and least apparent
A 500-mg dose will produce serum levels of about 15 µg/ml at 1 hr (8, 15). Much lower peak serum levels of cephalexin, in the order of 0.8 to 1.4 µg/ml, are obtained after a 500-mg oral dose (2, 10). In each instance, with the exception of cephaloglycin, a dose of 1,000 mg substantially increases the serum level of each of these cephalosporins.

For the sake of simplicity, if one accepts an MIC of 12.5 µg/ml in Mueller-Hinton agar as

with cephalexin. Line 4 of Fig. 5, derived by Wick utilizing the method of Bauer and co-workers (1) and a 1:100 dilution of an overnight broth culture, apparently yielded an inoculum size intermediate between that of the international collaborative study method and that used by us.

MIC values of cephaloglycin were determined with the pH of the medium at 6.6 because of the greater activity of this agent at an acidic pH than at a neutral or alkaline pH (2). Ronald and Turck (12) showed that there is no appreciable difference in MIC values of cephaloglycin obtained in Trypticase Soy Agar and nutrient agar. There were no published regression curves available with which to compare our data.

Serum levels of 10 to 12.5 µg/ml can be expected approximately 1 hr after a 500-mg intramuscular dose of cephalothin (5, 9, 18). A 500-mg oral dose of cephalexin in a fasting subject will yield a serum level of approximately 12.5 µg/ml 1 hr later (2, 6, 17). Cephaloridine administered intramuscularly in a 500-mg dose will produce serum levels of about 15 µg/ml at 1 hr (8, 15).

FIG. 3. Regression curve of cephaloridine for zone diameters and at MIC values. MIC values were determined in Mueller-Hinton agar in upper regression curve and in Trypticase Soy Agar in lower regression curve.

FIG. 4. Regression curve of cephaloglycin for zone diameters and at MIC values. MIC values were determined in Trypticase Soy Agar at pH 6.6.

FIG. 5. Comparison of regression curves of cephalothin, cephalexin, and cephaloridine as determined by (i) Wick (23), using international collaborative study methods; (ii) Sherris and associates (13); (iii) the present study; and (iv) Wick, using the method of Bauer and co-workers (1) with inoculum of 1:100 dilution of overnight broth culture. All data were obtained in Mueller-Hinton agar.
the criterion for susceptibility to cephalothin, cephalaxin, and cephaloridine and 25 to 50 μg/ml in Mueller-Hinton agar as the criterion for intermediate susceptibility for each of these agents, then one can construct a table with suggested interpretations of zone diameters (Table 1). In general, a minimal zone diameter of 17 mm with cephalothin and cephalaxin discs and a minimal zone diameter of 14 mm with a cephaloridine disc separates the susceptible population of organisms. As seen in Fig. 1–3, this definition is not entirely correct because a few strains with MIC values of less than 12.5 μg/ml have zone diameters less than the acceptable minimum for definition of susceptibility; also, a few strains with MIC values in excess of 12.5 μg/ml have zone diameters exceeding the acceptable minimum for definition of susceptibility. In general, those organisms demonstrating zone diameters of at least 17 mm with cephalothin also yield zone diameters of at least 17 mm with cephalaxin. Those organisms with zone diameters of at least 17 mm with cephalothin produced zone diameters of at least 14 mm with cephaloridine, except for the group D streptococci which were more susceptible to cephaloridine than to the other cephalosporins. Since systemic infections with group D streptococci are not apt to be treated with a cephalosporin, it is unlikely that routine susceptibility testing of the cephalosporins against this group of organisms would be carried out except when isolated from the urinary tract. It appears, therefore, that routine disc testing of cephalothin would also provide information on susceptibility to cephalaxin and cephaloridine.

Since cephaloglycin attains such low serum levels, it is difficult to interpret its regression lines in terms of applicability to treatment of systemic infection. Ronald and associates (11) reported the efficacy of this agent in eliminating bacteriuria originating from the lower urinary tract; however, they also pointed out its limitations in the treatment of renal bacteriuria or infections originating outside the urinary tract. The critical factor therefore does not appear to be the very high levels of cephaloglycin attainable in urine, but rather the site of infection. Determination of a minimal zone diameter to establish susceptibility must perhaps await further clinical experience with this cephalosporin.

In this study, there were few instances in which the zone diameters indicated susceptibility when the MIC values indicated resistance. A previous study (19) performed with broth-dilution methods pointed out the lack of correlation in a substantial number of instances between zone diameters and MIC values for E. coli, Klebsiella species, and Enterobacter species. Examination of Fig. 1–4 indicates that the correlation was considerably increased when zone diameters were compared with MIC values obtained in agar. There are probably two factors contributing to this difference. First, as Ericsson and Svartz-Malmberg (3) emphasized, heterogeneity in antibiologic susceptibility is obscured in a broth test because persistence of a few resistant cells provides sufficient inoculum to produce turbidity in broth, whereas on agar persistence of a very few discrete colonies can be readily differentiated from the confluent growth on agar produced by an original inoculum of 10^5 colony-forming units. In addition, as Wick (21) pointed out, the MIC of cephalothin rises abruptly between the 12th and the 24th hr in broth, which corresponds to the degradation of cephalothin. Those organisms surviving the first 12 hr of the broth-dilution test are therefore subjected to desacetylcephalothin or desacetylcephalothin lactone, each being less inhibitory than cephalothin (22). On agar, those few organisms surviving the first 12 hr cannot divide and thereby cause turbidity as it occurs in broth.

Calculation of regression curves allows a translation of the zone diameter into an approximate value for MIC. The manner in which zone diameters are determined and MIC values are obtained is tremendously important in providing laboratory data that are reproducible and have clinical significance.

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**Table 1. Suggested interpretation of zone diameters obtained with 30-μg discs of cephalothin, cephalaxin, and cephaloridine**

| Antibiotic        | Zone diameter of inhibition* |
|-------------------|-------------------------------|
|                   | Resistant | Intermediate | Susceptible |
| Cephalothin       | ≤11       | 12–16        | >17         |
| Cephalaxin        | ≤10       | 11–16        | >17         |
| Cephaloridine     | ≤9        | 10–13        | >14         |

*a Expressed to nearest milliliter.*
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