Comparison of the Antibacterial Spectra of Cephalexin and Cefaclor with Those of Cephalothin and Newer Cephalosporins: Reevaluation of the Class Representative Concept of Susceptibility Testing

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The validity of the class representative concept for in vitro susceptibility testing of older cephalosporins was reevaluated. Two oral cephalosporins, cephalexin and cefaclor, were compared with the established cephalosporin class representative, cephalothin, by using reference microdilution minimal inhibitory concentrations of 528 isolates of a wide variety of gram-positive and gram-negative bacterial pathogens. For each comparison, there were only 15 (2.8%) random major and very major interpretive discrepancies. Additional comparisons confirmed the need to test second-generation (cefamandole) and third-generation (cefotaxime) cephalosporins separately. These results provide reasonable assurance that the use of cephalothin as an in vitro predictor of qualitative bacterial susceptibility to these two oral cephalosporins remains an acceptable alternative to testing each antibiotic individually.

Since the clinical introduction of cephalexin in 1971, determination of bacterial susceptibility to this oral cephalosporin has been made on the basis of the zones of inhibition produced by disks containing 30 μg of cephalothin. This practice was encouraged by a U.S. Food and Drug Administration decision endorsing the concept of testing only one antibacterial agent from each class of closely related drugs (2, 3). The 30-μg cephalothin disk was recommended for testing bacterial susceptibility to all cephalosporins that were clinically available at that time, i.e., cephalothin, cephaloridine, cephaloglycin, and cephalexin (3, 6). In more recent years, a variety of other cephalosporins with essentially the same antimicrobial spectrum have become available, e.g., cefazolin, cephapirin, cephradine, cefaclor, and cefadroxil. These also are represented in the disk test by cephalothin (4–6).

Since the oral and parental cephalosporin antibiotics are used in distinctly different patient populations and different clinical infections, there may be an opportunity for differential development of resistance of organisms to one group of agents, but not to the other. If this should happen, the class susceptibility testing concept would no longer be legitimate. The

current study reevaluated the appropriateness of the cephalosporin class susceptibility testing concept with cephalothin and the two most commonly used oral agents, cefaclor and cephalexin. Additional studies were performed to confirm the need to test the susceptibility of cefamandole and third-generation (cefotaxime-like drugs) cephalosporins separately.

Recent clinical isolates were accumulated for testing by the microdilution procedure from routine cultures submitted to the Kaiser Foundation Laboratory (Oregon Region, Clackamas, Oreg.). Others were contributed by A. L. Barry (University of California, Davis Medical Center, Sacramento, Calif.), P. C. Fuchs (St. Vincent Hospital and Medical Center, Portland, Oreg.), T. L. Gavan (The Cleveland Clinic Foundation, Cleveland, Ohio), E. H. Gerlach (St. Francis Hospital, Wichita, Kans.), and H. M. Sommers (Northwestern Memorial Hospital, Chicago, Ill.). The 528 organisms included: Acinetobacter spp. (15), Citrobacter diversus (10), Citrobacter freundii (10), Enterobacter aerogenes (19), Enterobacter agglomerans (9), Enterobacter cloacae (19), Enterobacter gergoviae (2), enterococci (25), Escherichia coli (25), Haemophilus influenzae (1/2 β-lactamase positive) (40), Klebsiella pneumoniae (25), Morganella morganii (10), Neisseria gonorrhoeae (β-lactamase positive) (23), N. gonorrhoeae (β-lactamase nega-

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TABLE 1. Major interpretive errors utilizing the cephalothin MIC results (30-µg disks by inference) to predict susceptibility to cephalexin, cefaclor, cefamandole, and cefotaxime

Cephalosporin	Interpretive error[a]		
	Very major	Major	Total
Cephalexin	9 (1.7)	6 (1.1)	15 (2.8)
Cefaclor	4 (0.8)	11 (2.1)	15 (2.8)
Cefamandole	0 (0.0)	72 (13.6)	72 (13.6)
Cefotaxime	0 (0.0)	152 (26.6)	152 (26.6)

[a] Number of isolates. Numbers in parentheses are percents of all tested strains. Very major errors, susceptible by the cephalothin test, but resistant to the other cephalosporin; major error, cephalothin resistant and susceptible to the other drug.

tive) (22), *Neisseria meningitidis* (25), *Proteus mirabilis* (25), *Proteus vulgaris* (10), *Providencia rettgeri* (10), *Providencia stuartii* (20), *Pseudomonas aeruginosa* (30), *Pseudomonas* spp. (30), *Serratia marcescens* (24), *Staphylococcus aureus* (50), *S. aureus* (methicillin-resistant) (10), *Streptococcus pneumoniae* (20), and *Streptococcus pyogenes* (20).

Cephalothin, cephalexin, cefamandole, and cefaclor (Eli Lilly & Co, Indianapolis, Ind.) and cefotaxime (Hoechst-Rouseel Pharmaceuticals, Inc., Somerville, N.J.) were incorporated into divalent cation-supplemented Mueller-Hinton broth in serial twofold concentrations over a range of 64 to 0.06 µg/ml in 0.1-ml volumes in microdilution trays (7). Trays were inoculated with 5 × 10⁴ CFU/well and incubated aerobically for 18 to 24 h before interpretation. The minimal inhibition concentration (MIC) was defined as the lowest antibiotic concentration that prevented formation of macroscopically visible bacterial growth. The standard method proposed by the National Committee for Clinical Laboratory Standards, standard M7-P(7), was used throughout the study. The broth was further supplemented with 5% peptic digest of horse blood when fastidious streptococci, meningococci, or *H. influenzae* was tested. *N. gonorrhoeae* isolates were tested by an agar dilution procedure, using proteose peptone no. 3, with 1% hemoglobin and 1% Kellogg supplement.

Each isolate was tested for its ability to produce β-lactamase by adding one drop of nitrocefin (500 µg/ml in pH 7 phosphate buffer) to the growth control well (8). Nitrocefin hydrolysis tests were also performed after the microorganism had grown in the presence of inducing concentrations of cefoxitin (0.03 to 2.0 µg/ml) and cefsulodin (0.25 to 16 µg/ml). The results showed that 132 strains (25%) had a strong β-lactamase reaction without induction and 256

strains (48%) after exposure to the two enzyme-stable cephalosporins.

Table 1 summarizes the major interpretive errors by using the cephalothin MIC susceptibility results to predict susceptibility to cephalexin, cefaclor, cefamandole, and cefotaxime. These data assume the susceptible and resistant breakpoints for all cephalosporins to be ≤8.0 µg/ml and ≥32 µg/ml, respectively (7). Clearly, the use of the cephalothin MIC or, by inference, disk results (2, 4) produces acceptable predictive statistics for the oral cephalosporins (cephalexin and cefaclor). Only 2.8% combined major and very major errors were found for each durg, with 1.7% very major errors for cephalexin. The nine strains susceptible to cephalothin and resistant to cephalexin were two *H. influenzae*, two *P. mirabilis*, one *S. pneumoniae*, and four methicillin-resistant *S. aureus*. All of the cefaclor very major errors were with methicillin-resistant *S. aureus* strains. Since the National Committee for Clinical Laboratory Standards (6, 7) and other authorative groups (3) recommend that all methicillin-resistant *S. aureus* strains be reported as resistant to cephalosporins, the deletion of those four errors reduces the cephalexin and cefaclor very major discrepancies to 0.95% and nil, respectively. The major errors (false resistance) for cephalexin were three *E. coli* strains, two *E. agglomerans* strains, and one *P. rettgeri* strain.
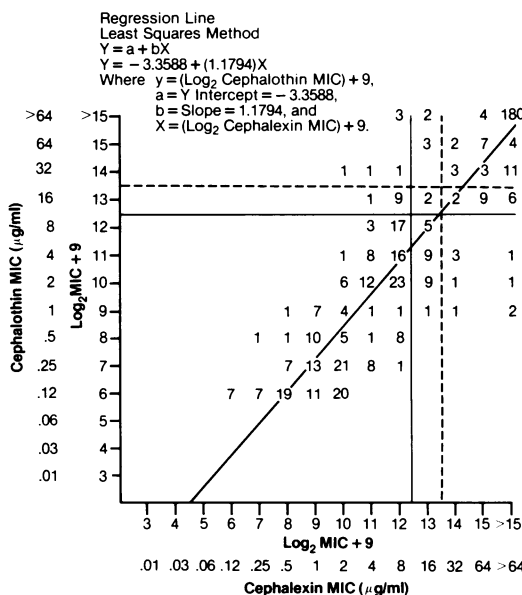

FIG. 1. Scattergram plot of the cephalothin MIC versus the cephalexin MIC (528 strains). Numerals indicate number of data points at each location.

Regression Line
Least Squares Method
$Y = a + bX$
$Y = 1.079 + (0.9105)X$
Where $y = (\text{Log}_2\ \text{Cephalothin MIC}) + 9$,
$a = Y\ \text{Intercept} = 1.079$,
$b = \text{Slope} = 0.9105$, and
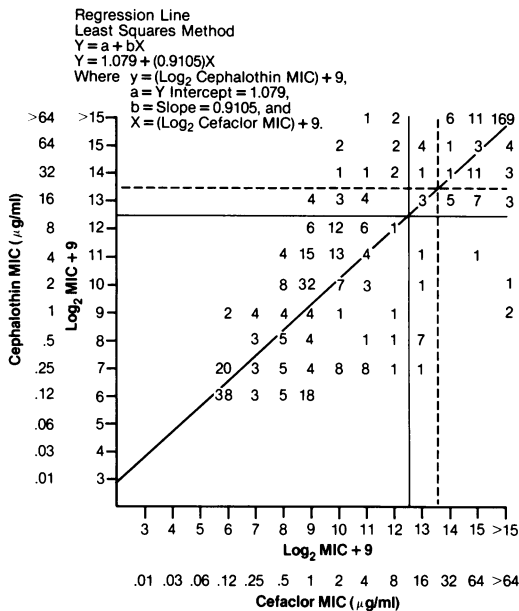$X = (\text{Log}_2\ \text{Cefaclor MIC}) + 9$.

FIG. 2. Scattergram plot of the cephalothin MIC versus the cefaclor MIC (528 strains). Numerals indicate number of data points at each location.

Second- and third-generation cephalosporins have very different spectra of antimicrobial activity, and their activities are grossly underestimated by cephalothin (Table 1). In this series of strains, 72 cephalothin-resistant isolates were fully susceptible to cefamandole, and 80 cephalothin- and cefamandole-resistant strains were susceptible to very low concentrations of the third-generation cephalosporin, cefotaxime.

Figures 1 and 2 plot the scattergrams of the oral cephalosporin MICs against cephalothin MICs. The comparison plot demonstrates cephalexin to be generally less active and cefaclor slightly more active than cephalothin. However, the spectra of activity remain essentially the same, with correlation coefficients of 0.92 and 0.93 for cephalexin and cefaclor, respectively. Note the small number of minor interpretive errors (intermediate for one of the two drugs) for cephalexin versus cephalothin (10.6%) and cefaclor versus cephalothin (7.7%).

The practice of class-representative testing of structurally related antibiotics having virtually identical antimicrobial activity spectra has been widely recommended for disk susceptibility testing (1–4, 6). However, for other test methods, such as prediluted microdilution and the semiautomated disk elution procedures, reporting susceptibility by antibiotic class is practiced in most laboratories without specific recommendation

by the instrument manufacturer or supplier of the test system. Whereas the various relatively new susceptibility testing systems are all correlated to either the disk method or a standardized broth dilution method (7) or both, we feel that this class practice can be applied to nearly all susceptibility procedures.

Care must be taken, however, to avoid the temptation to equate the absolute values of the MICs of these compounds. Because there are significant differences in potency among these compounds against various groups of organisms, it is only appropriate to equate the interpretive categories of susceptibility, e.g., susceptible, resistant, or intermediate (indeterminate).

These study results ensure that the use of cephalothin as an in vitro predictor of qualitative susceptibility of bacteria to cephalexin and cefaclor remains the alternative to testing each antibiotic. Long-term varied use of the older parenteral cephalosporins and their oral counterparts has not altered the validity of the cephalothin class disk. Conversely, the more recently released cephalosporins must be tested separately from cephalothin, yet other class representatives appear to be appropriate for second-generation cephalosporins (2, 4, 6) and possibly for some of the third-generation β-lactams (6).

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