CORRELATION OF AGAR-DISC-DIFFUSION TESTS WITH MINIMUM INHIBITORY CONCENTRATIONS OF CEFONICID (SK&F 75073) AND CEPHALOTHIN

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Cefonicid (SK&F 75073) is a new broad spectrum parenteral cephalosporin with high and prolonged serum levels[1]. The KIRBY-BAUER disc-diffusion test is commonly used in clinical laboratories to predict the antibiotic susceptibility of clinical isolates. In order to define the parameters to be used for interpretation of the disc-diffusion assay for cefonicid susceptibility, it was necessary to construct a regression line which would correlate minimum inhibitory concentration (MIC) with zone sizes. The ability of a 30-μg cefonicid disc to measure susceptibility of clinical bacterial isolates to this new cephalosporin was compared with that of the cephalothin disc.

The antibiotic discs, cefonicid, 30 μg and cephalothin, 30 μg, were purchased from BBL Microbiology Systems, Cockeysville, MD., U.S.A. The clinical isolates, a total of 134, were obtained from hospital laboratories in various areas of the U.S.A.

The disc-diffusion assay was that of BAUER and KIRBY, *et al.* 1966[2]. The bacterial strains were log phase cultures grown in Trypticase soy broth and adjusted to a standard optical density. The adjusted cell suspensions were swabbed on the surface of MUELLER-HINTON agar (BBL). Discs were placed on the surface in triplicate with a KIRBY-BAUER dispensor. Plates were incubated at 37°C overnight and zones of inhibition were recorded. An average of the three zone sizes obtained was used as a single value for each isolate for each antibiotic. Two reference strains, *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were included in each experiment as controls.

The minimal inhibitory concentrations were determined by two-fold dilution tests in MUELLER-HINTON agar at concentrations ranging from 0.1 to 100 μg/ml for each drug, according to the method described by ERICSSON and SHERRIS, 1971[3]. Approximately $10^2 \sim 10^3$ organisms were inoculated on the surface of the agar with a Steers' replicator. Plates were incubated at 37°C overnight.

The data relating MIC and zone diameters for each of the drugs were analyzed by plotting the log of the MIC for individual strains *vs.* the zone diameter. The average of the log MIC for each species was then plotted on the graph for fit.

Results and Discussion

Figs. 1 and 2 show the regression lines obtained by plotting log MIC *vs.* zone diameter for cefonicid and cephalothin, respectively. There was no statistical difference in the slopes of the regression lines. In logarithms, they were -0.0961 for cefonicid and -0.0861 for cephalothin. The regression line for cefonicid was similar to that previously reported for cephalothin[4]. However, at equivalent MIC values, the cefonicid disc gave zones of smaller diameter than that obtained with the cephalothin disc. A zone diameter of 14-mm with the cephalothin disc corresponds to an MIC of 25 μg/ml for cephalothin. However, a 14-mm zone diameter with the cefonicid disc corresponds to an MIC of 10.0 μg/ml for cefonicid, a level readily achievable in serum of patients.

The results of the KIRBY-BAUER disc susceptibility tests and their correlation with the sensitivities established by the agar-dilution tests are shown in Table 1. Using the same zone size diameters for susceptibility for both discs, the correlation of susceptibility by the KIRBY-BAUER assay with the MIC determinations is better for cefonicid than for cephalothin. The parameters used were a zone diameter of $\geq$18 mm for sensitive, 15$\sim$17 mm for intermediate, and $\leq$14 for resistance. The corresponding MIC values were grouped as follows: MIC, of $\leq$6.25 indicates sensitive, 12.5 indicates intermediate, and $\geq$25.0 indicates resistance.

An analysis of discrepancies in the disc susceptibility test's ability to measure the MIC for each drug is shown by further analyses of the data using the method of BARRY, *et al.*, 1978[5] (Table 2). It is obvious from the data in the table that the cefonicid and cephalothin discs are not interchangeable.

There was full agreement of the cefonicid disc data with the MIC data for cefonicid for 94.7%

Table 1. Percentage of microorganisms susceptible to cefonicid and cephalothin by KIRBY-BAUER and agar dilution assays[a].

Strains	No. of strains	Cefonicid						Cephalothin					
		S		I		R		S		I		R	
		KB[b]	MIC[c]	KB	MIC	KB	MIC	KB	MIC	KB	MIC	KB	MIC
S. aureus	15	93	100	0	0	7	0	100	100	0	0	0	0
E. coli	15	80	80	20	7	0	13	67	60	13	13	20	27
K. pneumoniae	15	93	93	0	7	7	0	87	87	7	7	7	7
P. mirabilis	15	100	100	0	0	0	0	93	93	0	0	7	7
Indole-positive *Proteus*	15	87	87	0	7	13	7	13	7	13	7	73	87
E. cloacae	15	100	100	0	0	0	0	40	27	27	7	33	67
E. aerogenes	15	100	100	0	0	0	0	20	20	33	27	47	53
Serratia	14	7	14	29	21	64	64	0	0	0	0	100	100
Rare Entero-bacteriaceae	15	87	87	0	0	13	13	47	27	13	20	40	53
Total strains	134	84	84	5	5	11	11	52	47	12	9	36	44

a) Data given as % of No. of strains.
b) KIRBY-BAUER (KB) assay, zone sizes $\geq$18 mm=S, 15~17 mm=I, $\leq$14 mm=R.
c) Agar dilution assay, MIC (μg/ml) of $\leq$6.25=S, 12.5=I, $\geq$25.0=R.

Fig. 1. Relationship of zone diameters (30-μg disc) and minimal inhibitory concentrations of cefonicid against clinical bacterial isolates.
SK&F 75073 MIC *vs.* zone size
●=Average of species

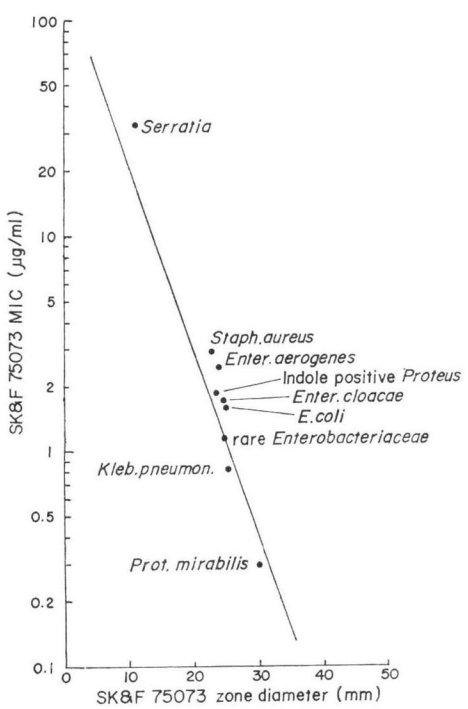

Fig. 2. Relationship of zone diameters (30-μg disc) and minimal inhibitory concentrations of cephalothin against clinical bacterial isolates.
Cephalothin MIC *vs.* zone size
●=Average of species

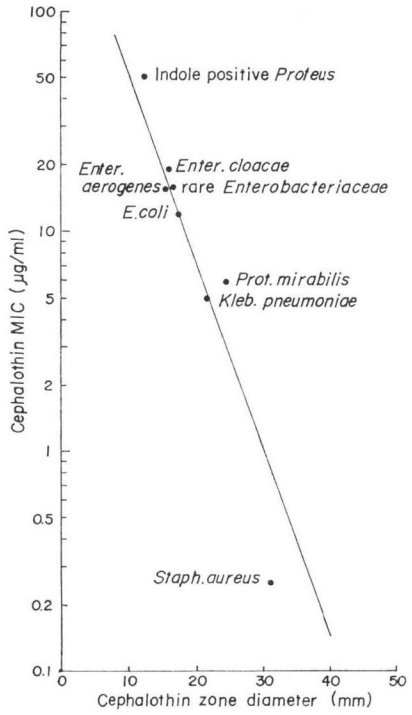

Table 2. Accuracy of disc tests for determining susceptibility to cefonicid and cephalothin[a].

30-μg Disc	MIC	% Full agreement	% Discrepancies		
			Minor	Major underestimation	Major overestimation
Cephalothin	Cephalothin	85.7	13.5	0.0	0.8
Cephalothin	Cefonicid	64.8	16.5	18.7	0.0
Cefonicid	Cefonicid	94.7	4.5	0.8	0.0
Cefonicid	Cephalothin	56.7	12.7	0.8	29.9

a) Interpretation of disc susceptibility test discrepancies.
Minor=I in one test and R or S in other
Major underestimation=R with disc and S by MIC
Major overestimation=S with disc and R by MIC

of the strains with no "major" discrepancies. However, the cephalothin disc predicted cefonicid MIC's in only 64.8% of the tests. Using the cephalothin disc to predict the cephalothin MIC, there was 85.7% full agreement and 0.8% major discrepancies (= 1 strain out of 134). The cefonicid discs could not be used to predict cephalothin susceptibility. As shown, there were 29.9% "major overestimation" of the sensitivity.

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