tant and those with MICs of 128 µg/ml as inter-
mediate in susceptibility (equivocal test results).
With those MIC breakpoints, the disk test was ac-
curately predictive, i.e. no false-susceptible disk test
results and only three false-resistant disk test
results were recorded.

Acknowledgement. These studies were made
possible by financial support from Zambon Cor-
poration, East Rutherford, New Jersey, USA.

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Comparison of Fixed Concentration
and Fixed Ratio Options for
Dilution Susceptibility Testing of
Gram-Negative Bacilli to Ampicillin
and Ampicillin/Sulbactam

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Ampicillin combined with sulbactam was tested
at both fixed ratio (2:1 and 1:1) and fixed sulbac-
tam concentrations (4 µg/ml, 8 µg/ml and 16 µg/ml) against 2440 consecutively isolated
gram-negative bacilli. Sulbactam significantly en-
hanced the spectrum of ampicillin activity. Over-
all, at 8 µg/ml ampicillin inhibited 50 % of the
Enterobacteriaceae isolates, whereas 69 % to
84 % of the isolates were inhibited by the various
sulbactam combinations. The widest spectrum of
activity for ampicillin/sulbactam was achieved by
testing at a fixed sulbactam concentration of
16 µg/ml, followed by the 1:1 ratio and the fixed
8 µg/ml (84 %, 76 % and 74 % inhibited, respec-
tively). The amount of sulbactam at the suscep-
tible breakpoint concentrations of ampicillin
markedly affected the percentage of susceptible
strains. Combinations that include 8 µg/ml of sul-
bactam are suggested for consideration.

The combination of a beta-lactam antibiotic, such
as ampicillin, with a beta-lactamase inhibitor,
such as sulbactam, has been useful in enhancing
the spectrum of activity of the penicillins (1-3).
The combination of ampicillin plus sulbactam is
administered intravenously in a 2:1 ratio and both
drugs are eliminated at similar rates. The addition

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of sulbactam broadens the in vitro antibacterial spectrum of ampicillin against Enterobacteriaceae, staphylococci and anaerobic bacteria (4–6). The combination has also proven to be effective in over 80% of the clinical infections due to these organisms (7–9).

The National Committee for Clinical Laboratory Standards (NCCLS) recommends that in vitro susceptibility to ampicillin/sulbactam should be determined by testing the two drugs in a 2:1 ratio (2 parts ampicillin to 1 part sulbactam) in dilution tests. The 2:1 ratio for the dilution tests is consistent with the ratio of the combination that is administered to adults, and approximates that achieved in serum (5, 10, 11). Dilution tests that utilize a fixed ratio suffer from the fact that excessive amounts of sulbactam are combined with high concentrations of ampicillin and unrealistically low concentrations of sulbactam are combined with low concentrations of ampicillin. Jones and Barry (11) evaluated the effect of fixed ratio combinations as well as the effect of adding fixed concentrations of sulbactam (4.0 and 8.0 μg/ml) to doubling dilutions of ampicillin in tests using a stock culture collection of 741 beta-lactam resistant bacteria (267 Enterobacteriaceae). They found that either fixed ratio (2:1 or 1:1 ampicillin to sulbactam) or fixed concentration (4.0 or 8.0 μg/ml) formulations resulted in comparably enhanced ampicillin activity.

In the present study, we have extended the comparative evaluation of fixed ratio (2:1 and 1:1) and fixed concentration (4.0, 8.0 or 16 μg/ml) ampicillin/sulbactam combinations to evaluate the in vitro activity of these combinations against a large collection of consecutively isolated gram-negative enteric bacilli from five medical centers. The 2:1 and 1:1 ratio combinations reflect the in vivo concentrations of ampicillin and sulbactam achievable under certain dosage conditions and the fixed sulbactam concentrations represent the peak concentrations that might be expected at the infection site at a time when irreversible beta-lactamase inhibition plays a significant role in determining the effectiveness of the ampicillin. This survey provides a database that actually reflects the performance of the MIC tests in the routine clinical laboratory.

Materials and Methods. Ampicillin and sulbactam were provided as standardized powders with certified potency by Pfizer-Roerig (New York, NY, USA). Each drug was diluted in cation-adjusted Mueller-Hinton broth (12) according to manufacturer’s recommendations for tests of aerobic bacteria.

Each testing facility performed broth microdilution susceptibility tests of ampicillin alone and in combination with sulbactam with all isolates of gram-negative enteric bacilli that were judged significant enough to be identified and tested by the routine methods employed in each laboratory.

Fresh clinical isolates were tested in each institution until at least 500 consecutive isolates of Enterobacteriaceae were evaluated. This provided an unbiased collection of 2440 clinical isolates encountered in the participating centers from November 1990 through January 1991 (Table 1).

Broth microdilution susceptibility testing was performed at all study sites according to the recommendations of the NCCLS (12). Serial two-fold dilutions of ampicillin were tested alone and in five combinations with sulbactam over a range of concentrations from 128 to 1.0 μg/ml. The ampicillin/sulbactam MIC combinations were fixed ratios of 2:1 and 1:1 or contained fixed sulbactam concentrations of 4.0 μg/ml, 8.0 μg/ml and 16 μg/ml. All strains were processed in cation-adjusted Mueller-Hinton broth (12). For comparison purposes, the recommended NCCLS breakpoints for susceptible, intermediate and resistant for ampicillin and the 2:1 ampicillin/sulbactam combinations (12) were applied to all combinations of ampicillin and sulbactam.

All participating laboratories tested 20 reference strains as well as standard quality control strains in order to document intra- and interlaboratory precision. Throughout the study, each laboratory performed at least 20 replicate tests with each of two quality control strains (Escherichia coli ATCC 25922 and Escherichia coli ATCC 35218). Such control data documented an acceptable level of reproducibility and accuracy.

Results and Discussion. Table 1 and Figure 1 summarize the broth dilution MIC results of ampicillin tested alone and combined with sulbactam in five different combinations against 2440 gram-negative clinical isolates. As observed previously (4–6, 11), the addition of sulbactam at any concentration had a marked effect on the activity of ampicillin against isolates of Enterobacteriaceae. Ampicillin MICs for the majority of isolates were at least one dilution interval lower when tested in the presence of sulbactam (fixed ratios or fixed concentrations). At the current NCCLS breakpoint of ≤8.0 μg/ml, ampicillin alone inhibited 50% of the isolates, whereas 69–84% of the isolates were inhibited by the different sulbactam combinations.