In Vitro Susceptibility of *Haemophilus influenzae* to Trimethoprim-Sulfamethoxazole

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Thirty-four strains of *Haemophilus influenzae* (20 ampicillin sensitive and 14 ampicillin resistant), mostly type b isolates from cerebrospinal fluid or blood, were tested for susceptibility to a combination of trimethoprim-sulfamethoxazole (TMP-SMZ) in a ratio of 1 part TMP to 19 parts SMZ. All strains were very susceptible to the TMP-SMZ combination, with minimal inhibitory concentrations ranging from 0.007–0.14 to 0.06–1.18 µg of TMP-SMZ per ml. There was little difference in the susceptibility of the ampicillin-sensitive and ampicillin-resistant strains to TMP-SMZ.

The recent occurrence of *Haemophilus influenzae* strains resistant to ampicillin in vitro (6, 8, 9, 11, 12) has prompted studies of the susceptibility of *H. influenzae* to other antimicrobial agents. Thirty-four strains of *H. influenzae*, 14 of which were resistant to ampicillin, were tested for their susceptibility to trimethoprim (TMP) and sulfamethoxazole (SMZ) in a combination of one part TMP to 19 parts SMZ. All strains were clinical isolates that had been submitted to the Center for Disease Control for confirmation of identification and/or antimicrobial susceptibility tests, or at our request. Most of the strains were isolated from cerebrospinal fluid or blood, and all except four were type b. All strains were tested for susceptibility to TMP-SMZ by both broth and agar dilution methods. Five percent lysed horse blood was added to both Mueller-Hinton broth and Muller-Hinton agar (BBL), and these were incubated overnight to neutralize the TMP-SMZ antagonizing substance of the media (3). Reduced nicotinamide adenine dinucleotide (Sigma Chemical Co.) was added to the medium in a concentration of 2.5 µg/ml. Inocula for the susceptibility tests were prepared by suspending the growth from an overnight chocolate agar plate culture in Mueller-Hinton broth and adjusting it to contain 10^6 colony-forming units per ml.

Although the strains were tested for susceptibility to TMP-SMZ by both broth and agar dilution methods, the end points of the broth dilution tests were not distinct because of trailing and could not be reproducibly determined by three bacteriologists in our laboratory. Therefore, the results reported here were obtained by the agar dilution method, in which the end points could be determined more reproducibly.

For the agar dilution test, Mueller-Hinton agar (BBL) was prepared to contain final concentrations of the TMP-SMZ (Burroughs-Wellcome Co.) combination ranging from 32 µg of TMP-608 µg of SMZ to 0.003 µg of TMP-0.07 µg of SMZ per ml in a serial twofold dilution series. Inocula were prepared as described above, diluted to contain 10^7 colony-forming units per ml, and inoculated onto the antimicrobial-containing agar with the Steers replicating apparatus (10). The final inoculum concentration delivered to the agar was approximately 10^6 colony-forming units. The inoculated plates were incubated at 35 C for 18 to 24 h. The minimal inhibitory concentrations (MICs) obtained by the agar dilution test are shown in Table 1. They ranged from 0.007–0.14 to 0.06–1.18 µg of TMP-SMZ per ml.

The ampicillin-sensitive strains, all of which were type b, had MICs ranging from 0.015–0.29 to 0.06–1.18 µg of TMP-SMZ per ml. The type b ampicillin-resistant strains had MICs ranging from 0.03–0.59 to 0.06–1.18 µg of TMP-SMZ per ml, whereas the ampicillin-resistant strains that were not type b, two of which were isolated from spinal fluid, had MICs ranging from 0.007–0.14 µg to 0.03–0.59 µg of TMP-SMZ per ml. Therefore, the MICs were similar for all the strains and indicated susceptibility of the strains to this combination of antimicrobial agents.

In general, results of other studies on the susceptibility of non-type b strains of *H. influenzae* (from the respiratory tract) to TMP-
SMZ agree with our results (1-3, 5). Reports of resistance among such strains (7) may have resulted from technical problems with the susceptibility testing procedure, in which trailing end points were interpreted incorrectly (S. R. M. Bushby and M. R. Bushby, Abstr. 8th Int. Congr. Chemother., Abstr. A-205, 1973).

Clinical experience with TMP-SMZ in this country is limited but increasing as indicated in the reports of a recent symposium (4). TMP-SMZ is active against a wide variety of organisms, and high concentrations of these agents have been demonstrated in body fluids (1, 4).

**LITERATURE CITED**