ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1978, p. 969-970
0066-4804/78/0013-0969$02.00/0
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Vol. 13, No. 6
Printed in U.S.A.

Susceptibility of *Haemophilus influenzae* Type b to Rifampin and Sulfisoxazole

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Received for publication 10 January 1978

A total of 100 and 97% of *Haemophilus influenzae* type b strains from major infections were susceptible, respectively, to levels of rifampin and sulfisoxazole attainable in saliva. It is theoretically feasible to eliminate *Haemophilus influenzae* from the nasopharynx with these drugs.

Clustering of cases of *Haemophilus influenzae* type b infection both inside and outside the family unit has been the subject of a recent review (9). A current survey suggests that the risk of major disease in household contacts of patients with serious *H. influenzae* infection may approach that seen with meningococcal sepsis (G. A. Filice, J. S. Andrews, Jr., M. D. Hudgins, and D. W. Fraser, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 17th, New York, N.Y., Abstr. 334, 1977). Chemoprophylaxis of such individuals has been recommended by several investigators (5, 7, 8) and attempted in a limited fashion by others (4, 6). Because rifampin and sulfonamides have been so effective in eliminating meningococci from the nasopharynx and in reducing the risk of secondary meningococcal disease in close contacts, we undertook to generate appropriate background data on the susceptibility of *H. influenzae* type b to these agents in the event that such a chemoprophylactic approach becomes necessary against this organism.

MATERIALS AND METHODS

Preparation of inoculum. Twenty-six blood and 72 cerebrospinal fluid isolates of *H. influenzae* type b, including four ampicillin-resistant strains, were tested. The isolates, collected between 1965 and 1977, were each derived from separate patients. All strains were grown on Levinthal agar for 24 h at 37°C before use. Cells were harvested with phosphate-buffered saline, pH 7.4, with 1% added gelatin, and the turbidity of the suspension was adjusted to a standard optical density of 0.2 as measured at 490 nm on a Spectronic 20 spectrophotometer (Bausch and Lomb, Inc., Rochester, N.Y.). This represented a concentration of approximately 3×10^8 organisms/ml. The cell suspension was further diluted 1:1,000 with phosphate-buffered saline to produce a final inoculum with the Steers replicator of 10^3 to 3×10^3 organisms.

Media. Susceptibility tests against rifampin were performed on Levinthal agar. For sulfisoxazole, diagnostic susceptibility test agar with 5% defibrinated, lysed horse blood and 0.33 mg of oxidized diphosphopyridine nucleotide per dl (Cozymase, Nutritional Biochemicals Corp., Cleveland, Ohio) was used. Both basic media were dispensed in 250-ml quantities in 500-ml Erlenmeyer flasks and sterilized by autoclaving, the supplements and drugs were added, and the well-mixed, melted agar was dispensed into sterile petri dishes (100 by 15 mm; Fisher Scientific Co., Pittsburgh, Pa.). Plates were cooled, stored at 4°C, and used within 48 h.

Preparation of antimicrobial solutions. Rifampin powder (Rifadin, Dow Chemicals Inc.), 10 mg was dissolved in methanol and diluted with 20% Levinthal broth to obtain stock solutions containing from 400 to 12.5 μg/ml. A 2.5-ml amount of appropriate drug solution (or Levinthal broth) was added to each 250 ml of melted agar to obtain final concentrations of rifampin from 4.0 to 0.125 μg/ml or drug-free medium. Sulfisoxazole (Gantrisin, sulfisoxazole diolamine injection, Hoffman-La Roche Ltd., Quebec), 400 mg/ml, was diluted with sterile distilled water to obtain stock solutions containing from 2,000 to 15.6 μg/ml. Working concentrations were prepared by agar dilution from 20 to 0.156 μg/ml. Drug-free controls were also prepared.

Susceptibility testing. A modification of the agar dilution-multiple inoculator method for *H. influenzae* susceptibility testing was used (3, 10). With the *H. influenzae* cell suspension prepared as above, agar plates were inoculated by using a Steers replicator. Plates were incubated at 37°C and examined for growth after 24 h of incubation. Complete absence of growth in at least two of three test cultures (all tests were performed in triplicate) was considered as the minimum inhibitory concentration (MIC). Controls included uninoculated sterility plates, drug- and solvent-free growth controls, and laboratory strains of *Staphylococcus aureus* and *Escherichia coli*.

RESULTS

All strains were susceptible to 0.5 μg of rifampin per ml (see Table 1). For most strains (98%) MICs were ≦0.25 μg/ml, and 20.4% were completely inhibited by ≦0.125 μg/ml. In the case of sulfisoxazole, MICs were ≦10 μg/ml for 95 of the 98 strains tested (97%). Three were

TABLE 1. *Susceptibility of H. influenzae to sulfisoxazole and rifampin*

Year isolated	No. of strains tested	No. of strains with MIC (μg/ml) of:								
		Rifampin			Sulfisoxazole					
		0.125 or under	0.25	0.5	0.625	1.25	2.5	5	10	20
1965	1		1			1				
1967	1		1			1				
1969	6	2	4		2	3		1		
1970	13	3	10		3	5	3	1	1	
1971	10	2	8		2	5	3			
1972	6	3	3		1	3	2			
1973	9		9		2	4	3			
1974	6	2	4		2	2	2			
1975	12		12		3	6	2	1		
1976	17	4	12	1	1	9	1	3	1	2
1977	17	4	12	1	1	12	1	1	1	1
Beta-lactamase-positive strains	4	1	3	0	2	1	0	0	1	0

relatively resistant. All four of the beta-lactamase-producing, ampicillin-resistant strains of *H. influenzae* were susceptible to 0.25 μg or less of rifampin per ml and to 10.0 μg or less of sulfisoxazole per ml. No definite pattern of a trend toward increased resistance to the two drugs was observed over the 12-year time span represented by the strains tested.

DISCUSSION

The effectiveness of an antibiotic in the elimination of the nasopharyngeal carrier state is determined by its ability to achieve salivary levels approaching the in vitro MIC for the infecting organism (1, 2). Salivary levels of sulfadiazine and rifampin after standard doses are in the ranges of 2 to 14 and 0.075 to 0.75 μg/ml, respectively (1, 2). For *H. influenzae* type b the MICs of the two drugs as determined here were 97% ≦10 μg/ml for sulfisoxazole and 100% ≦0.5 μg/ml for rifampin. It can be seen that the majority of strains have MICs within attainable salivary concentrations of both sulfisoxazole and rifampin, making the elimination of *H. influenzae* from the nasopharynx theoretically feasible. If nasopharyngeal colonization is the route by which household contacts of index cases acquire *H. influenzae,* as has been suggested (9), and if the risk of major disease in these infected contacts is confirmed (Filice et al., 17th ICAAC, 1977), then chemoprophylaxis, in the meningococcal tradition, is a logical next step. Data are presented to indicate that sulfisoxazole or rifampin deserves consideration for this task.

ACKNOWLEDGEMENT

This work was supported by an intramural grant from the Research Institute, The Hospital for Sick Children.

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