

Susceptibility of Shigellae to Mecillinam, Nalidixic Acid, Trimethoprim, and Five Other Antimicrobial Agents

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A total of 199 strains of shigella (1 *Shigella dysenteriae*, 15 *S. boydii*, 47 *S. flexneri*, and 136 *S. sonnei*) isolated in Malmö, Sweden, within a 3-year period (1977 through January 1980) were tested with the agar plate dilution method for susceptibility to commonly used and newer antimicrobial agents. Mecillinam, nalidixic acid, and trimethoprim had the best in vitro activity. *S. flexneri* dominated among strains resistant to three or more antimicrobial agents and were less susceptible to ampicillin, chloramphenicol, and doxycycline than other types studied. Sixty-four percent of the strains were resistant to sulfamethoxazole. In vitro, a synergistic effect with trimethoprim was shown only in strains susceptible to sulfamethoxazole. The amidinopenicillin mecillinam was highly active against shigellae. When resistance occurred, it was linked to ampicillin in 17 of 18 strains. The quinolones, here represented by nalidixic acid, might be the drugs of choice.

Shigella infections have not been endemic in Sweden for the last 2 to 3 decades. Since 1975 a doubling of the incidence to about 600 cases yearly has been noticed among returning tourists, foreign-aid workers, and immigrants. The strains isolated in Malmö originated in different parts of the world.

This study was designed to determine the in vitro antimicrobial susceptibility of shigella strains isolated in Malmö during the last 3 years, with special reference to the frequency of resistance to various antimicrobial agents, and, more importantly, to assess the susceptibilities to potentially effective agents, such as mecillinam, quinoline drugs, and trimethoprim.

MATERIALS AND METHODS

Bacterial strains. A total of 199 isolates (1 *Shigella dysenteriae*, 15 *S. boydii*, 47 *S. flexneri*, and 136 *S. sonnei*) were obtained in Malmö, Sweden, from patients with shigellosis from August 1976 through January 1980. After verification by biochemical tests and agglutination with commercial shigella antisera, all strains were confirmed by the National Bacteriological Laboratory, Stockholm, Sweden. The strains were stored in Stuart medium at 4°C until tested.

Culture media and antibiotics. The medium used was PDM-antibiotic susceptibility medium (PDM-ASM) agar from AB Biodisk, Solna, Sweden. Test compounds and sources were: ampicillin, DL, 634 (Astra, Södertälje, Sweden); chloramphenicol, lot 302982 (Dumex, Copenhagen, Denmark); doxycycline, batch 55004 k 8 (Rachelle Laboratories, Long Beach, Calif.); gentamicin, 5-N-4-1 (Schering Corp., Bloomfield, N.J.); mecillinam, batch GA 36375 (Leo Pharmaceuticals, Ballerup, Denmark); nalidixic acid, 214/556 LL1816 (Sterling Winthrop Dudley, Northumber-

land, England); streptomycin, batch 8CP247B (Glaxo, Greenford, Middlesex, England); sulfamethoxazole, batch 18810-51 (Kabi, Stockholm, Sweden); and trimethoprim, batch 20472-51 (Kabi). Standard solutions of these drugs were prepared (with correction for compound impurities) and subjected to twofold serial dilution before incorporation in PDM-ASM agar.

Susceptibility testing. The inoculum was derived from a 24-h culture of shigella grown on lactose-saccharose urea agar (9). Three to five colonies were transferred into a broth containing 0.5% beef extract (Difco Laboratories, Detroit, Mich.) 1% peptone, 0.1% glucose, 0.3% sodium chloride, and 0.2% sodium biphosphate in distilled water and incubated overnight at 37°C. The suspension was then diluted in this medium to give a density of 10⁶ to 10⁷ organisms per ml. With a multipoint inoculator (12), 2 to 3 µl of the bacterial suspension was applied to the surface of the antibiotic agar plates. These were incubated for 24 h at 37°C. Minimal inhibitory concentrations (MICs) were recorded as the lowest antibiotic concentrations that gave no visible growth. *Escherichia coli* (ATCC 25922) was included as a control strain. The breakpoints used are given in Table 1.

All strains were tested for in vitro synergism between sulfamethoxazole and trimethoprim by a paper disk diffusion method on PDM-ASM agar. Antibiotic disks (AB Biodisk) containing 1.2 µg of trimethoprim and 23.8 µg of sulfamethoxazole (1:20) were used together with separate disks containing 1.2 µg of trimethoprim and 23.8 µg of sulfamethoxazole, respectively. Synergism was said to be present if the zone diameter for sulfamethoxazole-plus-trimethoprim disk exceeded the zone diameter for the trimethoprim disk alone by 4 mm or more (11).

RESULTS

The range of MIC values for each of the nine antimicrobial agents tested and the concentra-

tions of these drugs required to inhibit 90% (MIC_{90}) and 50% of the test strains are presented in Table 1. Mecillinam, nalidixic acid, and trimethoprim were the most active drugs, with MIC_{90} concentrations well below therapeutic concentrations achievable in serum by normal oral dosages.

Strains in general were either totally susceptible or totally resistant to all drugs tested except ampicillin and doxycycline. Seventy-five percent of the strains had intermediate resistance to ampicillin, with MICs between 4 and 16 $\mu\text{g/ml}$, whereas mecillinam inhibited 91% of the strains at 0.125 $\mu\text{g/ml}$ and 8.5% at 8 to 32 $\mu\text{g/ml}$. One strain was not inhibited at 128 $\mu\text{g/ml}$.

Forty-four percent of the strains had intermediate resistance to doxycycline, with MICs between 1 and 4 $\mu\text{g/ml}$. The difference in susceptibility between trimethoprim (98%) and sulfamethoxazole (36%) was striking. Synergism was seen in no strain resistant to sulfamethoxazole but in all strains susceptible to both drugs.

All strains tested were susceptible to nalidixic acid; 98% had MICs below 2 $\mu\text{g/ml}$. Gentamicin inhibited 99.5% of the strains at the same concentration, but streptomycin inhibited only 49% at 8 $\mu\text{g/ml}$. Eighty-eight percent of the strains were susceptible to chloramphenicol.

Resistance patterns. Sixty-eight percent of the shigella strains were resistant to one or more antimicrobial agents. Less than 32% of the strains showed full susceptibility to all drugs tested. The multiple resistance patterns for the different strains are presented in Fig. 1. The most widespread multiple resistance was found among *S. flexneri* strains, two of which were not susceptible to seven of the drugs tested.

The resistance pattern for each drug tested is given in Table 2. The most common multiple resistance was to sulfamethoxazole-streptomycin-doxycycline. In strains resistant to four or more drugs, a pattern of ampicillin-chloramphenicol-mecillinam resistance was found. Four

strains were not susceptible to trimethoprim, ($MIC > 64 \mu\text{g/ml}$) and were also resistant to sulfamethoxazole. One of four was additionally resistant to streptomycin, doxycycline, ampicillin, chloramphenicol, and mecillinam.

DISCUSSION

The resistance of shigellae to sulfonamides is high. We found that 64% of the strains were resistant to sulfamethoxazole, comparable to the finding by Gordon et al. of 62% in 1975 (5) and by Haltalin and Nelson of 60% in 1965 (7). In spite of the resistance to sulfamethoxazole, several authors have described high susceptibility to the trimethoprim-sulfamethoxazole combination in vitro (1, 3, 5) and good clinical results (3). We believe that the efficacy of the trimethoprim-sulfamethoxazole combination in shigellosis is ascribable to trimethoprim alone in infections with sulfamethoxazole-resistant strains. The possible synergism between trimethoprim and sulfamethoxazole in shigella has been thoroughly examined by Jarvis and Scrimgeour (8) and Grimm (6), and, like us, none of these authors has found any synergistic effect in the

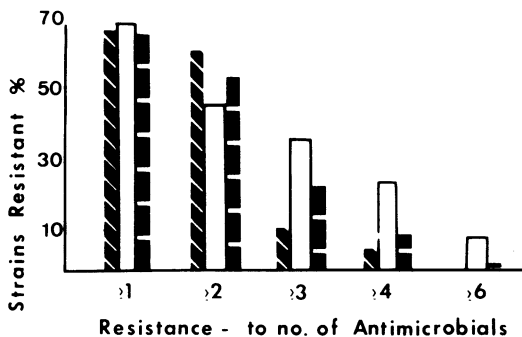


FIG. 1. Multiple resistance patterns for the different types of shigella. Symbols: Diagonal hatching, *S. dysenteriae plus S. boydii* (1 plus 15 strains); open bars, *S. flexneri* (47 strains); horizontal hatching, *S. sonnei* (136 strains).

TABLE 1. Susceptibility of 199 shigella strains to various antimicrobial drugs

Drug	Breakpoint ($\mu\text{g/ml}$)		Range	MIC ($\mu\text{g/ml}$ of medium)	
	Susceptible	Resistant		For % of strains	
				50	90
Ampicillin	≤ 2	> 16	1.0- > 128	8	> 128
Chloramphenicol	≤ 8	> 8	0.5- > 128	8	128
Doxycycline	≤ 1	> 4	0.5- > 64	2	64
Gentamicin	≤ 2	> 16	0.5- > 128	2	2
Mecillinam	≤ 2	> 2	0.125- > 128	0.125	0.125
Nalidixic acid	≤ 32	> 32	1.0-4	2	2
Streptomycin	≤ 8	> 64	8.0- > 128	32	> 128
Sulfamethoxazole	≤ 256	> 256	4.0- $> 1,064$	$> 1,064$	$> 1,064$
Trimethoprim	≤ 1	< 4	0.062- > 64	0.125	0.125

TABLE 2. Multiple resistance patterns for 199 strains of shigella

Resistance index ^a	No. resistant to:								Total
	Sulfamethoxazole	Streptomycin	Doxycycline	Ampicillin	Chloramphenicol	Mecillinam	Trimethoprim	Gentamicin	
0									63
1	28		1		1	1			31
2	51	50	1						51
3	27	27	22	1	1		3		27
4	2	7	7	5	5	2			7
5	13	10	13	13	8	8			13
6	5	5	5	5	5	5			5
7	2	2	2	2	2	2	1	1	2
Total	128	101	51	26	22	18	4	1	199

^aResistance to number of antimicrobial agents; 0, fully susceptible.

presence of sulfamethoxazole resistance. A controlled clinical comparison between the sulfamethoxazole-trimethoprim combination and trimethoprim alone in shigellosis is desirable.

One strain was resistant to mecillinam alone. The other 17 strains resistant to mecillinam were also resistant to at least three other drugs. Resistance was always linked to doxycycline and ampicillin. The association of mecillinam with these multiple resistance patterns warrants, in our opinion, restriction of its use in shigellosis despite the high in vitro susceptibility of shigella.

The rather uniform patterns of multiple resistance in our strains imply that the resistance probably is R-factor mediated. The linked resistance pattern between beta-lactam antibiotics is perhaps influenced by production of beta-lactamase.

The quinoline drugs are apparently not involved in plasmid-mediated transfer of antimicrobial resistance (2), which gives them a special advantage. As early as 1965, Moorhead and Parry concluded that nalidixic acid was a useful alternative in the treatment of Sonne dysentery (10). Our own experiences with nalidixic acid at 4 g daily for 7 days have been satisfactory in eradicating shigella from the stool in shigellosis and shigella carriers (Hansson et al., in press).

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