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Antimicrobial Susceptibilities of Shigellae Isolated in Houston, Texas, in 1974

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One hundred seventy-three strains of shigellae (113 *Shigella sonnei*, 56 *S. flexneri*, and four others) isolated in Houston during 1974 were tested for susceptibility to commonly used and newer antimicrobial agents. Forty-five percent of *S. sonnei* strains were susceptible to ampicillin, whereas 93% of *S. flexneri* strains were susceptible to that agent. *S. sonnei* and *S. flexneri* strains were equally susceptible to tetracycline (35 and 33%, respectively). All 173 strains were uniformly susceptible to quinoline drugs (cinoxacin, oxolinic acid, and nalidixic acid) and to trimethoprim-sulfamethoxazole. This study supports recent suggestions that the initial therapy of bacillary dysentery no longer should be ampicillin or tetracycline. It remains for field testing to determine whether quinoline agents or trimethoprim-sulfamethoxazole will be the treatment of choice.

Resistance of shigellae to a number of commonly used antimicrobial agents has prompted a search for new effective drugs in the treatment of shigellosis. Ampicillin (Am) has been considered the drug of choice for pediatric bacillary dysentery. Adults with shigellosis have generally been treated with either Am or tetracycline (Te). In light of recent reports of rapidly emerging multiple-resistant shigellae isolated in the United States, the use of Am and Te as primary drugs must be questioned (5, 11). Strains commonly isolated today reveal resistance to one or more of the following antimicrobial agents: Am, Te, streptomycin (Sm), chloramphenicol (C), and sulfonamides (3).

The present study was designed to determine the in vitro antimicrobial susceptibility patterns of shigella strains recently isolated in Houston, Tex. We were interested in documenting the frequency of resistance to commonly used antimicrobial agents, but more importantly in determining the susceptibilities to newer antimicrobial agents, the quinoline drugs and trimethoprim-sulfamethoxazole.

MATERIALS AND METHODS

Bacterial strains. One hundred seventy-three isolates of shigellae were studied. The strains were obtained and identified by the Houston City Health Department during 1974. Each culture was a single-colony primary isolate from a fecal specimen and/or a pure culture sent to the Health Department for evaluation. Storage was less than 12 months at 4 C or at room temperature. All strains were representative isolates from numerous individuals in Houston, and multiple strains from common source outbreaks or individual households were excluded from study. Strains were streaked by us on MacConkey agar (Difco) for isolations before inclusion in the study. In total, 113 strains of *Shigella sonnei*, 56 strains of *S. flexneri*, 3 strains of *S. dysenteriae*, and 1 strain of *S. boydii* were evaluated for their antimicrobial susceptibility patterns. *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) were obtained in lyophilized form from Bactrol Discs, set A, Difco Laboratories, Detroit, Mich., and were included as controls in the susceptibility testing.

Antimicrobial agent susceptibility disks. Disks for the following antimicrobial agents were purchased from Baltimore Biological Laboratories (BBL) in 400-disk supply lots: Te, 30 μg; Am, 10 μg; Sm, 10 μg; and nalidixic acid, 30 μg. Additional antimicrobials included trimethoprim-sulfamethoxazole (1.25:23.75 μg ratio) from Roche Laboratories, Div. Hoffmann-LaRoche Inc., Nutley, N.J.; cinoxacin, 30- and 100-μg disks (compound 64716) from Eli Lilly & Co., Indianapolis, Ind.; and oxolinic acid, 2-μg disks supplied by the Warner-Lambert Research Institute, Morris Plains, N.J.

Single disk agar diffusion susceptibility testing. Antimicrobial susceptibility tests on the 173 isolates were performed by using a modification of the Kirby-Bauer method. Four to five colonies showing the same morphology were picked from the MacConkey agar plate and inoculated into 5 ml of Trypticase soy broth (BBL). The cultures were incubated at 35 C for 2 to 5 h, and then their optical densities were adjusted to the barium sulfate standard's turbidity (0.5 ml of 0.048 M $BaCl_2$ plus 99.5 ml of

0.36 N H_2SO_4) with sterile Trypticase soy broth. A cotton swab was soaked in the adjusted broth culture and used to streak a 150-mm plate of freshly prepared Mueller-Hinton agar by using the lawn plate technique. Antimicrobial disks were applied with a multiple-disk dispenser (BBL) within 15 min and tapped down with sterile forceps to assure contact. The plates were inverted and incubated for 16 to 18 h at 35 C. Zone sizes were measured to the nearest millimeter with a standard centimeter ruler. Zone size was correlated with known values for susceptible and resistant response to each drug. Values for cinoxacin and oxolinic acid disks are as follows: CX-30, ≤13 is resistant, 14 through 18 is intermediate, ≥19 is susceptible; OA-2, ≤10 is resistant, 11 through 15 is intermediate, ≥16 is susceptible.

RESULTS

Antimicrobial susceptibility testing data on the 173 strains of shigellae are shown in Fig. 1. Strains in general were either totally susceptible or totally resistant, with intermediate susceptibility being unusual. All shigellae were susceptible to the quinoline drugs (cinoxacin, oxolinic acid, nalidixic acid) and to the trimethoprim-sulfamethoxazole combination drug. Eighty-eight percent of shigella strains were susceptible to C, 61% to Am, while 34 and 28% were inhibited by Te and Sm. Complete resistance (no zone of clearing) to Te, Sm, Am, and C was noted in 98% of inhibited strains. Figure 1 indicates comparable values of susceptibility of *S. sonnei* and *S. flexneri* to the three quinoline drugs (100% susceptible), Te (35 and 33% susceptible), Sm (30 and 24% susceptible), and trimethoprim-sulfamethoxazole (100% susceptible). The 30-μg cinoxacin disk gave a mean zone of inhibition of 24 mm (range 21 to 27), whereas the 100-μg disk was slightly more inhibitory where the zone size averaged 29 mm (range 24 to 33). Wide variation in susceptibility to Am and C was noted. Ninety-three percent of *S. flexneri* strains were susceptible to Am in contrast to only 45% with *S. sonnei*. *S. flexneri* strains showed a lower percentage of susceptibility to C when compared to *S. sonnei* (69 versus 98%).

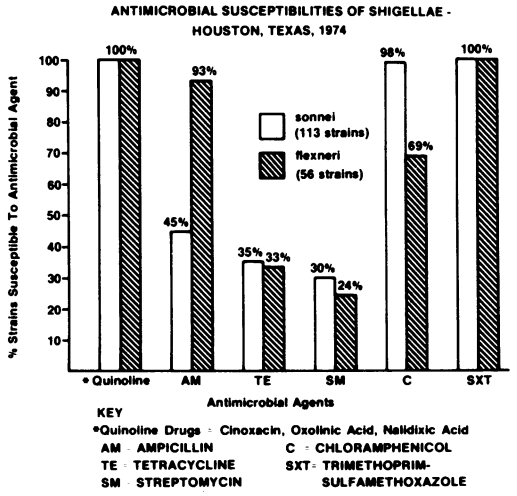

FIG. 1. *Antimicrobial susceptibility values determined by the Kirby-Bauer disk method.*

Multiple resistance was noted in a comparable number of *S. sonnei* and *S. flexneri* strains (63 and 61%) as shown in Table 1. Fifty-one percent of *S. sonnei* strains were resistant to three drugs, whereas *S. flexneri* strains revealed a lower but balanced rate of resistance to two or three drugs (29%). A review of two-drug resistance patterns shows both species resistant to the Te-Sm combination most often. The most common pattern of three-drug resistance was Am-Te-Sm with *S. sonnei* and C-Te-Sm with *S. flexneri*. Although only four strains of *S. boydii* and *S. dysenteriae* were examined, all strains were susceptible to the quinoline drugs and trimethoprim-sulfamethoxazole. The overall rate of resistance to two or more drugs (multiple-resistance) for all shigellae was 62%.

DISCUSSION

Acute diarrheal disease is a serious problem in the world today. The most common gram-negative bacterial pathogens in the United States are *Shigella* sp., *Salmonella* sp., and *E. coli*. Shigellosis is unique among diarrheal diseases in that clinical improvement unques-

TABLE 1. *Multiple antimicrobial resistance patterns of Shigella strains-Houston, 1974*

Patterns of multiple resistance	Resistance (%)		
	S. boydii and *S. dysenteriae* (4 strains)	*S. sonnei* (113 strains)	*S. flexneri* (56 strains)
One drug or more	75	72	73
Two drugs or more	50	63	61
Two drugs only:	0	11	29
Te, Sm	0	9	27
Am, Sm	0	2	2
Three drugs only:	50	51	29
Am, Te, Sm	25	50	2
C, Te, Sm	25	1	27
Four drugs only:	0	1	4
C, Am, Te, Sm	0	1	4

tionably follows appropriate antimicrobial therapy (7). Antimicrobial therapy in shigellosis, although of benefit to the patient in decreasing the duration of diarrhea and the excretion of shigellae in the stool, has recently come into question due to the normally self-limiting nature of the illness and because of repeated emergence of antimicrobial-resistant strains of shigellae. Most strains reveal in vitro multiple resistance to one or more commonly used agents: Te, Am, Sm, C, and sulfonamides. Resistance patterns to these drugs have previously been shown to be plasmid-controlled, and often resistance to multiple drugs is carried by the same plasmid (3).

Our data confirm the high degree of antimicrobial resistance of 173 shigella strains isolated in Houston during 1974 but reveal 100% susceptibility to several new and promising agents. Quinoline drugs (cinoxacin, oxolinic acid, nalidixic acid) and trimethoprim-sulfamethoxazole inhibited the growth of all strains. Studies done by D. Preston of Lilly Research Laboratories (personal communication) show all shigellae susceptible to 2 to 4 μg of cinoxacin per ml, which corresponds to the 19-mm or greater zone size with the 30-μg disk. Studies to correlate the minimal inhibitory concentrations of shigellae with their response to the 100-μg disk of cinoxacin are presently in progress.

Of particular interest were the differing susceptibility patterns between *S. sonnei* and *S. flexneri*. Forty-five percent of *S. sonnei* strains were susceptible to Am, whereas 98% were susceptible to C. On the other hand, 93% of *S. flexneri* strains were susceptible to Am, yet were less frequently inhibited by C where 69% of strains were found to be susceptible. Although C might be an efficacious drug due to the increasing frequency of susceptible *S. sonnei* strains, resistance would likely develop with increasing usage of C, and it would be difficult to justify the use of a potentially toxic drug in a normally self-limiting infection. Data reported by Gordon et al. (5) on in vitro susceptibility studies with 213 strains of shigellae isolated in Michigan reveal resistance patterns comparable with ours, with 100% susceptibility to oxolinic acid and trimethoprim-sulfamethoxazole and 97% susceptibility to nalidixic acid.

In view of the degree of antimicrobial resistance noted to commonly used drugs in the treatment of shigellosis, new antimicrobial agents or combinations deserve appropriate in vivo trials. The quinoline drugs (oxolinic acid, nalidixic acid) as well as trimethoprim-sulfamethoxazole are effective in vitro (12); however, they have been approved by the Food and Drug Administration only for treatment of urinary tract infection and not for shigellosis. Cinoxacin is a newer quinoline drug and is as yet unlicensed for use. Isolated in vivo studies with the quinoline drugs (2, 6) and with the trimethoprim-sulfamethoxazole combination (4, 8, 10) show adequate clinical response when compared to Am or furazolidone.

Development of resistance has been reported to occur during therapy of urinary tract infections with nalidixic acid and to a lesser extent with oxolinic acid (1). The quinoline drugs do have, however, theoretical advantage over commonly used drugs in that antimicrobial resistance does not appear to be R factor mediated (9), which should limit the spread of resistance if such strains would emerge. Currently we are comparing the efficacy of oxolinic acid with that of Am in the treatment of bacillary dysentery in field studies being conducted in Guatemala and in Mexico.

The study reported here and others cited question the use of Am and Te as preliminary antimicrobial agents in the treatment of the patient with shigellosis and highlight the need for evaluation of new agents such as the quinoline drugs and trimethoprim-sulfamethoxazole in clinical field trials. If adequate field studies demonstrate in vivo effectiveness, one of these newer compounds should become the treatment of choice for bacillary dysentery.

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