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Sulfamethoxazole-Trimethoprim-Resistant *Shigella flexneri* in Northeastern Brazil

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In contrast to prior experience in northeastern Brazil, three of four *Shigella flexneri* strains recently isolated from patients with acute inflammatory diarrhea in this setting were found to be resistant to sulfamethoxazole-trimethoprim. The resistant strains contained large, different plasmids, two of which were transferred with sulfamethoxazole-trimethoprim resistance to *Escherichia coli* K-12 recipient strains.

Sulfamethoxazole-trimethoprim (SXT) resistance among members of the family *Enterobacteriaceae* has been recognized for a decade, but resistance in *Shigella* spp. has been found infrequently (2; R. M. Bannatyne et al., Lancet i:425–426, 1980 [letter]; D. E. Taylor et al., Lancet i:426, 1980 [letter]). SXT is freely available in Brazil and is a common ingredient in many over-the-counter antidiarrheal medications. Perhaps because of the selective pressure caused by increasing, widespread use of this antibiotic combination, doubly resistant coliforms have emerged in recent years (5, 6). In the course of studies of inflammatory diarrhea at the Hospital das Clinicas in Fortaleza, Brazil, during January and February 1982, we isolated and performed antibiotic susceptibility tests on four *Shigella flexneri* strains and found that three of them were highly resistant to SXT. Over the period 1978 to 1980, we isolated 13 *Shigella* strains from the same area, none of which were resistant to SXT. We also report here the isolation of different large plasmids from each of these SXT-resistant *Shigella* strains and the transfer of combined resistance with two of these plasmids to recipient strains.

The strains were tested for susceptibility to trimethoprim and sulfamethoxazole by agar dilution on Mueller-Hinton agar with 10% lysed horse blood by using a Steers replicator device (12). Susceptibility to other antibiotics was determined on Sensititre plates (GIBCO Diagnostics, Lawrence, Mass.).

Transfer of SXT resistance to two nalidixic acid-resistant *Escherichia coli* K-12 strains, 185Nx (prototroph, Nal^r) and 711 (*lac-28 his-51 trp-30 proC23 phe* Nal^r), was achieved by collecting equal volumes of early-log-phase cultures for matings on nitrocellulose filters (11). The mating mixture was plated onto Mueller-Hinton agar with 10% lysed horse blood containing nalidixic acid alone (25 μg/ml) and onto plates containing sulfamethoxazole (304 μg/ml), trimethoprim (16 μg/ml), and nalidixic acid (25 μg/ml) (13). Any transconjugant colonies which resulted from the matings were confirmed as *E. coli* by the API biochemical identification system (Analytab Products, Plainview, N.Y.). Plasmid DNA was isolated by lysing the bacteria by the procedure of Hansen and Olsen (3), precipitation with polyethylene glycol, and cesium chloride-ethidium bromide ultracentrifugation. Plasmid DNA was separated and characterized by agarose gel electrophoresis as described by Meyers et al. (8).

Three SXT-resistant *S. flexneri* strains isolated from individuals without any recognized epidemiological link from different areas of town at different times and one SXT-resistant *Klebsiella pneumoniae* strain, designated LP, were mated with SXT-susceptible *E. coli* K-12 strains to determine whether SXT resistance could be transferred. Two of the three *Shigella* strains, LP (*S. flexneri* 1b) and FG (*S.* flexneri 2a), transferred SXT resistance to *E. coli* 185Nx at a frequency of approximately 10^{-8} and 10^{-9} per recipient cell, respectively. One *S. flexneri* strain (LP) transferred resistance to *E. coli* 711 at a frequency of approximately 10^{-10} per recipient cell. The resistant *K. pneumoniae* strain (LP) transferred large plasmids (different from those in the *S. flexneri* strain) and SXT resistance to *E. coli* 185Nx at an approximate rate of 10^{-8} per recipient cell. Appropriate controls revealed no evidence of mutation in either the donor or recipient strains.

The three *S. flexneri* strains were resistant to SXT (MIC > 608 and 32 μg/ml respectively), sulfamethoxazole (MIC ≥ 152 μg/ml), trimethoprim (MIC = 500 to 1,000 μg/ml), chloramphenicol, and tetracycline. Two of these strains were resistant to ampicillin (MIC > 128 μg/ml) as well. Two SXT-resistant *Enterobacteriaceae* strains were also isolated from the stools of two patients with SXT-resistant *Shigella* infections (*K. pneumoniae* LP and *E. coli* FG), and one SXT-resistant *E. coli* strain (RL) was isolated from a patient with diarrhea caused by *Campylobacter jejuni*.

The plasmid DNA from the original three *S. flexneri* strains, the SXT-resistant fecal *E. coli* strains FG and RL, and the four transconjugant strains resulting from matings of *S. flexneri* and *K. pneumoniae* with *E. coli* was extracted and then characterized by agarose gel electrophoresis. *S. flexneri* FG contained a large plasmid with an approximate molecular weight of 46,000, designated pKF1, in addition to two other bands of lower molecular weight. Only the large plasmid was transferred to *E. coli* 185Nx, resulting in the formation of a transconjugant strain having the pKF1 plasmid and SXT resistance. The SXT-resistant strain *E. coli* FG contained two large plasmids of molecular weights different from that of the *S. flexneri* plasmid pKF1. *S. flexneri* LP contained a different large plasmid, designated pKF2, with an approximate molecular weight of 30,000, and two faster-migrating bands. Again, only the large plasmid, pKF2, was transferred to both *E. coli* recipient strains, resulting in the

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formation of two SXT-resistant transconjugants. Although we did not isolate plasmid DNA from *K. pneumoniae* LP, SXT resistance was transferred by conjugation from this strain to *E. coli* 185Nx and was associated with the acquisition by the transconjugant of at least two large plasmids. *S. flexneri* MG contained yet another large plasmid, approximately 58 megadaltons in size, which did not transfer to the recipient strains, and there were also several other rapidly migrating bands. Two large plasmids, one of which was similar in size to pKF1, were recovered from the SXT-resistant *E. coli* strain RL isolated from a patient with diarrhea caused by *C. jejuni*.

Comparison of *Eco*RI and *Hin*dIII restriction patterns of total plasmid DNA, with two to nine fragments each, showed that there was no overall similarity among the large plasmids recovered from the SXT-resistant *S. flexneri* strains, but that the fragments corresponded precisely to those of the plasmids from each of the transconjugant strains. The small plasmids were not cleaved by the enzymes we used. Therefore, we could make a direct comparison of the fragments from the large plasmids in the original strains with those in the transconjugants. Thus, SXT resistance is associated with different plasmids in a number of different *Enterobacteriaceae* strains as well as in the *Shigella* strains. Furthermore, at least three different plasmids were transferred with SXT resistance to recipient strains in vitro.

Resistance to trimethoprim is becoming widespread, and this threatens the effectiveness of trimethoprim and SXT against infections such as shigellosis and typhoid fever. Particularly widespread amoung *Salmonella* spp. (perhaps related to the use of trimethoprim in treating animals) (7, 9, 10, 13), cotransferable SXT resistance is now being recognized among *Shigella* species from diverse areas (2; Bannatyne et al., letter; Taylor et al., letter) and is documented again here with three different plasmids in various species.

Shigella species frequently cause inflammatory diarrhea characterized by fever, tenesmus, and bloody, mucoid stools, with numerous polymorphonuclear neutrophils (4). Appropriate therapy with an absorbable agent to which the organism is susceptible in vitro results in rapid resolution of symptoms, including diarrhea, fever, and abdominal cramps (1, 14). The recent emergence of ampicillin- and now SXT-resistant strains may preclude effective therapy with these agents.

The transferable SXT resistance of the two *S. flexneri* and one *K. pneumoniae* strain was associated with three plasmids of different sizes. Therefore, this SXT resistance does not originate from a single promiscuous plasmid. Furthermore, the association of SXT resistance with more than one plasmid and the different *S. flexneri* subspecies involved indicate that this is not a single-source outbreak and that the problem may be more widespread than is currently recognized.

SXT resistance was cotransferred with both ampicillin and chloramphenicol resistance from *S. flexneri* FG to its transconjugant and was associated with the transfer of a single large plasmid. This finding raises still further concern about the continued efficacy of these widely used agents for shigellosis and typhoid fever.

Although we have not documented clinical failure or SXT in these patients; the appearance of *Shigella* spp. with multiple resistance capable of intergeneric transfer raises concerns about appropriate antimicrobial therapy for shigellosis and other enteric infections in this setting. These data also suggest that multiply resistant pathogens may be increasing in frequency and may pose clinical problems, especially in regions where over-the-counter antibiotics are in common use.

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