

Third-Generation Cephalosporin Resistance in *Shigella sonnei*, Argentina

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Shigella sonnei resistant to cefotaxime (but not to ceftazidime) was isolated for the first time in stool samples from a pediatric patient with vomiting and bloody diarrhea in northern Argentina. Microbiologic and biochemical tests confirmed the presence of an extended spectrum beta-lactamase displaying an apparent isoelectric point value of 8.2.

Shigellosis is an important public health problem, especially in developing countries. Antibiotic treatment of bacterial dysentery, aimed at resolving diarrhea or reducing its duration, is especially indicated whenever malnutrition is involved. First-line drugs include ampicillin and trimethoprim-sulfamethoxazole (TMP-SMX); however, multidrug-resistant (i.e., resistant to ampicillin, TMP-SMX, and chloramphenicol) *Shigella* isolates are ubiquitous (1-3). When epidemiologic data indicate a rise in resistance, fluoroquinolones may be used in adults and oral third-generation cephalosporins in children. Except for a single strain in Calcutta (4), *Shigella* have not obtained any extended-spectrum beta-lactamase (ESBL)(4), even though multidrug-resistance plasmids were described in this genus as early as the late 1950s (5,6).

A new family of ESBLs, displaying greater affinity for cefotaxime than ceftazidime (CTX-M), has appeared in various countries. In Argentina, CTX-M-2 is the most prevalent ESBL (7) and has been reported in several enterobacteria (8-10). Although very common in nosocomial pathogens, it has also been recovered from other enteric microorganisms (11). We describe our analysis of the first *Shigella sonnei* isolate resistant to cefotaxime (CTX) but not to ceftazidime.

The Study

The isolate was from stool samples from a 6-month-old girl, seen in the outpatient clinic of Hospital SAMIC Oberá in northern Argentina. The child had vomiting and laboratory-confirmed bloody diarrhea approximately 20 days after a 1-week hospital stay for diarrhea and primary malnutrition. During the hospital stay, she received gentamicin for 1 week, but no bacterial enteropathogen was isolated.

The child lived in Oberá, a subtropical area in Misiones that has no running water. In this region, *Shigella* spp. is three times more prevalent than *Salmonella* spp. and other enteropathogens as the cause of pediatric diarrhea, and *S. sonnei* resistance to ampicillin and TMP-SMX is approximately 43% and 74%, respectively.

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S. sonnei was isolated on eosin-methylene blue agar plates. The isolate did not produce gas and was hydrogen sulfide-negative and nonmotile. In triple sugar iron agar, it was negative for citrate, phenylalanine deaminase, and indol production. It was methyl-red positive, Voges-Proskauer negative, ornithine decarboxylase positive, arginine dihydrolyase negative, lysine decarboxylase negative, and urease (Christensen) negative. (All media were from Britania, Argentina.) Confirmatory serotyping was carried out with antisera from the Instituto Nacional de Microbiología Dr. Carlos Malbran.

Confirmatory susceptibility tests followed conventional methods (12,13). Briefly, MICs were determined by the agar dilution method, using Mueller-Hinton agar (Britania) and inoculums of 10⁴ CFU per spot; plates were incubated 18 hours at 35°C. *Escherichia coli* ATCC 25922 and *E. coli* ATCC 35218 were included as quality controls. Antibiotics tested were ampicillin, ampicillin + clavulanate (CLA), cefoxitin, cefotaxime (CTX), CTX + CLA (CTX/CLA), ceftazidime, and ceftazidime + CLA (ceftazidime/CLA); a fixed concentration of 4 µg/mL lithium CLA was used when combined with beta-lactam drugs. Antimicrobial drugs were provided by Argentia, Argentina (Ampicillin, CTX), Sigma Chemical Co., St. Louis, MO, USA (ceftazidime), Roemmers, Argentina (CLA) and Merck Sharp & Dohme, Argentina (cefoxitin). MICs were as follows: ampicillin, >1,024 µg/mL; ampicillin + CLA 16 µg/mL; cefoxitin, 2 µg/mL; CTX, 16 µg/mL; CTX/CLA, ≤ 0.063 µg/mL; ceftazidime, 1 µg/mL; and ceftazidime/CLA, ≤ 0.063 µg/mL.

Resistance to gentamicin, amikacin, and TMP-SMX was detected by agar diffusion (13); quality controls also included *E. faecalis* ATCC 29212.

Conclusions

The presence of an ESBL was confirmed by microbiologic and biochemical tests using different third-generation cephalosporins as substrates for the enzymes present in bacterial sonicates and an iodometric detection system (9). A microbiologic confirmation test for ESBLs was performed according to recommendations of the National Committee for Clinical Laboratory Standards for *E. coli* and *Klebsiella* spp. (13). After isoelectric focusing (9), crude bacterial extracts rendered two bands that hydrolyzed 500 µg/mL ampicillin

(isoelectric points 5.4 and 8.2), the latter also active on 1,000 µg/mL ceftriaxone. As this enzyme was likely CTX-M-2 (the band comigrated with authentic CTX-M-2 samples) and the first probably corresponded to TEM-1 (or another related enzyme), plasmid DNA obtained by the method of Birnboim and Doly (14) was used as the template for polymerase chain reaction amplification, with specific primers for CTX-M-2 (*bla*_{CTX-M-2} I: 5'-TTAATGATGACTCAGAGCATTC-3'; *bla*_{CTX-M-2} II: 5'-GATACCTCGCTCCATTTATTG-3') and TEM-1 (*bla*_{TEM-1} I: 5'-ATAAAATTCTTGAAGACGAAA-3'; *bla*_{TEM-1} II 5'-GACAGTTACCAATGCTTAATCA-3'). Two fragments of 0.9 kbp and 1.2 kbp were obtained; they showed 100% agreement with theoretical and experimentally obtained fragments from bona fide CTX-M-2 and TEM-1 producing strains, respectively (8,10).

No resistant *Shigella* was isolated from the other 124 pediatric patients admitted that month for nonsurgical (85 patients) or surgical reasons (29) nor was a nosocomial outbreak caused by a third-generation cephalosporin-resistant enterobacteria detected. The patient recovered. The isolated microorganism was not the likely cause for the patient's first hospitalization as it could not be isolated at that time. Likely alternatives for its acquisition are 1) intestinal selection or acquisition of a resistant enterobacterium and in vivo transference to a freshly acquired *Shigella* or 2) direct acquisition of the resistant strain from contaminated water (not sustained, as no other resistant *Shigella* was obtained from the same community in the following year).

These findings, which may portend the spread of serious resistance in *Shigella* throughout Argentina and beyond, suggest the need for susceptibility testing of all *Shigella* spp. whenever financially feasible.

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References

1. Mates A, Eyny D, Philo S. Antimicrobial resistance trends in *Shigella* serogroups isolated in Israel, 1990-1995. *Eur J Clin Microbiol Infect Dis* 2000;19:108-11.
2. Prats G, Mirelis B, Llovet T, Muñoz C, Miro E, Navarro F. Antibiotics resistance trends in enteropathogenic bacteria isolated in 1985-1987 and 1995-1998 in Barcelona. *Antimicrob Agents Chemother* 2000;44:1140-5.
3. Replogle ML, Fleming DW, Cieslak PR. Emergence of antimicrobial-resistant shigellosis in Oregon. *Clin Infect Dis* 2000;30:515-9.
4. Ahamed J, Kundu M. Molecular characterization of the SHV-11 β-lactamase of *Shigella dysenteriae*. *Antimicrob Agents Chemother* 1999;43:2081-3.
5. Watanabe T, Fukasawa T. Episome-mediated transfer of drug resistance in *Enterobacteriaceae*. *J Bacteriol* 1960;81:669-78.
6. Akiba T, Koyama T, Isshiki Y, Kimura S, Fukushima T. Studies on the mechanism of development of multiple drug-resistant *Shigella* strains. *Nihon Iji Shimpo* 1960;1866:45-50.
7. Radice M. Mecanismos de resistencia a antibióticos β-lactámicos: Análisis de las β-lactamasas de espectro expandido en *Escherichia coli* [dissertation]. Buenos Aires: University of Buenos Aires; 1998.
8. Bauernfeind A, Casellas JM, Goldberg M, Holley M, Jungwirth R, Mangold P, et al. A new plasmidic cefotaximase from patients infected with *Salmonella typhimurium*. *Infection* 1992;20:158-63.
9. Rossi A, Lopardo H, Woloj M, Picandet AM, Mariño M, Galas M, et al. Non-typhoid *Salmonella* spp. resistant to cefotaxime. *J Antimicrob Chemother* 1995;36:697-702.
10. Power P, Radice M, Barberis C, de Mier C, Mollerach M, Maltagliatti M, et al. Cefotaxime-hydrolysing β-lactamases in *Morganella morganii*. *Eur J Clin Microbiol Infect Dis* 1999;18:743-7.
11. Rossi A, Galas M, Binztein N, Rivas M, Caffer MI, Corso A, et al. Unusual multiresistant *Vibrio cholerae* O1 El Tor in Argentina. *Lancet* 1993;342:1172-3.
12. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard M7-A4, 4th edition, Vol. 19, No. 1. Wayne (PA): NCCLS; 1999.
13. National Committee for Clinical Laboratory Standards. Zone diameter interpretative standards and equivalent minimum inhibitory concentration (MIC) breakpoints for *Enterobacteriaceae*. Approved Standard M-2-A6, 6th edition, Vol. 19, No. 1. Wayne (PA): NCCLS; 1999.
14. Birnboim HC, Doly J. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res* 1979;7:1513.