Multidrug-Resistant Shigella Infections in Patients with Diarrhea, Cambodia, 2014–2015

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We observed multidrug resistance in 10 (91%) of 11 Shigella isolates from a diarrheal surveillance study in Cambodia. One isolate was resistant to fluoroquinolones and cephalosporins and showed decreased susceptibility to azithromycin. We found mutations in gyrA, parC, β-lactamase, and mphA genes. Multidrug resistance increases concern about shigellosis treatment options.

Shigellta is a major public health problem in developing countries. Antimicrobial therapy with fluoroquinolones is recommended to shorten the course of disease and fecal shedding. However, limitations on shigellosis treatment options have been a concern since 1993, when ciprofloxacin-resistant Shigella was documented (1), followed by reports of multidrug-resistant (MDR) Shigella and of Shigella that harbored extended-spectrum β-lactamase (ESBL) genes (2). We describe MDR Shigella isolated from patients with diarrhea in Cambodia during 2014–2015.

The Study

During July 2014–April 2015, we examined stool specimens collected from patients 3 months–5 years of age and 18–60 years of age who were seen for or admitted with acute diarrhea at 3 healthcare settings in Battambang, Cambodia, as part of ongoing hospital-based surveillance of diarrheal etiology. Stool specimens were processed for identification of enteric pathogens by standard microbiology, ELISA, and PCR. Shigella species were identified by standard biochemical tests and the API 20E system (bioMérieux, Marcy l’Étoile, France) and serotyped by commercial antisera (Denka Seiken Co, Ltd., Tokyo, Japan). Antimicrobial drug susceptibility testing was performed with the standard Kirby-Bauer disk diffusion method by using commercially available antimicrobial disks (Becton Dickinson, Franklin Lakes, NJ, USA). Antimicrobial drugs tested for susceptibility were ampicillin, azithromycin (AZM), cefotaxime (CTX), ceftazidime (CAZ), ceftriaxone (CRO), ciprofloxacin (CIP), nalidixic acid (NAL), tetracycline, and trimethoprim/sulfamethoxazole. Susceptibility results were interpreted according to Clinical and Laboratory Standards Institute guidelines (3). We used zone diameter interpretive standards for Enterobacteriaceae for all antimicrobial drugs tested, except AZM, for which we applied the standard for Staphylococcus spp. Shigella spp. were isolated from 11 (5%) of 212 diarrhea stool samples. Antimicrobial drug susceptibility testing showed that 10 (91%) of the 11 Shigella isolates were resistant to ampicillin, tetracycline, trimethoprim/sulfamethoxazole, and NAL. We selected the 10 MDR isolates for further characterization and determined MICs of AZM and CIP by Etest (bioMérieux). ESBL production was tested by usingNEG Combi Panel Type 50 on the MicroScan WalkAway plus System (Siemens Healthcare Diagnostics, Newark, DE, USA). PCR and sequencing were used to characterize resistance genes (gyrA and parC) in the quinolone-resistance determining region (QRDR), the AZM resistance gene (mphA), and β-lactamase genes (4–7).

Of the 10 MDR isolates, 2 were S. flexneri 2a; 1 was an S. flexneri 2 variant; 6 were S. flexneri 3a; and 1 was S. sonnei (Table 1). CIP resistance was detected in 5 (50%) of the 10 isolates. Sequence analysis showed mutations of gyrA and parC genes with the amino acid substitutions in the QRDR (Table 2). All NAL-resistant isolates susceptible to CIP had a single mutation in gyrA. Isolates resistant to both NAL and CIP contained multiple mutations in gyrA and parC.

The most common mechanism of quinolone resistance in the Shigella spp. was mutation of gyrA, typically at codon 83 or 87, and of parC at codon 80 (7). All isolates in our study had the common mutation in gyrA at position 83 (Ser83→Leu); 1 isolate had another common mutation at position 87 (Asp87→Gly). A mutation in parC at position 80 (Ser80→Ile), detected in the S. sonnei isolate, was previously reported in an S. dysenteriae serotype 1 isolate in India (7) and in Asia travel-associated S. sonnei and S. flexneri isolates in the United States (8). A mutation at position 57 (Ser57→Arg) was detected in all 4 CIP-resistant S. flexneri 3a isolates, but this mutation’s role in CIP resistance is unclear because position 57 is outside the QRDR region. Characterization of plasmid-mediated quinolone resistance (PMQR) genes should be further investigated because...
coexistence of mutations in the QRDR and PMQR genes has been reported in Shigella isolates with decreased susceptibility to fluoroquinolones (8). PMQR may facilitate the selection of QRDR mutations, resulting in higher levels of quinolone resistance.

No clinical breakpoints for AZM have been clearly defined for Shigella spp., but CDC’s National Antimicrobial Resistance Monitoring System for Enteric Bacteria (http://www.cdc.gov/narms/index.html) recommends using the term “decreased susceptibility” for reporting. We detected decreased susceptibility to AZM in S. flexneri 3a (isolate no. 9) with a MIC of 32 µg/mL. This isolate was found to carry the mphA gene encoding a macrolide 2′-phosphotransferase that inactivates macrolide antimicrobial drugs and has been reported to reduce AZM susceptibility in Shigella isolates (5). Emergence of decreased susceptibility to AZM may affect treatment options for shigellosis, especially for pediatric cases because ceftriaxone is administered parenterally by injection and fluoroquinolones are not encouraged for use in children.

We detected ≥1 β-lactamase gene in all 10 Shigella isolates; 2 isolates that were resistant to cephalosporins revealed ESBL production (Table 2). The 8 isolates that carried β-lactamase–producing genes TEM-1 or TEM-1 and OXA-1 were cephalosporin susceptible, suggesting that TEM-1 and OXA-1 may not play a role in increased resistance to third-generation cephalosporins. Of the remaining 2 isolates, 1 S. flexneri (isolate no. 9), which harbored CTX-M-27 and TEM-1, showed resistance to CRO and CTX but not to CAZ, and 1 S. sonnei (isolate no. 10), which carried CTX-M-55, was resistant to all cephalosporins tested.

### Table 1. Epidemiologic data of patients with multidrug-resistant Shigella, Cambodia, July 2014–April 2015

| Isolate no. | Organism | Isolate collection date | Patient age | Patient sex | Antimicrobial drugs taken before enrollment |
|-------------|----------|-------------------------|-------------|-------------|--------------------------------------------|
| 1           | S. flexneri 2a | 2015 Apr 3             | 3 y         | M           | No                                         |
| 2           | S. flexneri 2a | 2015 Apr 28            | 18 mo       | M           | No                                         |
| 3           | S. flexneri 2v | 2015 Apr 20            | 1 y         | F           | No                                         |
| 4           | S. flexneri 3a | 2015 Jan 22            | 1 y         | M           | Yes†                                      |
| 5           | S. flexneri 3a | 2015 Feb 20            | 6 mo        | M           | No                                         |
| 6           | S. flexneri 3a | 2014 Nov 17            | 4 y         | M           | No                                         |
| 7           | S. flexneri 3a | 2014 Nov 21            | 3 y         | M           | No                                         |
| 8           | S. flexneri 3a | 2014 Dec 22            | 2 y         | M           | No                                         |
| 9           | S. flexneri 3a | 2015 Feb 21            | 3 y         | M           | No                                         |
| 10          | S. sonnei   | 2015 Mar 11            | 2 y         | M           | No                                         |

*Unknown type of drug taken 1 time.
†250 mg amoxicillin taken for 3 d.

### Table 2. Antimicrobial susceptibility results and molecular characterization of resistance genes of Shigella isolates collected from patients in Cambodia, July 2014–April 2015

| Isolate no. | Organism | Antimicrobial resistance | CIP MIC, µg/mL† | Amino acid substitutions in QRDR | AZM MIC, µg/mL‡ | mphpA gene | ESBL confirmatory test | β-lactamase genes |
|-------------|----------|--------------------------|----------------|---------------------------------|----------------|-------------|-----------------------|-------------------|
| 1           | S. flexneri 2a | AMP-SXT-TET-NAL | 0.25 | Leu – – – – | 2.00 | Neg | Neg | TEM-1, TEM-1, OXA-1, OXA-1 |
| 2           | S. flexneri 2a | AMP-SXT-TET-NAL | 0.25 | Leu – – – – | 1.50 | Neg | Neg | TEM-1, TEM-1, OXA-1, OXA-1 |
| 3           | S. flexneri 2v | AMP-SXT-TET-NAL | 0.19 | Leu – – – – | 1.50 | Neg | Neg | TEM-1 |
| 4           | S. flexneri 3a | AMP-SXT-TET-NAL | 0.25 | Leu – – – – | 1.00 | Neg | Neg | TEM-1 |
| 5           | S. flexneri 3a | AMP-SXT-TET-NAL | 0.19 | Leu – – – – | 1.50 | Neg | Neg | TEM-1 |
| 6           | S. flexneri 3a | AMP-SXT-TET-NAL-CIP | 4.00 | Leu – Arg – | 0.75 | Neg | Neg | TEM-1 |
| 7           | S. flexneri 3a | AMP-SXT-TET-NAL-CIP | 4.00 | Leu – Arg – | 1.00 | Neg | Neg | TEM-1 |
| 8           | S. flexneri 3a | AMP-SXT-TET-NAL-CIP | 4.00 | Leu – Arg – | 1.00 | Neg | Neg | TEM-1 |
| 9           | S. flexneri 3a | AMP-SXT-TET-NAL-CIP-TEM-1 | 6.00 | Leu – Arg – | 32.00 | Pos | Pos | TEM-1, CTX-M-27 |
| 10          | S. sonnei   | AMP-SXT-TET-NAL-CIP-CTX-CAZ | 6.00 | Leu Gly Ile | 4.00 | Neg | Pos | CTX-M-55 |

*AMP, ampicillin; Arg, arginine; Asp, aspartate; AZM, azithromycin; CAZ, ceftazidime; CIP, ciprofloxacin; CRO, ceftriaxone; CTX, cefotaxime; ESBL, extended-spectrum β-lactamase; Gly, glycine; Ile, isoleucine; Leu, leucine; NAL, nalidixic acid; Neg, negative; Pos, positive; QRDR, quinolone-resistance determining region; Ser, serine; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; –, no amino acid substitutions found.
†CIP MIC interpretive criteria for Enterobacteriaceae is susceptible ≤1, resistant ≥4 µg/mL.
‡AZM MIC interpretive criteria for Salmonella enterica serovar Typhi is susceptible ≤16, resistant ≥32 µg/mL.
A key element that increased ceftazidimase activity was a single amino acid substitution from Asp to Gly at position 240; this substitution was identified in CTX-M-15, CTX-M-16, CTX-M-27, and CTX-M-32. CTX-M-27, first reported from France in 2003, differed from its parental enzyme, CTX-M-14, by substitution of Asp240Gly (9). Reports suggest that this Gly–240–harboring CTX-M-27 confers higher levels of resistance to CAZ in Escherichia coli infections, but we did not detect this characteristic in the Shigella isolates we examined. CTX-M-55 was first reported in ESBL-producing E. coli and Klebsiella pneumoniae isolates in Thailand in 2007; it was associated with high resistance to CRO, CTX, and CAZ (10) and was subsequently reported in other Asia countries, including Cambodia. Among fecal samples collected from children in Cambodia, 88% carried E. coli harboring ESBL genes containing blaCTX-M variants, including CTX-M-15, CTX-M-55, and CTX-M-14 (11). A case of ESBL-producing S. sonnei harboring CTX-M-55 was also reported in a woman traveling from Korea to China (12).

The CDC Health Alert Network has distributed a health advisory on CIP- and AZM-nonsusceptible Shigella infection in the United States (13). Three separate outbreaks of MDR shigellosis among men who have sex with men, international travelers, and children in daycare centers have been reported (13). We found 2 ESBL-producing, fluoroquinolone-resistant Shigella isolates. Moreover, S. flexneri 3a (isolate no. 9), which had decreased susceptibility to AZM, was also resistant to nearly all oral and parenteral drugs considered for shigellosis treatment. This isolate can ferment sorbitol, a feature found in 7% of Shigella spp. and possibly causing misidentification of Shigella spp. as other species (14). S. sonnei (isolate no. 10) belongs to biotype g (i.e., with biochemical reactions ONPG+ [α-nitrophenyl-β-D-galactopyranoside], rhamnose–, and xylose–), which has been shown to carry integrons with multiple gene cassettes, leading to multidrug resistance (15).

Conclusions

MDR Shigella is an emerging problem that raises concerns about shigellosis treatment worldwide, including in Cambodia. Health authorities should implement systematic surveillance of antimicrobial drug resistance and controlled antimicrobial drug use to increase understanding of the problem and minimize unnecessary antimicrobial drug use, which contributes to increased resistance.

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