Ciprofloxacin-Resistant Shigella sonnei among Men Who Have Sex with Men, Canada, 2010

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In 2010, we observed isolates with matching pulsed-field gel electrophoresis patterns from 13 cases of ciprofloxacin-resistant Shigella sonnei in Montréal. We report on the emergence of this resistance type and a study of resistance mechanisms. The investigation suggested local transmission among men who have sex with men associated with sex venues.

Shigella spp. are enteropathogen bacteria that are transmitted person-to-person and require a low infectious inoculum (1). Fluoroquinolones are among the first-choice antimicrobial drugs for treatment of Shigella spp. infections in adults (1), but resistance to these agents has been documented, primarily in Asia (2). Among men who have sex with men (MSM), Shigella spp. infection is, in most cases, sexually transmitted, and clusters are regularly reported (3–5). We investigated an outbreak of ciprofloxacin-resistant Shigella sonnei among MSM and studied its resistance mechanisms.

The Study

Laboratories report shigellosis to the Montreal public health department (Québec, Canada). When a cluster is suspected, isolates are sent to the provincial laboratory to conduct pulsed-field gel electrophoresis (PFGE) to identify links between patients.

In July 2010, microbiology services at the Hôpital Saint-Luc alerted the public health department to S. sonnei resistant to ciprofloxacin and trimethoprim/sulfamethoxazole and susceptible to ampicillin. The S. sonnei had been isolated 2 days apart from stool cultures of 2 HIV-positive MSM.

Public health officials sent a notice to physicians, clinics, and laboratories in Montréal to report the presence of ciprofloxacin-resistant S. sonnei among MSM and to describe the antimicrobial treatment with ampicillin or azithromycin, procedures for case reporting, and preventive measures (6). Confirmed cases were defined as infection by S. sonnei with resistance to ciprofloxacin and trimethoprim/sulfamethoxazole and susceptibility to ampicillin (later specified as pulsovar 72). Probable cases were defined as infection by S. sonnei with a resistance profile identical to that of confirmed cases but where PFGE was not conducted.

Retrospective searching of the notifiable disease database found ciprofloxacin-resistant S. sonnei with a different PFGE pattern that had been isolated in February 2010 from a female patient who had traveled to a country where shigellosis is highly prevalent. Hence, this case was not from this outbreak. The provincial laboratory searched their records to identify cases elsewhere in Québec. During June–October 2010, nine confirmed cases and 4 probable cases were identified in Montréal and the surrounding regions (Table 1). Most patients had an onset date from the end of June to mid-July 2010 (Figure 1). All 13 patients were interviewed. Most patients were men (11/13; 85%) with a mean age of 40 years (range 20–65 years). All male patients were MSM, and 4 (36%) of 11 reported being HIV positive. Travel to a European country during August 2010 was mentioned by 1 MSM patient. Eight (73%) of 11 MSM patients mentioned participation in anal sex or contact during the exposure period. The use of sex venues was indicated by 4/11 MSM patients, and 3 mentioned a common sex venue. In addition, 1 other MSM patient reported that his sex partners frequented the common sex venue. This suggests that unprotected anal sex, associated with local sex venues, was the primary mode of transmission.

Two female patients (45 and 50 years of age) were reported. S. sonnei was detected in a food sample from a restaurant where 1 female patient ate during the exposure period, but the isolate did not match the outbreak PFGE pattern and was not related to any known human patients. No epidemiologic links between the female and male patients could be identified.

The public health interventions included a weekly analysis of incident shigellosis infections, resistance profiles, and risk factors. Given the preponderance of infections among MSM visiting sex venues, kits of condoms, soap, and information on prevention were distributed.
at sex venues in August 2010 (3,6). Community-based organizations that work with MSM living with HIV/AIDS were contacted to disseminate information on preventive measures. As a potential effect, few cases were declared in September, although sporadic cases continued to appear until October 2010.

The resistance profile investigation identified 14 S. sonnei isolates from 13 patients by using commercial biochemical kit tests. Identification of S. sonnei from 9 patients was confirmed at the provincial laboratory. Antimicrobial susceptibility testing was done by agar dilution or disk diffusion method (7), Vitek 2 (bioMérieux, Marcy l’Étoile, France), or Etest (AB Biodisk, Solna, Sweden) (ampicillin, trimethoprim/sulfamethoxazole, and ciprofloxacin) for 14 isolates and by Etest (AB Biodisk) (azithromycin, cefotaxime, and tetracycline) and with nalidixic acid (30-μg disk) for 7 or 8 isolates. The susceptibility of S. sonnei isolates to antimicrobial agents is reported in Tables 1 and 2.

PFGE was done by the provincial laboratory according to international standards set by the US Centers for Disease Control and Prevention (8). The XbaI and BlnI patterns were interpreted using the standards of Tenover et al. (9). The Salmonella enterica serotype Braenderup strain (H9812) was used as the size marker in each gel (10). Band position tolerances and optimization values of 1% were used for all analyses. Similarity coefficient was obtained within BioNumerics (www.applied-maths.com/bionumerics/bionumerics.htm) by calculating Dice coefficients. Cluster analysis was done by using with the unweighted pair group method with arithmetic averages. The S. sonnei isolates from 9 patients for whom typing was done were indistinguishable for the 2 enzymes (Figure 2). PulseNet Canada accession numbers for the isolate from our study are SSOXAI.0067 and SSOBNI.0040 for the XbaI and BlnI patterns, respectively.

For the study of the mechanisms of drug resistance, bacterial DNA was extracted using MasterPure Complete DNA Purification Kit (Epicenter Biotechnologies, Madison, WI, USA). The gyrA and parC genes were analyzed by direct DNA sequencing procedures as described (11) on an ABI Prism Genetic Analyzer 3130xl (Applied Biosystems, Foster City, CA) and then compared with the nucleotide sequence of gyrA and parC from a susceptible S. sonnei strain (Genbank accession no. M80077). The gyrA and parC genes were amplified by PCR using the primers described in (11). The primers for gyrA were forward 5′-GGGATTACCTTCAAGATGGG-3′ and reverse 5′-GGCTTATTGCTGAGGCAGTT-3′. The primers for parC were forward 5′-GCTGGATAACTTGCGTGGG-3′ and reverse 5′-AAGCCTGCTTGGATGACAT-3′. The amplified products were sequenced by the Sanger method. The tetramers for gyra and parC were chosen as described in (11).

Table 1. Characteristics of 13 cases of ciprofloxacin-resistant Shigella sonnei and results of susceptibility testing, Montreal, Québec, Canada, June–October 2010*

| Patient ID | Patient age, y/sex | Stool sample date | Patient’s signs and symptoms | Antimicrobial agent | PFGE group |
|------------|--------------------|-------------------|-------------------------------|--------------------|------------|
|            |                    |                   |                               | Amp               | TMP/SMX    | Cip        |              |
| 1          | 52/M               | Jun 24            | Diarrhea                      | S R R R | 72         |
| 2          | 48/M               | Jun 26            | Nausea, vomiting, diarrhea, blood in stools, abdominal pain | S R R R | NT         |
| 3          | 22/M               | Jun 28            | Nausea, diarrhea, abdominal pain, fever | S R R | NT         |
| 4          | 38/M               | Jul 5             | Nausea, vomiting, diarrhea, abdominal pain, fever, fatigue | S R R | 72         |
| 5          | 52/M               | Jul 4             | Diarrhea, abdominal pain, fever | S R R | 72         |
| 6          | 32/M               | Jul 14            | Diarrhea                      | S R R R | NT         |
| 7          | 50/F               | Jul 11            | Nausea, diarrhea, blood in stools, abdominal pain, fever | S R R | 72         |
| 8          | 45/F               | Jul 14            | Diarrhea, blood in stools, abdominal pain | S R R R | 72         |
| 9          | 25/M               | Aug 30            | Diarrhea, blood in stools, abdominal pain | S R R | NT         |
| 10         | 50/M               | Sep 5             | Vomiting, diarrhea, abdominal pain, fever | S R R | 72         |
| 11         | 65/M               | Sep 8             | Nausea, vomiting, diarrhea, abdominal pain | S R R | 72         |
| 12         | 38/M               | Sep 23            | Diarrhea                      | S R R R | 72         |
| 13         | 20/M               | Oct 22            | Diarrhea, blood in stools, abdominal pain, fever | S R R | 72         |

*ID, identification; Amp, ampicillin; TMP/SMX, trimethoprim/sulfamethoxazole; Cip, ciprofloxacin; PFGE, pulsed-field gel electrophoresis; S, sensitive; R, resistant; NT, not typed.
Conclusions

Sporadic ciprofloxacin-resistant *Shigella* has been infrequently documented (2,12–14). In India, ciprofloxacin-resistant *S. dysenteriae, S. flexneri,* and *S. boydii* have been isolated since 2002, and their fluoroquinolone-resistant mechanisms have been determined (2). Ciprofloxacin-resistant *Shigella* remains rare and was found among 0.2% of isolates in the United States (2000–2009) (12) and 0.5% of isolates in Canada (1997–2000) (13). In the United States, 10 ciprofloxacin-resistant *Shigella* spp. isolates (6 *S. flexneri,* 3 *S. sonnei,* and 1 *Shigella* spp.) were documented by the National Antimicrobial Resistance Monitoring System (2000–2009) (12). In New York, NY, in 2006, ciprofloxacin resistance was detected among 4 *S. sonnei* and 1 *S. flexneri* acquired locally (14). In 2010 in South Carolina, ciprofloxacin-resistant *Shigella flexneri* 2a was isolated from 3 patients (15).

We report the suspected transmission of ciprofloxacin-resistant *S. sonnei,* among MSM in Montreal, Quebec. Some authors suggest the antimicrobial drug treatment of all patients infected with *Shigella* spp. (1), but others disagree with this recommendation (14). It is essential that physicians request bacterial stool cultures when *Shigella* spp. enteric infection is suspected in MSM even without blood in stools or fever.

Acknowledgments

We thank Michèle Tremblay, Elisabeth Lacombe, Patricia Hudson, Sylvie Habash, Suzanne Rajotte, Michel Poisson, Claude Lemioux, M.-D. Baptiste-Desruisseaux, Johanne Ismail, the medical microbiologists from Clinique l’Actuel and the hospitals Verdun and Maisonneuve-Rosemont, Pierre-Boucher, and Jean-Talon for their assistance.

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