

**Shigella spp. with Reduced Azithromycin Susceptibility, Quebec, Canada, 2012–2013**

Christiane Gaudreau, Sapha Barkati, Jean-Michel Leduc, Pierre A. Pilon, Julie Favreau, and Sadjia Bekal

During 2012–2013 in Montreal, Canada, 4 locally acquired *Shigella* spp. pulse types with the mph(A) gene and reduced susceptibility to azithromycin were identified from 9 men who have sex with men, 7 of whom were HIV infected. Counseling about prevention of enteric sexually transmitted infections might help slow transmission of these organisms.

*Shigella* spp. are transmitted directly from person to person or indirectly by low-inoculum infection (1). Among men who have sex with men (MSM), *Shigella* spp. are mostly transmitted sexually; clusters of such cases have been documented in Montreal and surrounding neighborhoods (2,3). Azithromycin is an alternative treatment for multidrug-resistant *Shigella* spp. infections in adults and children, but routine testing for azithromycin susceptibility is not yet standardized and recommended (1,4–6). In the United States, azithromycin MICs for 392 wild-type *Shigella* strains isolated in 2005–2006 were estimated to be 4–16 mg/L; the azithromycin MIC for 90% of the isolates was 8 mg/L (7).

**The Study**

In December 2012, the microbiology laboratory of the Centre Hospitalier de l’Université de Montréal–Hôpital Saint-Luc identified *Shigella* spp. with reduced susceptibility to azithromycin from 2 patients who had received this agent as treatment for shigellosis. The Montréal Public Health Department and Laboratoire de Santé Publique du Québec (LSPQ) were alerted. Retrospective and prospective laboratory surveillance was initiated to cover the period January 2011–April 2013. Laboratories routinely report shigellosis to the Montreal Public Health Department (Quebec, Canada).

Phenotypic identification of all *Shigella* spp. at the genus and species levels (8) was confirmed at LSPQ as described (9), after which serologic identification by slide agglutination (Denka Seiken Co., Ltd, Coventry, UK) was performed. Pulsed-field gel electrophoresis (PFGE) was performed at LSPQ according to international standards set by the US Centers for Disease Control and Prevention (10). Pulse types were determined by *Shigella* species, serotypes, and PFGE patterns. All *Shigella* spp. isolated during 2011–2013 underwent susceptibility testing for ampicillin, trimethoprim/sulfamethoxazole, and ceftriaxone by use of Vitek 2 (bioMérieux, Marcy l’Étoile, France) and for azithromycin and ciprofloxacin by use of Etest (AB Bio diagnostics, Solna, Sweden). *Shigella* spp. with elevated MICs for azithromycin were also tested by disk diffusion for 30 μg nalidixic acid and by Etest for tetracycline and chloramphenicol. Vitek 2 and Etest susceptibility testing was performed as recommended by the manufacturers, and quality control strains gave expected results. The mph(A) gene, which codes for the macrolide 2′-phosphotransferase, was detected by PCR, as described (11).

After receiving ethics approval from the Centre Hospitalier de l’Université de Montréal–Hôpital Saint-Luc, we reviewed hospital charts and public health investigation files of patients who were harboring *Shigella* spp. with decreased susceptibility to azithromycin. Differences were analyzed by using the Fisher exact 2-tailed test with Epi Info software, version 6.0 (Centers for Disease Control and Prevention, Atlanta, GA, USA). Statistical significance was set at p<0.05.

From January 1, 2011, through April 30, 2013, a total of 45 patients were infected by 46 *Shigella* spp. strains isolated from fecal samples, including 2 also isolated from blood. A total of 33 *Shigella* spp. isolates were acquired locally by 33 men, and 13 *Shigella* spp. isolates

**Table 1. Azithromycin susceptibility of 26 Shigella spp. isolates from 25 patients, Centre Hospitalier de l’Université de Montréal–Hôpital Saint-Luc, Montreal, Quebec, Canada, January 2012–April 2013**

<table>
<thead>
<tr>
<th>Azithromycin susceptibility</th>
<th>No. infections acquired locally</th>
<th>No. infections acquired abroad</th>
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</thead>
<tbody>
<tr>
<td>Reduced susceptibility</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Susceptible</td>
<td>7</td>
<td>9</td>
</tr>
</tbody>
</table>

\*p = 0.0039. All patients with locally-acquired *Shigella* and 3 patients with *Shigella* infections acquired abroad were men. One patient was infected successively with 2 *Shigella* species with reduced azithromycin susceptibility. For all patients infected with *Shigella* spp. with decreased azithromycin susceptibility, the strains were isolated from feces and none from blood.

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were acquired abroad, outside Canada, in the week before symptom onset, by 6 men and 7 women (p = 0.00003).

From January 2012 through April 2013, infection with 4 Shigella spp. pulse types with decreased azithromycin susceptibility was locally acquired by 9 patients (mean age 45 years, range 29–55 years) (Tables 1, 2). Among these patients, 1 HIV-positive man was infected successively with 2 Shigella species with reduced azithromycin susceptibility, 11 months apart, resulting in a total of 10 infections (Figure). All 9 men reported having had sex with men, and 7 were HIV positive. CD4 cell counts were 106 cells/L for 1 HIV-positive patient and 420–540 ´ 106 cells/L for the other 6. HIV viral load was <40 copies/mL with men, and 7 were HIV positive. CD4 cell counts were 640–9074 copies/mL for 3 of the 6 patients for whom data were available and 58–90,074 copies/mL for the other 3. During the 2011–2013 surveillance period, azithromycin MICs for 35 of 36 Shigella spp. isolates with no reduced azithromycin susceptibility were 2–8 mg/L, and the MIC for 1 isolate was 16 mg/L; this latter isolate was negative by PCR for mph(A), and the other 35 isolates were not tested. The 10 Shigella spp. isolates with reduced azithromycin susceptibility had azithromycin MICs >64 mg/L and were positive for the mph(A) gene by PCR. The 3 Sh. flexneri and 1 Sh. sonnei pulse types were susceptible to nalidixic acid, ciprofloxacin, and ceftriaxone (Table 2); 3 pulse types were resistant to ampicillin, trimethoprim/sulfamethoxazole, and chloramphenicol; and 4 pulse types were resistant to tetracycline (Table 2). During 2012–2013, Shigella spp. with reduced azithromycin susceptibility represented 57.1% of 7 locally acquired pulse types (data not shown). Pulse-Net Canada XbaI and BlnI pattern designations were SFXAXAI.0105/SFXBNI.0017 for S. flexneri type 2a pulsovar 15 and 16, respectively; SFXAXAI.0193/SFXBNI.0084 for S. flexneri type 3a pulsovar 6; SSOXAI.0395/SSOBNI.0020 for S. sonnei pulsovar 101; and SSOXAI.0174/SSOBNI.0176

Figure. Distribution of Shigella spp. infections by sample date and years, Montreal, Quebec, Canada, January 2011–April 2013.
for Shigella sonnei pulsovar 105. No PFGE matches were identified in isolates from other Canada provinces.

Conclusions
During 2012–2013, at the Centre Hospitalier de l’Université de Montréal–Hôpital Saint-Luc, 10 infections with 1 of the 4 Shigella spp. pulse types with reduced azithromycin susceptibility were documented for 9 MSM, 7 of whom were HIV positive. These 4 locally acquired Shigella pulse types had increased azithromycin MICs of ≥64 mg/L and were positive by PCR for mph(A). This gene, which encodes macrolide-inactivating 2′-phosphotransferase, occurs on various plasmids (7). It has been documented in many aerobic gram-negative rods, such as Escherichia coli and Shigella spp. (14). This gene was harbored by all Shigella spp. with azithromycin MICs >16 mg/L (7,13–15). Azithromycin treatment failure has been reported for patients who received this drug for infection with such isolates (14). In this study, the acquisition of this gene by >50% of locally acquired Shigella spp. pulse types, infecting MSM over 15 months, is a concern in view of the potentially rapid development of reduced Shigella spp. susceptibility to azithromycin. For facilitation of clinical decision making and surveillance, azithromycin susceptibility breakpoint points for Enterobacteriaceae should be standardized (12). MSM should be counseled about prevention of enteric sexually transmitted infections; prevention measures include handwashing and using barriers during oral, anal, and genital sex (2,3). Such counseling might lead to behavior changes that might help slow the transmission of enteric sexually transmitted infections, including Shigella spp. infections with reduced azithromycin susceptibility.

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Dr Gaudreau is a clinical microbiologist and infectious diseases physician at Centre Hospitalier de l’Université de Montréal–Hôpital Saint-Luc in Montreal and a clinical titular professor at the Département de Microbiologie, Infectiologie et Immunologie de l’Université de Montréal. Her main research interests are epidemiology and antimicrobial drug susceptibility of enteric bacteria.

References

Address for correspondence: Christiane Gaudreau, Microbiologie Médicale et Infectiologie, CHUM-Hôpital Saint-Luc, 1058 rue Saint-Denis, Montreal, Quebec, Canada H2X 3J4; email: christiane.gaudreau.chum@ssss.gouv.qc.ca