Laboratory based surveillance of travel-related *Shigella sonnei* and *Shigella flexneri* in Alberta from 2002 to 2007

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**Abstract**

Between 2002 and 2007, travel related cases of *Shigella sonnei* and *S. flexneri* in Alberta, Canada were acquired from Central America, the Indian subcontinent and North America. Of this group, resistance to ciprofloxacin and nalidixic acid was identified in isolates from patients who had travelled to the Indian subcontinent. This study provides a Canadian perspective to a growing body of literature linking ciprofloxacin and nalidixic acid resistance to travel to the Indian subcontinent.

*Shigella* is a common cause of diarrheal illness in North America with a rate of 2.0 per 100,000 in Canada [1] and a rate of 3.2 per 100,000 in the United States [2,3]. Imported cases of *Shigella* infections have been reported in developed countries following travel to a foreign or developing country [4,5] and may be impacted by factors including socio-economic factors [6], food distribution networks [5] and microbiologic factors [7]. Across multiple geographic regions, high rates of antimicrobial resistance to multiple agents (e.g. sulfonamides, tetracycline, chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole) have limited the choices for empiric antimicrobial therapy required to manage *Shigella* infections and reduce fecal excretion of the bacteria [8-10] with descriptions of shifting species dominance and changes in antimicrobial susceptibility [10,11]. Generally, *Shigella flexneri* and *Shigella sonnei* are the dominant species and are heavily impacted by changes in antimicrobial susceptibility [12,13].

This study identifies the global regions associated with travel-related cases of *S. flexneri* and *S. sonnei* in Alberta, Canada and compares antibiotic resistance patterns of these isolates for 2002 to 2007 inclusive.

Specimens collected 2002-2007 (inclusive) from *S. flexneri* and *S. sonnei* infections in Alberta, Canada were included for study. Data collected at time of specimen submission included: date of specimen collection, outbreak association if present, travel history and antibiogram (data source-ProvLab Information Systems; Communicable Disease Report at Alberta Health and Wellness). Outbreaks were defined by public health officials as ≥2 epidemiologically related cases. Each outbreak was assigned a unique incident number. Repeat isolates received within six months of original case infections were excluded. Only one representative case for each outbreak was included, unless the isolates had different antibiotic susceptibility patterns. Based on travel history the origin of an isolate was grouped into corresponding regions and continents. Regions included in the study represented major travel destinations for individuals living in Canada. Domestic exposures were defined as "travel within North America."

**Isolate confirmation**

Presumptive *Shigella* isolates were confirmed using conventional biochemical tests [14]. Serotyping was done for *S. flexneri* and phagetyping was done for *S. sonnei*.

Serotyping was performed using commercially available antisera (Denka Seiken USA Inc., Campbell, CA) for *S. flexneri* and the following serotypes (STs) were determined: 1-4, 6, SH-101, SH-104, and variants x or y [14]. Phage typing was performed on *S. sonnei* isolates following standard procedures at the National Microbiology Laboratory in Winnipeg, Manitoba [15]. For 2002 and 2003, there were representative but fewer numbers of
isolates were available for testing. For example, in 2002 and 2003, only 24% and 58% of representative isolates were available respectively. From 2004-2007, representative isolates for each case of infection were available for susceptibility testing: 2004 (100%), 2005 (100%), 2006 (89%), 2007 (91%).

Susceptibility testing

Susceptibility testing was performed using Sensititre panels (Trek Diagnostic Systems, Cleveland, OH) against the following antimicrobial agents:

- amikacin (AMI)
- amoxicillin/clavulanic acid (AMC)
- ampicillin (AMP)
- cefoxitin (FOX)
- ceftriaxone (AXO)
- chloramphenicol (CHL)
- ciprofloxacin (CIP)
- gentamicin (GEN)
- kanamycin (KAN)
- nalidixic acid (NAL)
- streptomycin (STR)
- tetracycline (TET)
- sulfisoxazole (SSS)
- trimethoprim/sulfamethoxazole (SXT)

The minimum inhibitory concentrations (MIC) and breakpoints were determined in accordance with guidelines established by the Clinical and Laboratory Standards Institute (CLSI) [16,17].

Data analysis

GraphPad Prism 5 software (GraphPad Software, Inc. La Jolla, CA) was used for statistical analysis.

Between 2002-2007, 578 Shigella isolates were received and confirmed by ProvLab. The overall

| Table 1 Travel history and frequency of antimicrobial resistance of Shigella isolates in Alberta, 2002-2007⁴ |
|---------------------------------|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|
|                                 | North America | Central America | South America | Africa | Middle East | Indian subcontinent | Far East | Asia | Unknown | Western Hemisphere | Eastern Hemisphere |
| Shigella flexneri                | N = 14        | N = 53          | N = 6         | N = 27 | N = 37       | N = 8               | N = 17   | N = 73 | N = 74 |
| Streptomycin                    | 7(50)         | 30(57)          | 3(50)         | 12(44) | 2100         | 32(86)             | 6(75)    | 14(82) | 40(55) | 52(70)               |
| Ampicillin                      | 7(50)         | 39(74)          | 4(67)         | 22(81) | 2100         | 23(62)             | 7(88)    | 14(82) | 50(68) | 54(73)               |
| Trimethoprim-sulfamethoxazole   | 7(50)         | 21(40)          | 3(50)         | 17(63) | 2100         | 26(70)             | 6(75)    | 14(82) | 31(42) | 51(69)               |
| Sulfisoxazole                   | 7(50)         | 25(47)          | 3(50)         | 21(78) | 2100         | 26(70)             | 8(100)   | 14(82) | 35(48) | 57(77)               |
| Chloramphenicol                 | 8(57)         | 35(66)          | 4(67)         | 22(81) | 2100         | 24(65)             | 6(75)    | 12(71) | 47(64) | 54(73)               |
| Ciprofloxacin                   | 1(7)          | 0(0)            | 0(0)          | 0(0)   | 0(0)         | 7(19)              | 0(0)     | 0(0)   | 1(1)   | 7(9)                 |
| Nalidixic acid                  | 1(7)          | 0(0)            | 0(0)          | 0(0)   | 0(0)         | 21(57)             | 0(0)     | 3(18)  | 1(1)   | 21(28)               |
| Tetracycline                    | 13(93)        | 51(96)          | 6(100)        | 25(93) | 2100         | 37(100)            | 6(75)    | 17(100)| 70(96) | 70(95)               |
| Shigella sonnei                 | N = 35        | N = 87          | N = 14        | N = 16 | N = 25       | N = 32              | N = 31   | N = 136| N = 55 |
| Gentamicin                      | 0(0)          | 0(0)            | 1(7)          | 0(0)   | 0(0)         | 0(0)               | 0(0)     | 1(3)   | 1(1)   | 0(0)                 |
| Streptomycin                    | 34(97)        | 71(82)          | 7(50)         | 16(100)| 2100         | 24(96)             | 10(83)   | 25(81) | 109(80)| 52(95)               |
| Ampicillin                      | 8(25)         | 30(34)          | 9(64)         | 16(0)  | 0(0)         | 11(4)              | 2(17)    | 19(61)| 47(35) | 4(7)                 |
| Amoxicillin/clavulanic acid     | 0(0)          | 0(0)            | 0(0)          | 0(0)   | 0(0)         | 0(0)               | 0(0)     | 1(3)   | 0(0)   | 0(0)                 |
| Cefuroxime                      | 0(0)          | 0(0)            | 0(0)          | 0(0)   | 0(0)         | 0(0)               | 0(0)     | 2(6)   | 0(0)   | 0(0)                 |
| Ceftriaxone                     | 0(0)          | 0(0)            | 0(0)          | 0(0)   | 0(0)         | 0(0)               | 0(0)     | 2(6)   | 0(0)   | 0(0)                 |
| Trimethoprim-sulfamethoxazole   | 26(74)        | 62(71)          | 14(100)       | 16(100)| 2100         | 24(96)             | 11(82)   | 19(61)| 99(73) | 53(96)               |
| Sulfisoxazole                   | 30(86)        | 64(74)          | 14(100)       | 15(94) | 2100         | 25(100)            | 10(83)   | 24(77)| 105(77)| 52(95)               |
| Chloramphenicol                 | 1(3)          | 0(0)            | 8(57)         | 0(0)   | 0(0)         | 14(4)              | 18(0)    | 0(0)   | 9(7)   | 2(4)                 |
| Ciprofloxacin                   | 0(0)          | 0(0)            | 0(0)          | 0(0)   | 0(0)         | 0(0)               | 0(0)     | 0(0)   | 0(0)   | 0(0)                 |
| Nalidixic acid                  | 4(11)         | 4(5)            | 0(0)          | 0(0)   | 0(0)         | 20(80)             | 0(0)     | 5(16) | 8(6)   | 20(36)               |
| Tetracycline                    | 23(66)        | 57(66)          | 7(50)         | 15(94) | 2100         | 25(100)            | 9(75)    | 14(45)| 91(67) | 51(93)               |

⁴Data for antimicrobial susceptible isolates are not shown.
distribution of species included: \( S. \) \( \text{sonnei} \) 54.7% (\( n = 316 \)); \( S. \) \( \text{flexneri} \) 33.9% (\( n = 196 \)); \( S. \) \( \text{boydii} \) 7.6% (\( n = 44 \)); \( S. \) \( \text{dysenteriae} \) 3.8% (\( n = 22 \)). Twenty nine \( S. \) \( \text{flexneri} \) and 79 \( S. \) \( \text{sonnei} \) were not archived (stored and cataloged); three \( S. \) \( \text{flexneri} \) could not be cultured; 3 were not archived (stored and cataloged); 3 did not grow. The majority of travel cases for \( S. \) \( \text{flexneri} \) were from Central America (32.3% [53/164]), the Indian subcontinent (22.6% [37/164]) and North America (8.5% [14/164]). The majority of \( S. \) \( \text{sonnei} \) cases were from Central America (39.2% [87/222]), North America (15.8% [35/222]), and the Indian subcontinent (11.3% [25/222]).

Of the 196 \( S. \) \( \text{flexneri} \) isolates, as described above 164 were available for analysis, while 29 were not archived and 3 did not grow. The most common ST for \( S. \) \( \text{flexneri} \) was ST2 (37.8% [62/164]) with 40.3% (25/62) of the ST2 isolates originating from Central America.

The most common phage type for \( S. \) \( \text{sonnei} \) was S1 (65.8% [146/222]) with (38.4% [56/146] of S1 isolates from Central America.

Of the 196 \( S. \) \( \text{flexneri} \) isolates, as described above 164 were available for analysis, while 29 were not archived and 3 did not grow. The most common ST for \( S. \) \( \text{flexneri} \) was ST2 (37.8% [62/164]) with 40.3% (25/62) of the ST2 isolates originating from Central America. Of the \( S. \) \( \text{flexneri} \) isolates from the Indian subcontinent the two most common STs were ST2 (40.5% [15/37]) and ST6 (35.1% [13/37]). The most common phage type for \( S. \) \( \text{sonnei} \) was S1 (65.8% [146/222]) with (38.4% [56/146] of S1 isolates from Central America.

Only 1.2% (\( n = 2 \)) \( S. \) \( \text{flexneri} \) and 8.1% (\( n = 18 \)) \( S. \) \( \text{sonnei} \) isolates were pan-susceptible to all antibiotics tested. All \( S. \) \( \text{flexneri} \) isolates were susceptible to AMI, GEN, AMC, KAN, FOX, TIO, AXO. All the \( S. \) \( \text{sonnei} \) were

| S. flexneri | Total per year (n) |
|------------|------------------|
| 2002       | 10 4 2 2 < = 0.25 |
| 2003       | 28 2 >32 8 < = 0.25 |
| 2004       | 38 2 >32 8 < = 0.25 |
| 2005       | 35 2 >32 8 < = 0.25 |
| 2006       | 22 2 >32 8 < = 0.25 |
| 2007       | 31 2 >32 8 < = 0.25 |

| S. sonnei | Total per year (n) |
|-----------|------------------|
| 2002      | 12 2 32 4 < = 0.25 |
| 2003      | 27 2 32 4 < = 0.25 |
| 2004      | 35 2 2 4 < = 0.25 |
| 2005      | 60 2 2 4 < = 0.25 |
| 2006      | 26 2 2 2 < = 0.25 |
| 2007      | 19 2 2 2 < = 0.25 |

Table 2 Median MICs of antimicrobial agents in \( S. \) \( \text{flexneri} \) and \( S. \) \( \text{sonnei} \) per year

| AMI | AMP | AMC | AXO | CHL | CIP | SXT | FOX | GEN | KAN | NAL | SSS | STR | TET | TIO |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 2002| 10 4 2 2 < = 0.25 |
| 2003| 28 2 >32 8 < = 0.25 |
| 2004| 38 2 >32 8 < = 0.25 |
| 2005| 35 2 >32 8 < = 0.25 |
| 2006| 22 2 >32 8 < = 0.25 |
| 2007| 31 2 >32 8 < = 0.25 |

| amikacin (AMI), amoxicillin/clavulanic acid (AMC), ampicillin (AMP), cefoxitin (FOX), ceftiofur (TIO), ceftizoxime (AXO), chloramphenicol (CHL), ciprofloxacin (CIP), gentamicin (GEN), kanamycin (KAN), nalidixic acid (NAL), streptomycin (STR), tetracycline (TET), sulfisoxazole (SSS), trimethoprim/sulfamethoxazole (SXT).
resistant to AMP, CHL, NAL, STR, TET and SXT (Table 1).

When median MICs were analyzed for all agents the following changes were identified as in Table 2. For *S. flexneri* median MICs were within two dilutions for most agents over the study period. Exceptions were for the following agents; AMP (increase), CHL (increase), SXT (increase and following drop), and SSS (decrease). For *S. sonnei*, median MICs were within two dilutions for most agents over the study period with the following exceptions; exception of AMP (decrease).

When data was combined for all years, the NAL and CIP resistance was 20.1% (33/164) and 14.9% (33/222) for *S. flexneri* and *S. sonnei* respectively. CIP resistance was identified only in *S. flexneri* isolates (4.9%, 8/164) when averaged over the six-year study period (Fisher’s exact test, \( p = 0.001 \)) (Figure 1a and 1b) CIP resistance in *S. flexneri* was not steady but instead was most evident in the years 2005, 2006, and 2007 (Figure 1a). Combined CIP and NAL resistance was related to travel to the Indian subcontinent for *S. flexneri* (84.8%, 28/37) and *S. sonnei* (80.0%, 20/25) (Fisher’s exact test, \( p < 0.0001 \)). The proportion of antibiotic resistance was constant over six years except for *S. sonnei*, where AMP resistance decreased from 83% in 2002 to 11% in 2007 (\( p < 0.0001, \chi^2 = 36.52, df = 5 \)) and NAL resistance increased from 0% in 2002 to 30% in 2007 (\( p = 0.0168, \chi^2 = 13.82, df = 5 \)).

**Figure 1** Frequency of antimicrobial resistance of study isolates from Alberta 2002-2007: 1 a) *S. flexneri* (n = 164); and 1b) *S. sonnei* (n = 222)
At the study onset, treatment guidelines suggested a fluoroquinolone for acute traveler’s diarrhea regardless of travel location. It is possible that some CIP resistance was underestimated in 2002-2003 due to the smaller number of isolates tested. By 2009, treatment guidelines for acute traveler’s diarrhea (outside of Latin America and Africa) suggested azithromycin or a fluoroquinolone [18,19]. Data also suggests that azithromycin resistance may be emerging and resistance rates of 16% have been recently described in Bangladesh [20]. These studies indicate that travel to the Indian subcontinent, in patients returning to Western Canada with traveler’s diarrhea should be determined to guide initial empiric treatment options; especially for severe infections because the association of *S. flexneri* and *S. sonnei* isolates from this region with fluoroquinolone and potential macrolide resistance [13,21]. Although CIP resistance was described only in *S. flexneri*, we should remain vigilant for developing *gyrA* and *parC* mutations as well as the presence of plasmid mediated quinolone resistance determinants (PMQR) genes that may lead to increasing rates of CIP resistance in travel-related *Shigella* isolates which are beginning to emerge globally [4,22].

There are multiple factors that may have lead to CIP and NAL resistance in *Shigella* species originating from the Indian subcontinent [21]. It is possible that part of this emerging resistance may be associated with the increasing dominance of specific STs or clones of *Shigella*. Both this study and other work have identified a dominance of *S. flexneri* STs 2 and 6 in isolates of Indian origin and cases of traveler’s diarrhea associated with the Indian subcontinent [23]. One factor driving multi-drug resistance in the Indian subcontinent may be the emergence of specific clones within these dominant STs [24]. Therefore, the identification of clonal groups within Alberta strains may be a powerful tool for tracking the development of drug-resistance in *Shigella* isolates from future cases of traveler’s diarrhea.

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**Authors’ contributions**

SJD, CL, MA, CF, and ML participated in data analysis and interpretation of susceptibility and travel data, drafted and revised paper, and made follow-up revisions to submission. CL, CF performed susceptibility testing on isolates, and interpreted/analyzed this data. CL collated, analyzed, and interpreted travel history data. LS, BF participated in susceptibility testing on isolates, and reviewed paper. KS, DE collaborated for travel history data, and reviewed/editied paper. ML, CL conceived study design. All authors read and approved the final manuscript draft.

**Competing interests**

The authors declare that they have no competing interests.

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**References**

1. Demczuk W, Ng LK, Woodward D, Ahmed R, Clark C, Tabor H, Dare K, Ciampa N, Muckle A. Laboratory Surveillance Data for Enteric Pathogens in Canada: Annual Summary 2006. Winnipeg, Manitoba, Canada: Public Health Agency of Canada, National Microbiology Laboratory, 2007.

2. Centers for Disease Control and Prevention: *Shigella* Surveillance: Annual Summary, 2004. Atlanta, Georgia, USA: US Department of Health and Human Services, 2005.

3. Centers for Disease Control and Prevention: National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS): *Human Isolates Final Report, 2004*. Atlanta, Georgia, USA: U.S. Department of Health and Human Services, CDC, 2007.

4. Iumiya H, Tada Y, Ito K, Monta-Ishihara T, Oindsay M, Terajima J, Watanabe H. Characterization of *Shigella sonnei* isolates from travel-associated cases in Japan. *J Med Microbiol* 2009, 58:1496-1491.

5. Gaynor K, Park SY, Kanenaka R, Colinéres R, Mintz E, Ram PK, Kitutani P, Nakata M, Wedel S, Boxrud D, Jennings D, Yoshida H, Tosa ka N, He H, Ching-Lee M, Effier PV. International foodborne outbreak of *Shigella sonnei* infection in airline passengers. *Epidemiol Infect* 2009, 137:335-341.

6. Simonsen J, Frisch M, Helb et al. Socioeconomic risk factors for bacterial gastrointestinal infections. *Epidemiology* 2008, 19:282-290.

7. Todd EC, Greig JD, Bartleson CA, Michaels B. Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 4. Infective doses and pathogen carriage. *J Food Prot*, 2008, 71:2359-2373.

8. Niyoji SK: Increasing antimicrobial resistance-an emerging problem in the treatment of shigellosis. *Clin Microbiol Infect* 2007, 13:1141-1143.

9. Nelson JD, Kursmüller H, Jackson GH, Woodman E. Trimethoprim-sulfamethoxazole therapy for shigellosis. *JAMA* 1976, 235:1239-1243.

10. Vrints M, Mainiaux E, Van ME, Collard JM, Bertrand S. Surveillance of antibiotic susceptibility patterns among *Shigella sonnei* strains isolated in Belgium during the 18-year period 1990 to 2007. *J Clin Microbiol* 2009, 47:1379-1385.

11. Vinh H, Nhu NT, Nga TV, Duy PT, Campbell JI, Hoang NV, Boni MF, My PV, Pany C, Nga TT, Van Minh P, Thyu CT, Diep TS, Phuong Le T, Chinh MT, Loan HT, Tham NT, Lanh MN, Mong BL, Anh VT, Bay PV, Chau NV, Farrar J, Baker S. A changing picture of shigellosis in southern Vietnam: shifting species dominance, antimicrobial susceptibility and clinical presentation. *BMC Infect Dis* 2009, 9:204.

12. Haulka K, Sittonen A. Emerging resistance to newer antimicrobial agents among *Shigella* isolated from Finnish foreign travellers. *Epidemiol Infect* 2008, 136:476-482.

13. Srinivasan H, Baijayanti M, Raksha Y. Magnitude of drug resistant Shigellosis: a report from Bangalore. *Indian J Med Microbiol* 2009, 27:358-360.

14. American Society for Microbiology. *Manual of Clinical Microbiology*. Washington, DC: American Society for Microbiology, 8 2003.

15. Ahmed SF, Riddle MS, Wierzba TF, Messih IA, Monteville MR, Sanders JW, Klena JD. Epidemiology and genetic characterization of *Shigella flexneri* strains isolated from three paediatric populations in Egypt (2000-2004). *Epidemiol Infect* 2006, 134:1237-1248.

16. Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically: Approved Standard. *Wayne, Pennsylvania, USA: Clinical and Laboratory Standards Institute*, 7 2006.

17. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 17th Informational Supplement. *Wayne, Pennsylvania, USA: Clinical and Laboratory Standards Institute*, 2007.
18. Sanford Guide to Antimicrobial Therapy 2000. Hyde Park, VT: Antimicrobial Therapy Inc, 30 2000.
19. Sanford Guide to Antimicrobial Therapy 2009. Sperryville, VT: Antimicrobial Therapy Inc, 39 2009.
20. Rahman M, Shoma S, Rashid H, El AS, Baqui AH, Siddique AK, Nair GB, Sack DA. Increasing spectrum in antimicrobial resistance of *Shigella* isolates in Bangladesh: resistance to azithromycin and ceftriaxone and decreased susceptibility to ciprofloxacin. *J Health Popul Nutr* 2007, 25:158-167.
21. Mensa L, Marco F, Vila J, Gascon J, Ruiz J. Quinolone resistance among *Shigella* spp. isolated from travellers returning from India. *Clin Microbiol Infect* 2008, 14:279-281.
22. Pu XY, Pan JC, Wang HQ, Zhang W, Huang ZC, Gu YM. Characterization of fluoroquinolone-resistant *Shigella flexneri* in Hangzhou area of China. *J Antimicrob Chemother* 2009, 63:917-920.
23. Dutta S, Rajendran K, Ray S, Chatterjee A, Dutta P, Nair GB, Bhattacharya SK, Yoshida S. Shifting serotypes, plasmid profile analysis and antimicrobial resistance pattern of *Shigella* strains isolated from Kolkata, India during 1995-2000. *Epidemiol Infect* 2002, 129:235-243.
24. Pazhani GP, Niyogi SK, Singh AK, Sen B, Taneja N, Kundu M, Yamasaki S, Ramamurthy T. Molecular characterization of multidrug-resistant *Shigella* species isolated from epidemic and endemic cases of shigellosis in India. *J Med Microbiol* 2008, 57:856-863.

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