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ANTIBIOTIC RESISTANCE OF *SHIGELLA* SPP., *SALMONELLA* SPP., AND *YERSINIA* SPP. ISOLATED IN QUEBEC

Salmonella spp., *Shigella* spp., and *Yersinia* spp. are important enteropathogenic bacteria. Antibiotic treatment is normally indicated for infections caused by *Shigella* spp. and only in the case of severe infections for *Salmonella* and *Yersinia*. Studies from a number of countries have reported the development of resistance of these bacteria to certain antibiotics[1,2]. The new quinolones, such as ciprofloxacin, have proven effective in vitro against the usual enteropathogens[3]. There are no recent Quebec data on the antibiotic resistance of these enteropathogenic bacteria.

Methods

The purpose of this study was to demonstrate the susceptibility of 125 strains of *Salmonella* spp., 118 of *Shigella* spp., and 53 of *Yersinia* spp. isolated from 296 patients at *Hôpital Saint-Luc*, Montreal, between 1991 to 1995. Susceptibility to ampicillin, trimethoprim/sulfamethoxazole (TMP/SMX), and ciprofloxacin was determined using standardized disk diffusion (1991 to 1992) and agar dilution (1993 to 1995) methods[4]. Twenty percent of the strains of *Salmonella* spp. were isolated from blood cultures. Of these, one was identified as *S. typhi* and two were identified as *S. paratyphi* A. The remaining strains were identified serologically as belonging to group B — 47 strains, group C — 42 strains, group D — 25 strains, group E — five strains, and group G — one strain. Two strains could not be typed serologically. Of the 118 *Shigella* strains investigated, these were identified as belonging to *S. dysenteriae* — one strain, *S. flexneri* — 77 strains, *S. boydii* — five strains, and *S. sonnei* — 35 strains. Of the 53 *Yersinia* strains identified, one belonged to *Y. frederiksenii* and the remaining 52 strains belonged to the *Y. enterocolitica*.

Table 1 shows the number and percentage of strains that were resistant or multiresistant to the antibiotics tested. None of the *Shigella* or *Salmonella* strains were resistant to ciprofloxacin and none of the *Yersinia* spp. strains were resistant to TMP/SMX or ciprofloxacin.

Table 1
Antibiotic resistance of enteropathogenic bacteria

	Shigella spp. 118 strains* No. (%) R**	*Salmonella* spp. 125 strains* No. (%) R**	*Yersinia* spp. 53 strains* No. (%) R**
Ampicillin	74 (62.7%)	5 (4%)	49 (92.5%)
TMP/SMX***	31 (26.3%)	1 (0.8%)	0 (0%)
Ciprofloxacin	0 (0%)	0 (0%)	0 (0%)
Ampicillin and TMP/SMX	19 (16.1%)	1 (0.8%)	0 (0%)

* One strain per patient ** Resistance *** Trimethoprime/sulfamethoxazole

Table 2 shows the number and percentage of *Shigella* strains that were resistant or multiresistant to ampicillin or to TMP/SMX. The one *S. dysenteria*e strain was resistant to TMP/SMX. Of the five *S. boydii* strains, one was resistant to ampicillin and to TMP/SMX. The *S. flexneri* strains were significantly more resistant to ampicillin than were those of *S. sonnei*, at 79.2% and 34.3%, respectively (p = 0.0000037). The *S. sonnei* strains were significantly more resistant to TMP/SMX than were those of *S. flexneri*, at 42.9% and 18.2%, respectively (p = 0.0057). Resistance to ampicillin and to TMP/SMX was observed in 13% and 22.9% of the *S. flexneri* and *S. sonnei* strains, respectively.

Discussion

An Ontario study of 598 *Shigella* strains isolated in 1990 found that 39% to 89% were resistant to ampicillin and 27% to 38% were resistant to TMP/SMX[5]. None of the investigated strains were resistant to ciprofloxacin. An American study of 798 *Salmonella* strains, isolated between 1989 and 1990, found 13% were resistant to ampicillin, 15% were resistant to TMP/SMX, and 0% were

Table 2
Antibiotic resistance of *Shigella*

	S. dysenteriae 1 strain* No. (%) R**	*S. flexneri* 77 strains* No. (%) R**	*S. boydii* 5 strains* No. (%) R**	*S. sonnei* 35 strains* No. (%) R**	Total 118 strains* No. (%) R**
Ampicillin	0	61 (79.2%)[a)]	1 (20%)	12 (34.3%)	**74 (62.7%)**
TMP/SMX***	1	14 (18.2%)	1 (20%)	15 (42.9%)[b)]	**31 (26.3%)**
Ampicillin and TMP/SMX	0	10 (13%)	1 (20%)	8 (22.9%)	**19 (16.1%)**

* One strain per patient ** Resistance *** Trimethoprime/sulfamethoxazole

a) p = 0.0000037
b) p = 0.0057 (chi-square test)

resistant to ciprofloxacin[6]. The *Yersinia* spp. are normally reported as being resistant to ampicillin, but susceptible to TMP/SMX and ciprofloxacin[7].

In this study, as in a previous one[3], resistance to ciprofloxacin in *Shigella* spp., *Salmonella* spp., and *Yersinia* spp. was not observed; a high proportion of the *Shigella* strains, but relatively few of the *Salmonella*, were resistant to ampicillin, TMP/SMX, or to both. The *S. flexneri* strains were significantly more resistant to ampicillin but significantly less resistant to TMP/SMX than were those of *S. sonnei*. The majority of the *Yersinia* strains were resistant to ampicillin but none were resistant to TMP/SMX or ciprofloxacin. Continuous surveillance of antibiotic susceptibility is essential in monitoring the evolution of their susceptibility profiles and in determining which antibiotics may be of value in the treatment of these bacterial infections.

Acknowledgements

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Source: *C Gaudreau, MD, P Turgeon, MD, Medical Microbiology and Infectious Diseases Unit, Hôpital Saint-Luc, Montreal, QC.*

International Notes

SHIGELLA VACCINE RESEARCH AND DEVELOPMENT

At the end of 1996, a meeting was organized at the World Health Organization (WHO), Geneva, by the Steering Committee on Diarrhoeal Disease Vaccines (Global Programme for Vaccines and Immunization), which has placed *Shigella* vaccine development first in line of its priorities.

Background on shigellosis

Shigellosis causes about 600,000 deaths every year globally. The increasing number of infected persons in some areas of the world, in addition to the emergence of strains resistant to multiple antibiotics, emphasizes the need for an effective vaccine. In developing countries, the major burden of *Shigella* infection is among children 1 to 4 years of age, but during *Shigella dysenteriae* type 1 epidemics all age groups are affected. Various surveys carried out in treatment centres show that *Shigella* is associated with 5% to 15% of cases of diarrhea and 30% to 50% of cases of dysentery. *S. flexneri* serotypes (serotype 2a is the most common) predominate as agents of endemic shigellosis. *S. dysenteriae* 1 (Shiga bacillus) has been an important cause of epidemic dysentery in Latin America, Asia, and Africa since the 1960s. Epidemics are characterized by severe clinical disease, high case fatality, person-to-person spread, and multiple antibiotic resistance. *Shigella* persists as a public-health problem in several sub-populations in industrialized countries, including children in

day-care centres, individuals in custodial institutions, migrant workers, selected groups of American Indians in reservations, and travellers to developing countries.

The need for a vaccine against shigellosis is obvious in view of *Shigella*'s repeatedly demonstrated tendency to acquire antibiotic resistance. In the 1940s, *Shigella* acquired resistance to sulfa drugs, in the 1950s to tetracycline and chloramphenicol, in the 1970s to ampicillin, and in the late 1980s to trimethoprim/sulfamethoxazole.

Candidate vaccines under development

The primary role of a *Shigella* vaccine would be to protect against clinical disease. An additional benefit would be to interfere with infection and colonization. The most important *Shigella* strains to be targeted for vaccine development are *S. flexneri* 2a, *S. dysenteriae* 1, and *S. sonnei*. However, the possible emergence of new serotypes has been emphasized. The emergence of *S. flexneri* serotypes 1, 3, and 6 has been observed in various countries. In addition, improved sanitation and socioeconomic development in several emerging economies have been accompanied by a switch towards a predominance of *S. sonnei*.

Live attenuated strains: Two prototype attenuated vaccine strains of *S. flexneri* 2a and *S. dysenteriae* 1 have been developed in the United States. The two strains are safe, highly immunogenic in stimulating secretory IgA (sIgA) antibodies and protective in the guinea-pig challenge model (Sereny test). Preliminary phase I clinical trials with one of these strains have recently begun.

Another attenuated mutant *S. flexneri* 2a vaccine strain has been developed in France. It resulted in a double attenuation of its capacity to move intra- and inter-cellularly, and of its survival within tissues. After being tested in macaque monkeys and shown to be protective in 75% of vaccinated animals, double-blind, placebo-controlled studies on the safety and the immunogenicity of this candidate vaccine were recently conducted in volunteers in the United States. The dose used was shown to be safe and immunogenic. Volunteers had high levels of circulating antibody-secreting cells, similar to those observed in clinical shigellosis. These encouraging results supported an expanded phase I study initiated in July 1996 and which is currently ongoing.

Other candidate vaccines have been developed in Sweden, based on the auxotrophic mutant of *S. flexneri* Y strain, which were well tolerated and highly protective in monkeys. They elicited species-specific antibody responses directed not only against serotype Y, but also against serotypes 1b and 2a, leading to cross-protection. In a safety and immunogenicity trial in Swedish volunteers, they were shown to be safe and to elicit significant anti-lypopolysaccaride (LPS) peripheral and secretory antibody responses, and significant increases in anti-LPS-specific peripheral antibody-secreting cells. The advantages of a *Shigella* vaccine based on serotype Y include possible cross-protection as well as a possible widened spectrum of protection via conversion to the other sero-types by phage transduction. Protection elicited by these different strains remains to be tested in humans.

Another approach, based on expression of LPS in a live attenuated vector, is under development in Mexico. To develop a candidate vaccine against *S. dysenteriae* 1, gene clusters, which code for *S. dysenteriae* 1 O-antigen biosynthesis have been manipulated and introduced in the vector. The resulting vaccine is able to express O-antigen, producing a good level of protective antibodies in animal models and yielding 47% full protection and 53% partial protection (later onset of disease and/or less severe symptoms) against challenge with the wild-type strain. A derivative of the previous strain which expresses the Shiga toxin B- subunit-HlyA fusion and the O-antigen of *S. dysenteriae* 1 was also constructed. Immunogenicity and safety should be further assessed in human volunteers.

A similar approach has been taken in Switzerland. In this case, the strains used as a carrier for the expression of O-antigen were two already licensed vaccine strains. A first generation cholera vaccine vector-based strain, named CH3, that expresses both host-encoded (Inaba) and heterologous (*S. sonnei*) O serotype (bivalent strain) showed weak but significant immunogenicity in human volunteers. A clinical trial is planned for the end of 1997 with one or two strains selected for properties that should ensure optimal survival of the vaccines in the gastrointestinal tract. Using the same approach, a candidate *S. dysenteriae* type 1 vaccine strain has also been constructed.

Subunit vaccines

Conjugate vaccines: An evaluation of a *S. sonnei*-recombinant exoprotein A (rEPA) and *S. flexneri* 2a-rEPA parenteral conjugate vaccines has been conducted in Israel. A phase II (double-blind, randomized, vaccine-controlled study) performed in 192 volunteers revealed that the vaccines were safe and highly immunogenic. Four years after a single dose of the *S. sonnei* conjugate, 50% of the vaccinees still had significantly higher levels of anti-LPS antibodies compared with the pre-vaccination stage. The protective efficacy of a single injection of the *S. sonnei* conjugate has been further evaluated among Israeli military recruits naturally exposed to *Shigella* during their training cycles under field conditions. During outbreaks of *S. sonnei* infection which occurred among volunteers 71 to 155 days after immunization, the *S. sonnei* conjugate showed 74% protective efficacy. The data indicate that a single injection of the *S. sonnei* conjugate may confer type-specific protection against shigellosis caused by *S. sonnei*.

Proteosome vaccines: Proteosomes are multimolecular vesicles formed from *Neisseria* outer membrane proteins that are used as mucosal vaccines to induce systemic and mucosal immune responses. After nasal or oral immunization in mice or guinea pigs with *S. flexneri* or *S. sonnei* proteosomes, high levels of antibodies against LPS are induced in intestinal and lung secretions as well as in serum. Monkey studies showed that intratracheal administration was more efficient than nasal spray, and nasal spray more efficient than oral delivery. Good manufacturing practice (GMP) production of *S. sonnei* and *S. flexneri* vaccines has yielded candidate vaccines for human clinical trials. The initial phase I trial of the proteosome *S. sonnei* vaccine, a dose-escalating study, showed a dose-dependent immune response after two intranasal spray administrations, whereas oral immunization gave only minimal antibody-secreting cell responses. A phase I trial of *S. flexneri* 2a proteosome vaccine is planned, as well as expanded nasal or nasal-oral combination prime and boost studies.

***Shigella* nucleoprotein (ribosomal) vaccine:** This approach has been taken in the United States. Parenteral vaccination with the non-covalent complexes of O-polysaccharide and ribosomal particles from *Shigella* induces an intense systemic O-antibody response in experimental animals. It also elicits a significant response of the secretory immune system with IgA antibodies

appearing in tears, milk (guinea pigs), bile (rats), and saliva (monkeys). One subcutaneous injection of the vaccine protects 70% to 90% of animals from the challenge with homologous *Shigella* in the guinea-pig model. Lyophilized *Shigella* ribosomal vaccine is very stable and can be produced at low cost. A GMP protocol for vaccine production on a large scale is now under development.

Recommendations

Based on the data presented and on discussions, a consensus was reached on several major points that should be taken into account for accelerating *Shigella* vaccine development.

Disease burden and need of a vaccine: A general consensus of the meeting was that development and availability of *Shigella* vaccines should be a major public-health priority. Because industrialized and developing countries have different dominant serotypes, different vaccines (or components) may need to be developed to cover strains in different geographic areas. A special emphasis was placed on the need for development of a vaccine against *S. dysenteriae* type 1.

Epidemiologic situation: Although many epidemiologic data are available, more work is needed to establish the incidence, prevalence, disease burden, and serotype distribution of shigellosis in many areas of the world so that country, regional, and global estimates can be made. Manufacturers need these data, which may be difficult for them to obtain directly. The potential areas to be selected for these trials should ideally meet the following requirements: a stable population, a well-functioning health-care delivery system, and suitable laboratories for conducting microbiologic evaluation. The need for a monograph summarizing available epidemiologic data was stressed; this will be produced under the auspices of the steering committee.

Vaccine strategies: It was recommended that all the different vaccine approaches developed so far should continue to be evaluated. There are no data to suggest that any of them should be stopped because of its ineffectiveness. In any case, the efficacy of a candidate vaccine directed against one of the major serotypes should be demonstrated. The development of a trivalent vaccine directed against the three major strains responsible for the disease (*S. flexneri* 2a, *S. dysenteriae* 1, and *S. sonnei*) would then be undertaken. Priority should be given to the development of a *S. dysenteriae* 1 vaccine. Special note should be made of the ethical problem related to the evaluation of the protective efficacy of such a candidate vaccine, since this type of vaccine cannot be evaluated in many volunteers challenged with a virulent strain expressing the toxin.

Features for an ideal vaccine: An ideal vaccine should be easy to administer, preferably orally, although parenteral vaccines should not be discarded if all the following requirements are met: well-tolerated; able to induce a high-level, long-term protection after a single dose; multivalent; directed against the most representative *Shigella* serotypes causing endemic and epidemic infections; and easy to manufacture.

WHO Editorial Note

It is important to stress that a consensus was reached among researchers, vaccine manufacturers, representatives of the Expanded Programme on Immunization, and public-health decision-makers from developing countries, with regard to the urgent need for an effective and safe vaccine against diarrhea due to *Shigella*, particularly since the emergence of multiple antibiotic resistance is such a serious problem.

Additional information is available upon request, addressed to the Vaccine Research and Development unit, WHO (tel. 41 22 791 2698 — E-mail Ivanoffb@who.ch).

Source: *WHO Weekly Epidemiological Record, Vol 72, No 11, 1997.*

RESPIRATORY VIRUS SURVEILLANCE
FluWatch Project

Standardized rates of influenza-like illness (ILI) reported to FluWatch indicate a second rise in influenza activity beginning in mid-February (Figure 1). The increased activity seen in Nova Scotia and New Brunswick was most notable; the bi-monthly standardized ILI rates from weeks 8 to 12 exceeded 80 per 1,000 patients seen. Similar rates were reported in Quebec, Saskatchewan, and Alberta, where higher ILI activity has been noted throughout the 1996 to 1997 influenza season (Figure 2).

The secondary wave in influenza activity has been associated with influenza B. The number of B isolates reported to the Laboratory Centre for Disease Control (LCDC) increased in January and the last 2 weeks of February; influenza B was more commonly reported than influenza A. All of the B isolates typed at LCDC have been B/Beijing/184/93-like.

Figure 1
Standardized rates of reported ILI across Canada by 2-week periods, reported to FluWatch, 13 January - 23 March 1997

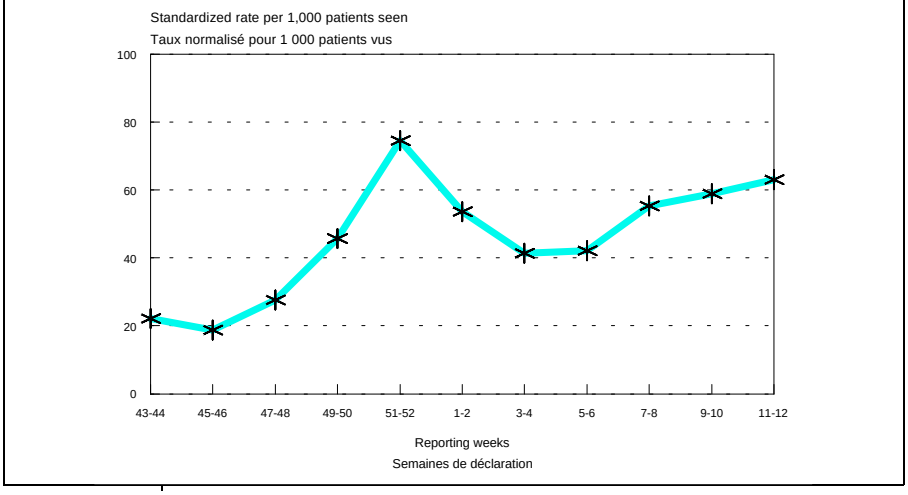


Figure 2
ILI reported to FluWatch, 1 October 1996 - 24 March 1997

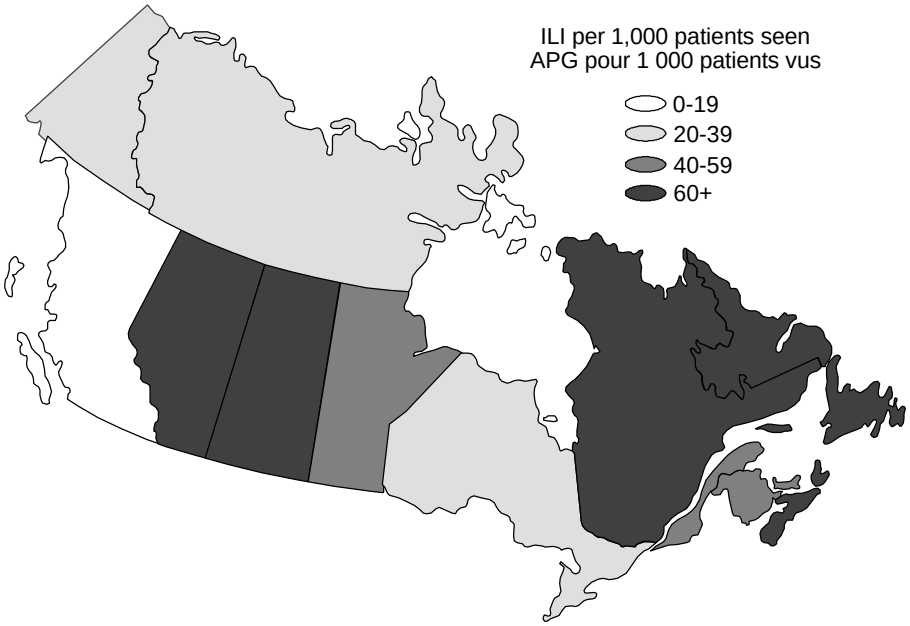
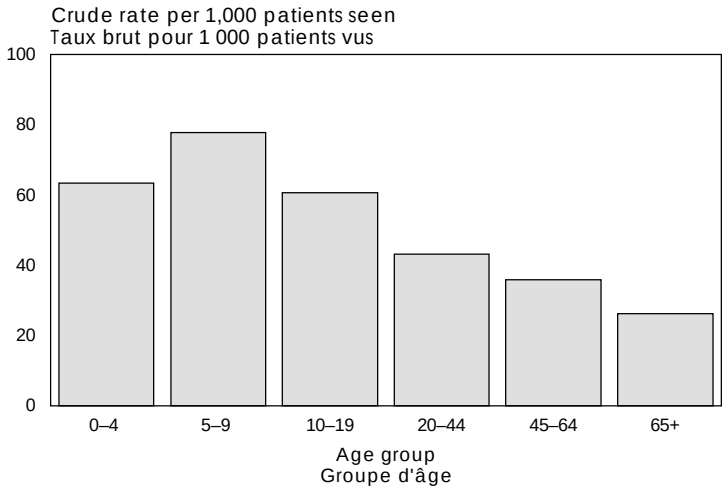


Figure 3
Cumulative rates of ILI across Canada by age group, reported to FluWatch, 13 January - 23 March 1997



Source: *Division of Disease Surveillance, Bureau of Infectious Diseases, LCDC, Ottawa, ON.*

The distribution of cases has shifted. The highest rates of ILI are now seen in children < 10 years of age; earlier in the season, the highest rates were among persons 10 to 44 years old (Figure 3).

The current pattern of influenza activity seen in Canada is similar to what has been reported elsewhere in the northern hemisphere.

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