Regional, Seasonal, and Antimicrobial Resistance Distributions of *Salmonella* Typhimurium in Canada

A Multi-Provincial Study

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ABSTRACT

Background: This study was conducted to describe the geographical and seasonal distributions of reported human *Salmonella* Typhimurium (ST) definitive type 104 (DT104) cases, to compare these characteristics to those of non-DT104 cases, and to investigate specific antimicrobial resistance (AMR) patterns in four Canadian provinces.

Methods: All laboratory-confirmed ST cases originating from passive reporting in Alberta, British Columbia, and Saskatchewan, and every second case in Ontario identified from December 1999 through November 2000 were investigated.

Results: A total of 470 human *Salmonella* Typhimurium cases were identified during the study period. DT104 was the most common phage type, although its incidence varied by province. The proportion of DT104 cases living in urban Ontario, British Columbia and Saskatchewan did not differ from the general population, but in Alberta, the DT104 cases were more likely to live in rural areas. Overall, DT104 isolates were more often R-type ACSSuT compared to non-DT104 cases, and R-type AKSSuT was often associated with DT208. DT104 cases displayed no seasonality whereas non-DT104 cases were more frequent in the summer than in the winter.

Interpretation: Our results suggest that DT104 and non-DT104 cases vary by province, urban vs. rural residential status and by resistance patterns. Lack of seasonality in the DT104 cases may indicate a lesser influence of the agro-environmental route (i.e., farm – manure – water and direct contact) compared to the agro-food route (i.e., farm – animals – food) for these infections. Strain characterization and integration of surveillance information related to ST from animal, food and humans is warranted.

MeSH terms: *Salmonella* typhimurium; spatial distribution; drug resistance, microbial

La traduction du résumé se trouve à la fin de l'article.

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The transmission of many bacterial zoonotic enteric diseases such as salmonellosis, campylobacteriosis, and Shiga toxin-producing *Escherichia coli* infections involves various interconnected pathways linking the environment, animals, and human populations. For human salmonellosis, the main recognized modes of transmission are contaminated food and water and direct contact with an infected person or animal.^{1,2}

Among the various enteric microbial pathogens transmitted through multiple and dynamic pathways, *Salmonella enterica* serovar Typhimurium (ST) definitive type 104 (DT104) infections have caused significant public health concern in various countries due to their increased incidence in the last decade and an association with antimicrobial resistance (AMR).^{3,4} Although substantial research has been conducted to understand the general epidemiology, pathogenesis, and bacteriology of *Salmonella* and ST infections in Canada and elsewhere, only fragmented information arising from population-based studies and describing geographical and temporal distributions of DT104 in Canada have been published.⁵⁻¹⁶

The objectives of this study, conducted in four Canadian provinces in 1999-2000, were to describe the geographical and seasonal distributions of reported human ST DT104 cases, to compare these characteristics to those of ST non-DT104 cases, and to investigate specific AMR patterns by province.

METHODS

Data

Every laboratory-confirmed ST case originating from passive reporting in Alberta, British Columbia, and Saskatchewan, and every second case in Ontario (due to higher load of submissions) identified from December 1999 through November 2000 were eligible for inclusion. Latitude and longitude coordinates were assigned to each case using the centroid of their residential postal code.¹⁷ Cases who resided in the classes “urban core” and “urban fringe” based on the 1996 Statistics Canada classes of urbanicity¹⁸ were categorized as urban. Cases who resided in the classes “rural fringe”, “urban area outside Consolidated Metropolitan Areas (CMA)” and “rural area outside CMAs” were categorized as rural. Records were excluded upon failure

of geographical referencing. In an attempt to focus our study to cases who had likely acquired their infections in Canada, where data were available, we excluded those who had travelled outside Canada during the five days prior to diarrhea onset. The 1996 census of Canada was used to calculate the total population living in urban and rural areas for each participating province.¹⁹

Laboratory methods

Laboratory analyses were performed at the National Laboratory for Enteric Pathogens and Foodborne Diseases (Winnipeg) by the microtitre dilution method (Sensititre™, Trek Diagnostics, Westlake, OH). ST strains that demonstrated resistance to ampicillin, chloramphenicol, streptomycin, sulphamethoxazole, and tetracycline were labelled as R-type ACSSuT, and those with resistance to ampicillin, kanamycin, streptomycin, sulphamethoxazole, and tetracycline were labelled as AKSSuT.³

Analysis

Data analysis was conducted using Epi-Info software.²⁰ The 95% Confidence intervals (CI) for means and proportions (Fisher's exact test) were calculated using the PEPI program.²¹ Means and proportions were deemed significantly different if there were no overlaps in their CIs.

RESULTS

Laboratory and epidemiological information was available for 535 cases (197 DT104 and 338 non-DT104). After excluding cases who travelled outside Canada (n=38), had missing postal codes (n=26), or missing *Salmonella* typing status (n=1), 173 DT104 cases and 297 non-DT104 cases remained in the dataset (total=470). Travel information was missing for 22% of DT104 cases and 19% of non-DT104 cases. The age and gender distributions of DT104 (mean age = 24.0; CI: 20.7-27.2) (50.9% male; CI: 43.2-58.5) and non-DT104 cases (mean age = 22.1; CI: 18.4-23.4) (50.8% male; CI: 45.0-56.6) did not significantly differ. Of the non-DT104 cases, 21.5% were DT208, 14.8% were DT124, and 63.6% were made up of 50 other phage types. Overall, DT124 and DT208 cases comprised 23.0% of all study cases.

TABLE I

Urban Distribution of Reported ST Cases by Province

Province	Status	Urban (%)	95% CI	Total
Ontario	DT104	75	(78.1%)	96
	non-DT104	79	(61.2%)	129
	Pop1996	8,007,718	(74.5%)	10,753,573
Alberta	DT104	31	(54.4%)	57
	non-DT104	47	(47.5%)	99
	Pop1996	1,859,790	(69.0%)	2,696,826
BC	DT104	15	(88.2%)	17
	non-DT104	41	(71.9%)	57
	Pop1996	2,824,991	(75.8%)	3,724,500
SK	DT104	3	(100.0%)	3
	non-DT104	6	(50.0%)	12
	Pop1996	513,216	(51.8%)	990,237
All	DT104	124	(71.7%)	173
	non-DT104	173	(58.2%)	297
	Pop1996	13,205,715	(72.7%)	18,165,136

Geographical distributions

DT104 was the most common phage type in every province, except for Saskatchewan where an equal number of cases were DT1. The proportion of DT104 cases varied across provinces with Ontario presenting the highest proportion (42.7%) followed by Alberta (36.5%), British Columbia (23.0%), and Saskatchewan (20.0%). These proportions were significantly different (Table I) between Ontario and British Columbia and between Alberta and British Columbia. DT208 was the second most common phage type in Alberta and British Columbia (28.8% and 16.2%, respectively), although this phage type was less common in Ontario (2.7%), where DT124 was the second most common phage type (15.5%) as it was in Saskatchewan (6.7%).

In 1996, Ontario, Alberta, and British Columbia had similar proportions of their general populations who lived in urban areas (urbanicity: 74.5%, 69.0%, 75.8%, respectively); however, the population of Saskatchewan was comparatively more rural with only 51.8% of the population living in urban areas (Table I). Overall, DT104 cases (71.7%) were more likely to live in urban areas compared to non-DT104 cases (58.2%). Although this difference was also apparent in the point estimates of each province, the results were not significant. For all provinces combined, the urbanicity of DT104 cases did not differ from the general population; however, in Alberta, the urbanicity of DT104 cases was lower (54.4%) than for the general provincial population (69.0%). In general, and for the provinces of Ontario and Alberta, the urbanicity of

non-DT104 cases was significantly lower than that of the general population.

AMR patterns

Overall, the proportion of DT104 cases infected with strains resistant to ACSSuT (74.0%) was significantly higher than for non-DT104 cases (10.4%) (Table II). This finding was noted for each province individually, but significant only within Ontario and Alberta. Among DT104 isolates, 92.7% were R-type ACSSuT in Ontario compared to 50.9% in Alberta, 47.1% in British Columbia and 66.7% in Saskatchewan. None of the 44 DT124, 10.9% of the DT208 and 12.7% of the other non-DT104 isolates were R-type ACSSuT.

Non-DT104 isolates were more commonly resistant to AKSSuT compared to DT104 isolates in Alberta (46.5% vs. 7.0%) and British Columbia (36.8% vs. 0%). In contrast, AKSSuT resistance was more common among DT104 cases (26.0%) than non-DT104 cases (6.2%) in Ontario. Furthermore, 100% of DT104 R-type AKSSuT isolates in Ontario were additionally resistant to chloramphenicol, compared to only 50% of the non-DT104 R-type AKSSuT isolates in the other three provinces. For all provinces combined, although 82.8% of the DT208 isolates were R-type AKSSuT, none of the 44 DT124 isolates and 12.2% of all isolates of other non-DT104 phage types were R-type AKSSuT.

Seasonal distribution

We observed DT104 cases with similar relative frequencies during each season,

TABLE II

Distribution of AMR Patterns by Phage-type Status and by Province

Province	Status	ACSSuT(%)	95% CI	AKSSuT(%)	95% CI	Total
Ontario	DT104	89 (92.7%)	[85.55; 97.02]	25 (26.0%)	[17.62; 36.00]	96
	non-DT104	5 (3.9%)	[1.27; 8.81]	8 (6.2%)	[2.72; 11.85]	129
	124	— (0.0%)		0 (0.0%)		35
	208	— (0.0%)		4 (66.7%)		6
	other	5 (5.7%)		4 (4.5%)		88
Alberta	DT104	29 (50.9%)	[37.29; 64.37]	4 (7.0%)	[1.95; 17.00]	57
	non-DT104	13 (13.1%)	[7.18; 21.41]	46 (46.5%)	[36.38; 56.77]	99
	124	— (0.0%)		0 (0.0%)		5
	208	6 (13.3%)		39 (86.7%)		45
	other	7 (14.3%)		7 (14.3%)		49
BC	DT104	8 (47.1%)	[22.98; 72.18]	0 (0.0%)	[0.00; 19.51]	17
	non-DT104	12 (21.1%)	[11.38; 33.89]	21 (36.8%)	[24.47; 50.66]	57
	124	— (0.0%)		0 (0.0%)		3
	208	— (0.0%)		9 (75.0%)		12
	other	12 (28.6%)		12 (28.6%)		42
SK	DT104	2 (66.7%)	[9.43; 99.16]	0 (0.0%)	[0.00; 70.76]	3
	non-DT104	1 (8.3%)	[0.21; 38.48]	1 (8.3%)	[0.21; 38.48]	12
	124	— (0.0%)		0 (0.0%)		1
	208	1 (100.0%)		1 (100.0%)		1
	other	— (0.0%)		0 (0.0%)		10
All	DT104	128 (74.0%)	[66.79; 80.35]	29 (16.8%)	[11.52; 23.18]	173
	non-DT104	31 (10.4%)	[7.20; 14.49]	76 (25.6%)	[20.72; 30.95]	297
	124	— (0.0%)		— (0.0%)		44
	208	7 (10.9%)		53 (82.8%)		64
	other	24 (12.7%)		23 (12.2%)		189
Total		159 (33.8%)	[29.56; 38.31]	105 (22.3%)	[18.65; 26.38]	470

Figure 1. Seasonality of human reported cases of ST DT104 and ST non-DT104 cases (all provinces).

* Significance for the seasonal difference is shown with non-overlapping confidence intervals around the point estimates.

ranging from 22% to 28% of total study cases (Figure 1). However, the distribution of non-DT104 cases, including DT208 and DT124 (data not shown), showed a marked seasonal cycle with lower relative frequency of cases being

reported in winter (11.6%; CI: 8.3-15.9) and higher during the summer (40.5%; CI: 35.0-46.3). The proportion of non-DT104 cases reported in the winter were significantly lower than for DT104 cases.

DISCUSSION

Geographical distributions

We noted that in all provinces combined, the rate of urbanicity among non-DT104 cases (58.2%) was significantly lower than for the general population. Overall, these proportions were unexpectedly different from DT104 cases, for which the rate of urbanicity was estimated to be 13.5% higher (71.7%) than for the non-DT104 cases and similar to the rate of urbanicity in the general population. This finding might be attributed to predisposing factors specific to DT104 infections in people living in urban areas or, when comparing to non-DT104 infections, might be explained by a greater importance of the food transmission route (relative to waterborne or direct contact) for ST DT104 organisms. However, this relative difference in case distribution might also be related to different food habits (e.g., local production or processing of meat, food safety awareness, education, diet) in rural versus urban communities or by the presence of relatively more immunologically challenged or naïve populations located mostly in urban areas; however, these factors were not measured in the present study.

ST DT208 cases were observed most frequently in Alberta (with 70.3% of all

DT208 cases studied) where they comprised 29% of all ST cases. Provincial-level surveillance of ST phage types is necessary to better understand the relationship between food, livestock, environmental factors and human salmonellosis in Canada. For Ontario, the largest group of non-DT104 cases was DT124. To our knowledge, no epidemiological information has been published about this phage type in Canada. Nevertheless, it seems prudent to investigate further the risk factors associated with this phage type, which represented 27% of the non-DT104 ST cases reported in Ontario during this study period.

Season

Another contrasting element in the epidemiology of ST DT104 vs. non-DT104 cases is underlined by their temporal distributions. DT104 cases displayed no seasonality whereas non-DT104 cases peaked in the summer and exhibited a lower incidence in the winter, a phenomenon observed for many other microbial enteric pathogens associated with agro-environmental factors.^{22,23} Researchers have proposed various reasons for this predictable cycle, including higher recreational water exposure, increased activity relating to cooking outside, and greater microbial contamination of the environment from livestock manure due to fertilization of agricultural land and animals on pasture.^{24,25} The seasonality of the incidence of ST non-DT104 cases may indicate a true change over time in the occurrence of organisms of these different phage type groups in the environment. Alternatively, we hypothesize that the lack of seasonality in the incidence of human DT104 cases may indicate a lesser influence of the agro-environmental route (i.e., farm – manure – water and direct contact) compared to the agro-food route (i.e., farm – animals – food) for these infections. This would be consistent with finding cases living in urban areas in greater proportion for DT104 infections when compared to non-DT104 type.

Antimicrobial resistance

Overall, DT104 isolates were much more likely to be R-type ACSSuT compared to non-DT104. This proportion was higher in Ontario (91.9%) than observed for

British Columbia where the ACSSuT pattern comprised 19% of ST DT104 isolates in 1997-1998.¹⁴ However, the difference in AMR patterns between provinces is not well understood.¹⁶ R-type AKSSuT was often associated with DT208 and this was noted in Alberta and British Columbia in particular. In Ontario, all of the DT104 R-type AKSSuT isolates were additionally resistant to chloramphenicol compared to only half of DT104 R-type AKSSuT isolates in the other provinces. None of the DT124 cases were R-type ACSSuT or AKSSuT.

In England and Wales, in a comparison of antimicrobial susceptibility in non-typhoidal *Salmonellae* from humans and food animals, resistance was observed in DT104 and DT208 isolates.²⁶ The incidence of R-type ACSSuT was similar for all animal species (i.e., cattle, sheep, pigs and poultry) and was almost identical to that in human isolates. Establishing long-term nation-wide initiatives to describe antimicrobial use and integrated monitoring of resistance patterns in food, animals, and humans would certainly provide considerable value in better understanding the public health importance of these various AMR patterns and the extent of their link with animal sources.

Limitations

Although the main characteristics of DT104 and non-DT104 ST cases reported through their respective disease surveillance programs are assumed to represent the population of cases arising from the same underlying population, annual frequencies of reportable enteric zoonoses are known to suffer under-reporting. We must also note that the geographical location of cases was defined using the residential postal codes. Although this is a recognized geo-referencing method used for surveillance databases, this cannot, however, be seen as a precise measure of contact with agriculture activities nor closeness to urban centres. Results presented in this study are descriptive in nature. Further multivariate analyses, including an attempt to take possible socio-demographic factors into consideration, are warranted.

Our results may reflect different contamination levels of DT104 and non-DT104 strains in food, reservoir animals (wild and domestic), water sources, and/or

a variable proportion of susceptible populations with important determinants linked to the geography. There is, however, no current comprehensive animal and environmental surveillance data to verify these conclusions. Ongoing multi-provincial initiatives should be encouraged to better understand the reasons for these observed regional and temporal variations. As such, the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) was initiated in 2002 to monitor trends in antimicrobial use and the development of resistance in selected bacterial organisms from human, animal and food sources across Canada.²⁷

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APPENDIX

The Multi-Provincial *Salmonella* Typhimurium Case-Control Study Steering Committee

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RÉSUMÉ

Contexte : Notre étude vise à décrire la répartition géographique et saisonnière des cas humains déclarés d'infections par *Salmonella* typhimurium type définitif (DT) 104, à comparer ces caractéristiques à celles des autres salmonelloses à *S. typhimurium* et à examiner les patrons de résistance aux antimicrobiens dans quatre provinces canadiennes.

Méthode : Nous avons examiné tous les cas de *S. typhimurium* confirmés en laboratoire déclarés dans les rapports de surveillance passive de l'Alberta, de la Colombie-Britannique et de la Saskatchewan, et un cas sur deux déclaré en Ontario entre décembre 1999 et novembre 2000.

Résultats : En tout, il y a eu 470 notifications d'infections humaines à *S. typhimurium* pendant la période de l'étude. Le lysotype DT104 était le plus commun, bien que sa fréquence ait varié d'une province à l'autre. La proportion des personnes infectées par DT104 vivant en milieu urbain en Ontario, en Colombie-Britannique et en Saskatchewan était la même que dans la population générale, mais en Alberta, les personnes infectées par DT104 étaient plus susceptibles de vivre en milieu rural. Dans l'ensemble, les isolats de DT104 présentaient plus souvent le phénotype de résistance ACSSuT que les autres lysotypes de *S. typhimurium*, et le phénotype de résistance AKSSuT était souvent associé au lysotype DT208. Les cas d'infections par *S. typhimurium* DT104 ne présentaient aucun cycle saisonnier, tandis que les autres lysotypes étaient plus fréquents l'été que l'hiver.

Interprétation : Nos résultats donnent à penser que les cas d'infections par DT104 et par les autres lysotypes de *S. typhimurium* varient selon la province, la résidence (en milieu urbain ou rural) et les patrons de résistance. L'absence de cycles saisonniers dans les cas d'infections par DT104 pourrait être le signe d'une moindre influence de la voie de transmission agroenvironnementale (ferme - lisier - eau et contact direct) par rapport à la voie agroalimentaire (ferme - animaux - aliments) pour ces infections. Cela confirme l'utilité de la caractérisation des souches et de l'intégration des données de surveillance de *S. typhimurium* chez les animaux, dans les aliments et chez les humains.

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