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Utilization of a molecular serotyping method for *Salmonella enterica* in a routine laboratory in Alberta Canada

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ABSTRACT

Salmonella is one of the most common enteric pathogens related to foodborne illness. Alberta's Provincial Laboratory for Public Health (ProvLab) provides Outbreak and Surveillance support by performing serotyping. The Check&Trace *Salmonella*™ (CTS) assay (Check-Points, Netherlands), a commercial DNA microarray system, can determine the serotype designation of a *Salmonella* isolate with automated interpretation. Here we evaluate 1028 *Salmonella* isolates of human clinical or environmental sources in Alberta, Canada with the CTS assay. CTS was able to assign a serovar to 98.7% of the most frequently occurring human clinical strains in Alberta (82.5% overall), and 71.7% of isolates which were inconclusive by conventional methods. There was 99.7% concordance in environmental isolates. The CTS database has potential to expand to identify rare serovars. With the anticipated shift to molecular methods for identification, CTS provides an easy transition and demonstrates ease-of-use and reduces the turn-around-time of a reported result significantly compared to classical serotyping.

1. Introduction

Salmonella is one of the most common enteric pathogens causing diarrhea, fever and abdominal cramps and can lead to hospitalization or death. Salmonellosis continues to be one of the highest healthcare burdens in Canada and the US with reported case incidence rates of 19.67 per 100,000 patient year and 15.45 per 100,000 population respectively (Thomas et al., 2015; Crim et al., 2015). Transmission is often through consumption of contaminated foods and the source in outbreaks are frequently associated with eggs and meats. In Canada, nontyphoidal *Salmonella* is estimated to be responsible for the second highest foodborne mortality rate next to *Listeria* of all bacterial etiologies (Belanger et al., 2015).

The Provincial Laboratory for Public Health (ProvLab) in Alberta, Canada is involved with isolate identification and serotyping which is critical to support outbreak investigations and national surveillance initiatives. ProvLab works closely with Alberta public health authorities and with Alberta Agriculture and Forestry (AAF) to support foodborne illness investigations across the province. ProvLab provides serotyping for isolates obtained from stools and food samples submitted for public health investigations; as well as over 800 *Salmonella* isolates annually from human clinical cases submitted by Alberta's diagnostic laboratories. Data generated from the clinical isolates feeds into surveillance systems such as FoodNet Canada (http://www.phac-aspc.gc.ca/foodnetcanada/index-eng.php) and molecular typing results such as pulsed-field gel electrophoresis are uploaded to PulseNet Canada (http://www.pulsenetinternational.org/networks/canada/) coordinated by the Public Health Agency of Canada.

Salmonella serotypes are determined through the White-Kauffmann-Le Minor (WKL, formerly "Kauffmann-White") scheme (Grimont and Weill, 2007), which is the current gold- standard reference method. However, this method is time consuming, subjective and can take up to 4 or 5 days for phase expression for some serovars. Serotyping isolates with incomplete antigenic formula (including *Salmonella enterica* subsp. *enterica* 4,[5],12:i:-) is found in nearly 10% of isolates received at the ProvLab. This can be due to variability of expression of somatic and/or flagellar antigens between strains, and also the availability of high quality antisera. Interpretation of the results requires visual interpretation and considerable expertise. For these reasons, conventional serotyping is often only available at provincial or national reference laboratories and results are further delayed by shipping time from the submitting laboratory.

Abbreviations: CTS, Check&Trace *Salmonella*™ assay; AAF, Alberta Agriculture and Forestry; PHAC-NML, Public Health Agency of Canada-National Microbiology Laboratory.

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The Check&Trace *Salmonella* (CTS) platform (Check-Points, Netherlands) is a commercial microarray system based on genomic markers which can genetically identify the WKL serotype designation of a large variety of *Salmonella* isolates. The principle of the assay is based on a DNA hybridization array system where the software assigns a numeric 'genovar number' to each array pattern. The genovar number will associate with a serovar identity if recognized by the software. If the specific serovar is not available in the CTS database, alternately, the 'genovar number' is provided (Wattiau et al., 2008).

In this study, we evaluate 1) the CTS platform for serotyping *Salmonella enterica* isolates from human and environmental sources in parallel with conventional serotyping: the WKL scheme and 2) its application in a routine clinical microbiology laboratory setting.

2. Materials and methods

2.1. Isolate selection

2.1.1. Pilot study

Salmonella isolates from human clinical cases previously submitted and serotyped at ProvLab from 2006 to 2012 were selected, representing common, difficult to type or unusual serovars. At least four isolates representing each of the most frequent serovars from human cases in Alberta were included (Table 1). In addition, a blind panel of 25 isolates was provided by the Public Health Agency of Canada-National Microbiology Laboratory (PHAC-NML) in Winnipeg, Manitoba, Canada. The panel (Table 1) contained 8 of the most commonly isolated human serovars, 10 other serovars, 4 difficult-to-type isolates (due to antigen expression and antibody variability) and 3 unusual serovars rarely found in Canada.

2.1.2. Clinical comparative evaluation

Two hundred and seventy-two *Salmonella enterica* isolates from human cases (a 'case' is defined as the first clinical isolation of *Salmonella* species from the same patient within six months) over a one-year period (April 15, 2013–Apr 14, 2014) were included in the evaluation (Table 1). In the case of an outbreak setting, only one isolate per serovar within the particular outbreak was selected. To avoid over-representation of common serovars, a maximum of three isolates per serovar per month were included. In addition, isolates of *Salmonella* Enteritidis and Typhimurium were restricted for testing on CTS after the pilot phase as both serovars comprised the majority of isolates submitted to ProvLab. All human isolates received during this period with incomplete antigenic formulae where a serovar could not be decided based on the WKL scheme were referred to the PHAC-NML for confirmation. This included all *Salmonella* 4,[5],12:i:- isolates. An additional 38 archived clinical isolates that were difficult-to-type or unusual serovars were also included.

2.1.3. Environmental comparative evaluation

A parallel comparison of conventional versus molecular serotyping was performed for a one-year period for *Salmonella enterica* isolated from animal/environmental sources for a total of 334 isolates (Table 1). *Salmonella* was isolated from samples submitted to AAF for disease investigation or surveillance programs from March 26, 2013–March 25, 2014.

2.1.4. Exclusivity isolates

The following non-*Salmonella* enteric organisms isolated from clinical samples were tested on CTS to confirm that non-specific patterns would not be generated by the arrays: *E. coli* ($n = 1$), *E. coli* O157:H7 ($n = 1$) *Citrobacter* sp. ($n = 2$) *Shigella* sp. ($n = 2$) and *Yersinia enterocolitica* ($n = 1$).

2.2. Conventional serotyping

Salmonella isolates of human and environmental sources were grown as pure 18–24 h cultures on tryptic soy broth agar (TSBA, ProvLab Alberta) following initial subculture onto Sheep Blood Agar plate (BAP) from frozen culture collection. An acriflavin slide agglutination test was done to screen for O-rough isolates. Antigenic formulae and serovar were determined using the WKL scheme. Where required, phase inversion was induced to determine the second phase. Commercially available *Salmonella* antisera (Statens Serum Institut, Denmark)

Table 1
Human clinical and environmental *Salmonella* isolate selection used in the evaluation.

Human sources							Environmental sources		
Salmonella enterica subsp. *enterica* serovar group	Annual ave (%)	Pilot study	NML blind panel	Clinical comparative evaluation	Additional archived isolates	Total human clinical	*Salmonella enterica* subsp. *enterica* serovar group	Annual ave (%)	Environmental comparative evaluation
Top 20 most frequent	839 (84.5)	102	8	272	4	386	Top 20 most frequent[a]	302 (84.1)	334
S. Enteritidis	384 (38.7)	5	1	15	0	21	*S.* Kentucky	79 (22.0)	70
S. Typhimurium	103 (10.4)	5	1	19	0	25	*S.* Enteritidis	50 (13.9)	49
S. Heidelberg	82 (8.3)	7	0	22	0	29	*S.* Typhimurium	28 (7.8)	62
S. 4,[5],12:i:-	60 (6.0)	4	1	47	3	55	*S.* Mbandaka	27 (7.5)	29
S. Newport	24 (2.4)	4	1	15	0	20	*S.* Heidelberg	21 (5.8)	12
S. Typhi	22 (2.2)	5	0	20	0	25	*S.* Senftenberg	16 (4.5)	12
S. Infantis	22 (2.2)	7	1	11	1	20	*S.* Montevideo	10 (2.8)	11
S. Saintpaul	22 (2.2)	5	0	13	0	18	*S.* Infantis	9 (2.5)	9
S. Agona	14 (1.4)	5	0	12	0	17	*S.* Agona	8 (2.2)	5
S. Javiana	13 (1.3)	5	1	13	0	19	*S.* Schwarzengrund	7 (1.9)	18
S. Paratyphi A	11 (1.1)	5	0	10	0	15	*S.* Rissen	7 (1.9)	10
S. Braenderup	11 (1.1)	5	0	7	0	12	*S.* 4,[5],12:i:-	6 (1.7)	12
S. Paratyphi B var Java	10 (1.0)	5	0	9	0	14	*S.* Braenderup	6 (1.7)	12
S. Oranienburg	10 (1.0)	5	0	9	0	14	*S.* Brandenburg	5 (1.4)	0
S. Stanley	9 (0.9)	5	1	12	0	18	*S.* Alachua	5 (1.4)	5
S. Hadar	9 (0.9)	6	0	2	0	8	*S.* Cubana	4 (1.1)	3
S. Thompson	9 (0.9)	4	1	9	0	14	*S.* Ohio	4 (1.1)	2
S. Schwarzengrund	9 (0.9)	5	0	17	0	22	*S.* Dublin	4 (1.1)	8
S. Muenchen	8 (0.8)	5	0	5	0	10	*S.* Johannesburg	3 (0.8)	0
S. Panama	7 (0.7)	5	0	5	0	10	*S.* Hadar	3 (0.8)	5
Other serovars	112 (11.3)	66	10	107	32	215	Other serovars	29 (8.1)	24
Incomplete antigenic formulae	30 (3.0)	18	4	22	2	46	Incomplete antigenic formulae	27 (7.5)	11
Non-*enterica* subsp.	12 (1.2)	4	3	5	0	12	Non-*enterica* subsp.	1 (0.2)	0

[a] Based on isolates submitted from Alberta clinical laboratories and Alberta Agriculture and Forestry 2009–2013.