C-1460

Ongoing Evolution in the Epidemiology and Antimicrobial Resistance of *Streptococcus pneumoniae* in Canada in the PCV-13 Era: SAVE 2013

H.J. ADAM[1,2,3], A. GOLDEN[2], M. GILMOUR[1,2], M. BAXTER[2,3], I. MARTIN[4], K.A. NICHOL[1,3], W. DEMCZUK[4], R. VASHISHT[2,3], J.A. KARLOWSKY[1,2,3], D.J. HOBAN[1,2,3], and G.G. ZHANEL[2,3]

[1]Diagnostic Services Manitoba, [2]University of Manitoba, [3]Canadian Antimicrobial Resistance Alliance (CARA), and [4]National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada

Dr. Heather Adam
MS673-820 Sherbrook St.,
Winnipeg, MB R3A 1R9
Phone: 204-787-8678
Email: hadam@dsmanitoba.ca

ABSTRACT

Background: The SAVE study (SPN Serotyping and Antimicrobial Susceptibility: Assessment for Vaccine Efficacy in Canada) is an annual, ongoing study that was initiated in 2011, after PCV-13 was introduced in Canada.
Methods: In collaboration between CARA and the National Microbiology Laboratory, the SAVE study collected 3700 invasive isolates in 2011-13 from across Canada (1356, 1206, and 1138 in 2011, 2012, and 2013, respectively). Serotyping was performed using the Quellung reaction (Statens Serum Institute, Copenhagen, Denmark). Susceptibility testing (AST) was performed in accordance with CLSI methods. Changes in serotype (ST) distribution and multi-drug resistance (MDR) rates between 2011 and 2013 were assessed for statistical significance.
Results: In 2013, 4.6%, 14.9%, and 33.6% of the currently circulating SPN ST are contained in PCV-7, PHiD-CV, and PCV-13, respectively. The susceptibility results of the 10 most common STs circulating in 2013 are shown below.

Serotype (N)	% Susceptible								% MDR
	PEN (iv, M)	PEN (iv, NM)	CRO (M)	CRO (NM)	CLR	LEV	SXT	DOX	
22F (125)	99.2	99.2	99.2	98.2	71.2	99.2	98.4	100	0
19A (120)	71.2	92.4	77.1	94.9	38.1	100	68.6	67	26.7
7F (115)	100	100	100	100	98.2	100	100	95.5	0
3 (88)	98.8	100	100	100	96.3	100	97.6	89	2.3
12F (58)	100	100	100	100	17.2	100	98.3	94.8	1.7
8 (52)	98.1	100	98.1	100	98.1	100	94.2	92.3	1.9
33F (49)	98.0	100	100	100	14.3	100	21.4	92.9	10.2
11A (49)	97.9	100	100	100	77.1	97.9	85.4	100	0
9N (46)	97.8	100	100	100	91.3	100	100	95.7	0
10A (37)	94.3	100	100	100	94.3	100	91.4	94.3	0

M, meningitis; NM, nonmeningitis; PEN, penicillin; CRO, ceftriaxone; CLR, clarithromycin; LEV, levofloxacin; SXT, trimethoprim-sulfamethoxazole; DOX, doxycycline; MDR, multi-drug resistance [resistance to ≥ 3 antibiotic classes (penicillin resistance defined as MIC ≥ 2 µg/ml)]

Significant changes (P<0.05) in ST prevalence between 2011 and 2013 were noted in STs 5 (0.8% vs. 0.2%), 7F (19.9% vs. 10.1%), 8 (2.8% vs. 4.6%), 10A (1.3% vs. 3.3%), 11A (2.8% vs. 4.3%), 22F (7.8% vs. 11%), 31 (0.2% vs. 1.0%), 33A (1.7% vs. 0%), 33F (1.8% vs. 4.3%), and 35B (1.5% vs. 2.7%). Current MDR was noted in STs 3 (2.3%), 6B/C (33.3/14.7%), 8 (1.9%), 12F (1.7%), 14 (33.3%), 15A (22.5%), 17F (10%), 19A/F (26.7/31.3%), 23F (25%), 33F (10.2%), 35A (50%), and 38 (6.7%). MDR SPN rates decreased from 8.5% in 2011 to 5.7% in 2013 (P=0.02).
Conclusion: In 2013, 33.6% of all circulating SPN and 66.2% of MDR SPN in Canada are ST included in PCV-13. Significant changes in the epidemiology and AST patterns continue to occur in SPN in Canada, warranting ongoing surveillance.

BACKGROUND

The introduction of Prevnar® (PCV-7), a 7-valent pneumococcal conjugate vaccine, was effective in reducing systemic infections due to *Streptococcus pneumoniae* in children as well as reducing the incidence of recurrent upper respiratory tract infections in children.[1,2] However, the emergence of non-PCV-7 *S. pneumoniae* serotypes in Canada, particularly multi-drug resistant strains, is an ongoing issue.

Subsequently, two newer pneumococcal conjugate vaccines have been introduced in Canada: Synflorix™ (PHiD-CV) and Prevnar®13 (PCV-13). The broader serotype coverage and critical inclusion of serotype 19A in PCV-13 offers an important advancement in the protection of Canadian children against invasive *S. pneumoniae* infections. Due to the enhanced coverage of the predominant serotypes in North America, current immunization guidelines recommend the routine use of PCV-13.[3,4]

The *S. pneumoniae* Serotyping and Antimicrobial Susceptibility: Assessment for Vaccine Efficacy in Canada (SAVE) study began in 2011 to assess the *S. pneumoniae* serotypes and their antimicrobial susceptibility patterns in Canada after the introduction of the PCV-13 vaccine. Changes in serotype (ST) distribution and multi-drug resistance (MDR) rates between 2011 and 2013 were assessed to evaluate the evolution of serotypes and antimicrobial resistance subsequent to the introduction of PCV-13 in Canada.

MATERIALS & METHODS

Isolate Collection:

S. pneumoniae isolated from sterile sites are forwarded from Canadian public health laboratories [Canadian Public Health Laboratory Network (CPHLN)] to the National Microbiology Laboratory - Public Health Agency of Canada. Through a collaboration between the Canadian Antimicrobial Resistance Alliance (CARA) and the National Microbiology Laboratory – Public Health Agency of Canada and subsequent to the permission of the submitting CPHLN sites, the *S. pneumoniae* isolates were forwarded to CARA. A total of 3700 invasive *S. pneumoniae* isolates from across Canada were included in the SAVE study as part of this collaboration (Jan. 1, 2011 – Dec. 31, 2013).

Antimicrobial Susceptibility Testing:

Antimicrobial susceptibility testing was performed using custom designed antimicrobial susceptibility panels using CLSI methods. These antimicrobials were obtained as laboratory grade powders from their respective manufacturers or commercial sources. The MICs of the antimicrobial agents for the isolates were determined by the broth microdilution method, which was performed in adherence to all CLSI practices and quality control measures, and interpreted utilizing CLSI criteria (M7-A9, M100-S23).

Multi-drug resistance was defined as resistance to ≥3 antimicrobial classes (penicillin MIC ≥ 2 µg/mL).

Serotyping:

Serotyping was performed using the Quellung reaction using pool, group, type and factor commercial antisera (Statens Serum Institute, Copenhagen, Denmark) and supplemental molecular serotyping was performed with the US Centre for Disease Control's PCR multiplex method (http://www.cdc.gov/ncidod/biotech/strep/pcr.htm). Isolates for which a serotype was not determined by PCR and a Quellung reaction was not observed were confirmed as *S. pneumoniae* by *rpo*B gene sequencing.

RESULTS

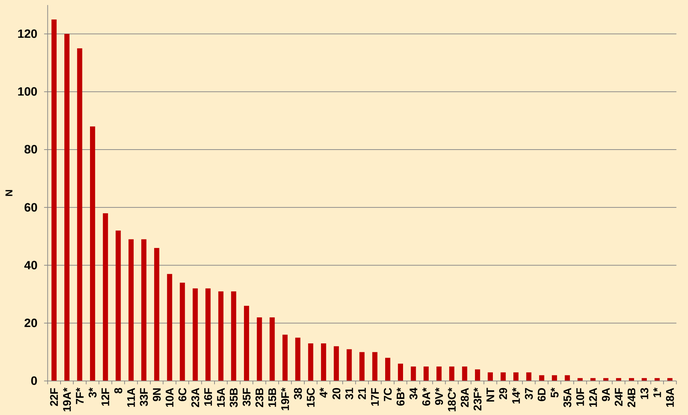

Figure 1. ***Streptococcus pneumoniae*** **Serotype Distribution in Canada (2013).**
*, PCV-13 Serotypes

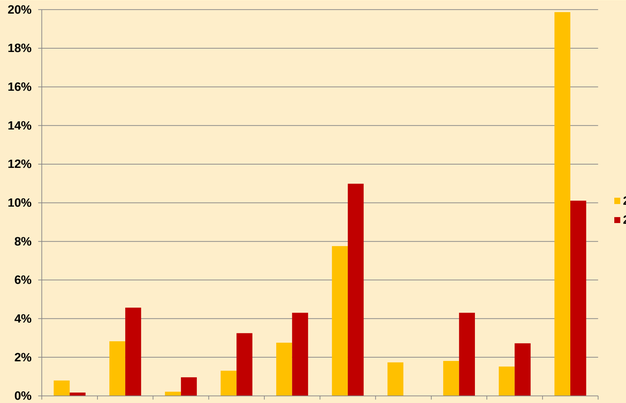

Figure 2. Serotypes demonstrating Significant Changes (P<0.05) in Canada between 2011 and 2013.
*, PCV-13 Serotypes

Table 1. Antimicrobial Susceptibility for the 10 Most Common Serotypes Circulating in Canada (SAVE 2013).

Serotype (N)	% Susceptible							
	PEN (M)	PEN (NM)	CRO (M)	CRO (NM)	CLR	LEV	SXT	DOX
22F (125)	99.2	99.2	99.2	98.2	71.2	99.2	98.4	100
19A (120)	71.2	92.4	77.1	94.9	38.1	100	68.6	67
7F (115)	100	100	100	100	98.2	100	100	95.5
3 (88)	98.8	100	100	100	96.3	100	97.6	89
12F (58)	100	100	100	100	17.2	100	98.3	94.8
8 (52)	98.1	100	98.1	100	98.1	100	94.2	92.3
33F (49)	98.0	100	100	100	14.3	100	21.4	92.9
11A (49)	97.9	100	100	100	77.1	97.9	85.4	100
9N (46)	97.8	100	100	100	91.3	100	100	95.7
10A (37)	94.3	100	100	100	94.3	100	91.4	94.3

M, meningitis; NM, nonmeningitis; PEN, penicillin; CRO, ceftriaxone; CLR, clarithromycin; LEV, levofloxacin; SXT, trimethoprim-sulfamethoxazole; DOX, doxycycline

Table 2. Antimicrobial Susceptibility of *S. pneumoniae* in Canada for All Serotypes and PCV-13 Serotypes (SAVE 2013).

Antimicrobial Agent (CLSI Interpretive Criteria)	% Susceptible	
	All serotypes (n=1101)	PCV-13 serotypes (n=369)
Penicillin (iv, nonmeningitis)	99.0	97.3
Penicillin (iv, meningitis)	89.8	85.6
Penicillin (oral, Penicillin V)	89.8	85.6
Ceftriaxone (nonmeningitis)	99.3	98.1
Ceftriaxone (meningitis)	96.5	90.5
Clarithromycin	73.0	74.5
Levofloxacin	99.3	98.6
Trimethoprim-Sulfamethoxazole	86.0	83.7
Doxycycline	89.2	83.2

MDR was observed in serotypes 3 (2.3%), 6B/C (33.3/14.7%), 8 (1.9%), 12F (1.7%), 14 (33.3%), 15A (22.5%), 17F (10%), 19A/F (26.7/31.3%), 23F (25%), 33F (10.2%), 35A (50%), and 38 (6.7%) in 2013.

Of the MDR *S. pneumoniae* in SAVE 2013, there were 26 isolates resistant to 3 classes of antibiotics, 7 resistant to 4 classes of antibiotics, 31 resistant to 5 classes of antibiotics, and 1 resistant to 7 classes of antibiotics. The most common MDR phenotypes demonstrated resistance to clarithromycin, clindamycin, doxycycline, penicillin, and trimethoprim-sulfamethoxazole (n=31) and clarithromycin, clindamycin, and doxycycline (n=21).

MDR *S. pneumoniae* rates decreased from 8.5% in 2011 to 5.7% in 2013 (P=0.02).

Table 3. Demographics of the Common (n≥5) Multi-drug Resistant *S. pneumoniae* by Serotype in Canada (SAVE 2013)

Serotype (N)	Geographic Region *	Age Group (years)							Region Total
		0-<1	1-<2	2-<6	6-<18	18-<50	50-<65	≥65	
19A (28)	West	1				5	1	2	9
	Central		1		3	3	4	6	17
	East			1			1		2
15A (7)	West					1			1
	Central	1					1	4	6
	East								0
19F (5)	West								0
	Central							4	4
	East		1						1
33F (5)	West								0
	Central	1		1			2	1	5
	East								0
6C (5)	West								0
	Central			1			2	2	5
	East								0

*, West (Saskatchewan, Manitoba); Central (Ontario, Quebec); East (Prince Edward Island, Nova Scotia, New Brunswick, Newfoundland and Labrador); [a.] No age data available for 4 additional serotype 19A isolates : 1 from Central, 3 from East

CONCLUSIONS

1. In 2013, 33.6% of all circulating *S. pneumoniae* and 66.2% of MDR *S. pneumoniae* in Canada are serotypes in PCV-13.
2. The most commonly circulating serotypes are 22F, 19A, 7F, 3, 12F, 8, 33F, 11A, 9N, and 10A. Between 2011 and 2013, statistically significant reductions in the prevalence of vaccine serotypes 5 and 7F,were observed. Among non-vaccine serotypes, significant reductions in serotype 33A and increases in serotypes 8, 10A, 11A, 22F, 31, 33F, and 35B occurred.
3. In 2013, multidrug resistance was observed in serotypes 3, 6B/C, 8, 12F, 14, 15A, 17F, 19A/F, 23F, 33F, 35A, and 38. Rates of multidrug resistance in *S. pneumoniae* significantly decreased from 8.5% in 2011 to 5.7% in 2013.
4. Significant changes in the epidemiology and antimicrobial susceptibility patterns continue to occur in *S. pneumoniae* in Canada, warranting ongoing surveillance.

ACKNOWLEDGMENTS

We sincerely thank the participating Canadian Public Health Laboratory Network (CPHLN) sites: Saskatchewan Disease Control Laboratory (Regina, SK), Cadham Provincial Laboratory (Winnipeg, MB), Ontario Provincial Laboratory (Etobicoke, ON), Quebec Public Health Laboratory (Ste-Anne-de-Bellevue, QC), Queen Elizabeth Hospital Laboratory Medicine (Charlottetown, PEI), Horizon Health Network - Zone 3 (Fredericton, NB), Microbiology Section, IWK Health Center (Halifax, NS), and Newfoundland Public Health Laboratory (St. John's, NL).

Support for this study was provided in part by the University of Manitoba, Health Sciences Centre and the National Microbiology Laboratory in Winnipeg, Manitoba, Canada and Pfizer Canada.

REFERENCES

1. Bettinger, J.A., D.W. Scheifele, J.D. Kellner, S.A. Halperin, W. Vaudry, B. Law, and G. Tyrrell for Members of the Canadian Immunization Monitoring Program, Active (IMPACT). 2010. The effect of routine vaccination on invasive pneumococcal infections in Canadian children, Immunization Monitoring Program, Active 2000-2007. Vaccine. 28:2130-2136.
2. Centers for Disease Control and Prevention. 2005. Direct and indirect effects of routine vaccination of children with 7-valent pneumococcal conjugate vaccine on incidence of invasive pneumococcal disease – United States, 1998-2003. MMWR Morb. Mortal. Wkly. Rep. 54: 893-897.
3. National Advisory Committee on Immunization. 2010. Update on the use of conjugate vaccines in childhood. Can. Commun. Dis. Rep. 36: 1-21.
4. [CDC] Centers for Disease Control and Prevention. 2010. Prevention of pneumococcal disease among infants and children - Use of 13-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm. Rep. 59(RR-11): 1-18.

54th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, September 6-9, 2014