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SAVE Study: *Streptococcus pneumoniae* Serotyping and Antimicrobial Susceptibility: Assessment for Vaccine Efficacy in Canada, 2011-2015

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ABSTRACT

Background: The SAVE study is an annual study initiated in 2011, after PCV-13 was introduced in Canada. The study aims to detect antimicrobial susceptibility changes, overall and by serotype, in *S. pneumoniae* (SPN) overtime.

Methods: In collaboration between CARA, selected public health laboratories and the National Microbiology Laboratory, the SAVE study collected 6207 invasive isolates in 2011-15 from selected jurisdictions across Canada (1379, 1285, 1138, 1210 and 1195 in 2011, 2012, 2013, 2014 and 2015, 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 2015 were assessed for statistical significance.

Results: In 2015, 25.3% of the SPN STs collected as part of the SAVE study were contained in PCV-13; however, considerable variability was noted by study age group (0-<1 year: 14.3% - 6-<18 years: 34.4%). The susceptibility results of the 10 most common STs in 2015 are shown below.

Serotype (N)	% Susceptible								% MDR
	PEN (iv, M)	PEN (iv, NM)	CRO (M)	CRO (NM)	CLR	LVX	SXT	DOX	
22F (101)	99.0	100	100	100	66.3	100	100	96.0	3.0
3 (96)	100	100	100	100	96.8	100	100	91.5	3.1
19A (91)	71.4	87.9	80.2	96.7	29.7	100	69.2	68.1	27.5
12F (68)	98.5	100	100	100	52.9	100	98.5	95.6	1.5
33F (65)	100	100	100	100	23.4	100	28.1	89.1	3.1
9N (64)	96.9	100	98.4	100	92.2	100	93.8	98.4	1.6
8 (58)	98.3	100	100	100	100	100	100	98.3	0
7F (49)	100	100	100	100	100	100	100	95.9	0
11A (45)	97.8	100	100	100	75.6	100	80.0	95.6	0
15A (40)*	27.6	100	100	100	24.1	100	86.2	17.2	40.0
20 (40)*	100	100	100	100	95.0	100	100	100	0

M, meningitis; NM, nonmeningitis; PEN, penicillin; CRO, ceftriaxone; CLR, clarithromycin; LVX, levofloxacin; SXT, trimethoprim-sulfamethoxazole; DOX, doxycycline; MDR, multi-drug resistance [resistance to ≥ 3 antibiotic classes (penicillin resistance defined as MIC ≥ 2 µg/ml)]; *, serotype 15A and 20 both ranked as the 10th most common serotype.

Significant changes (P<0.05) in ST prevalence were observed among the isolates tested between 2011 and 2015 with decreases of STs 7F, 19A and 33A and increases of STs 7C, 8, 9N, 10A, 20, 24F, 29, 31, 33F, 35B, and 38. Current MDR was noted in STs 3 (3.1%), 6B/C (50/2.7%), 9N (1.6%), 12F (1.5%), 14 (28.6%), 15A (40%), 19A/F (27.5/18.2%), 22F (3.0%), 23F (50%), 33F (3.1%) and 35B (5.7%). MDR SPN rates decreased from 8.6% in 2011 to 5.6% in 2015 (P=0.0041).

Conclusion: In 2015, 25.3% of all SPN and 57.1% of MDR SPN are STs included in PCV-13. The ongoing changes in epidemiology and AST patterns in SPN in Canada underscore the need for continued 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 was of significant concern. Subsequently, newer pneumococcal conjugate vaccines were developed with enhanced serotype coverage, including 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. Current immunization guidelines recommend the routine use of PCV-13 in North America.[3,4] The predominant serotypes and their antimicrobial susceptibility patterns are expected to continue to evolve over time.

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 2015 were assessed to evaluate the evolution of serotypes and antimicrobial resistance subsequent to the introduction of PCV-13 in Canada.

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.

MATERIALS & METHODS

Isolate Collection:

S. pneumoniae isolated from sterile sites are forwarded from the 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 select submitting CPHLN sites (as detailed in the acknowledgments), the *S. pneumoniae* isolates were forwarded to CARA. A total of 6207 invasive *S. pneumoniae* isolates from across Canada were included in the SAVE study as part of this collaboration (Jan. 1, 2011 – Dec. 31, 2015) The annual number of *S. pneumoniae* collected were 1379, 1285, 1138, 1210 and 1195 in 2011, 2012, 2013, 2014 and 2015, respectively.

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 27th Edition).

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 supplementary 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.

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CONCLUSIONS

1. In 2015, 25.3% of all circulating *S. pneumoniae* and 57.1% of MDR *S. pneumoniae* in Canada are serotypes in PCV-13.
2. The most commonly circulating serotypes in the 2015 SAVE study are 22F, 3, 19A, 12F, 33F, 9N, 8, 7F, 11A, 15A and 20.
3. Between 2011 and 2015, statistically significant reductions in the prevalence of vaccine serotypes 7F and 19A were observed. Among non-vaccine serotypes, significant reductions in serotype 33A and increases in serotypes 7C, 8, 9N, 10A, 20, 24F, 29, 31, 33F, 35B, 35F and 38 occurred.
4. In 2015, multidrug resistance was observed in serotypes 3, 6B, 6C, 9N, 12F, 14, 15A, 19A, 19F, 22F, 23F, 33F, and 35B.
5. Rates of multidrug resistance in *S. pneumoniae* significantly decreased from 8.5% in 2011 to 5.6% in 2015 (P=0.0041).
6. Overall, 379 MDR *S. pneumoniae* have been collected. The majority of the MDR *S. pneumoniae* are serotypes 15A (26%) and 19A (41%).
7. Significant changes in the epidemiology and antimicrobial susceptibility patterns continue to occur in *S. pneumoniae* in Canada, warranting ongoing study.

RESULTS

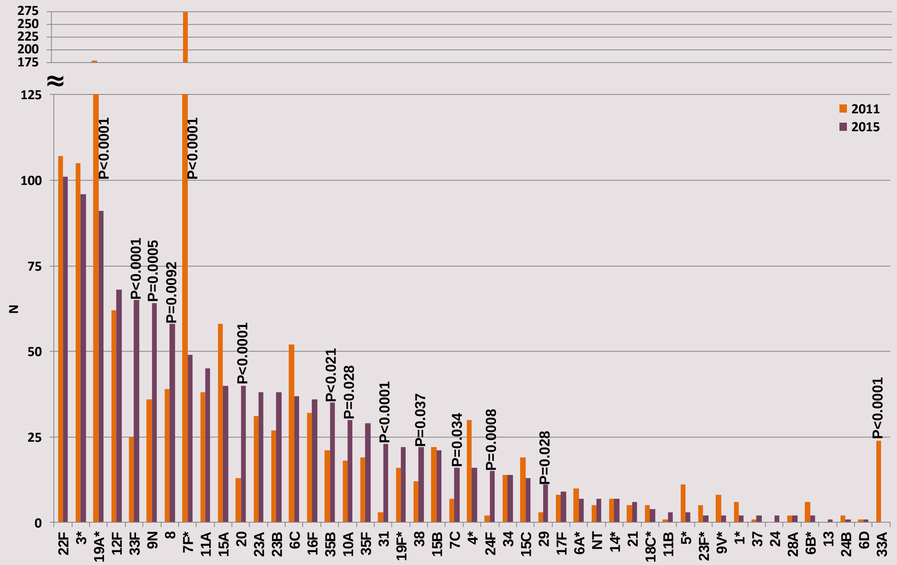

Figure 1. *S. pneumoniae* Serotype Distribution in 2015 compared to 2011
* PCV-13 Serotypes

Proportion of SAVE Isolates Contained in PCV-13:

In 2015, 25.3% of the *S. pneumoniae* collected as part of SAVE were serotypes contained in PCV-13. Regional variation of serotypes was noted as 20%, 26.4% and 29.2% of the isolates were PCV-13 serotypes in the West, Central and Eastern parts of Canada, respectively. Variability in the proportion of *S. pneumoniae* contained in PCV-13 by age group was also noted: 14.3% in 0-<1 years, 16.7% in 1-<2 years, 15.2% in 2-<6 years, 34.4% in 6-<18 years, 28% in 18-<50 years, 29.1% in 50-<65 years and 22.9% in ≥65 years.

Antimicrobial Susceptibility Rates:

The antimicrobial susceptibility rates for all *S. pneumoniae* and PCV-13 serotypes in 2015 was as follows: penicillin (iv, nonmeningitis) 99.1% and 96.3%, penicillin (iv, meningitis and oral) 89.5% and 85.6%, ceftriaxone (nonmeningitis) 99.7% and 99.0%, ceftriaxone (meningitis) 97.3% and 91.0%, clarithromycin 74.9% and 72.2%, levofloxacin 99.7% and 100%, trimethoprim-sulfamethoxazole 87.4% and 85.0%, and doxycycline 90.2% and 83.6%.

Multidrug Resistance:

Current (2015) MDR was noted in serotypes 3 (3.1%), 6B (50%), 6C (2.7%), 9N (1.6%), 12F (1.5%), 14 (28.6%), 15A (40%), 19A (27.5%), 19F (18.2%), 22F (3.0%), 23F (50%), 33F (3.1%) and 35B (5.7%).

Of the 63 MDR *S. pneumoniae* in SAVE 2015, there were 28 isolates resistant to 3 antibiotic classes, 15 resistant to 4 antibiotic classes and 20 resistant to 5 antibiotic classes.

The most common MDR phenotypes demonstrated resistance to clarithromycin, clindamycin, and doxycycline (n=23; predominantly serotype 15A, n=14), and clarithromycin, clindamycin, doxycycline, penicillin, and trimethoprim-sulfamethoxazole (n=20 predominantly serotype 19A, n=17).

Table 1. Annual Prevalence of Multi-drug Resistance in *S. pneumoniae* in Canada, 2011-2015

	SAVE Study Year					P-value, 2011 versus 2015
	2011	2012	2013	2014	2015	
***S. pneumoniae* isolates (N)**	1379	1285	1138	1210	1195	N/A
MDR Rate	8.6%	6.8%	6.0%	4.1%	5.6%	P = 0.0041

N/A, not applicable

Table 2. Demographics of the Common (n≥4) Multi-drug Resistant *S. pneumoniae* by Serotype in Canada (2015)

Serotype (N)	Geographic Region*	Age Group (years)						Region Total
		0-<1	1-<2	2-<6	6-<18	18-<50	50-<65	≥65	
19A (25)	West				3	2	2	2	9
	Central				2	1		5	8
	East		1		4	1		5	8
15A (6)	West		1					1	2
	Central		1			2		9	12
	East					1		1	2
19F (4)	West								0
	Central				1			1	2
	East							2	2

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

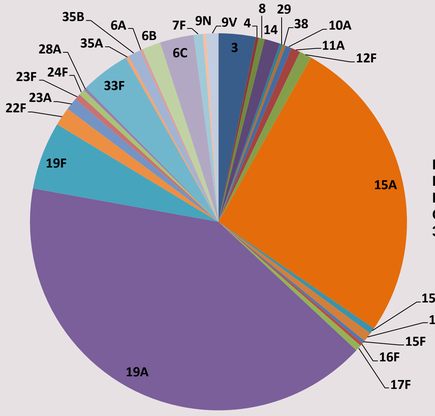

Figure 2. Serotype Distribution of Multi-drug Resistant *S. pneumoniae* in Canada, 2011 – 2015 (N = 379)

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