

Antimicrobial Susceptibility Patterns of Common Invasive *Streptococcus pneumoniae* Serotypes in Canada: SAVE 2011 - 2016

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Introduction

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 multidrug 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 multidrug resistance (MDR) rates between 2011 and 2016 were assessed to evaluate the evolution of serotypes and antimicrobial resistance subsequent to the introduction of PCV-13 in Canada.

Materials and 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 7416 invasive *S. pneumoniae* isolates from across Canada were included in the SAVE study as part of this collaboration (Jan. 1, 2011 – Dec. 31, 2016). The annual number of *S. pneumoniae* collected were 1379, 1285, 1138, 1210, 1196 and 1208 in 2011, 2012, 2013, 2014, 2015 and 2016, 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 (5,6).

Multidrug resistance was defined as resistance to ≥ 3 antimicrobial classes (penicillin MIC $\geq 2 \mu\text{g/mL}$).

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 *rpoB* gene sequencing.

Results

Table 1. Antimicrobial Susceptibilities for the Top 10 Serotypes of *S. pneumoniae* in SAVE 2016

Serotype (N)	% Susceptible									% MDR
	PEN (iv, M)	PEN (iv, NM)	CRO (M)	CRO (NM)	CLR	LVX	SXT	DOX		
3 (123)	99.2	100	100	100	95	100	98.3	87.5		2.5
22F (111)	100	100	100	100	60	100	99.1	100		0
8 (74)	100	100	100	100	100	100	98.6	94.6		0
19A (68)	83.8	100	100	100	45.6	100	85.3	83.8		11.8
9N (67)	98.5	100	100	100	95.5	100	100	100		0
12F (60)	98.3	100	100	100	71.7	98.3	96.7	98.3		0
33F (49)	100	100	100	100	18.4	100	10.2	100		0
11A (46)	100	100	100	100	66.7	100	82.2	97.8		2.2
23B (43)	62.8	100	100	100	90.7	100	72.1	88.4		0
23A (42)	61	100	100	100	82.9	100	100	68.3		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 $\geq 2 \mu\text{g/ml}$)]

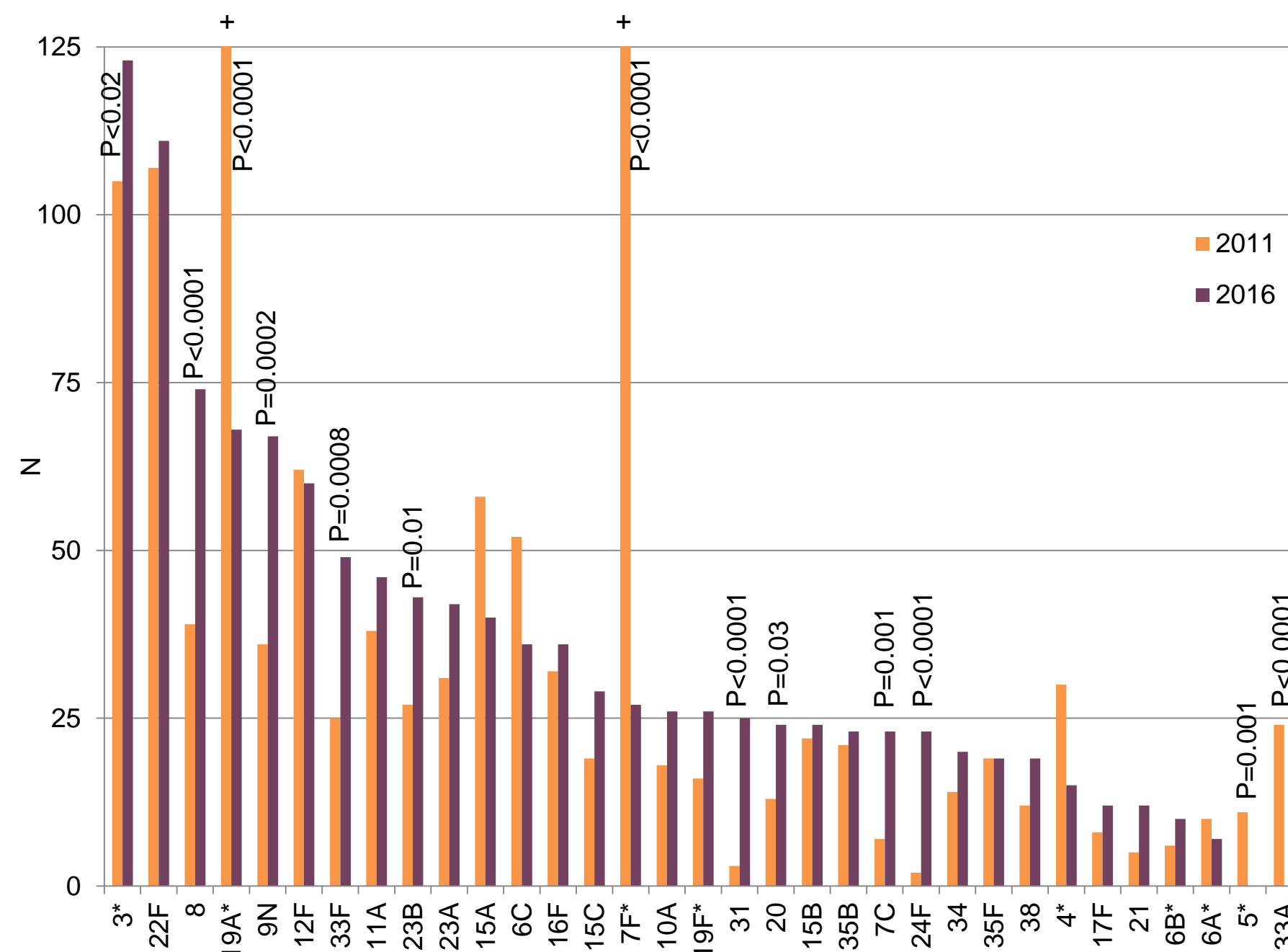


Figure 1. *S. pneumoniae* Serotype Distribution in 2016 compared to 2011 (for serotypes with ≥ 10 in either year)

* , PCV-13 Serotypes; +, 19A N=179 in 2011, 7F N=274 in 2016; 9V: N=8 in 2011 vs N=1 in 2016 ($P=0.04$)

Proportion of SAVE Isolates Contained in PCV-13

In 2016, 24.5% of the *S. pneumoniae* collected as part of SAVE were serotypes contained in PCV-13. Regional variation of serotypes was noted as 14.4%, 28.4% and 19.1% 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: 24.3% in 0-<1 years, 23.5% in 1-<2 years, 17.5% in 2-<6 years, 18.2% in 6-<18 years, 24.4% in 18-<50 years, 29.4% in 50-<65 years and 21.9% in ≥ 65 years.

Antimicrobial Susceptibility Rates

The antimicrobial susceptibility rates for all *S. pneumoniae* and PCV-13 serotypes in 2016 was as follows: penicillin (iv, nonmeningitis) 100% and 100%, penicillin (iv, meningitis and oral) 87.9% and 86.6%, ceftriaxone (nonmeningitis) 99.7% and 99.7%, ceftriaxone (meningitis) 98.4% and 96.9%, clarithromycin 77.3% and 77.0%, levofloxacin 99.7% and 99.7%, trimethoprim-sulfamethoxazole 86.5% and 89.3%, and doxycycline 90.8% and 84.5%.

Multidrug Resistance

Current (2016) MDR was noted in serotypes 3 (2.5%), 6B/C (40/5.7%), 9V (100%), 11A (2.2%), 14 (14.3%), 15A (69.6%), 19A/F (11.8/19.2%), 34 (5%), 35A (100%) and 35B (4.3%).

Of the 45 MDR *S. pneumoniae* in SAVE 2016, 29 isolates were resistant to 3 antibiotic classes, 11 resistant to 4 antibiotic classes and 5 resistant to 5 antibiotic classes. The most common MDR phenotype demonstrated resistance to clarithromycin, clindamycin, and doxycycline (n=24; predominantly serotype 15A, n=13).

Table 2. Annual Prevalence of MDR in *S. pneumoniae* in Canada, 2011-2016

	SAVE Study Year						P-value, 2011 to 2016
	2011	2012	2013	2014	2015	2016	
<i>S. pneumoniae</i> isolates (N)	1379	1285	1138	1210	1196	1208	N/A
MDR Rate	8.6%	6.8%	6.0%	4.1%	5.6%	3.9%	$P < 0.0001$

N/A, not applicable

Table 3. Demographics of the Common ($N \geq 5$) MDR *S. pneumoniae* by Serotype in Canada (2016)

Serotype (N)	Geographic Region *	Age Group (years)						Region Total	
		0-<1	1-<2	2-<6	6-<18	18-<50	50-<65		≥ 65
15A (16)	West						3	1	4
	Central					1	3	2	6
	East								6
19A (8)	West				1				2
	Central					1		5	6
	East								0
19F (5)	West								0
	Central				1			3	4
	East					1			1

* West (Saskatchewan, Manitoba); Central (Ontario, Quebec); East (Prince Edward Island, Nova Scotia, New Brunswick, Newfoundland and Labrador)

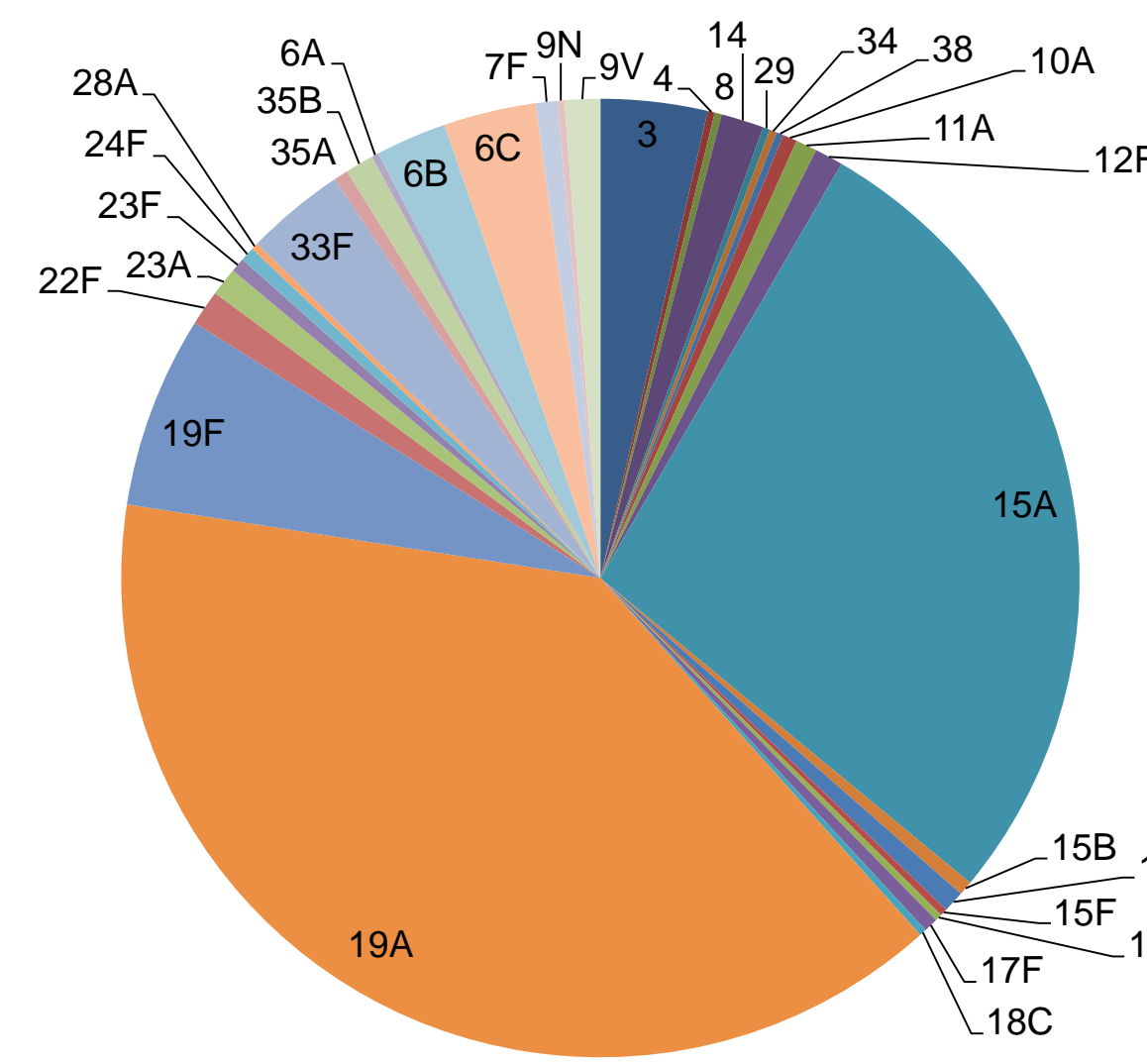


Figure 2. Serotype Distribution of MDR *S. pneumoniae* in Canada, 2011 - 2016 (N = 424)

Conclusions

- In 2016, 24.5% of all circulating *S. pneumoniae* and 51.1% of MDR *S. pneumoniae* in Canada were serotypes in PCV-13.
- The most commonly circulating serotypes in the 2016 SAVE study were 3, 22F, 8, 19A, 9N, 12F, 33F, 11A, 23B, and 23A.
- Between 2011 and 2016, statistically significant reductions in the prevalence of 5, 7F, 9V, 19A and 33A were observed. Increases in serotypes 3, 7C, 8, 9N, 20, 23B, 24F, 31 and 33F were noted during the study.
- In 2016, multidrug resistance was observed in serotypes 3, 6B, 6C, 9V, 11A, 14, 15A, 19A, 19F, 34, 35A and 35B.
- Rates of multidrug resistance in *S. pneumoniae* significantly decreased from 8.5% in 2011 to 3.9% in 2016 ($P < 0.0001$).
- Overall, 424 MDR *S. pneumoniae* have been collected. The majority of the MDR *S. pneumoniae* are serotypes 15A (27.1%) and 19A (38.4%).
- The change in serotype distribution, specifically the decreased prevalence of 19A, subsequent to the introduction of PCV13 may account for the decreased rate of MDR in *S. pneumoniae* in Canada.

Acknowledgements

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.

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