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The Impact of PCV-13: Changes in the Epidemiology and Antimicrobial Resistance of *Streptococcus pneumoniae* (SPN) in Canada, 2007-9 versus 2011-12

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], D.J. HOBAN[1,2,3], and G.G. ZHANEL[2,3]

[1]Diagnostic Services of 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

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ABSTRACT

Background: Two studies evaluating the serotypes (ST) of invasive SPN circulating in Canada were recently conducted: 1) Baseline Epidemiology of SPN ST (BESST) study (2007-2009) and the SPN Serotyping and Antimicrobial Susceptibility: Assessment for Vaccine Efficacy in Canada (SAVE) study (2011-12). SAVE was initiated after PCV-13 was introduced in Canada.

Methods: In BESST, 400 SPN were obtained as part of CANWARD (a national study) between 2007 and 2009. In collaboration with the National Microbiology Laboratory, the SAVE study collected 2571 isolates in 2011-12 from across Canada. Serotyping was performed using the Quellung reaction (Statens Serum Institute, Copenhagen, Denmark). Susceptibility testing (AST) was performed in accordance with CLSI methods.

Results: The 2011-12 SPN isolates covered by PCV-7, PHiD-CV, and PCV-13 were 5.2%, 24.2%, and 44.3%, respectively. A comparison of the susceptibility results in BESST and SAVE for the 10 most common STs circulating in 2011-12 are shown below.

ST (n)[a]	Antimicrobial Susceptibility (BESST: 2007-9 / SAVE: 2011-12)								
	%S								% MDR
	PEN (iv, M)	PEN (iv, NM)	CRO (M)	CRO (NM)	CLR	LVX	SXT	DOX	
7F (452)	100/99	100/100	100/100	100/100	100/97	100/100	100/100	100/98	0/1
19A (297)	54/62	98/82	98/78	100/91	73/40	100/99	78/68	85/70	14/26
22F (236)	96/99	100/100	100/100	100/100	96/78	100/99	100/100	100/99	0/1
3 (156)	100/100	100/100	100/100	100/100	95/95	100/100	100/98	100/93	0/2
12F (112)	100/100	100/100	100/100	100/100	45/32	100/100	100/97	100/99	0/0
15A (100)	67/33	100/100	100/93	100/100	42/19	100/100	100/93	42/22	33/69
6C (90)	100/82	100/100	100/98	100/100	75/80	100/100	100/87	100/93	0/6
11A (80)	100/100	100/100	100/100	100/100	92/71	100/100	100/78	100/100	0/0
9N (76)	100/100	100/100	100/100	100/100	100/93	100/100	100/96	100/97	0/0
23A (68)	63/71	100/100	100/100	100/100	100/87	100/99	91/93	100/84	0/3

[a], n in SAVE 2011-12; M, meningitis; NM, nonmeningitis; PEN, penicillin; CRO, ceftriaxone; CLR, clarithromycin; LVX, levofloxacin; SXT, trimethoprim-sulfamethoxazole; DOX, doxycycline; MDR, multi-drug resistance [resistance ≥3 antimicrobial classes (penicillin resistance defined as MIC ≥ 2 µg/mL)]

Significant changes in ST prevalence between BESST and SAVE were noted in STs 4 (6% vs. 1.7%, P<0.0001), 5 (8.3% vs. 0.5%, P<0.0001), 7F (5.5% vs. 17.9%, P<0.0001), 9V (3.5% vs. 0.6%, P<0.0001), 14 (3% vs. 0.3%, P<0.0001), 18C (1.5% vs. 0.5%, P=0.03), 19F (2.8% vs. 1%, P=0.01), 33A (0% vs. 1.1%, P=0.03), and 35A (0.5% vs. 0%, P=0.02). 13 (3%) and 179 (7%) MDR SPN were isolated in BESST and SAVE, respectively (P=0.003).

Conclusion: In 2011-2012, PCV-13 provided coverage of 44.3% of all SPN and 54.2% of MDR SPN. 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 Baseline Epidemiology of *S. pneumoniae* serotype (BESST) study assessed the circulating serotypes of *S. pneumoniae* from all age groups in Canada between 2007 and 2009, prior to the introduction of PCV-13.[5] 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. The results of the BESST and SAVE studies were compared to assess the evolution of serotypes and antimicrobial resistance subsequent to the introduction of PCV-13 in Canada.

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MATERIALS & METHODS

Isolate Collection:

BESST: Invasive *S. pneumoniae* isolates (n=400) were collected as part of the CANWARD study (an annual national surveillance study) from patients in 15 tertiary-care centres across Canada between 2007 and 2009, inclusive.[5]

SAVE: Invasive *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 2571 *S. pneumoniae* isolates from across Canada were included in the SAVE study as part of this collaboration (Jan. 1, 2011 – Dec. 31, 2012).

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. Stock solutions were prepared and dilutions made as described by the Clinical Laboratory Standards Institute.[6] Following two subcultures from frozen stock, the MICs of the antimicrobial agents for the isolates were determined by the broth microdilution method and interpreted utilizing CLSI criteria.[7] Briefly, 96-well custom designed microtitre plates containing doubling antibiotic dilutions in 100µl/well of cation adjusted Mueller-Hinton broth with lysed horse blood (2-5% V/V) were inoculated to achieve a final concentration of approximately 5 x 10^5 CFU/ml and incubated in ambient air for 24 hours prior to reading. Colony counts were performed periodically to confirm inocula. Quality control was performed using a variety of ATCC QC organisms including *S. pneumoniae* 49619.

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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RESULTS

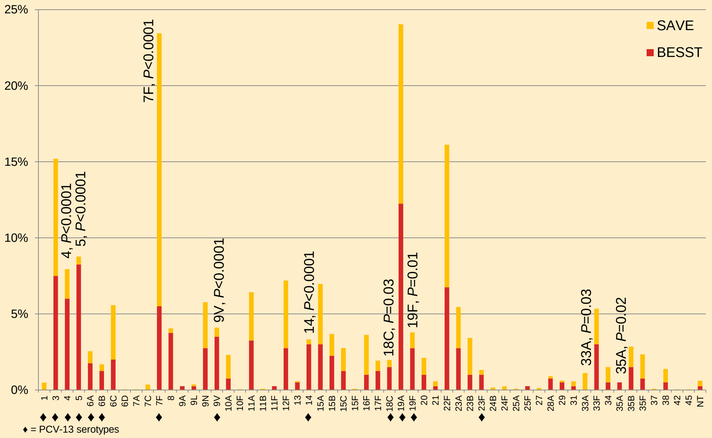

Figure 1. Changes in Serotype Distribution in Canada, post PCV-13.

Table 1. Changes in Antimicrobial Susceptibility, BESST (2007-2009) vs. SAVE (2011/12), for the 10 Most Common Serotypes Circulating in Canada in 2011-12.

ST (n)[a]	Antimicrobial Susceptibility (BESST: 2007-9 / SAVE: 2011-12)								
	%S								% MDR
	PEN (iv, M)	PEN (iv, NM)	CRO (M)	CRO (NM)	CLR	LVX	SXT	DOX	
7F (452)	100/99	100/100	100/100	100/100	100/97	100/100	100/100	100/98	0/1
19A (297)	54/62	98/82	98/78	100/91	73/40	100/99	78/68	85/70	14/26
22F (236)	96/99	100/100	100/100	100/100	96/78	100/99	100/100	100/99	0/1
3 (156)	100/100	100/100	100/100	100/100	95/95	100/100	100/98	100/93	0/2
12F (112)	100/100	100/100	100/100	100/100	45/32	100/100	100/97	100/99	0/0
15A (100)	67/33	100/100	100/93	100/100	42/19	100/100	100/93	42/22	33/69
6C (90)	100/82	100/100	100/98	100/100	75/80	100/100	100/87	100/93	0/6
11A (80)	100/100	100/100	100/100	100/100	92/71	100/100	100/78	100/100	0/0
9N (76)	100/100	100/100	100/100	100/100	100/93	100/100	100/96	100/97	0/0
23A (68)	63/71	100/100	100/100	100/100	100/87	100/99	91/93	100/84	0/3

[a], n in SAVE 2011-12; M, meningitis; NM, nonmeningitis; PEN, penicillin; CRO, ceftriaxone; CLR, clarithromycin; LVX, levofloxacin; SXT, trimethoprim-sulfamethoxazole; DOX, doxycycline; MDR, multi-drug resistance [resistance ≥3 antimicrobial classes (penicillin resistance defined as MIC ≥ 2 µg/mL)]; changes in resistance rates ≥10% between BESST and SAVE are indicated in orange.

Table 2. Current Antimicrobial Susceptibility of *S. pneumoniae* in Canada for All Serotypes and PCV-13 Serotypes (SAVE 2012).

Antimicrobial Agent (CLSI Interpretive Criteria)	% Susceptible	
	All serotypes (n=1155)	PCV-13 serotypes (n=461)
Penicillin (iv, nonmeningitis)	98.1	95.4
Penicillin (iv, meningitis)	89.2	87.0
Penicillin (oral, Penicillin V)	89.2	87.0
Ceftriaxone (nonmeningitis)	99.1	97.8
Ceftriaxone (meningitis)	96.4	93.3
Clarithromycin	74.0	75.9
Levofloxacin	99.2	99.3
Trimethoprim-Sulfamethoxazole	88.5	88.3
Doxycycline	89.0	86.8

Table 3. Multi-drug Resistance (MDR) Phenotypes by *S. pneumoniae* Serotype (SAVE 2011/12).

Serotype (N)	Age groups (years) by Region [West; Central; East]																				
	0 to <1			1 to <2			2 to <6			6 to <18			18 to <50			50 to <65			≥65		
	W	C	E	W	C	E	W	C	E	W	C	E	W	C	E	W	C	E	W	C	E
15A (61)	1	1	0	0	3	0	0	1	0	0	0	0	3	6	0	0	14	1	1	28	2
19A (76)	3	1	0	2	5	1	0	7	2	0	2	0	8	6	1	2	7	6	3	14	6
Total (137)	6			11			10			2			24			30			54		

N, n in SAVE 2011-12; W, West (Saskatchewan and Manitoba); C, Central (Ontario and Quebec); E, East (Prince Edward Island, New Brunswick, Nova Scotia, and Newfoundland)

In SAVE 2011/12, 2571 *S. pneumoniae* were collected (1365 in 2011 and 1206 in 2012). Regarding gender and age, there were 1317 (54.2%) *S. pneumoniae* collected from males; there were 130 (5.2%) *S. pneumoniae* collected from children 0 to <1 years, 89 (3.6%) from children 1 to <2 years, 129 (5.2%) from children 2 to <6 years, 104 (4.2%) from children 6 to <18 years, 528 (21.3%) from adults 18 to <50 years, 624 (25.2%) from adults 50 to <65 years, and 873 (35.2%) from adults ≥65 years. There were 553 (21.9%) isolates collected from the west, 1702 (67.5%) from central Canada, and 265 (10.5%) from the eastern provinces.

The most common serotypes in SAVE 2011/12 were: 7F (n=452, 20.3%), 19A (n=297, 11.8%), 22F (n=236, 9.2%), 3 (n=156, 6.2%), 12F (n=112, 4.4%), 15A (n=100, 4.0%), 6C (n=90, 3.6%), 11A (n=80, 3.2%), 9N (n=76, 3.0%), and 23A (n=68, 2.7%). Comparatively, the most common serotypes in BESST were: 19A (n=49, 12.3%), 5 (n=33, 8.3%), 3 (n=30, 7.5%), 22F (n=27, 6.8%), 4 (n=24, 6%), 7F (n=22, 5.5%), 8 (n=15, 3.8%), 9V (n=14, 3.5%), and 11A (n=13, 3.3%).

Thirteen (3%) and 179 (7%) multi-drug resistant (MDR) *S. pneumoniae* were isolated in BESST and SAVE, respectively (P=0.003). Of the MDR *S. pneumoniae* in SAVE, there were 132 isolates resistant to 3 classes of antibiotics, 42 resistant to 4 classes of antibiotics, 4 resistant to 5 classes of antibiotics, and 1 resistant to 6 classes of antibiotics. The most common MDR phenotypes demonstrated resistance to clarithromycin, clindamycin, and doxycycline (n=88), clarithromycin, clindamycin, and trimethoprim-sulfamethoxazole (n=32), and clarithromycin, clindamycin, doxycycline, and trimethoprim-sulfamethoxazole (n=22). Multi-drug resistance was seen most frequently in serotypes 15A and 19A (Table 3).

CONCLUSIONS

1. PCV-13 provided coverage of 44.3% of invasive Canadian isolates tested by CARA in 2011 and 2012. Comparatively, PCV-7 and PHiD-CV provided coverage of 5.1% and 24.1% of the isolates, respectively.
2. The coverage of 2011/12 multidrug resistant *S. pneumoniae* isolates in Canada by PCV-13 was 54.2%.
3. A comparison of the BESST and SAVE studies demonstrated that post-PCV-13 introduction in Canada, the prevalence of 7F and 33A have increased and multidrug resistance has increased, particularly in serotypes 15A and 19A.
4. Common multidrug resistant *S. pneumoniae*, 15A and 19A, are seen across Canada and in all age groups.
5. Significant changes in the epidemiology and susceptibility patterns of *S. pneumoniae* were noted following the introduction of PCV-13 in Canada. These changes warrant ongoing surveillance to assist future vaccine development and to guide empiric therapy.