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Multi-drug Resistance in the Most Prevalent Invasive *Streptococcus pneumoniae* (SPN) Serotypes (STs) Post PCV-13 Introduction in Canada

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REVISED ABSTRACT

Background: The SPN Serotyping and Antimicrobial Susceptibility: Assessment for Vaccine Efficacy in Canada (SAVE) study began in 2011 to assess the SPN antimicrobial susceptibility patterns in Canada after the introduction of PCV-13.
Methods: The National Microbiology Laboratory receives and tests invasive SPN from the provincial health laboratories in Canada for ST analysis. In 2011, the SAVE study at CARA collaboratively received 1255 SPN from 8 provinces for antimicrobial susceptibility testing. Serotyping was performed using the Quellung reaction using pool, group, type and factor commercial antisera (Statens Serum Institute, Copenhagen, Denmark). Susceptibility testing was performed utilizing custom-designed broth microdilution panels in accordance with CLSI methods.
Results: The ST isolates found in PCV-7, PHiD-CV, and PCV-13 were 5.8%, 27.4%, and 49.2%, respectively. The susceptibilities for the 10 most common STs are below.

Serotype (n)[a]	Antimicrobial Susceptibility (%S)								% MDR
	PEN (iv, M)	PEN (iv, NM)	CRO (M)	CRO (NM)	CLR	LVX	SXT	DOX	
7F (253)	98.8	100	99.6	100	96.8	100	99.6	99.2	0.8
19A (162)	61.7	81.5	79	90.1	47.5	98.8	69.1	81.5	26.5
3 (99)	100	100	100	100	97	100	97	99	1
22F (95)	99	100	100	100	77.9	99	100	100	0
12F (59)	100	100	100	100	39	100	96.6	100	0
6C (47)	85.1	100	97.9	100	80.9	100	91.5	93.6	2.1
15A (38)	18.4	100	94.7	100	18.4	100	94.7	21	76.3
11A (35)	100	100	100	100	74.3	100	71.4	100	0
8 (34)	97.1	100	100	100	97.1	100	97.1	97.1	2.9
9N (32)	100	100	100	100	100	100	96.9	100	0

[a], n for which complete susceptibility results were available; 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)]

89 (7.2%) isolates were MDR. MDR was observed in STs 3 (1; 1%), 6C (1; 2.1%), 7F (2; 0.8%), 8 (1; 2.9%); 9V (2; 40%), 14 (1; 14.3%), 15A (29; 76.3%), 15B (2; 10.5%), 15C (1; 5.9), 15F (1; 100%), 16F (1; 3.6%), 19A (43; 26.5%), 19F (3; 18.7%), and 35B (1; 5%). The most common MDR pattern was resistance to CLR, clindamycin and DOX (44; 49.4%). MDR 19A were observed in all Canadian regions and in various age groups [6/123 (4.9%) in 0 - <2 years (yrs); 4/120 (3.3%) in 2 - 17 yrs; 20/578 (3.5%) in 18 - 64 yrs; 13/430 (3.0%) in ≥65 yrs].
Conclusions: Compared to pre-2011 studies, MDR rates have increased slightly. The most common MDR STs were 15A and 19A. PCV-13 provided coverage of 49.2% of invasive Canadian isolates and 58.4% of MDR isolates in 2011.

BACKGROUND

Antibiotic resistance in *Streptococcus pneumoniae* is a global concern. Respiratory and systemic isolates of *S. pneumoniae* are commonly resistant to penicillins, macrolides, tetracyclines, sulfonamides and fluoroquinolones and frequently multi-drug resistant. Prevnar® (PCV-7: 4, 6B, 9V, 14, 18C, 19F, 23F) is a conjugate vaccine that has been shown both in Canada and the United States to be effective in reducing systemic infections due to *S. pneumoniae* in children as well as reducing the incidence of recurrent upper respiratory tract infections in children.[1,2] However, the increase of PCV-7 related and PCV-7 non-related *S. pneumoniae* serotypes in Canada is an emerging issue.

Two newer pneumococcal conjugate vaccines have been introduced in Canada: Synflorix™ (PHiD-CV: PCV-7 + 1, 5, 7F) and Prevnar®13 (PCV-13: PCV-7 + 1, 3, 5, 6A, 7F and 19A). 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.[3] Due to the enhanced coverage of the predominant serotypes in North America, current NACI (National Advisory Committee on Immunization) and ACIP (the Centers for Disease Control and Prevention Advisory Committee on Immunization Practices) guidelines recommend the routine use of PCV-13 in children.[4,5]

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 PCV-13 vaccine.

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MATERIALS & METHODS

Isolate Collection:
Invasive *S. pneumoniae* isolated from sterile sites are forwarded from provincial public health microbiology laboratories [Canadian Public Health Laboratory Network (CPHLN)] to the Streptococcus Unit at the National Microbiology Laboratory (NML; Public Health Agency of Canada). Through a collaboration between the Canadian Antimicrobial Resistance Alliance (CARA) and the NML and subsequent to the permission of the submitting CPHLN provincial laboratories, the *S. pneumoniae* isolates were forwarded to CARA. A total of 1,255 *S. pneumoniae* isolates from across Canada were included in the SAVE study as part of this collaboration. Isolates were collected from January 1, 2011 – December 31, 2011, inclusive.

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 R: 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).

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RESULTS

Table 1. Antimicrobial susceptibilities for the 10 most common *S. pneumoniae* serotypes isolated in Canada in 2011.

Serotype (n)[a]	Antimicrobial Susceptibility (%S)						
	Penicillin (iv, meningitis*)	Penicillin (iv, nonmeningitis)	Ceftriaxone (meningitis)	Ceftriaxone (nonmeningitis)	Clarithromycin	Levofloxacin	Trimethoprim-sulfamethoxazole	Doxycycline[b]
7F (253)	98.8	100	99.6	100	96.8	100	99.6	99.2
19A (162)	61.7	81.5	79	90.1	47.5	98.8	69.1	81.5
3 (99)	100	100	100	100	97	100	97	99
22F (95)	99	100	100	100	77.9	99	100	100
12F (59)	100	100	100	100	39	100	96.6	100
6C (47)	85.1	100	97.9	100	80.9	100	91.5	93.6
15A (38)	18.4	100	94.7	100	18.4	100	94.7	21
11A (35)	100	100	100	100	74.3	100	71.4	100
8 (34)	97.1	100	100	100	97.1	100	97.1	97.1
9N (32)	100	100	100	100	100	100	96.9	100

[a], n for which complete susceptibility results were available; [b], doxycycline interpreted with CLSI breakpoints for tetracycline; *, meningitis S breakpoint = oral S breakpoint

Table 2. Antimicrobial susceptibilities overall and for serotypes included in PCV-7, PHiD-CV, and PCV-13.

Antimicrobial Agent (CLSI Interpretative Criteria)	Antimicrobial Susceptibility (%S)			
	All Serotypes (n=1244)[a]	PCV-7 Serotypes (n=71)[a]	PHiD-CV Serotypes (n=341)[a]	PCV-13 Serotypes (n=614)[a]
Penicillin (iv, nonmeningitis)	97.4	97.2	99.4	94.8
Penicillin (iv, meningitis*)	87.0	80.3	95.0	86.5
Ceftriaxone (nonmeningitis)	98.6	97.2	99.4	97.1
Ceftriaxone (meningitis)	95.9	88.7	97.4	93.0
Clarithromycin	77.5	77.5	93.0	80.4
Levofloxacin	99.6	100	100	99.7
Trimethoprim-sulfamethoxazole	87.5	87.3	94.7	88.1
Doxycycline[b]	92.8	83.1	95.9	92.7

[a], n for which complete susceptibility results were available; [b], doxycycline interpreted with CLSI breakpoints for tetracycline; *, meningitis S breakpoint = oral S breakpoint

Table 3. Multi-drug resistance (MDR) phenotypes by *S. pneumoniae* serotype.

MDR Phenotype	Serotype														Total MDR by phenotype
	3	8	14	15A	15B	15C	15F	16F	19A	19F	35B	6C	7F	9V	
MDR: 3 antimicrobial classes															
Chloramphenicol/ Clarithromycin/ Clindamycin	1														1
Clarithromycin/ Clindamycin/ Trimethoprim-sulfamethoxazole									1						1
Clarithromycin/ Clindamycin/ Doxycycline		1		27	1	1	1	1	9	1		1	1		44
Clarithromycin/ Clindamycin/ Levofloxacin									1						1
Clarithromycin/ Doxycycline/ Trimethoprim-sulfamethoxazole													1		1
Clarithromycin/ Penicillin/ Trimethoprim-sulfamethoxazole									4		1			1	6
Clarithromycin/ Clindamycin/ Penicillin			1												1
MDR: 4 antimicrobial classes															
Clarithromycin/ Clindamycin/ Trimethoprim-sulfamethoxazole/ Penicillin									26	2					28
Chloramphenicol/ Clarithromycin/ Trimethoprim-sulfamethoxazole / Penicillin									1						1
Clarithromycin/ Doxycycline/ Trimethoprim-sulfamethoxazole/ Penicillin														1	1
Clarithromycin/ Clindamycin/ Doxycycline/ Penicillin				2											2
MDR: 5 antimicrobial classes															
Clarithromycin/ Clindamycin/ Levofloxacin/ Trimethoprim-sulfamethoxazole/ Penicillin									1						1
Chloramphenicol/ Clarithromycin/ Clindamycin/ Trimethoprim-sulfamethoxazole/ Penicillin					1										1
Total MDR by serotype	1	1	1	29	2	1	1	1	43	3	1	1	2	2	89

Table 4. Demographics of the common (n>5) multi-drug resistant (MDR) *S. pneumoniae* by serotype.

Serotype (MDR n)	Region			Age group (years)				
	West	Central	East	0 - < 2	2 - 17	18 – 49	50 - 64	≥ 65
15A (29)	3	26	0	4	0	5	9	11
19A (43)	12	23	8	6	4	12	8	13

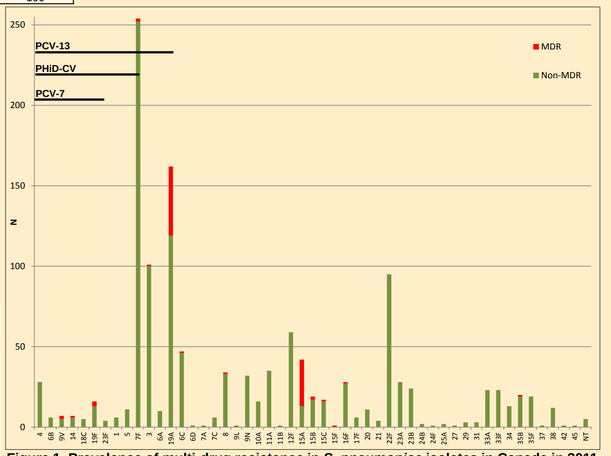

Figure 1. Prevalence of multi-drug resistance in *S. pneumoniae* isolates in Canada in 2011.

CONCLUSIONS

1. PCV-13 provided coverage of 49.2% of invasive Canadian isolates tested by CARA from 2011. Comparatively, PCV-7 and PHiD-CV provided coverage of 5.8% and 27.4% of the isolates, respectively.
2. The coverage of multi-drug resistant *S. pneumoniae* isolates in Canada by the conjugate vaccines is: PCV-7: 6.7%; PHiD-CV: 9.0%; PCV-13: 58.4%.
3. Common multi-drug resistant *S. pneumoniae*, 15A and 19A, are seen across Canada and in all age groups.
4. The identities of the most common serotypes of *S. pneumoniae* circulating in Canada after the introduction of PCV-13 are important to assess on an ongoing basis to predict the efficacy of PCV-13 in various age groups. As well, it is important to know what the antimicrobial susceptibility profiles are of common *S. pneumoniae* serotypes to guide empiric and directed treatments.

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