

Analysis of MDR in the predominant *Streptococcus pneumoniae* serotypes in Canada: the SAVE study, 2011–2020

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Objectives: To investigate the levels of MDR in the predominant serotypes of invasive *Streptococcus pneumoniae* isolated in Canada over a 10 year period.

Methods: All isolates were serotyped and had antimicrobial susceptibility testing performed, in accordance with CLSI guidelines (M07–11 Ed., 2018). Complete susceptibility profiles were available for 13 712 isolates. MDR was defined as resistance to three or more classes of antimicrobial agents (penicillin MIC ≥ 2 mg/L defined as resistant). Serotypes were determined by Quellung reaction.

Results: In total, 14 138 invasive isolates of *S. pneumoniae* were tested in the SAVE study (*S. pneumoniae* Serotyping and Antimicrobial Susceptibility: Assessment for Vaccine Efficacy in Canada), a collaboration between the Canadian Antimicrobial Resistance Alliance and Public Health Agency of Canada—National Microbiology Laboratory. The rate of MDR *S. pneumoniae* in SAVE was 6.6% (902/13 712). Annual rates of MDR *S. pneumoniae* decreased between 2011 and 2015 (8.5% to 5.7%) and increased between 2016 and 2020 (3.9% to 9.4%). Serotypes 19A and 15A were the most common serotypes demonstrating MDR (25.4% and 23.5% of the MDR isolates, respectively); however, the serotype diversity index increased from 0.7 in 2011 to 0.9 in 2020 with a statistically significant linear increasing trend ($P < 0.001$). In 2020, MDR isolates were frequently serotypes 4 and 12F in addition to serotypes 15A and 19A. In 2020, 27.3%, 45.5%, 50.5%, 65.7% and 68.7% of invasive MDR *S. pneumoniae* were serotypes included in the PCV10, PCV13, PCV15, PCV20 and PPSV23 vaccines, respectively.

Conclusions: Although current vaccine coverage of MDR *S. pneumoniae* in Canada is high, the increasing diversity of serotypes observed among the MDR isolates highlights the ability of *S. pneumoniae* to rapidly evolve.

Introduction

Streptococcus pneumoniae is an important pathogen worldwide because it is a common cause of respiratory infections, including community-acquired pneumonia and acute otitis media, and the causative agent of invasive pneumococcal disease (IPD). The overall incidence rate of IPD in Canada was stable from 2009 to 2019, with annual rates between 9.0 and 10.9 cases per 100 000 population.¹ The incidence rate decreased significantly to 5.9 cases per 100 000 population in 2020.¹ This decreased rate has been attributed to public health measures related to the COVID-19 pandemic.¹

Pneumovax[®], a 7-valent pneumococcal conjugate vaccine (PCV7) (serotypes 4, 6B, 9V, 14, 18C, 19F and 23F), was developed to give vaccine coverage for the seven commonest serotypes causing

pneumococcal disease in children at the time, of the approximately 100 now recognized serotypes.^{1,2} Studies in North America demonstrated that the vaccination of children with PCV7 resulted in significant reductions in invasive infections due to *S. pneumoniae* as well as reductions in the incidence of recurrent upper respiratory tract infections in children.^{2–4} Serotype replacement with non-vaccine serotypes, particularly MDR serotype 19A, was rapidly observed following the introduction of PCV7.^{2,3} Subsequently, newer pneumococcal conjugate vaccines were developed with enhanced serotype coverage, including SYNFLORIX[™] (PCV10) (PCV7 serotypes plus serotypes 1, 5 and 7F) and Pnevna[®]13 (PCV13) (PCV7 serotypes plus serotypes 1, 3, 5, 6A, 7F and 19A). The immunization guidelines in North America were updated in 2010 to recommend the routine use of PCV13 in infants,^{5,6} and the replacement of PCV7 with PCV13

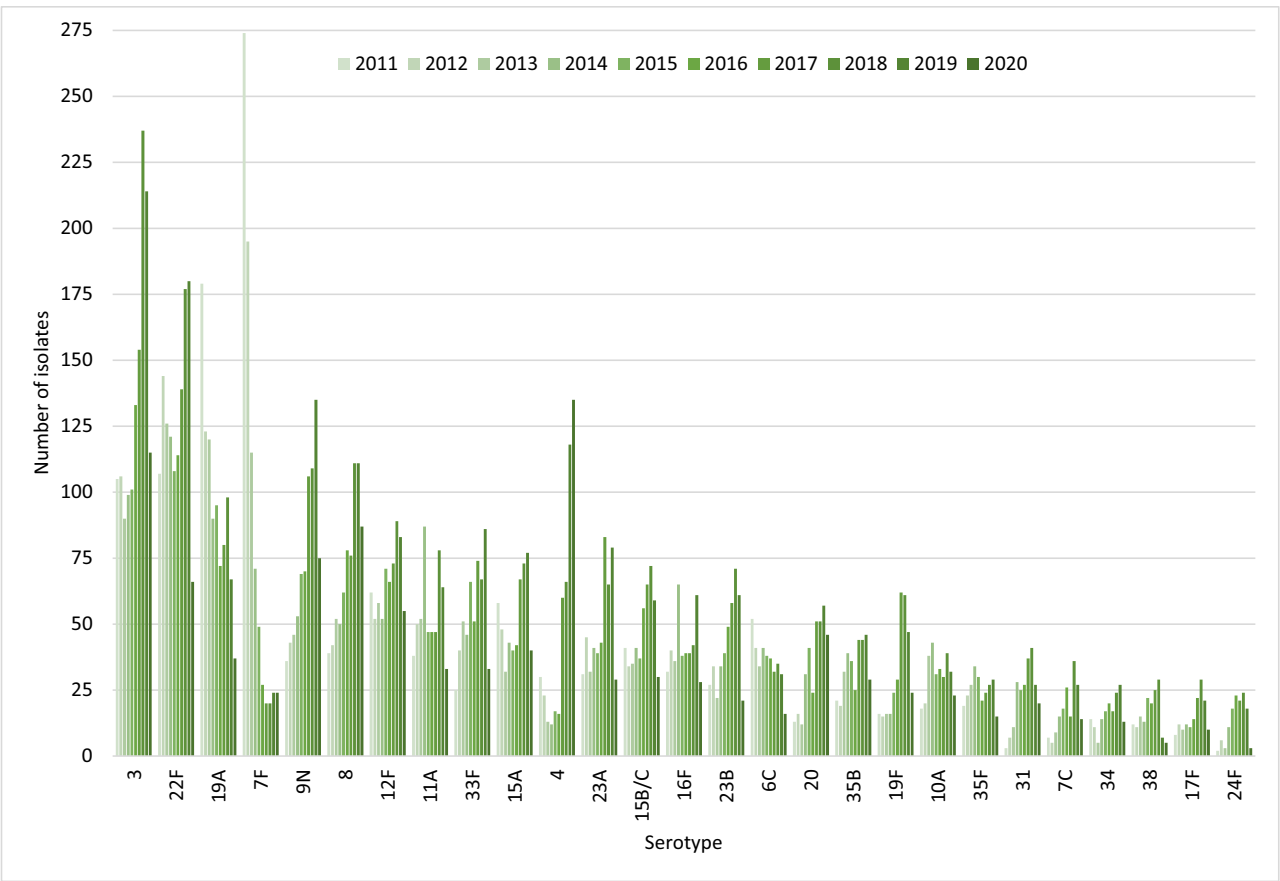


Figure 1. Annual serotype distribution of invasive *S. pneumoniae* isolates ($n \geq 100$) collected in the SAVE study, 2011–2020. Serotypes for which less than 100 isolates were collected between 2011 and 2020 include: 9V ($n=92$), 21 ($n=82$), 6A ($n=76$), 14 ($n=64$), 18C ($n=62$), non-typeable ($n=61$), 29 ($n=53$), 6B ($n=43$), 28A ($n=39$), 13 ($n=35$), 33A ($n=28$), 23F ($n=27$), 1 ($n=19$), 5 ($n=17$), 37 ($n=14$), 24B ($n=14$), 9L ($n=9$), 11B ($n=8$), 12A ($n=8$), 10F ($n=7$), six isolates each of serotypes 6D, 7B and 22A, five isolates each of serotypes 9A and 27, four isolates each of serogroup/serotypes 2, 24, 24A and 35A, three isolates each of serotypes 10B, 18A, 18F, 25A and 35D, two isolates each of serotypes 11D, 17A and 45, and one isolate each of serotypes 7A, 15F, 32A, 33C, 35C and 42. This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.

as part of routine infant vaccinations was completed in Canada by early 2011.¹ PCV13 was also demonstrated to prevent pneumococcal pneumonia and IPD by vaccine-related serotypes in adults.⁷ Further pneumococcal conjugate vaccine development has led to the availability of VAXNEUVANCE™, a 15-valent vaccine (PCV15) (PCV13 serotypes plus serotypes 22F and 33F) and PREVNAR 20™, a 20-valent vaccine (PCV20) (PCV13 serotypes plus serotypes 8, 10A, 11A, 12F, 15B, 22F and 33F). In late 2021 and 2022, the US Advisory Committee on Immunization Practices and the Canadian National Advisory Committee on Immunization recommended the use of PCV20 or PCV15, followed by the pneumococcal polysaccharide vaccine PNEUMOVAX®23 (PPSV23) (PCV20 serotypes plus serotypes 2, 6B, 9N, 17F and 20 minus 6A), for adults 65 years of age and older as well as younger adults with specific underlying medical conditions putting them at higher risk for IPD.^{8,9}

Despite the overall success of the vaccine programmes, treatment concerns have remained because many serotypes that were commonly penicillin-resistant or MDR, such as 15A and 19A, have persisted or increased with the changing epidemiology in the vaccine era. Drug-resistant *S. pneumoniae* has been

identified as a serious threat in the USA by the CDC,¹⁰ whereas the WHO¹¹ identified penicillin-non-susceptible *S. pneumoniae* as a pathogen of high priority.

The *S. pneumoniae* Serotyping and Antimicrobial Susceptibility: Assessment for Vaccine Efficacy in Canada (SAVE) study is an annual study that began in 2011. The purpose of this study was to evaluate changes in antimicrobial resistance, particularly MDR, in relation to serotype evolution in Canada, subsequent to the introduction of PCV13 and as higher valency vaccines have been developed.

Materials and methods

Bacterial isolates

From January 2011 to December 2020, *S. pneumoniae* isolates from sterile body sites by participating Canadian provincial public health and hospital laboratories were forwarded to the Public Health Agency of Canada—National Microbiology Laboratory (PHAC-NML) in Winnipeg, Canada. As part of an ongoing collaboration between the Canadian Antimicrobial Resistance Alliance (CARA) and PHAC-NML, PHAC-NML forwarded their collection of invasive isolates of *S. pneumoniae* from 8 of the 10

Canadian provincial public health laboratories (Saskatchewan, Manitoba, Ontario, Quebec, Nova Scotia, Prince Edward Island, Newfoundland and Labrador, and a portion of isolates collected from New Brunswick) to CARA for antimicrobial susceptibility testing.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed in the Department of Clinical Microbiology at the Winnipeg Health Sciences Centre using the reference CLSI broth microdilution method¹² with custom-designed, in-house prepared, 96-well microtitre panels containing doubling-dilutions of antimicrobial agents in cation-adjusted Mueller–Hinton broth supplemented to a final concentration of 4% lysed horse blood. Isolates were tested against penicillin, ceftriaxone, clarithromycin, clindamycin, levofloxacin, linezolid, trimethoprim/sulfamethoxazole, doxycycline, tigecycline, chloramphenicol and vancomycin in all study years. MICs were interpreted as susceptible, intermediate or resistant using CLSI MIC breakpoints for all antimicrobial agents.¹³ MDR was defined as resistance to three or more antimicrobial agents selected as antimicrobial class markers (penicillin, clarithromycin, clindamycin, doxycycline, levofloxacin, trimethoprim/sulfamethoxazole and chloramphenicol). In MDR calculations, penicillin resistance was defined using the CLSI breakpoint for oral penicillin V (MIC ≥ 2 mg/L).

Of the 14 138 study isolates, complete susceptibility profiles for all seven antimicrobial agents used in the MDR definition were generated for 13 712 isolates. The number of isolates with complete antimicrobial susceptibility testing profiles for the MDR-definition antimicrobials per year was 1362 isolates in 2011, 1230 isolates in 2012, 1118 isolates in 2013, 1219 isolates in 2014, 1215 isolates in 2015, 1227 isolates in 2016, 1562 isolates in 2017, 1882 isolates in 2018, 1849 isolates in 2019, and 1048 isolates in 2020.

Serotyping

Serotyping was performed using the Quellung reaction using pool, group, type and factor commercial antisera (SSI Diagnostica; Statens Serum Institute, Copenhagen, Denmark).¹⁴ Isolate identity for which a serotype was not determined by a Quellung reaction was confirmed as *S. pneumoniae* by *rpoB* gene sequencing.¹⁵ Serotypes 15B and 15C were grouped as 15B/C due to the reported reversible switching between these serotypes.^{16,17}

Statistical analysis

Trends in the proportion of serotypes and MDR rates throughout the study were assessed for statistically significant differences ($P < 0.05$) using the Cochran–Armitage test for trend. The annual diversity of the MDR *S. pneumoniae* serotypes was assessed by Simpson's Diversity Index.

Results

In total, 14 138 invasive isolates of *S. pneumoniae* collected as part of the SAVE study between 2011 and 2020 were forwarded to the CARA. The annual numbers of isolates were: 1379 isolates from 2011, 1285 from 2012, 1160 from 2013, 1274 from 2014, 1262 from 2015, 1284 from 2016, 1598 from 2017, 1930 from 2018, 1893 from 2019, and 1073 from 2020. The annual serotype distribution of invasive isolates of *S. pneumoniae* collected as part of the SAVE study in Canada between 2011 and 2020 is presented in Figure 1 and Table S1 (available as [Supplementary data](#) at JAC Online). The 10 most common serotypes in the SAVE study overall ($n = 14\,138$) were 3 (1354/9.6%), 22F (1282/9.1%), 19A (961/6.8%), 7F (819/5.8%), 9N (742/5.3%), 8 (708/5.0%), 12F (661/4.7%), 11A (543/3.8%), 33F (539/3.8%) and 15A (520/3.7%). In the 2020 study year ($n = 1073$), the most common serotypes

Table 1. The annual proportion of invasive *S. pneumoniae* isolates with serotypes contained in the pneumococcal conjugate vaccines, SAVE 2011–2020

Phenotype	Vaccine	Study total, % (n)	Year, % (n)									
			2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
All isolates ($n = 14\,138$)	PCV10	13.7 (1943)	26.7 (368)	20.6 (265)	14.7 (170)	9.3 (118)	9.0 (113)	7.9 (102)	11.0 (176)	10.2 (196)	12.1 (229)	19.2 (206)
	PCV13	30.7 (4334)	48.0 (662)	39.6 (509)	33.3 (386)	24.7 (315)	25.0 (316)	24.5 (315)	26.0 (415)	28.1 (542)	27.2 (514)	33.6 (360)
	PCV15	43.5 (6155)	57.6 (794)	53.9 (693)	48.5 (563)	37.8 (482)	38.8 (490)	37.4 (480)	39.3 (628)	40.7 (786)	41.2 (780)	42.8 (459)
	PCV20	62.6 (8844)	71.9 (992)	69.3 (891)	68.8 (798)	59.3 (755)	58.5 (738)	59.2 (760)	57.5 (919)	60.9 (1175)	59.6 (1129)	64.0 (687)
	PPSV23	70.8 (10005)	75.3 (1039)	73.7 (947)	74.1 (860)	66.2 (843)	67.5 (852)	67.0 (860)	68.5 (1094)	70.3 (1356)	70.7 (1338)	76.0 (816)
MDR ($n = 902$)	PCV10	16.2 (146)	8.6 (10)	10.7 (9)	13.4 (9)	16.7 (8)	13.0 (9)	25.0 (12)	20.8 (22)	12.1 (15)	17.7 (25)	27.3 (27)
	PCV13	48.0 (433)	54.3 (63)	53.6 (45)	64.2 (43)	52.1 (25)	55.1 (38)	50.0 (24)	44.3 (47)	44.4 (55)	34.0 (48)	45.5 (45)
	PCV15	53.3 (481)	56.0 (65)	61.9 (52)	71.6 (48)	56.3 (27)	65.2 (45)	50.0 (24)	50.9 (54)	49.2 (61)	39.0 (55)	50.5 (50)
	PCV20	59.5 (537)	60.3 (70)	64.3 (54)	76.1 (51)	60.4 (29)	68.1 (47)	52.1 (25)	52.8 (56)	58.9 (73)	47.5 (67)	65.7 (65)
	PPSV23	60.8 (548)	59.5 (69)	64.3 (54)	77.6 (52)	60.4 (29)	71.0 (49)	52.1 (25)	53.8 (57)	59.7 (74)	50.4 (71)	68.7 (68)

Table 2. Antimicrobial susceptibility testing results for the 10 most common serotypes of invasive *S. pneumoniae*, SAVE 2011–2020

Serotype (n) ^a	% Susceptible/MIC ₉₀ (mg/L)								% MDR
	Penicillin (IV, meningitis)	Penicillin (IV, non-meningitis)	Ceftriaxone (meningitis)	Ceftriaxone (non-meningitis)	Clarithromycin	Levofloxacin	SXT ^b	Doxycycline	
3 (1312)	99.5/≤0.03	100/≤0.03	99.9/≤0.12	100/≤0.12	94.1/≤0.03	100/1	98.6/0.25	87.7/2	4.0
22F (1279)	99.3/≤0.03	99.8/≤0.03	99.8/≤0.12	99.8/≤0.12	65.1/4	99.4/1	98.8/0.25	98.6/≤0.25	1.6
19A (957)	70.9/4	87.6/4	82.8/1	95.2/1	37.7/≥32	99.7/1	72.8/8	71.8/4	23.9
7F (807)	99.1/≤0.03	100/≤0.03	99.9/≤0.12	100/≤0.12	97.3/≤0.03	99.8/1	99.5/0.25	97.0/≤0.25	0.4
9N (740)	97.0/≤0.03	100/≤0.03	99.6/≤0.12	100/≤0.12	92.0/0.06	99.6/1	96.4/0.5	97.3/≤0.25	1.1
8 (704)	99.4/≤0.03	100/≤0.03	99.9/≤0.12	100/≤0.12	99.2/≤0.03	100/1	99.2/0.25	97.0/≤0.25	0.1
12F (661)	99.4/≤0.03	100/≤0.03	99.9/≤0.12	100/≤0.12	45.1/8	99.9/1	94.1/0.5	93.3/≤0.25	4.2
11A (541)	94.8/≤0.03	99.8/≤0.03	98.9/≤0.12	99.5/≤0.12	67.3/4	99.6/1	82.3/8	96.9/≤0.25	2.8
33F (538)	99.8/≤0.03	100/≤0.03	100/≤0.12	100/≤0.12	20.3/8	100/1	22.7/8	90.3/≤0.25	5.0
15A (377)	39.4/2	100/2	92.6/0.5	100/0.5	29.2/≥32	99.2/1	92.6/0.5	31.8/16	56.2

^an of each serotype for which complete susceptibility data were available.
^bSXT, trimethoprim/sulfamethoxazole.

Table 3. Demographics of the MDR invasive *S. pneumoniae* isolates in the SAVE study, 2011–2020

Patient demographic	SAVE	MDR isolates	
	n	n	% of SAVE
All isolates	13 712	902	6.6
Patient age, ^a years			
0 to <1	356	19	5.3
1 to <2	405	34	8.4
2 to <6	521	30	5.8
6 to <18	388	21	5.4
18 to <50	2792	160	5.7
50 to <65	3801	230	6.1
≥65	5212	388	7.4
Patient biological sex ^b			
Female	5904	399	6.8
Male	7158	461	6.4
Geographical region ^c			
Western Canada	2989	149	5.0
Central Canada	9291	649	7.0
Eastern Canada	1432	104	7.3

^aThere were 237 isolates with unknown patient age.
^bThere were 650 isolates with unknown patient biological sex.
^cWestern Canada included isolates from Manitoba and Saskatchewan, Central Canada included isolates from Ontario and Quebec, and Eastern Canada included isolates from Nova Scotia, New Brunswick, Prince Edward Island, and Newfoundland and Labrador.

were 4 (135/12.6%), 3 (115/10.7%), 8 (87/8.1%), 9N (75/7.0%), 22F (66/6.2%), 12F (55/5.1%), 20 (46/4.3%), 15A (40/3.7%), 19A (37/3.4%), 33F (33/3.1%) and 11A (33/3.1%).
Between 2011 and 2020, statistically significant reductions in the overall temporal trend of the annual proportion of serotypes 1 ($P<0.0001$), 5 ($P<0.0001$), 6A ($P=0.001$), 6B ($P=0.04$), 6C

($P<0.0001$), 7F ($P<0.0001$), 19A ($P<0.0001$), 23F ($P=0.01$), 33A ($P<0.0001$) and 37 ($P=0.04$) were observed. Statistically significant increases in serotypes 3 ($P<0.0001$), 7C ($P=0.0001$), 8 ($P<0.0001$), 9N ($P<0.0001$), 17F ($P=0.04$), 19F ($P<0.0001$), 20 ($P<0.0001$), 23A ($P=0.02$), 23B ($P=0.02$), 24A ($P=0.008$), 31 ($P<0.0001$), 33F ($P=0.02$) and 35D ($P=0.009$) were also observed. Serotypes 9V ($P=0.001$) and 24F ($P=0.008$) demonstrated significant bidirectional changes, with serotype 9V decreasing and then increasing and serotype 24F increasing and then decreasing throughout the study. Notable changes in the rank order of the top 10 serotypes were observed during the study. Serotype 7F ranked as the most common serotype in 2011 and 2012 but fell out of the top 10 starting in 2016. Serotype 19A ranked between the 1st and 3rd most common serotype from 2011 to 2015 and decreased over time to the 10th and 9th most common serotypes in 2019 and 2020. Serotype 9N ranked 9th or 10th from 2011 to 2013 and rose to 3rd or 4th in 2017 to 2020.
The overall proportions of invasive *S. pneumoniae* isolates collected in Canada as part of the SAVE study that were serotypes contained in PCV10, PCV13, PCV15, PCV20 and PPSV23 are shown in Table 1.
The antimicrobial susceptibility testing results for the 10 most common serotypes of invasive *S. pneumoniae* in Canada are presented in Table 2. Reduced susceptibility rates were observed in serotype 19A for penicillin (70.9% based on the IV meningitis breakpoint), ceftriaxone (82.8% based on the IV meningitis breakpoint), clarithromycin (37.7%), doxycycline (71.8%) and trimethoprim/sulfamethoxazole (72.8%). Reduced susceptibility rates were also observed for clarithromycin in serotype 12F isolates (45.1%) and 22F isolates (65.1%), clarithromycin (67.3%) and trimethoprim/sulfamethoxazole (82.3%) in serotype 11A isolates, clarithromycin (20.3%) and trimethoprim/sulfamethoxazole (22.7%) in serotype 33F isolates, and penicillin (39.4% based on the IV meningitis breakpoint), clarithromycin (29.2%) and doxycycline (31.8%) in serotype 15A isolates. All isolates were susceptible to vancomycin. Among the top 10 serotypes, MDR rates ≥5% were observed for serotypes 15A (56.2%), 19A

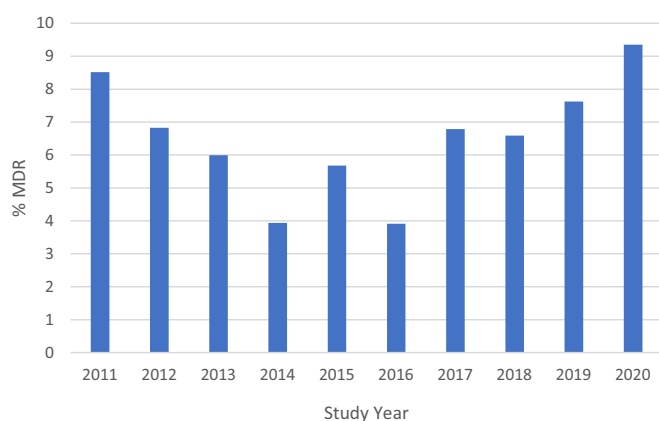


Figure 2. Annual rates of MDR in invasive *S. pneumoniae* isolates collected in the SAVE study, 2011–2020. This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.

(23.9%) and 33F (5.0%). Serotypes that were isolated less frequently in the study but for which MDR was observed in >5% of isolates included 6A (6.6%, $n=5/76$), 6B (37.2%, $n=16/43$), 6C (6.2%, $n=22/355$), 6D (16.7%, $n=1/6$), 7B (66.7%, $n=4/6$), 9A (20.0%, $n=1/5$), 9V (11.8%, $n=10/85$), 13 (14.3%, $n=5/35$), 14 (34.9%, $n=22/63$), 15F (100%, $n=1/1$), 17A (50.0%, $n=1/2$), 19F (20.9%, $n=64/306$), 22A (16.7%, $n=1/6$), 23A (10.4%, $n=50/482$), 23F (14.8%, $n=4/27$), 24F (8.1%, $n=7/86$), 35A (50.0%, $n=2/4$), 35B (5.7%, $n=19/335$) and 35D (66.7%, $n=2/3$).

Between 2011 and 2020, 902 (6.6%) MDR *S. pneumoniae* were isolated as part of SAVE. The patient demographics are presented in Table 3. The annual rates of MDR in *S. pneumoniae* are portrayed in Figure 2. There was no statistically significant trend in the annual rates of MDR in *S. pneumoniae* over the entire study of 2011 to 2020; however, there was a significant decrease from 8.5% ($n=116$) in 2011 to 5.7% ($n=69$) in 2015 ($P<0.0001$) followed by a significant increase from 3.9% ($n=48$) in 2016 to 9.4% ($n=99$) in 2020 ($P<0.0001$).

MDR *S. pneumoniae* most commonly demonstrated resistance to antimicrobial agents from three classes (452, 50.1%). Resistance to antimicrobial agents from four, five, six and seven classes was identified in 235 (26.1%), 188 (20.8%), 26 (2.9%) and 1 (0.1%) of the MDR *S. pneumoniae*, respectively (Table S2). The most common pattern was resistance to clarithromycin, clindamycin and doxycycline (329, 36.5% of all MDR isolates). Notably, the second most common pattern was resistance to antimicrobial agents from five classes: clarithromycin, clindamycin, doxycycline, penicillin and trimethoprim/sulfamethoxazole (163, 18.1% of all MDR isolates) (Table S2). Levofloxacin resistance was observed in only 2.3% ($n=21$) of the MDR isolates. The most common associations of serotype and specific resistance patterns among the MDR *S. pneumoniae* were 15A ($n=143$) and 23A ($n=45$) resistant to clarithromycin, clindamycin and doxycycline, 3 ($n=45$) resistant to chloramphenicol, clarithromycin, clindamycin and doxycycline, and 19A ($n=130$) resistant to clarithromycin, clindamycin, doxycycline, penicillin and trimethoprim/sulfamethoxazole (Table S2).

MDR was observed in 44 serotypes of the *S. pneumoniae* invasive isolates as shown in Figure 3(a). The predominant serotypes among the MDR isolates were 19A (229, 25.4%) and 15A (212,

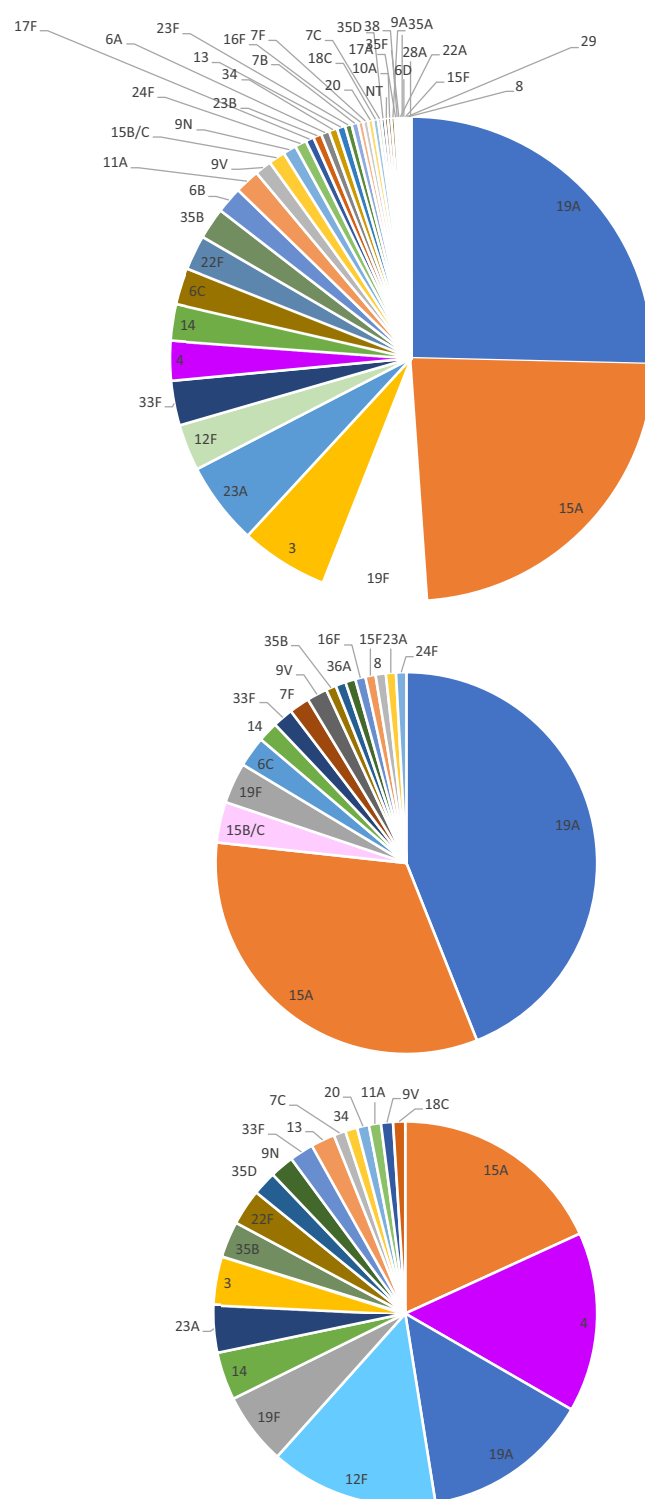


Figure 3. Serotype distribution of MDR in invasive *S. pneumoniae* isolates collected in the SAVE study in (a) 2011–2020 ($n=902$), (b) 2011 ($n=116$) and (c) 2020 ($n=99$). This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.

23.5%). Serotypes 15A and 19A ranked as two of the top three serotypes of the MDR isolates every year of the study; however, the serotype diversity index increased from 0.7 in 2011 to 0.9 in

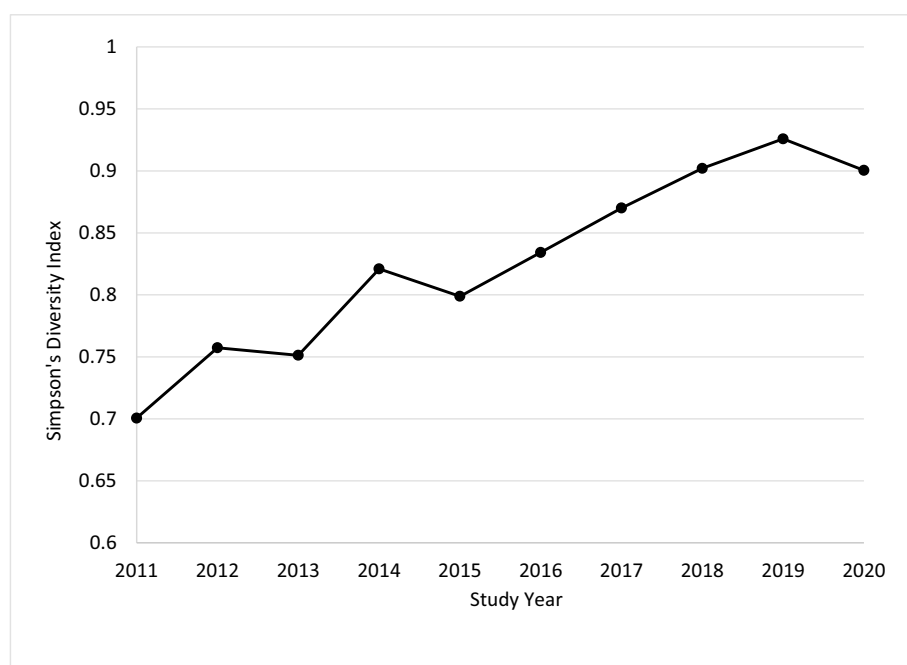


Figure 4. Annual diversity of invasive MDR *S. pneumoniae* serotypes, calculated by the Simpson Diversity Index, in the SAVE study, 2011–2020.

2020, with a statistically significant linear increasing trend ($P < 0.001$), as shown in Figure 4. In 2011, serotypes 15A and 19A represented 32.8% and 44.0% of all invasive MDR *S. pneumoniae* isolates, respectively (Figure 3b). In 2020, serotypes 15A and 19A represented 18.2% and 14.1% of all invasive MDR *S. pneumoniae* isolates, respectively (Figure 3c).

Discussion

The 10 predominant *S. pneumoniae* serotypes identified throughout the SAVE study, in order of frequency of isolation, were 3, 22F, 19A, 7F, 9N, 8, 12F, 11A, 33F and 15A, consistent with a meta-analysis by Balsells et al.,¹⁸ which reported that for countries that had introduced PCV10 or PCV13, the most common serotypes in paediatric IPD cases included 22F, 12F, 15C, 24F and 33F. Some regional differences were noted, with serotypes 22F, 33F, 15B, 38 and 35B being most common in North America and 12F, 10A, 24F, 22F and 15C being most common in Europe.¹⁸ Among adults with IPD in Japan in the years following PCV13 introduction, the predominant serotypes were 12F, 3, 23A, 19A, 10A, 6C and 22F.¹⁹

Throughout the 10 years evaluated in the SAVE study, statistically significant reductions were noted in PCV13-contained serotypes 1, 5, 6A, 6B, 7F, 19A and 23F. Significant decreases were also noted in serotype 6C, likely due to protection from cross-functional antibodies to 6A²⁰ in PCV13. The post-PCV13 reduction in serotypes 7F and 19A observed in this study was also noted very early on in the USA.²¹ Notably, PCV13-related serotypes 3 and 19F increased significantly throughout the SAVE study, although by rank order 19F did not appear in the top 10 serotypes. A predominance of serotypes 3 and 19F was also observed in countries where PCV10 was used.²² Between 2011 and 2020, significant increases were observed in serotypes that are included in

the higher valency conjugate vaccines, including 8 (PCV20) and 33F (PCV15 and PCV20), in the SAVE study. Among non-conjugate vaccine serotypes, statistically significant increases were observed in serotypes 7C, 9N, 17F, 20, 23A, 23B, 24A, 31 and 35D. Similar changes in serotype distribution were described in a report from the active surveillance of IPD in Israel when comparing the time periods before pneumococcal conjugate vaccine introduction (2004–2008) and following significant use of PCV13 (2016–2019). The authors describe significant decreases in PCV13 serotypes 1, 5, 6A, 6B, 7F, 9V, 14, 18, 19A, 19F and 23F and significant increases in serotypes 2, 8 and 12F among all age groups.²³ A number of additional serotypes noted to have increased in the SAVE study were also shown to have increasing trends, but not reaching statistical significance in Israel, including serotypes 9N, 17F and 33F.²³ Country-specific differences were also observed as the rate of serotype 3 was found to be stable throughout the use of pneumococcal conjugate vaccines in Israel²³ whereas we observed a significant increase in Canada. A continued predominance of serotype 3 has been reported in a number of studies around the world.^{18,19,21,22,24}

A post-PCV13 licensure study on serotype-specific vaccine effectiveness demonstrated 73% overall vaccine effectiveness for the serotypes included in PCV13 that were not previously in PCV7, but serotype-specific variability was noted.²⁵ Vaccine effectiveness was lowest for serotype 3 (26%) and highest for serotypes 6A (98%) and 7F (91%), with lower but significant effectiveness reported for serotype 19A (67%).²⁵ The strong vaccine effectiveness against serotypes 6A, 7F and 19A may have positively contributed to the significant reductions observed in the proportion of isolates with serotypes 6A, 6C (due to cross-protection),²⁰ 7F and 19A in the SAVE study. The low vaccine effectiveness for serotype 3 may explain the continued high annual incidence of this serotype in Canada.¹ Studies with PCV15

demonstrated improved immunogenicity for serotype 3; however, it remains to be determined if the *in vitro* results will translate to improved clinical outcomes.²⁶

During the most recently analysed SAVE study year of 2020, 19.2%, 33.6%, 42.8%, 64.0% and 76.0% of *S. pneumoniae* were serotypes included in the PCV10, PCV13, PCV15, PCV20 and PPSV23 vaccines, respectively. The current proportion of serotypes contained in the PCV13 vaccine (33.6%) is lower than the 54.3% reported in a pre-PCV13 surveillance study performed by CARA between 2007 and 2009²⁷ but greater than the 25.4% noted in the 2015 SAVE study year.²⁸ The proportions of isolates with serotypes contained in the vaccines were higher in the SAVE study than those reported for adult cases of IPD in Japan: 2.8%, 28.2% and 61.0% with PCV7, PCV13 and PPSV23 serotypes, respectively.¹⁹ A meta-analysis of paediatric IPD cases reported that in countries using PCV10 or PCV13, the proportion of serotypes contained in PCV7 was 12.5%, with an additional 9.2% including the other three serotypes in PCV10, 49.1% in PCV13, with an additional inclusion of approximately 9.8% with the other two PCV15 serotypes and 25.4% with the other seven PCV20 serotypes.¹⁸ The results from the 24 countries included in the meta-analysis are similar to the results of the SAVE study, although regional differences were noted. The North America-specific results were even more similar to those observed in the SAVE study, with the proportion of serotypes contained in PCV7 reported by Balsells *et al.*¹⁸ as 3.4%, with an additional 7.9% including the other three serotypes in PCV10, 37.3% in PCV13, with an additional inclusion of approximately 11.8% with the other two PCV15 serotypes and 25.9% with the other seven PCV20 serotypes.

Among the most common serotypes of invasive *S. pneumoniae* isolated in this study, high rates of antimicrobial non-susceptibility with multiple agents were most notable for serotypes 15A and 19A, with other serotypes noted to have decreased susceptibility to specific antimicrobial agents. Increased rates of non-susceptibility to penicillin and erythromycin in serotypes 15A and 19A were observed in the USA following the introduction of PCV-7.³ Following the introduction of PCV13 in France, decreased susceptibility to penicillin and erythromycin was frequently observed in serotypes 15A, 24F and 35B but not in 12F.²⁹ Serotype 15A had the lowest penicillin susceptibility rate (39.4% with the IV meningitis breakpoint) of all the serotypes analysed in the SAVE study. Penicillin-non-susceptibility was commonly observed in serotypes 15A and 19A identified from cases of IPD in Norway.³⁰ Clarithromycin non-susceptibility was very commonly observed in our Canadian study, with high rates reported for serotypes 11A, 12F, 15A, 19A, 22F and 33F. Erythromycin resistance was most commonly observed in serotypes 14, 15A and 19A in Norway.³⁰ In addition to the reduced susceptibility to penicillin and clarithromycin, serotype 15A isolates were frequently non-susceptible to doxycycline, and serotype 19A isolates demonstrated high levels of non-susceptibility to doxycycline and trimethoprim/sulfamethoxazole in the SAVE study. Tetracycline and doxycycline resistance was most commonly noted in serotypes 15A, 19A and 19F, and resistance to trimethoprim/sulfamethoxazole was most frequently observed with serotypes 9V, 19A and 24F in Norway.³⁰ Tetracycline resistance was common in serotypes 12F, 15A and 24F isolates, and resistance to trimethoprim/sulfamethoxazole was most frequently observed with serotype 24F in

France.²⁹ In contrast, the 12F isolates in our study were generally susceptible to doxycycline (93.3%). Serotype 33F isolates in the SAVE study demonstrated very low susceptibility rates to trimethoprim/sulfamethoxazole (22.7%). The existence of a clone of trimethoprim/sulfamethoxazole-resistant serotype 33F (ST100) was reported in the study of IPD in Norway.³⁰

Overall, MDR was observed in 6.6% (902/13 712) of the invasive *S. pneumoniae* isolates in the SAVE study. MDR isolates were identified from patients of all age groups, biological sexes and geographical regions of the country. The MDR rate was higher than that reported from the Public Health England surveillance, where 3.5% of *S. pneumoniae* isolates from blood and CSF infections demonstrated 'triple resistance' (erythromycin and tetracycline resistant and penicillin non-susceptible) during the years 2005 to 2014.²⁴ No statistically significant trend in the annual rates of MDR was demonstrated for the SAVE study (2011–2020); however, a significant decrease in MDR was noted during the first 5 years of the study (2011–2015) with a subsequent increase observed in the subsequent five study years (2016 to 2020). Although the increase did not reach statistical significance, the incidence of MDR *S. pneumoniae* also increased in Norway between 2004 and 2016.³⁰

Throughout the study, MDR was observed in 44 serotypes. Serotypes 15A and 19A represented 48.9% of all MDR isolates. The emergence of MDR 19A and 15A isolates has been well documented.^{24,30,31} Although serotypes 15A and 19A were predominant, a significant increase in the diversity of serotypes among the MDR isolates was demonstrated throughout our study. In 2011, serotypes 15A and 19A represented 76.7% of the MDR isolates and any other identified serotype represented less than 5% of the isolates. Conversely, in 2020, serotypes 15A and 19A represented only 32.3% of the MDR isolates because there were five serotypes that each represented more than 5% of the MDR isolates: 15A > 4 > 19A = 12F > 19F. Clonal spread of the MDR serotype 15A-ST63 has been described for our isolates³² and in international studies.³⁰ Other MDR serotypes, such as 14, 19A and 19F, have been shown to be represented by diverse STs.^{24,30} Although MDR serotype 24F isolates were uncommon in our study, they are emerging globally. A WGS-based assessment of international serotype 24F isolates demonstrated that three lineages have emerged, with Global Pneumococcal Sequencing (GPS) lineage GPSC10 associated with MDR.³³ Internationally, lineage GPSC10 expressed 17 serotypes, which may allow it to adapt under vaccine pressure.³³ GPSC10 was shown to have rapidly switched from serotype 19A to 24F after the introduction of PCV13 in Spain.³³ The existence of diverse serotype variants allowed the lineage to survive and expand,³³ which highlights the importance of the increased diversity of MDR *S. pneumoniae* circulating in Canada.

Lo *et al.*³³ noted that the differences in GPS lineages in specific countries may be attributable to antibiotic pressure because the susceptibility patterns of the predominant lineages of serotype 24F could be correlated with the country's antibiotic usage. The MDR GPSC10 lineage demonstrated resistance to penicillin, erythromycin and tetracycline and had intermediate MICs to trimethoprim/sulfamethoxazole.³³ Resistance to the antimicrobial classes represented by penicillin, erythromycin and tetracycline was observed in 31.4% of the MDR isolates in the SAVE study. Overall, the most common MDR pattern observed in the SAVE study was

resistance to clarithromycin, clindamycin and doxycycline. This specific phenotype represented 36.5% of the MDR isolates and was a component of the phenotype of 82.8% of the MDR isolates in this study. The frequent association of resistance between clarithromycin and clindamycin reflects the high prevalence of the *erm*(B) resistance determinant in the *S. pneumoniae* identified in the SAVE study.³²

During the most recently analysed SAVE study year of 2020, 27.3%, 45.5%, 50.5%, 65.7% and 68.7% MDR *S. pneumoniae* were serotypes included in the PCV10, PCV13, PCV15, PCV20 and PPSV23 vaccines, respectively. The percentage of MDR isolates with serotypes included in PCV13 is significantly lower than that reported in our pre-PCV13 study where 88% of MDR isolates were PCV13 serotypes.²⁷

The limitations of this study are the lack of participation of all provinces in Canada, and as such we can only demonstrate changes in the proportion of serotypes and the MDR isolates causing IPD from 8 of the 10 Canadian provinces. The specific antimicrobial agents tested were not universally consistent with the comparator studies; antimicrobial susceptibility results and the MDR comparisons were made using representative agents for an antimicrobial class.

Our analysis of invasive MDR *S. pneumoniae* isolates as part of the SAVE study has highlighted a number of key findings that may affect antimicrobial resistance patterns and vaccination strategies in the future. In the first 5 years of the SAVE study (2011–2015), MDR rates decreased, as the introduction of PCV13 was associated with a reduction in the proportion of isolates identified with the resistant 19A serotype. However, MDR rates increased from 2016 to 2020 such that there was no observed trend in rates across the entire study. The MDR isolates in the most recent years of the SAVE study included a greater diversity of serotypes than in previous years, potentially demonstrating population changes as *S. pneumoniae* continues to evolve under both vaccine and antibiotic selective pressure. The observations made with the SAVE study provide critical information to support the ongoing development of serotype-based vaccines.

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Transparency declarations

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Disclaimer

The opinions expressed in this article are those of the authors and do not necessarily represent those of Merck Canada Inc. or Pfizer Canada Inc.

Supplementary data

Tables S1 and S2 are available as [Supplementary data](#) at JAC Online.

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