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Analysis of Multidrug Resistance in the Predominant *Streptococcus pneumoniae* Serotypes in Canada: The SAVE Study, 2011-2015

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Synopsis

Objectives: This study assessed multidrug resistant (MDR) invasive isolates of *Streptococcus pneumoniae*, in relation to the serotype evolution, in Canada between 2011 and 2015 as part of the annual SAVE study.

Methods: As part of a collaboration between the Canadian Antimicrobial Resistance Alliance and Public Health Agency of Canada-National Microbiology Laboratory, 6207 invasive isolates of *S. pneumoniae* were evaluated. All isolates were serotyped and had antimicrobial susceptibility testing performed, in accordance with CLSI guidelines (M07-A10, 2015). Complete susceptibility profiles were available for 6001 isolates. MDR was defined as resistance to three or more classes of antimicrobial agents (with penicillin MIC ≥2 mg/L defined as resistant).

Results: The overall rate of MDR *S. pneumoniae* was 6.2% (372/6001) in SAVE; decreasing significantly from 8.5% in 2011 to 5.6% in 2015 (P=0.0041). MDR was observed in 32 serotypes with serotypes 15A and 19A predominating (26.6% and 41.7% of the MDR isolates, respectively). The overall proportion of serotypes 19A, 7F and 33A decreased significantly (P<0.0001) throughout the study. The annual proportion of serotypes 7C, 8, 9N, 10A, 20, 24F, 29, 31, 33F, 35B and 38 increased throughout the study; however, among those increasing serotypes, MDR was only notable (>5%) for 24F and 33F.

Conclusions: In 2015, 56.3% of invasive MDR *S. pneumoniae* were serotypes included in the PCV-13 vaccine. PCV-13 includes the most commonly identified serotype 19A, however other increasingly important MDR serotypes such as 15A, 24F and 33F, are notably not in the currently used vaccines.

Introduction

Streptococcus pneumoniae is recognized as an important pathogen worldwide as it is a common cause of respiratory infections, including community-acquired pneumonia, and the causative agent of Invasive Pneumococcal Disease (IPD). The overall incidence rate of IPD in Canada had been reported as remaining relatively stable with an average of 9.6 cases per 100 000 population during the time period of 2009 to 2014.[1,2] However, the crude incidence rate of IPD in Canada decreased significantly by 2014 to 8.9 cases per 100 000 population with notable decreases in incidence in children less than 5 years of age.[1,3]

Prevnar®, a 7-valent pneumococcal conjugate vaccine (PCV-7) including serotypes 4, 6B, 9V, 14, 18C, 19F and 23F, was incorporated in routine vaccination schedules in all Canadian provinces between 2002 and 2006.[4] The use of PCV-7 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 in North America.[4,5,6] Increases in non-vaccine serotypes, particularly MDR serotype 19A, were rapidly observed following the introduction of PCV-7.[4,5] Subsequently, newer pneumococcal conjugate vaccines were developed with enhanced serotype coverage, including Synflorix™, with the additional inclusion of serotypes 1, 5 and 7F (PCV-10), and Prevnar®13, which includes serotypes 1, 3, 5, 6A, 7F and 19A (PCV-13). The broader serotype coverage and the critical inclusion of serotype 19A in PCV-13 offered an important advancement in the protection of children against invasive *S. pneumoniae* infections. The immunization guidelines were updated in 2010 to recommend the routine use of PCV-13 in infant vaccine schedules in North America.[7,8] The replacement of PCV-7 with PCV-13 as part of routine infant vaccinations was completed in Canada by early-2011.[2]

Subsequent to the successful implementation of conjugate vaccines in the routine infant immunization programs, the efficacy of PCV-13 in the prevention of IPD and community-acquired

pneumonia in immunocompetent adults was evaluated. The CAPiTA study demonstrated efficacy in the prevention of pneumococcal pneumonia and IPD for *S. pneumoniae* with serotypes included in PCV-13.[9] Accordingly, the immunization recommendations were updated in North America to include routine administration of PCV-13 to healthy adults ≥65 years.[3,10]

Despite the overall success of the vaccine programs, treatment concerns have remained as 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. Penicillin resistance was commonly observed in serotypes 19A, 19F and 35B in the United States shortly after the introduction of PCV-13.[11] Resistance to one or more antibiotics in emerging non-PCV-13 serotypes was recently observed in serotypes 12F, 15A, 24F and 35B in France.[12] Similarly, a high prevalence of MDR non-vaccine serotypes were observed in Bulgaria five years after the introduction of PCV-10.[13]

The *S. pneumoniae* Serotyping and Antimicrobial Susceptibility: Assessment for Vaccine Efficacy in Canada (SAVE) study is an annual study which began in 2011. The purpose of this study was to evaluate changes in antimicrobial resistance, particularly multidrug resistance, in relation to serotype evolution in Canada between 2011 and 2015, subsequent to the introduction of PCV-13.

Materials and methods

Bacterial isolates

From January 2011 to December 2015, *S. pneumoniae* isolated 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 eight 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. For the SAVE study, regional analysis were conducted as Western (Saskatchewan and Manitoba, *n*=1352), Central (Ontario and Quebec, *n*=4107) and Eastern (New Brunswick, Nova Scotia, Prince Edward Island and Newfoundland and Labrador, *n*=748).

In total, 6207 invasive isolates of *S. pneumoniae* collected as part of the SAVE study between 2011 and 2015 were forwarded to the CARA. Patient gender and age information was available for 5980 (96.3%) and 6072 (97.8%) of the isolates, respectively. The annual numbers of isolates were: 1379 isolates from 2011, 1285 from 2012, 1138 from 2013, 1210 from 2014, and 1195 from 2015.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed in the Department of Clinical Microbiology at the Winnipeg Health Sciences Centre using the standard CLSI broth microdilution method[14,15] 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. All isolates were tested against penicillin, ceftriaxone, cefuroxime, clarithromycin, clindamycin, telithromycin, levofloxacin, moxifloxacin, linezolid, trimethoprim/sulfamethoxazole, doxycycline, tigecycline, chloramphenicol and vancomycin. MICs were interpreted as susceptible, intermediate or resistant using CLSI MIC breakpoints for all antimicrobial agents.[15] Multidrug-resistant (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 6207 invasive isolates of *S. pneumoniae* received by CARA for

antimicrobial susceptibility testing, complete susceptibility profiles for all 14 antimicrobial agents were generated for 6001 isolates; the remaining 206 isolates failed to grow or generated incomplete susceptibility profiles. The number of isolates with complete antimicrobial susceptibility testing profiles per year was 1362 isolates in 2011, 1230 isolates in 2012, 1099 isolates in 2013, 1159 isolates in 2014 and 1151 isolates in 2015.

Serotyping

Serotyping was performed using the Quellung reaction using pool, group, type and factor commercial antisera (Statens Serum Institute, Copenhagen, Denmark).[16] Isolate identity for which a serotype was not determined by a Quellung reaction was confirmed as *S. pneumoniae* by *rpoB* gene sequencing.[17]

Statistical Analysis

Changes in serotype distribution and multidrug resistance rates between 2011 and 2015 were assessed for statistically significant differences (P <0.05) using a two-tailed Fisher's exact test (α=0.05). The statistical significance of differences (P <0.05) in the proportion of isolates included in the current vaccine formulations were assessed using the Chi square test due to the large sample size.

Results

The annual serotype distribution of invasive isolates of *S. pneumoniae* collected as part of the SAVE study in Canada between 2011 and 2015 is presented in Figure 1. The most common serotypes in the SAVE study overall (n=6207) were 7F (704/11.3%), 19A (599/9.7%), 22F (593/9.6%), 3(491/7.9%), 12F (291/4.7%), 11A (264/4.3%), 9N (240/3.9%), 8 (239/3.9%), 33F (223/3.6%), and 15A (217/3.5%). In the 2015 study year (n=1195), the most common serotypes were 22F (101/8.5%), 3 (96/8.0%), 19A

(91/7.6%), 12F (67/5.6%), 33F (65/5.4%), 9N (64/5.4%), 8 (58/4.9%), 7F (49/4.1%), 11A (45/3.8%), 15A (40/3.3%) and 20 (40/3.3%).

Between 2011 and 2015, statistically significant reductions in the prevalence of serotypes 7F (*P*<0.0001), 19A (*P*<0.0001) and 33A (*P*<0.0001) were observed. Statistically significant increases in serotypes 7C (*P*=0.034), 8 (*P*=0.0092), 9N (*P*=0.0005), 10A (*P*=0.028), 20 (*P*<0.0001), 24F (*P*=0.0008), 29 (*P*=0.028), 31 (*P*<0.0001), 33F (*P*<0.0001), 35B (*P*=0.021) and 38 (*P*=0.037) were observed during this time period. Notable changes in the rank order of the top ten serotypes were observed during the study. Serotype 7F ranked as the most common serotype in 2011 but fell to eighth in 2015 whereas serotype 19A was only reduced from ranking second to third most common serotype. Serotypes 22F and 3 rose in ranking from third and fourth to first and second, respectively. Serotype 33F ranked as the ninth most common serotype in 2011 and rose to the fifth position in 2015.

The overall proportion of invasive *S. pneumoniae* isolates collected in Canada as part of the SAVE study that were serotypes contained in PCV-7, PCV-10 and PCV-13 are shown in Table 1.

The antimicrobial susceptibility testing results for the ten most common serotypes of invasive *S. pneumoniae* in Canada are presented in Table 2. Reduced susceptibility rates were observed in serotype 19A for penicillin (68.1% based on the IV meningitis breakpoint), ceftriaxone (79.7% based on the IV meningitis breakpoint), clarithromycin (37.6%), doxycycline (69.3%) and trimethoprim/sulfamethoxazole (69.6%). Reduced susceptibility rates were also observed for clarithromycin in serotype 22F isolates (72.9%) and 12F isolates (37.8%), clarithromycin (73.0%) and trimethoprim/sulfamethoxazole (81.4%) in serotype 11A isolates, clarithromycin (21.6%) and trimethoprim/sulfamethoxazole (32.0%) in serotype 33F isolates, and penicillin (38.7%), clarithromycin (22.7%) and doxycycline (23.3%) in serotype 15A isolates. All isolates were susceptible to vancomycin.[18] Among the top ten serotypes, multidrug resistance rates greater than five percent were observed for serotypes 15A (57.6%), 19A (26.0%) and

33F (6.3%). Serotypes that were isolated less frequently in the study but for which multidrug resistance was observed in more than five percent of isolates included 6B (30%, *n*=6/20), 9V (22.2%, *n*=4/18), 14 (21.7%, *n*=5/23), 15F (100%, *n*=1/1), 19F (26.8%, *n*=22/82), 23F (11.1%, *n*=2/18), 28A (5.9%, *n*=1/17) and 35A (50%, *n*=1/2).

Between 2011 and 2015, 372 (6.2%) MDR *S. pneumoniae* were isolated as part of SAVE. The annual rates of multidrug resistance in *S. pneumoniae* are portrayed in Figure 2. There was a significant decrease in multidrug resistance in *S. pneumoniae* from 8.5% (*n*=116) in 2011 to 5.6% (*n*=64) in 2015 (*P*=0.004) with the lowest proportion seen in 2014 (3.9%, *n*=45).

MDR *S. pneumoniae* isolates were identified from all regions of the country with the following distribution (*n* / % by region): Western (71 / 5.3%), Central (247 / 6.0%), and Eastern (54 / 7.2%). Among the age groups, MDR *S. pneumoniae* represented 5.3% (12/228) of the isolates from children 0 - <1 years of age, 11.8% (24/203) of those aged 1 - <2 years, 6.8% (17/251) of those aged 2 - <6 years, 4.5% (9/198) of those aged 6 - <18 years, 5.4% (67/1233) of adults 18 - < 50 years, 5.1% (83/1627) of those aged 50 - <65 years, and 6.6% (155/2332) of the elderly ≥ 65 years. The rate of multidrug resistance was significantly higher in the 1 - <2 year age category than all other combined age groups (*P*=0.002). Similar numbers of MDR isolates were identified from men and women (MDR *n* / total *n* by gender [%]): 185/3213 (5.8%) and 176/2767 (6.4%).

The MDR *S. pneumoniae* most commonly demonstrated resistance to antimicrobial agents from three classes (184, 49.5%) , with the most common pattern overall of resistance to clarithromycin, clindamycin and doxycycline (150, 40.3%) (Supplementary data, Table 1). Notably, the second most common pattern was resistance to antimicrobial agents from five classes: clarithromycin, clindamycin, doxycycline, penicillin and trimethoprim/sulfamethoxazole (110, 29.6%). Levofloxacin resistance was

only observed in 2% (*n*=8) of the MDR isolates and only in 2 isolates with an MDR phenotype that included penicillin.

Multidrug resistance was observed in 32 serotypes of the *S. pneumoniae* invasive isolates as shown in Figure 3. The predominant serotypes among the MDR isolates were 15A (99, 26.6%) and 19A (155, 41.7%). The proportion of invasive MDR *S. pneumoniae* isolates of the SAVE study contained in PCV-7, PCV-10 and PCV-13 are shown in Table 1.

The demographics of the most common serotypes demonstrating multidrug resistance are presented in Table 3. The table includes information on serotypes for which more than 10 MDR isolates were identified throughout the study, which includes serotypes 3, 6C, 15A, 19A, 19F and 33F. MDR serotypes 3, 6C and 33F were only observed in Central Canada. MDR serotype 3 isolates were only observed in adults while multidrug resistance was noted in children and adults in the other serotypes.

The most common associations of serotype and specific resistance patterns among the MDR *S. pneumoniae* were 19A resistant to clarithromycin, clindamycin, doxycycline, penicillin and trimethoprim/sulfamethoxazole (*n*=97) and 15A resistant to clarithromycin, clindamycin and doxycycline (*n*=81) (Supplementary data, Table 1). A single *S. pneumoniae* resistant to seven antimicrobial agents was isolated during this study, which was a serotype 23F from a blood culture of a 64 year old female in 2013 from Central Canada.

Discussion

The ten predominant *S. pneumoniae* serotypes identified throughout the SAVE study, in order of frequency of isolation, were 7F, 19A, 22F, 3, 12F, 11A, 9N, 8 and 33F, similar to those reported in the United Kingdom and the United States.[11,19] The serotypes included in the top ten were fairly consistent

198 throughout the five years of study; however, the ranking order changed somewhat with reductions in 7F
199 and 19A and increases of serotypes 3, 22F and 33F. The annual proportion of serotypes 7F and 19A, two
200 of the serotypes in PCV-13 that were not in PCV-7, decreased significantly during the course of the
201 study. Among non-vaccine serotypes, statistically significant reductions in serotype 33A and increases in
202 serotypes 7C, 8, 9N, 10A, 20, 24F, 29, 31, 33F, 35B and 38 occurred. Interestingly, the most common
203 serotypes in IPD cases in Bulgaria, where PCV-10 was used instead of PCV-13, were comparable with
204 serotypes 3, 19F and 7F predominating.[13] The post-PCV-13 reduction in serotypes 7F and 19A and the
205 increase of serotype 35B observed in this study was also noted very early on in the United States.[11]

206 A study on serotype-specific vaccine effectiveness post-licensure of PCV-13 demonstrated 73%
207 vaccine effectiveness for the serotypes included in PCV-13 that were not previously in PCV-7.[20] Of the
208 PCV-13 specific serotypes, vaccine effectiveness was lowest for serotype 3 (26%) and highest for
209 serotype 6A (98%) and 7F (91%).[20] The effectiveness against serotype 19A was significant at 67% but
210 notably lower than some of the other serotypes.[20] The low vaccine effectiveness for serotype 3 may
211 explain the ongoing high ranking of this serotype in this study as well as a recent study reported from
212 the United Kingdom, which noted decreasing trends for all PCV-13 serotypes except serotype 3 following
213 the routine use of the vaccine.[19] A continued predominance of serotype 3 has been reported in a
214 number of studies around the world.[11,13,19] Similarly, the high vaccine effectiveness against serotypes 7F
215 and 19A may have positively contributed to the significant reductions observed in the proportion of
216 isolates with serotypes 7F and 19A in the SAVE study. The small number of serotype 6A isolates
217 collected during this study may have precluded a notable vaccine effect despite the high effectiveness
218 reported for PCV-13.

219 During the most recently analyzed SAVE study year of 2015, only 25.4% of the *S. pneumoniae*
220 were serotypes included in PCV-13. This is a significant reduction from the 54.3% reported for invasive

221 *S. pneumoniae* isolates as part of a pre-PCV-13 surveillance study performed by CARA between 2007 and
222 2009.[21] The high proportion of non-vaccine serotypes causing IPD after only a few years of PCV-13 use is
223 similar to the epidemiology observed following the implementation of PCV-7.

224 Among the most common serotypes of invasive *S. pneumoniae* isolated in this study, high rates
225 of antimicrobial resistance were most notable for serotypes 15A and 19A. An increase in the rates of
226 penicillin and erythromycin non-susceptibility in serotypes 15A and 19A was observed in the United
227 States following the introduction of PCV-7.[5] In France, decreased susceptibility to penicillin and
228 erythromycin resistance post-PCV-13 implementation was frequently observed in serotypes 15A, 24F
229 and 35B but not in 12F.[12] The decreased penicillin susceptibility reported for serotype 15A isolates in
230 France is consistent with our study in which serotype 15A had the lowest penicillin susceptibility rate
231 (38.7% with the IV meningitis breakpoint) of all the serotypes analyzed. Clarithromycin resistance was
232 very commonly observed in our Canadian study with high rates reported for serotypes 11A, 12F, 22F and
233 33F. In addition to penicillin and clarithromycin resistance, serotype 15A isolates were frequently
234 resistant to doxycycline and serotype 19A isolates demonstrated high levels of resistance to doxycycline
235 and trimethoprim/sulfamethoxazole. In France, tetracycline resistance was common in serotypes 12F,
236 15A and 24F isolates while resistance to trimethoprim/sulfamethoxazole was most frequently observed
237 with serotype 24F.[12] In contrast, the 12F isolates in our study were generally susceptible to doxycycline.

238 Despite the consistent reports of some serotype-specific susceptibility patterns, such as the high
239 levels of penicillin non-susceptibility in serotype 15A, a number of differences in serotype-specific
240 susceptibility patterns have been observed in various regions of the world. Differences in antimicrobial
241 prescribing practices between countries are likely one of many contributing factors to these
242 observations.[12] A significant association between high rates of outpatient antibiotic prescribing and an
243 increased proportion of IPD caused by non-susceptible *S. pneumoniae* was documented in the United

States.[22] Another recent study conducted in the United States demonstrated that regional variability in the rates of penicillin resistance in non-PCV-13 serotypes was influenced by multiple factors including the geographic heterogeneity in serotype distribution and the serotype-specific differences in rates of penicillin resistance.[23]

Subsequent to the introduction of PCV-13 in Canada, rates of multidrug resistance in *S. pneumoniae* decreased significantly from 8.5% in 2011 to 5.6% in 2015 (*P*=0.004). Similar rates of multidrug resistance were reported in the United Kingdom with 6.2% of invasive isolates tested between 2005 and 2014 demonstrating resistance to penicillin, erythromycin and tetracycline.[19] In that study, multidrug resistance in invasive isolates was most commonly observed, with rates greater than 5%, in serotypes 6B, 15A, 19A and 19F.[19] These observations are consistent with those in our study where a large proportion of the commonly isolated serotypes 15A, 19A and 33F and the infrequently isolated 6B, 9V, 14, 15F, 19F, 23F, 28A and 35A demonstrated a MDR phenotype. MDR serotypes circulating in Bulgaria post-PCV-10 were similar and included 6A, 6C, 15A, 19A, 19F and 23A.[13] The similarity of the predominant MDR serotypes is notable despite the selective pressure of a different vaccine. The overall decrease in multidrug resistance noted in our study was likely driven by the decrease in the proportion of circulating strains that were 19A, which was a predominant serotype and is frequently MDR. This conclusion is consistent with other large scale studies evaluating antimicrobial resistance in *S. pneumoniae* subsequent to PCV-13.[12]

Between 2011 and 2015, only 56.7% of the MDR *S. pneumoniae* evaluated in this study were PCV-13 serotypes. Although this proportion is lower than that reported in our pre-PCV-13 study where 88% of MDR isolates were PCV-13 serotypes[21], it remained consistent throughout the SAVE study.

MDR *S. pneumoniae* were isolated from all regions of Canada included in the SAVE study with slightly higher rates observed in the Eastern region. MDR isolates were also collected from patients of

all ages; however, the rate of multidrug resistance was significantly higher for children 1 to <2 years of age than in all other age groups (*P*=0.002). Children under the age of 2 are frequently prescribed antimicrobials for the treatment of common infections, such as otitis media,[24] which may provide the selective pressure contributing to the high rates of multidrug resistance observed within this age group.

Among the MDR isolates, the most common resistance pattern observed was resistance to clarithromycin, clindamycin and doxycycline. This specific phenotype represented 40.3% of the MDR isolates and was a component of the phenotype of 86.6% of the MDR isolates in this study. The frequent association of resistance between clarithromycin and clindamycin reflects the prevalence of the *erm*(B) resistance determinant in the *S. pneumoniae* in this study.[25] Janoir *et al.* reported that the predominant MDR phenotype post-PCV-13 in France was resistance to penicillin, erythromycin and tetracycline.[12] Although that unique phenotype was rare in our study, representing less than 1% of the isolates, MDR phenotypes that included resistance to penicillin, clarithromycin and doxycycline were observed in 41% of the isolates. Importantly, levofloxacin, which is one of the recommended antimicrobial agents for the treatment of penicillin-resistant isolates, was only observed in 2% of the MDR isolates and very rarely in association with penicillin resistance.

The most significant limitations of this study are the lack of participation of all provinces in Canada and that it does not represent incidence data. Accordingly, we can only demonstrate changes in the proportion of serotypes causing IPD from the eight Canadian provinces. The lack of isolates from British Columbia and Alberta results in an underrepresentation of Western region isolates and may skew the regional analysis. Additionally, the specific antimicrobial agents tested were not universally consistent with the comparator studies. Antimicrobial susceptibility results and the MDR comparisons were made using agents representing an antimicrobial class. For example, many of the comparator

studies reported erythromycin and tetracycline results instead of the clarithromycin and doxycycline reported in the SAVE study.

A significantly greater proportion of the invasive *S. pneumoniae* isolated as part of the SAVE study overall, as well as the subset of isolates that were MDR, were serotypes included in PCV-13 compared to the other currently available vaccines ($P<0.0001$). However, only 25.4% of *S. pneumoniae* overall and 56.3% of the MDR isolates were PCV-13 serotypes in the 2015 SAVE study collection. These epidemiological shifts are critical to monitor in order to provide optimal empiric therapy and guide future vaccine development. The SAVE study has highlighted a number of critical serotypes to monitor including serotype 33F, which is increasing in frequency, is included in the top ten most common serotypes and demonstrates multidrug resistance in more than 5% of isolates. Other notable serotypes are 24F, of which more than 5% of isolates are MDR and is increasing in frequency, as well as serotypes 15A and 19A that continue to predominate in both frequency of isolation and rates of multidrug resistance.

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Disclaimer

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

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Table 1. The annual proportion of invasive *S. pneumoniae* isolates with serotypes contained in the pneumococcal conjugate vaccines, SAVE 2011-2015.

Phenotype	Vaccine	Study Total (% [*n*])	Year (% [*n*])					2011 vs 2015 (*P*)
			2011	2012	2013	2014	2015	
All isolates	PCV7	4.7 (291)	5.6 (77)	4.9 (63)	4.6 (52)	3.6 (44)	4.6 (55)	0.3
	PCV10	16.6 (1029)	26.7 (368)	20.6 (265)	14.9 (170)	9.7 (117)	9.1 (109)	<0.0001
	PCV13	34.9 (2164)	48.0 (662)	39.6 (509)	33.7 (383)	25.4 (307)	25.4 (303)	<0.0001
MDR	PCV7	10.8 (40)	6.9 (8)	9.6 (8)	12.5 (8)	17.8 (8)	12.5 (8)	0.3
	PCV10	11.6 (43)	8.6 (10)	10.8 (9)	12.5 (8)	17.8 (8)	12.5 (8)	0.4
	PCV13	56.7 (211)	54.3 (63)	54.2 (45)	65.6 (42)	55.6 (25)	56.3 (36)	0.9

398 **Table 2.** Antimicrobial susceptibility testing results for the ten most common serotypes of invasive *S. pneumoniae*, SAVE 2011-2015.

Serotype (n[a])	% Susceptible/MIC_{90} (mg/L)								%MDR[b]
	Penicillin (IV, meningitis)	Penicillin (IV, nonmeningitis)	Ceftriaxone (meningitis)	Ceftriaxone (nonmeningitis)	Clarithromycin	Levofloxacin	SXT[c]	Doxycycline	
7F (694)	99.3/≤0.03	100/≤0.03	99.9/≤0.12	100/≤0.12	97.3/≤0.03	99.7/1	99.6/0.5	96.5/≤0.25	0.4
19A (596)	68.1/4	86.1/4	79.7/1	94.1/1	37.6/>32	99.7/1	69.6/8	69.3/4	26.0
22F (591)	99.5/≤0.03	99.8/≤0.03	99.8/≤0.12	99.8/≤0.12	72.9/2	98.8/1	99.0/0.25	99.0/≤0.25	0.8
3 (457)	99.8/≤0.03	100/≤0.03	100/≤0.12	100/≤0.12	96.1/≤0.03	100/1	98.9/≤0.12	89.3/1	2.6
12F (291)	99.7/≤0.03	100/≤0.03	100/≤0.12	100/≤0.12	37.8/4	100/1	98.3/0.5	97.3/≤0.25	1.4
11A (263)	98.5/≤0.03	100/≤0.03	99.2/≤0.12	100/≤0.12	73.0/2	99.6/1	81.4/8	98.5/≤0.25	0.8
9N (240)	98.3/≤0.03	100/≤0.03	99.6/≤0.12	100/≤0.12	91.7/0.25	100/1	96.7/0.5	97.9/≤0.25	0.4
8 (238)	98.7/≤0.03	100/≤0.03	99.6/≤0.12	100/≤0.12	99.2/≤0.03	100/1	98.7/0.25	95.4/≤0.25	0.4
33F (222)	99.5/≤0.03	100/≤0.03	100/≤0.12	100/≤0.12	21.6/16	100/1	32.0/2	84.7/0.5	6.3

15A (172)	38.7/1	100/1	94.8/0.5	100/0.5	22.7/>32	100/1	91.9/0.5	23.3/16	57.6

399 [a]*n* of each serotype for which complete suscpetibility data was available, [b]MDR, multidrug reistance; [c]SXT, trimethoprim/sulfamethoxazole

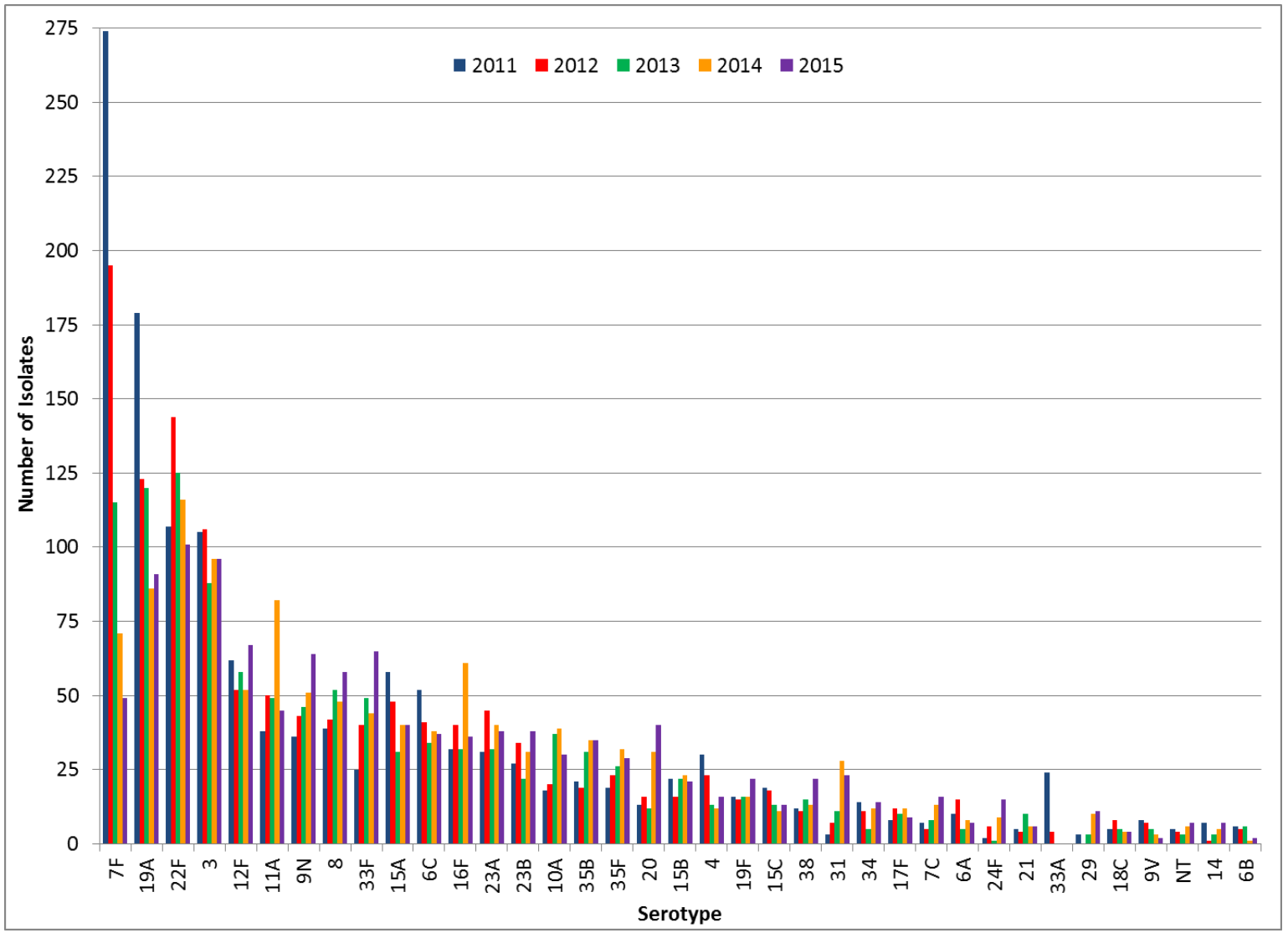
400 **Table 3.** Demographics of the common (*n*>10) multidrug resistant invasive *S. pneumoniae* isolates by

401 serotype, SAVE 2011 – 2015.

Serotype (*n*)	Canadian Region	Age Group (years)						
		0-<1	1-<2	2-<6	6-<18	18-<50	50-<65	≥65
3 (12)	Western							
	Central					2	3	7
	Eastern							
6C (11)	Western							
	Central			1		1	4	4
	Eastern							1
15A (99)	Western	1	1			5	1	3
	Central	2	4	2		8	18	46
	Eastern						2	6
19A (155)*	Western	4	4	1	2	18	8	9
	Central	2	6	7	5	12	13	28
	Eastern		2	3		6	9	11
19F (22)	Western	1					4	
	Central			1		1		9
	Eastern		1				3	2
33F (14)	Western							
	Central	1	3	2		1	4	3
	Eastern							

402 *The age was unknown for 5 MDR isolates of 19A

403 **Figure 1.** Annual serotype distribution of invasive *S. pneumoniae* isolates (*n*≥20) collected in the SAVE study, 2011 – 2015.



404

Serotypes for which less than 20 isolates were collected between 2011 and 2015 include: 23F (*n*=18), 1 (*n*=17), 5 (*n*=17), 28A (*n*=17), 13 (*n*=11), 37 (*n*=10), 24B (*n*=8), 11B (*n*=7), 6D (*n*=5), 9L (*n*=3), 10F (*n*=3), 24 (*n*=3), 27 (*n*=3), 25A (*n*=2), 35A (*n*=2), 22A (*n*=2), and 1 isolate each of serotypes 7A, 7B, 9A, 10B, 12A, 15F, 18A, 35C, 42 and 45.

Figure 2. Annual rates of multidrug resistance in invasive *S. pneumoniae* isolates collected in the SAVE study, 2011 – 2015.

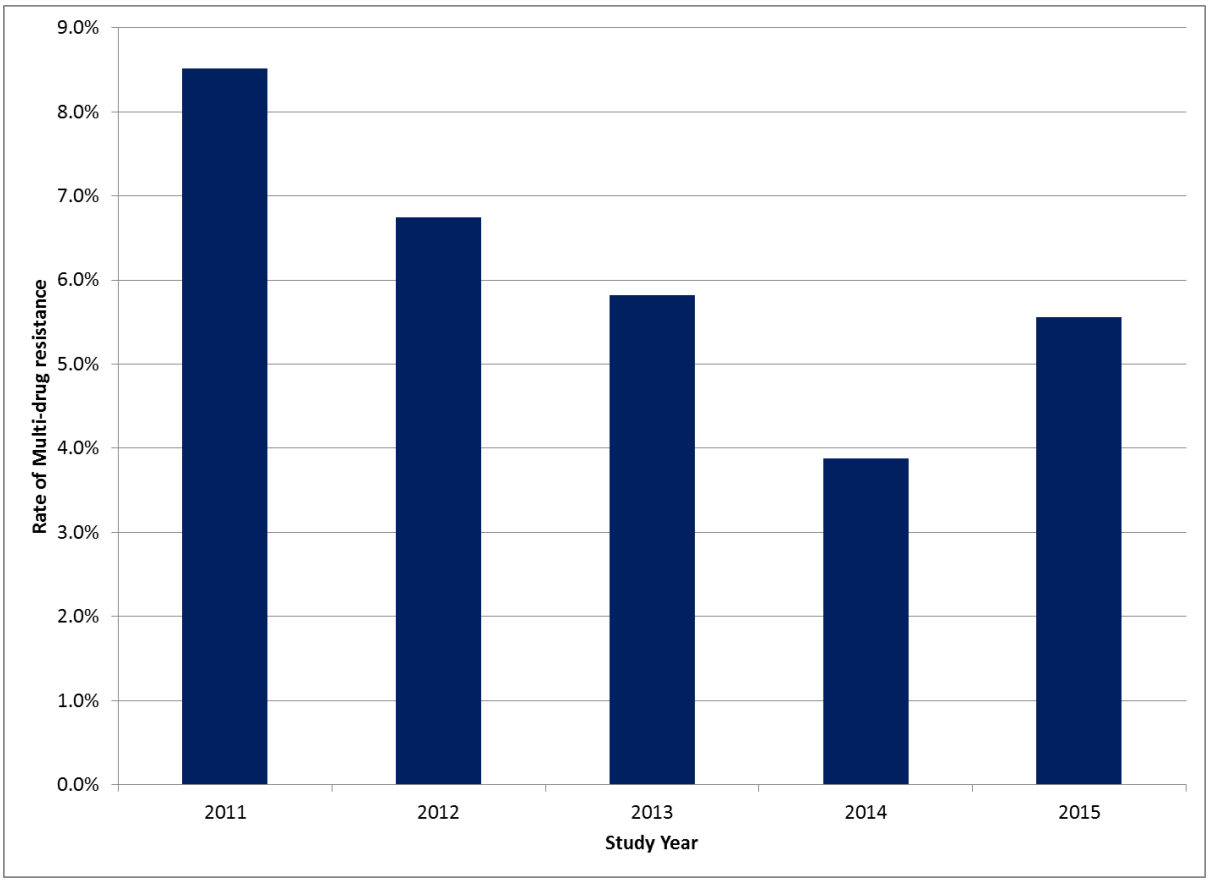


Figure 3. Serotype distribution of multidrug resistance in invasive *S. pneumoniae* isolates collected in the SAVE study, 2011 – 2015 (*n*=372).