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

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6 Heather J. Adam<sup>1,2\*</sup>, Alyssa R. Golden<sup>1</sup>, James A. Karlowsky<sup>1,2</sup>, Melanie R. Baxter<sup>1</sup>, Kim A. Nichol<sup>2</sup>, Irene  
7 Martin<sup>3</sup>, Walter Demczuk<sup>3</sup>, Michael R. Mulvey<sup>3</sup>, Matthew W. Gilmour<sup>1,3</sup>, Daryl J. Hoban<sup>1,2</sup>, George G.  
8 Zhanel<sup>1</sup>, on behalf of the Canadian Antimicrobial Resistance Alliance (CARA) <sup>†</sup>

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10 <sup>1</sup>*Department of Medical Microbiology, Max Rady College of Medicine, University of Manitoba, Room 543*  
11 *- 745 Bannatyne Avenue, Winnipeg, Manitoba, R3E 0J9, Canada;*

12 <sup>2</sup>*Clinical Microbiology, Diagnostic Services Manitoba, MS673-820 Sherbrook Street, Winnipeg,*  
13 *Manitoba, R3A 1R9, Canada;*

14 <sup>3</sup>*National Microbiology Laboratory, Public Health Agency of Canada, 1015 Arlington St, Winnipeg,*  
15 *Manitoba, R3E 3M4, Canada*

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20 **Corresponding author:** Telephone: 204-787-8678; Fax: 204-787-4699; Email: [hadam@dsmanitoba.ca](mailto:hadam@dsmanitoba.ca)

21

22 **Synopsis**

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

26 **Methods:** As part of a collaboration between the Canadian Antimicrobial Resistance Alliance and Public  
27 Health Agency of Canada-National Microbiology Laboratory, 6207 invasive isolates of *S. pneumoniae*  
28 were evaluated. All isolates were serotyped and had antimicrobial susceptibility testing performed, in  
29 accordance with CLSI guidelines (M07-A10, 2015). Complete susceptibility profiles were available for  
30 6001 isolates. MDR was defined as resistance to three or more classes of antimicrobial agents (with  
31 penicillin MIC  $\geq 2$  mg/L defined as resistant).

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

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

41

42 **Introduction**

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

50         Pevnar<sup>®</sup>, a 7-valent pneumococcal conjugate vaccine (PCV-7) including serotypes 4, 6B, 9V, 14,  
51 18C, 19F and 23F, was incorporated in routine vaccination schedules in all Canadian provinces between  
52 2002 and 2006.<sup>4</sup> The use of PCV-7 resulted in significant reductions in invasive infections due to *S.*  
53 *pneumoniae* as well as reductions in the incidence of recurrent upper respiratory tract infections in  
54 children in North America.<sup>4,5,6</sup> Increases in non-vaccine serotypes, particularly MDR serotype 19A, were  
55 rapidly observed following the introduction of PCV-7.<sup>4,5</sup> Subsequently, newer pneumococcal conjugate  
56 vaccines were developed with enhanced serotype coverage, including Synflorix<sup>™</sup>, with the additional  
57 inclusion of serotypes 1, 5 and 7F (PCV-10), and Pevnar<sup>®</sup>13, which includes serotypes 1, 3, 5, 6A, 7F and  
58 19A (PCV-13). The broader serotype coverage and the critical inclusion of serotype 19A in PCV-13  
59 offered an important advancement in the protection of children against invasive *S. pneumoniae*  
60 infections. The immunization guidelines were updated in 2010 to recommend the routine use of PCV-13  
61 in infant vaccine schedules in North America.<sup>7,8</sup> The replacement of PCV-7 with PCV-13 as part of  
62 routine infant vaccinations was completed in Canada by early-2011.<sup>2</sup>

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

65 pneumonia in immunocompetent adults was evaluated. The CAPiTA study demonstrated efficacy in the  
66 prevention of pneumococcal pneumonia and IPD for *S. pneumoniae* with serotypes included in PCV-13.<sup>9</sup>  
67 Accordingly, the immunization recommendations were updated in North America to include routine  
68 administration of PCV-13 to healthy adults  $\geq 65$  years.<sup>3,10</sup>

69 Despite the overall success of the vaccine programs, treatment concerns have remained as  
70 many serotypes that were commonly penicillin-resistant or MDR, such as 15A and 19A, have persisted or  
71 increased with the changing epidemiology in the vaccine era. Penicillin resistance was commonly  
72 observed in serotypes 19A, 19F and 35B in the United States shortly after the introduction of PCV-13.<sup>11</sup>  
73 Resistance to one or more antibiotics in emerging non-PCV-13 serotypes was recently observed in  
74 serotypes 12F, 15A, 24F and 35B in France.<sup>12</sup> Similarly, a high prevalence of MDR non-vaccine serotypes  
75 were observed in Bulgaria five years after the introduction of PCV-10.<sup>13</sup>

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

80

## 81 **Materials and methods**

### 82 ***Bacterial isolates***

83 From January 2011 to December 2015, *S. pneumoniae* isolated from sterile body sites by participating  
84 Canadian provincial public health and hospital laboratories were forwarded to the Public Health Agency  
85 of Canada-National Microbiology Laboratory (PHAC-NML) in Winnipeg, Canada. As part of an ongoing  
86 collaboration between the Canadian Antimicrobial Resistance Alliance (CARA) and PHAC-NML, PHAC-

87 NML forwarded their collection of invasive isolates of *S. pneumoniae* from eight provincial public health  
88 laboratories (Saskatchewan, Manitoba, Ontario, Quebec, Nova Scotia, Prince Edward Island,  
89 Newfoundland and Labrador, and a portion of isolates collected from New Brunswick) to CARA for  
90 antimicrobial susceptibility testing. For the SAVE study, regional analysis were conducted as Western  
91 (Saskatchewan and Manitoba,  $n=1352$ ), Central (Ontario and Quebec,  $n=4107$ ) and Eastern (New  
92 Brunswick, Nova Scotia, Prince Edward Island and Newfoundland and Labrador,  $n=748$ ).

93 In total, 6207 invasive isolates of *S. pneumoniae* collected as part of the SAVE study between  
94 2011 and 2015 were forwarded to the CARA. Patient gender and age information was available for 5980  
95 (96.3%) and 6072 (97.8%) of the isolates, respectively. The annual numbers of isolates were: 1379  
96 isolates from 2011, 1285 from 2012, 1138 from 2013, 1210 from 2014, and 1195 from 2015.

#### 97 **Antimicrobial susceptibility testing**

98 Antimicrobial susceptibility testing was performed in the Department of Clinical Microbiology at the  
99 Winnipeg Health Sciences Centre using the standard CLSI broth microdilution method<sup>14,15</sup> with custom-  
100 designed, in-house prepared, 96-well microtitre panels containing doubling-dilutions of antimicrobial  
101 agents in cation-adjusted Mueller-Hinton broth supplemented to a final concentration of 4% lysed horse  
102 blood. All isolates were tested against penicillin, ceftriaxone, cefuroxime, clarithromycin, clindamycin,  
103 telithromycin, levofloxacin, moxifloxacin, linezolid, trimethoprim/sulfamethoxazole, doxycycline,  
104 tigecycline, chloramphenicol and vancomycin. MICs were interpreted as susceptible, intermediate or  
105 resistant using CLSI MIC breakpoints for all antimicrobial agents.<sup>15</sup> Multidrug-resistant (MDR) was  
106 defined as resistance to three or more antimicrobial agents selected as antimicrobial class markers  
107 (penicillin, clarithromycin, clindamycin, doxycycline, levofloxacin, trimethoprim/sulfamethoxazole and  
108 chloramphenicol). In MDR calculations, penicillin resistance was defined using the CLSI breakpoint for  
109 oral penicillin V (MIC,  $\geq 2$  mg/L). Of the 6207 invasive isolates of *S. pneumoniae* received by CARA for

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

### 115 **Serotyping**

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

### 119 **Statistical Analysis**

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

124

### 125 **Results**

126 The annual serotype distribution of invasive isolates of *S. pneumoniae* collected as part of the SAVE  
127 study in Canada between 2011 and 2015 is presented in Figure 1. The most common serotypes in the  
128 SAVE study overall ( $n = 6207$ ) were 7F (704/11.3%), 19A (599/9.7%), 22F (593/9.6%), 3 (491/7.9%), 12F  
129 (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  
130 2015 study year ( $n = 1195$ ), the most common serotypes were 22F (101/8.5%), 3 (96/8.0%), 19A

131 (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  
132 (40/3.3%) and 20 (40/3.3%).

133           Between 2011 and 2015, statistically significant reductions in the prevalence of serotypes 7F  
134 ( $P<0.0001$ ), 19A ( $P<0.0001$ ) and 33A ( $P<0.0001$ ) were observed. Statistically significant increases in  
135 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  
136 ( $P=0.028$ ), 31 ( $P<0.0001$ ), 33F ( $P<0.0001$ ), 35B ( $P=0.021$ ) and 38 ( $P=0.037$ ) were observed during this  
137 time period. Notable changes in the rank order of the top ten serotypes were observed during the  
138 study. Serotype 7F ranked as the most common serotype in 2011 but fell to eighth in 2015 whereas  
139 serotype 19A was only reduced from ranking second to third most common serotype. Serotypes 22F  
140 and 3 rose in ranking from third and fourth to first and second, respectively. Serotype 33F ranked as the  
141 ninth most common serotype in 2011 and rose to the fifth position in 2015.

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

144           The antimicrobial susceptibility testing results for the ten most common serotypes of invasive *S.*  
145 *pneumoniae* in Canada are presented in Table 2. Reduced susceptibility rates were observed in serotype  
146 19A for penicillin (68.1% based on the IV meningitis breakpoint), ceftriaxone (79.7% based on the IV  
147 meningitis breakpoint), clarithromycin (37.6%), doxycycline (69.3%) and trimethoprim/sulfamethoxazole  
148 (69.6%). Reduced susceptibility rates were also observed for clarithromycin in serotype 22F isolates  
149 (72.9%) and 12F isolates (37.8%), clarithromycin (73.0%) and trimethoprim/sulfamethoxazole (81.4%) in  
150 serotype 11A isolates, clarithromycin (21.6%) and trimethoprim/sulfamethoxazole (32.0%) in serotype  
151 33F isolates, and penicillin (38.7%), clarithromycin (22.7%) and doxycycline (23.3%) in serotype 15A  
152 isolates. All isolates were susceptible to vancomycin.<sup>18</sup> Among the top ten serotypes, multidrug  
153 resistance rates greater than five percent were observed for serotypes 15A (57.6%), 19A (26.0%) and



154 33F (6.3%). Serotypes that were isolated less frequently in the study but for which multidrug resistance  
155 was observed in more than five percent of isolates included 6B (30%,  $n=6/20$ ), 9V (22.2%,  $n=4/18$ ), 14  
156 (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  
157 35A (50%,  $n=1/2$ ).

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

162 MDR *S. pneumoniae* isolates were identified from all regions of the country with the following  
163 distribution ( $n / \%$  by region): Western (71 / 5.3%), Central (247 / 6.0%), and Eastern (54 / 7.2%). Among  
164 the age groups, MDR *S. pneumoniae* represented 5.3% (12/228) of the isolates from children 0 - <1 years  
165 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)  
166 of those aged 6 - <18 years, 5.4% (67/1233) of adults 18 - < 50 years, 5.1% (83/1627) of those aged 50 -  
167 <65 years, and 6.6% (155/2332) of the elderly  $\geq 65$  years. The rate of multidrug resistance was  
168 significantly higher in the 1 - <2 year age category than all other combined age groups ( $P=0.002$ ). Similar  
169 numbers of MDR isolates were identified from men and women (MDR  $n /$  total  $n$  by gender [%]):  
170 185/3213 (5.8%) and 176/2767 (6.4%).

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

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

178 Multidrug resistance was observed in 32 serotypes of the *S. pneumoniae* invasive isolates as  
179 shown in Figure 3. The predominant serotypes among the MDR isolates were 15A (99, 26.6%) and 19A  
180 (155, 41.7%). The proportion of invasive MDR *S. pneumoniae* isolates of the SAVE study contained in  
181 PCV-7, PCV-10 and PCV-13 are shown in Table 1.

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

187 The most common associations of serotype and specific resistance patterns among the MDR *S.*  
188 *pneumoniae* were 19A resistant to clarithromycin, clindamycin, doxycycline, penicillin and  
189 trimethoprim/sulfamethoxazole ( $n=97$ ) and 15A resistant to clarithromycin, clindamycin and doxycycline  
190 ( $n=81$ ) (Supplementary data, Table 1). A single *S. pneumoniae* resistant to seven antimicrobial agents  
191 was isolated during this study, which was a serotype 23F from a blood culture of a 64 year old female in  
192 2013 from Central Canada.

193

## 194 **Discussion**

195 The ten predominant *S. pneumoniae* serotypes identified throughout the SAVE study, in order of  
196 frequency of isolation, were 7F, 19A, 22F, 3, 12F, 11A, 9N, 8 and 33F, similar to those reported in the  
197 United Kingdom and the United States.<sup>11,19</sup> 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.<sup>13</sup> 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.<sup>11</sup>

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.<sup>20</sup> 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%).<sup>20</sup> The effectiveness against serotype 19A was significant at 67% but  
210 notably lower than some of the other serotypes.<sup>20</sup> 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.<sup>19</sup> A continued predominance of serotype 3 has been reported in a  
214 number of studies around the world.<sup>11,13,19</sup> 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.<sup>21</sup> 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.<sup>5</sup> 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.<sup>12</sup> 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.<sup>12</sup> 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.<sup>12</sup> 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

244 States.<sup>22</sup> Another recent study conducted in the United States demonstrated that regional variability in  
245 the rates of penicillin resistance in non-PCV-13 serotypes was influenced by multiple factors including  
246 the geographic heterogeneity in serotype distribution and the serotype-specific differences in rates of  
247 penicillin resistance.<sup>23</sup>

248           Subsequent to the introduction of PCV-13 in Canada, rates of multidrug resistance in *S.*  
249 *pneumoniae* decreased significantly from 8.5% in 2011 to 5.6% in 2015 ( $P=0.004$ ). Similar rates of  
250 multidrug resistance were reported in the United Kingdom with 6.2% of invasive isolates tested between  
251 2005 and 2014 demonstrating resistance to penicillin, erythromycin and tetracycline.<sup>19</sup> In that study,  
252 multidrug resistance in invasive isolates was most commonly observed, with rates greater than 5%, in  
253 serotypes 6B, 15A, 19A and 19F.<sup>19</sup> These observations are consistent with those in our study where a  
254 large proportion of the commonly isolated serotypes 15A, 19A and 33F and the infrequently isolated 6B,  
255 9V, 14, 15F, 19F, 23F, 28A and 35A demonstrated a MDR phenotype. MDR serotypes circulating in  
256 Bulgaria post-PCV-10 were similar and included 6A, 6C, 15A, 19A, 19F and 23A.<sup>13</sup> The similarity of the  
257 predominant MDR serotypes is notable despite the selective pressure of a different vaccine. The overall  
258 decrease in multidrug resistance noted in our study was likely driven by the decrease in the proportion  
259 of circulating strains that were 19A, which was a predominant serotype and is frequently MDR. This  
260 conclusion is consistent with other large scale studies evaluating antimicrobial resistance in *S.*  
261 *pneumoniae* subsequent to PCV-13.<sup>12</sup>

262           Between 2011 and 2015, only 56.7% of the MDR *S. pneumoniae* evaluated in this study were  
263 PCV-13 serotypes. Although this proportion is lower than that reported in our pre-PCV-13 study where  
264 88% of MDR isolates were PCV-13 serotypes<sup>21</sup>, it remained consistent throughout the SAVE study.

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

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

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

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

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

291 A significantly greater proportion of the invasive *S. pneumoniae* isolated as part of the SAVE  
292 study overall, as well as the subset of isolates that were MDR, were serotypes included in PCV-13  
293 compared to the other currently available vaccines ( $P<0.0001$ ). However, only 25.4% of *S. pneumoniae*  
294 overall and 56.3% of the MDR isolates were PCV-13 serotypes in the 2015 SAVE study collection. These  
295 epidemiological shifts are critical to monitor in order to provide optimal empiric therapy and guide  
296 future vaccine development. The SAVE study has highlighted a number of critical serotypes to monitor  
297 including serotype 33F, which is increasing in frequency, is included in the top ten most common  
298 serotypes and demonstrates multidrug resistance in more than 5% of isolates. Other notable serotypes  
299 are 24F, of which more than 5% of isolates are MDR and is increasing in frequency, as well as serotypes  
300 15A and 19A that continue to predominate in both frequency of isolation and rates of multidrug  
301 resistance.

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### 319 **Disclaimer**

320 The opinions expressed in this paper are those of the authors, and do not necessarily represent those of  
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396 **Table 1.** The annual proportion of invasive *S. pneumoniae* isolates with serotypes contained in the  
 397 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
		16.6 (1029)	26.7 (368)	20.6 (265)	14.9 (170)	9.7 (117)	9.1 (109)	<0.0001
	PCV10	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 <sup>a</sup> )	% Susceptible/MIC <sub>90</sub> (mg/L)								%MDR <sup>b</sup>
	Penicillin (IV, meningitis)	Penicillin (IV, nonmeningitis)	Ceftriaxone (meningitis)	Ceftriaxone (nonmeningitis)	Clarithromycin	Levofloxacin	SXT <sup>c</sup>	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

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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
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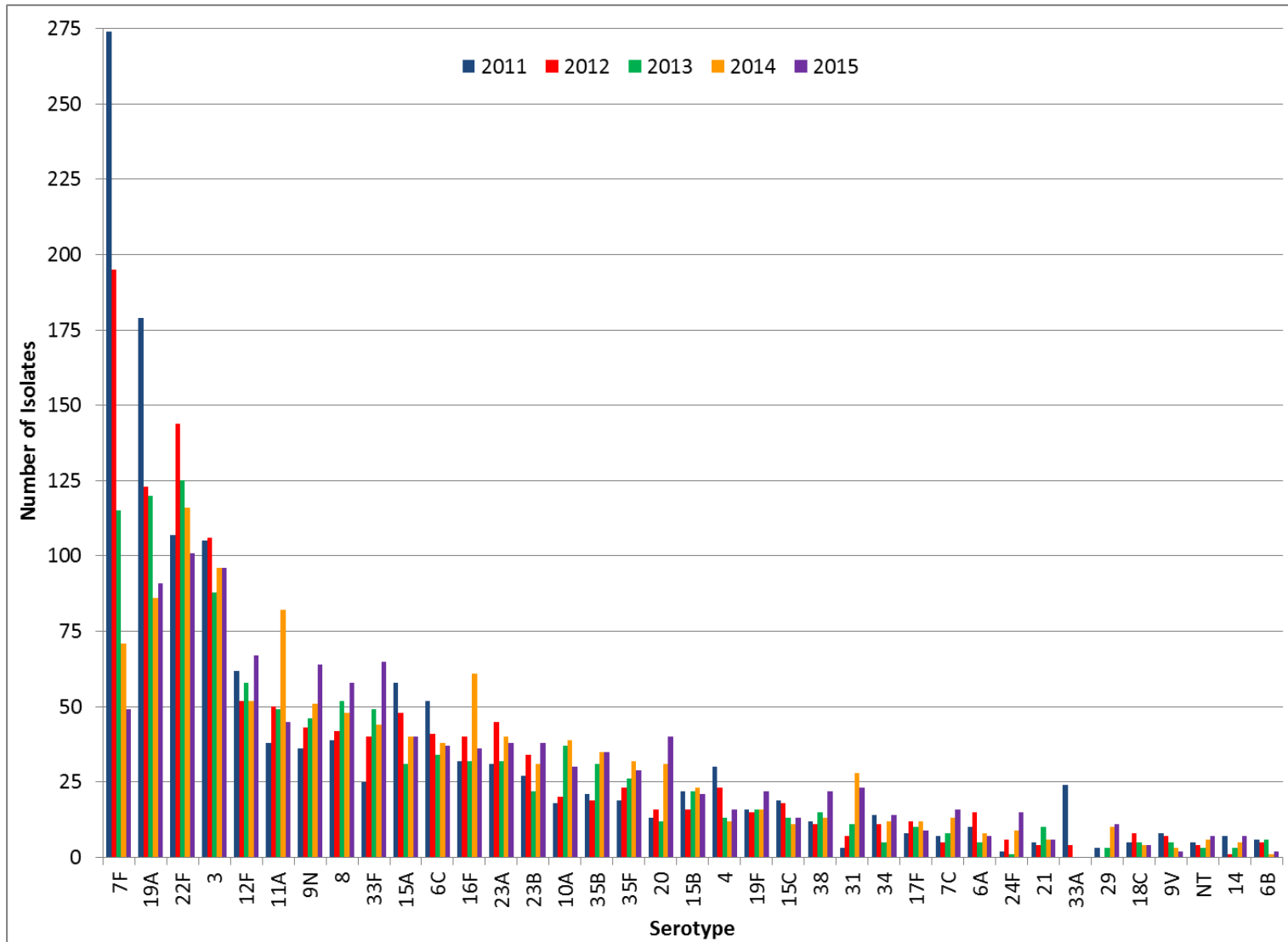
399 <sup>a</sup>*n* of each serotype for which complete susceptibility data was available, <sup>b</sup>MDR, multidrug resistance; <sup>c</sup>SXT, trimethoprim/sulfamethoxazole

400 **Table 3.** Demographics of the common ( $n>10$ ) multidrug resistant invasive *S. pneumoniae* isolates by  
 401 serotype, SAVE 2011 – 2015.

Serotype ( <i>n</i> )	Canadian		Age Group (years)						
	Region		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

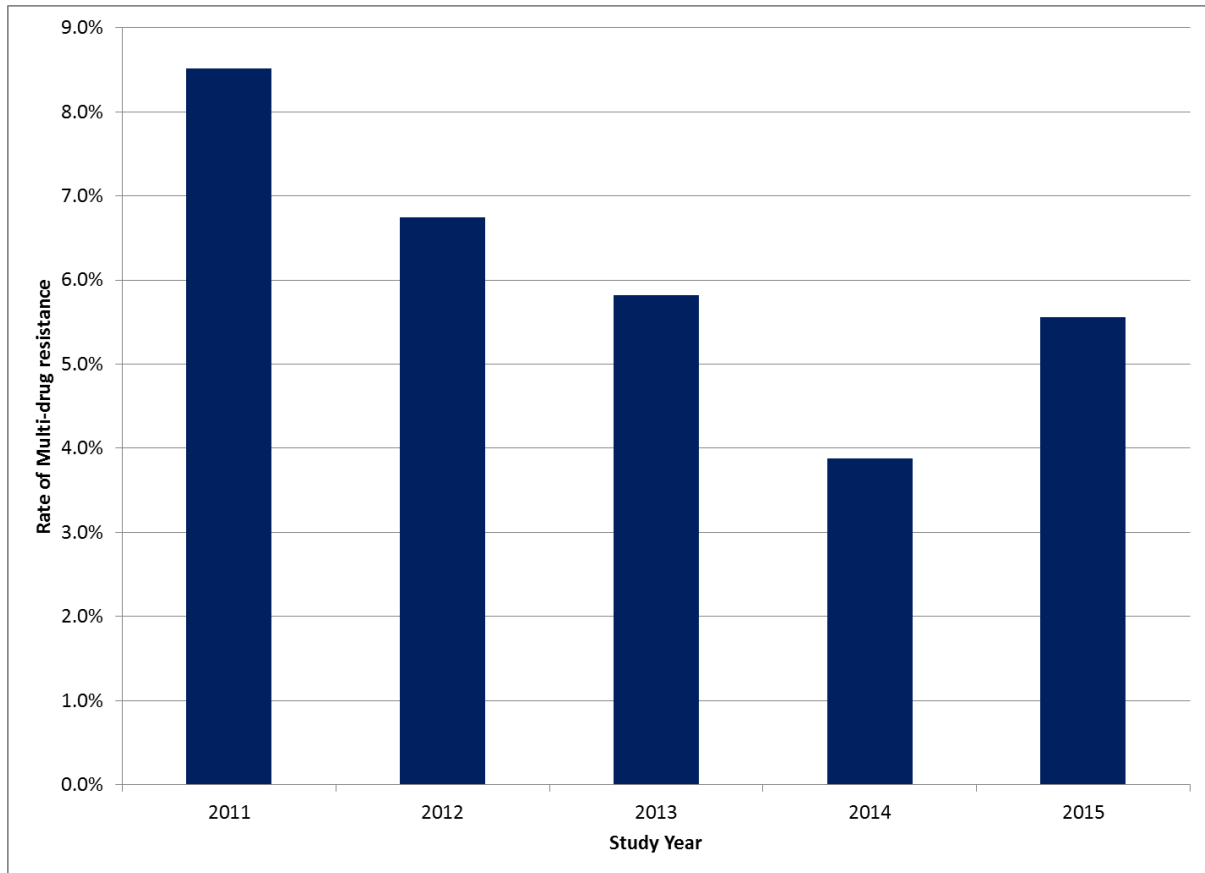
403 **Figure 1.** Annual serotype distribution of invasive *S. pneumoniae* isolates ( $n \geq 20$ ) collected in the SAVE study, 2011 – 2015.





405 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 ( $n=11$ ),  
406 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  
407 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 ( $n=372$ ).

