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Antimicrobial Susceptibility Testing of Invasive Isolates of *Streptococcus pneumoniae* from Canadian patients: The SAVE Study, 2011-2015

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Synopsis

Objectives: To assess antimicrobial susceptibility for 14 agents tested against 6001 invasive isolates of *Streptococcus pneumoniae* cultured from invasive patient samples from 2011 to 2015 as a part of the annual SAVE study.

Methods: Isolates of *S. pneumoniae* were tested using the standard CLSI broth microdilution method (M07-A10, 2015) with MICs interpreted by CLSI M100 27th Edition (2017) MIC breakpoints.

Results: From 2011 to 2015, small but significant increases ($P<0.05$) in percent susceptibility for penicillin (interpreted by all three CLSI MIC breakpoint criteria) (1.7 – 3.2%), clindamycin (3.1%) and ceftriaxone (interpreted by non-meningitis and meningitis CLSI MIC breakpoint criteria) (1.1 – 1.5%) were observed. Susceptibility rates for clarithromycin and other commonly tested antimicrobial agents remained unchanged ($P>0.05$) over the five-year period. Isolates with a MDR phenotype (resistance to three or more antimicrobial agent classes) decreased significantly ($P <0.001$) from 8.5% in 2011 to 5.6% in 2015. Antimicrobial susceptiblity rates were not generally associated ($P>0.05$) with patient gender (exception: clarithromycin) but were associated ($P<0.05$) with patient age (chloramphenicol and clindamycin) or specimen source (penicillin, doxycycline, trimethoprim/sulfamethoxazole and clindamycin), as well as geographic location in Canada and concurrent resistance to penicillin or clarithromycin.

Conclusions: The *in vitro* susceptibility of invasive isolates of *S. pneumoniae* in Canada increased to penicillin, clindamycin and ceftriaxone from 2011 to 2015 coincident with a significant decrease in MDR phenotypes.

Introduction

Streptococcus pneumoniae is a leading cause of both invasive (e.g., bacteremia, meningitis) and non-invasive (e.g., pneumonia, otitis media) infections.[1] Invasive pneumococcal disease (IPD) produces substantial patient morbidity and mortality, particularly among the very young (<5 years), the elderly (≥65 years) and immunocompromised individuals. In addition to the aforementioned risk factors for IPD, both carriage of, and infection with, antimicrobial-resistant *S. pneumoniae* is associated with previous antimicrobial use, institutionalization, and community or household exposure to antimicrobial-resistant isolates.[2] Resistance arising following previous antimicrobial use is more dependent on the time elapsed since the last antimicrobial exposure rather than on the duration of therapy; the association between elapsed time and resistance was stronger for macrolides than other antimicrobial classes.[2]

In patients with pneumococcal infection, particularly IPD, adequate antimicrobial therapy reduces morbidity and mortality, particularly when administered early in the course of disease.[2] The success of empiric antimicrobial therapy is continuously challenged by the threat of increasing antimicrobial resistance, serious adverse events, and collateral damage to patients' colonizing flora. The development of new antimicrobial agents with novel mechanisms of action and attempting to minimize the use of currently available agents through antimicrobial stewardship are two important strategies intended to subvert the spread of antimicrobial resistance.

Vaccination is a proven means of reducing the incidence of IPD and antimicrobial resistance associated with serotypes included in the vaccine by reducing the transmission of resistant isolates.[1,3-7] In June 2001, the 7-valent (4, 6B, 9V, 14, 18C, 19F, 23F) conjugate vaccine (PCV-7) was licensed for use in Canada and universal infant (children <2 years of age)

PCV-7 immunization programs were introduced in all Canadian provinces and territories between 2002 and 2006.[1] As anticipated, the Canadian Immunization Monitoring Program, Active (IMPACT) reported a significant decrease in the number of cases of IPD between 2000 and 2007 (a 48% decrease overall and 56% in children <5 years old)[1] with the greatest decreases in incidence of IPD, and rates of antimicrobial resistance, occurring in children <2 years of age.[1,3,4] At the same time, increases in non-vaccine serotypes (e.g., 19A) as causes of IPD and sources of antimicrobial resistance were observed and offset some of the reductions in PCV-7 serotypes.[1,5] Bettinger *et al*. reported that although the absolute number of reported IPD cases caused by serotypes in PCV-7 decreased 87.5%, overall the proportion of penicillin-resistant isolates remained unchanged at 17% and cefotaxime/ceftriaxone resistance remained unchanged at 2% annually.[1] Subsequently, in 2010, a 13-valent polyvalent conjugate vaccine (PCV-13) targeting additional serotypes (1, 3, 5, 6A, 7F, 19A) was introduced in Canada and by mid-2011, all Canadian provinces and territories had incorporated PCV-13 into their routine immunization schedule. Prior to PCV-13 introduction in Canada, Adam *et al*. reported that 54.3% of circulating serotypes causing IPD in 2007-2009 would be covered by PCV-13.[6] Demczuk *et al*. later reported that from 2010 to 2014, PCV-13 serotypes declined in Canada, overall, from 55% of the isolates in 2010 to 43% in 2012 to 31% in 2014; by patient age, PCV serotype reductions were from 54 to 43% for children aged ≥5 years, from 66 to 41% for children <5 years old and from 63 to 42% for children aged <2 years.[7,8] The rate of decrease in IPD serotypes in children following the introduction of PCV-13 was less dramatic than that observed for PCV-7 over a comparable time period.[7] Serotype 22F has been the most common replacement serotype following use of PCV-13, increasing from 7% to 11%.[8] Similar results have been observed in the United States for children <5 years of age where the use of PCV-7 and PCV-13 has also been

widespread; they observed a 90% decline in IPD in children <5 years of age and a 50% decline in adults between 1998 and 2015.[9] As a result of the use of PCV-7 and PCV-13 in Canada, the overall incidence of IPD decreased from 9.8 to 8.9 cases per 100 000 population between 2009 and 2014. In 2014 in Canada, rates of IPD were highest in infants <1 year of age (16.9 cases per 100 000 population), children 1-4 years of age (11.0 cases per 100 000 population), and in patients 60 years of age and older (21.5 cases per 100 000 population.[10,11]

Despite the availability and use of pneumococcal conjugate vaccines in Canada, invasive infections continue to occur. Therefore, access to current antimicrobial surveillance data such as that generated by the ongoing SAVE study in Canada remains important to clinicians, antimicrobial stewardship programs, infection control practitioners, antimicrobial formulary committees, clinical laboratory scientists, governments, academic scientists involved in drug discovery and the pharmaceutical industry as this data can improve the delivery of effective antimicrobial therapy (reducing discordant empiric therapy that results in increased rates of morbidity and mortality), determine the impact of immunization programs, provide the impetus to revise empiric therapy guidelines and help to prioritize future antimicrobial agent development agendas.[2] The SAVE study is an annual surveillance program that collects and characterizes invasive isolates of *S. pneumoniae* submitted by select provincial public health and hospital laboratories across Canada. In the current study, invasive isolates of *S. pneumoniae* collected from 2011 to 2015, inclusive, by the SAVE study were tested for their susceptibilities to a panel of 14 antimicrobial agents using the standard CLSI broth microdilution method.[12,13] Because comparative statistical analyses of factors associated with antimicrobial resistance have not been extensively performed using Canadian pneumococcal isolates, data from the SAVE study were also analyzed to evaluate the activities of several anti-pneumococcal agents on the basis of

116 factors such as patient age, patient gender, isolate specimen source, geographic region and MIC
117 interpretative category for penicillin and clarithromycin.
118
119 **Materials and methods**
120 ***Bacterial isolates***
121 From January 2011 to December 2015, *S. pneumoniae* isolated from sterile body sites by
122 participating Canadian provincial public health and hospital laboratories were forwarded to the
123 Public Health Agency of Canada-National Microbiology Laboratory (PHAC-NML) in Winnipeg,
124 Canada. As part of an ongoing collaboration between the Canadian Antimicrobial Resistance
125 Alliance (CARA) and PHAC-NML, PHAC-NML forwarded their collection of invasive isolates
126 of *S. pneumoniae* eight provincial public health laboratories (Saskatchewan, Manitoba, Ontario,
127 Quebec, Nova Scotia, Prince Edward Island, Newfoundland and Labrador, and a portion of
128 isolates collected from New Brunswick) to CARA for antimicrobial susceptibility testing. For
129 the SAVE study, regional analysis were conducted as Western (Saskatchewan and Manitoba,
130 *n*=1352), Central (Ontario and Quebec, *n*=4107) and Eastern (New Brunswick, Nova Scotia,
131 Prince Edward Island, and Newfoundland and Labrador, *n*=748).
132 In total, 6207 invasive isolates of *S. pneumoniae* collected as part of the SAVE study
133 between 2011 and 2015 were forwarded to the CARA for antimicrobial susceptibility testing.
134 Patient gender and age information was available for 5980 (96.3%) and 6072 (97.8%) of the
135 isolates. The annual numbers of isolates were: 1379 isolates from 2011, 1285 from 2012, 1138
136 from 2013, 1210 from 2014, and 1195 from 2015.
137 ***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[12,13] 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 except tigecycline for which FDA MIC breakpoints were used (susceptible, ≤0.06 mg/L).[14] 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).[13] 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.

Statistical analysis

Antimicrobial susceptibility rates between 2011 and 2015 and the associations between patient demographic or isolate factors and resistance to antimicrobial agents were assessed for statistically significant differences (P <0.05) using the 2-tailed Chi-square test.

Results

From 2011 to 2015, small but significant increases ($P<0.05$) in percent susceptibility for penicillin (by all three MIC breakpoint criteria) (1.7 – 3.2%), clindamycin (3.1% increase) and ceftriaxone (by non-meningitis and meningitis MIC breakpoint criteria) (1.1 – 1.5% increase) were observed for invasive isolates of *S. pneumoniae* included in the SAVE study (Table 1). Susceptibility rates for all other antimicrobial agents tested remained unchanged ($P>0.05$) over the five-year period/ (Table 1). In the clarithromycin subset analysis, significant differences ($P<0.05$) in the prevalence of putative *mef*[A] (i.e., M phenotype/efflux/low-level macrolide resistance; MICs of 1-32 mg/L; n=1157 [19.3% of all isolates]) and putative *erm*[B] (i.e., target site methylation/high-level macrolide resistance; MICs of ≥64 mg/L; n=240 [4.0% of all isolates]) phenotypes were not identified across the five-year period from 2011 to 2015 (data not shown)[15]

The majority (71.5%; 4289/6001) of all isolates of invasive *S. pneumoniae* tested from 2011 to 2015 were pan-susceptible to the panel of seven antimicrobial agents used in MDR analysis (Table 2). Of isolates demonstrating resistance to at least one antimicrobial agent, 62.1% (1064/1712) were resistant to only a single antimicrobial agent. A MDR phenotype was demonstrated by 6.2% (372/6001) of all isolates tested. The most common MDR phenotypes were concurrent resistance to clarithromycin, doxycycline and clindamycin (n=150; 40.3% of MDR isolates), concurrent resistance to clarithromycin, doxycycline, clindamycin, penicillin and trimethoprim/sulfamethoxazole (n=110; 29.6% of MDR isolates) and concurrent resistance to clarithromycin, doxycycline, clindamycin and trimethoprim/sulfamethoxazole (n=18; 4.8% of MDR isolates) (as described elsewhere in this supplement).[16] The rank order of frequency of resistance to specific antimicrobial agent classes

among MDR isolates of invasive *S. pneumoniae* was: clarithromycin > doxycycline ≈ clindamycin > trimethoprim/sulfamethoxazole ≈ penicillin >> chloramphenicol > levofloxacin (Table 2). The rate of MDR among invasive isolates of *S. pneumoniae* decreased significantly (P <0.001) from 8.5% in 2011 to 5.6% 2015, with the lowest rates seen during 2014 at 3.9%.

Rates of antimicrobial resistance in invasive isolates of *S. pneumoniae* were significantly associated with patient age for chloramphenicol and clindamycin (P <0.05) and approached clinical significance for penicillin (P=0.057) (Table 3). The penicillin resistance rates were highest (4.3%) and the chloramphenicol resistance rates were lowest (0.6%) among children less than 18 years of age. Clindamycin resistance rates were higher for children less than 18 years of age and adults greater than 64 years of age compared to patients in the 18 to 64 year age category. None of the other agents (clarithromycin, doxycycline and trimethoprim/sulfamethoxazole) demonstrated a significant association with patient age. Patient gender was associated with resistance to clarithromycin and approached clinical significance for penicillin (P=0.075). Specimen source was associated with resistance for all antimicrobial agents except clarithromycin and chloramphenicol. Blood isolates generally had the lowest percent resistance rates and sterile body fluids (other than blood and cerebrospinal fluid) had the highest percent resistance rates. Geographic region was also associated with resistance for all antimicrobial agents except clarithromycin and clindamycin, although the results approached signficance for these agents. Resistance to penicillin and trimethoprim/sulfamethoxazole was more common among isolates from western and eastern Canada than for isolates from central Canada while resistance to chloramphenicol was highest in central Canada. Resistance to doxycyline was more common among isolates from central and eastern Canada than from the western region. Penicillin resistance and clarithromycin resistance were associated with each

other and with resistance to other antimicrobial agents (doxycycline, trimethoprim/sulfamethoxazole, chloramphenicol and clindamycin).

Discussion

Increases in antimicrobial resistance in *S. pneumoniae* is the result of the expansion of successful clones as well as the introduction of new clonal types.[17-21] Previous observations provide strong evidence that the spread of penicillin-, macrolide-, trimethoprim/sulfamethoxazole-, fluoroquinolone-resistant and MDR *S. pneumoniae* is often driven by the dissemination of a few successful clones and that the use of non-fluoroquinolone antimicrobials (β-lactams, macrolides and trimethoprim/sulfamethoxazole) may lead to resistance to all three antimicrobial classes, given the propensity for these resistances to associate in clinical isolates.[17-19,22] Given that PCV-7 included serotypes that were frequently associated with non-susceptibility to penicillin and other antimicrobial agents, its use facilitated changes in the epidemiology of antimicrobial resistance in Canada.[1,3]

In Canada, penicillin-resistant and MDR *S. pneumoniae* were rarely isolated (<5%) prior to 1990.[23,24] From the 1990s to 2000, rates of penicillin-non-susceptibility in invasive isolates of pneumococci in Canada increased significantly to as high as 30% of isolates in some studies.[23,25-29] The introduction of PCV-7 did not have an effect on the prevalence of fluoroquinolone (levofloxacin, moxifloxacin) resistance in pneumococci as resistance to respiratory fluoroquinolones has not been associated with clonal spread and remained at very low levels (<2%) from 1998 to 2009.[30] However, in the same study, fluoroquinolone resistance was associated with living in central or eastern Canada, patient age >64 years, respiratory tract isolate, hospitals with greater numbers of beds, and isolates with penicillin MICs >1 mg/L.[30] In

the Canadian province of Alberta, from 2000 to 2006, overall, PCV-7 serotypes decreased 61% accompanied by a significant decline in non-susceptibility of *S. pneumoniae* isolates to penicillin from 14% in 2000 to 4.6% in 2006; non-susceptibility to erythromycin also decreased from 8.8% (2000) to 5.8% (2006).[3] Bettinger *et al*. showed a decrease in vaccine serotypes from 2000 to 2007 but no decrease in the proportion of invasive pneumococcal isolates that were penicillin-resistant and ceftriaxone/cefotaxime-resistant.[1] The ABC Surveillance Program in the USA determined the rates of IPD caused by antibiotic non-susceptible pneumococci for the regions surveyed; rates of penicillin-non-susceptible *S. pneumoniae* dropped from a high of 6.3/100 000 in 1999 to 2.7/100 000 in 2004 (a drop of 57%).[31] The greatest effect was seen in children <2 years of age with a decrease in penicillin-non-susceptible *S. pneumoniae* of 81%.[32] Demczuk *et al*. noted no significant changes in antimicrobial resistance rates between isolates collected during 2011 and those collected in 2012 despite a concurrent decrease in relative proportions of the generally resistant serotype 19A; they noted higher resistance rates for PCV-13 serotypes than for non-PCV-13 serotypes for the majority of tested antimicrobial agents and that the highest rates of resistance were to clarithromycin (23.3%) and penicillin using intravenous meningitis breakpoints (12.4%) while resistance was lower for clindamycin (8%) and trimethoprim/sulfamethoxazole (6%).[7]

The primary limitation of this study is the underrepresentation of British Columbia and Alberta, two Canadian provinces who do not participate in the SAVE study. The regional analyses may be affected by the limited representation of data from the Western provinces.

S. pneumoniae is a remarkably adaptable pathogen as demonstrated by the emergence of replacement serotypes following PCV-7 and PCV-13 introduction. The isolates tested in the current study were from 2011-2015, following the introduction of PCV-13 in Canada and

included both PCV-13 and non-PCV-13 serotypes.[16] The recent report by Olarte *et al*. of increasing incidence of MDR serotype 35B disease underscores the limitations of pneumococcal vaccines that target the polysaccharide capsule.[32] Clearly, vaccination, and replacement serotypes influence the *in vitro* susceptibilities of invasive ***S. pneumoniae*** in Canada, and elsewhere. We conclude that *in vitro* susceptibilities of invasive isolates of ***S. pneumoniae*** increased from 2011 to 2015 for penicillin, clindamycin and ceftriaxone and that isolates with a MDR phenotype decreased over the same time. Antimicrobial resistance rates were generally not associated with patient gender but were associated with patient age, specimen source, geographic location in Canada, and concurrent resistance to penicillin or clarithromycin for some agents. Our observations are certainly the result of conjugate pneumococcal vaccine use in Canada and demonstrate that vaccination may serve as one approach to lowering antimicrobial resistance among invasive isolates of *S. pneumoniae*. However, appropriate use of antimicrobial agents and ongoing surveillance are required to carefully monitor resistance trends by both categorical results and MIC distributions. Equally important is careful analysis of surveillance data in terms of factors associated with resistance and other associated trends, so that resistance and susceptibility and their consequences are neither over- nor under-estimated. Such analyses must be performed at national, regional, and institutional levels to guide physicians sufficiently in their selection of empiric therapies for patients. To enhance the protection provided by the pneumococcal conjugate vaccines, new formulations need to continue to be developed as antimicrobial-resistant replacement serotypes continue to emerge.

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Transparency Declaration

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Disclaimer

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

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Table 1. Annual antimicrobial susceptibility testing results for 14 antimicrobial agents tested against invasive isolates of *S. pneumoniae* as part of the SAVE study from 2011 to 2015

	Year[a]					
	2011	2012	2013	2014	2015	
Antimicrobial agent	Percent susceptible/percent resistant/MIC_{90} (mg/L)					*P*[b]
Penicillin (IV, nonmeningitis)	97.3/0/0.12	98.1/0.1/0.12	99.0/0.1/0.12	99.1/0.1/0.06	99.0/0/0.12	0.001
Penicillin (IV, meningitis)	86.3/13.7/0.12	89.1/10.9/0.12	89.9/10.1/0.12	91.0/9.0/0.06	89.5/10.5/0.12	0.017
Penicillin (oral, penicillin V)	86.3/4.2/0.12	89.1/3.1/0.12	89.9/3.5/0.12	91.0/2.1/0.06	89.5/3.0/0.12	0.017
Ceftriaxone (nonmeningitis)	98.6/0.2/≤0.12	99.2/0.2/≤0.12	99.3/0.2/≤0.12	99.8/0.1/≤0.12	99.7/0/≤0.12	0.002
Ceftriaxone (meningitis)	95.8/1.4/≤0.12	96.5/0.8/≤0.12	96.5/0.7/≤0.12	97.6/0.2/≤0.12	97.3/0.3/≤0.12	0.050
Cefuroxime (parenteral)	94.7/5.1/≤0.25	95.7/4.1/≤0.25	94.2/4.9/≤0.25	94.7/5.1/≤0.25	94.0/5.5/≤0.25	0.487
Cefuroxime (oral)	94.9/4.8/≤0.25	95.9/3.7/≤0.25	95.1/3.7/≤0.25	94.9/3.7/≤0.25	94.5/4.1/≤0.25	0.721
Clarithromycin	76.8/22.5/8	74.2/23.7/4	73.1/25.1/4	76.6/22.2/2	74.9/23.1/2	0.282
Clindamycin	90.7/8.9/≤0.12	93.6/6.3/≤0.12	93.3/5.8/≤0.12	94.9/4.6/≤0.12	93.8/6.0/≤0.12	0.004
Telithromycin	99.9/0/0.12	100/0/0.12	100/0/0.12	100/0/0.12	100/0/0.12	1
Levofloxacin	99.6/0.4/1	99.3/0.6/1	99.4/0.5/1	99.0/0.9/1	99.7/0.3/1	0.762
Moxifloxacin	99.6/0.2/0.25	99.3/0.4/0.25	99.5/0/0.25	99.1/0.8/0.25	99.7/0.1/0.12	1
Linezolid	100/0/1	100/0/2	100/0/2	100/0/1	100/0/1	1
Trimethoprim/sulfamethoxazole	87.2/5.8/1	88.1/5.7/1	86.2/7.6/1	89.3/5.9/1	87.4/6.3/1	0.904
Doxycycline	88.5/10.9/2	89.2/10.2/1	89.4/9.8/0.5	91.1/8.0/≤0.25	90.2/8.7/≤0.25	0.175
Tigecycline	100/0/0.03	100/0/0.03	100/0/0.03	100/0/0.03	100/0/0.03	1
Chloramphenicol	99.0/1.0/4	97.7/2.3/4	99.0/1.0/4	96.8/3.2/4	99.0/1.0/4	1
Vancomycin	100/0/0.5	100/0/0.5	100/0/0.25	100/0/0.25	100/0/0.25	1

[a] A total of 6001 isolates of *S. pneumoniae* were available for antimicrobial susceptibility testing from 2001 to 2015. The number of isolates tested per year was 1362 isolates in 2011, 1230 isolates in 2012, 1099 isolates in 2013, 1159 isolates in 2014, and 1151 isolates in 2015.
[b] *P* values generated by comparing antimicrobial susceptibility rates for 2011 versus 2015.

Table 2. Resistance to one or more antimicrobial agents among invasive isolates of *S. pneumoniae* in the SAVE study from 2011 to 2015 (cumulative data)

Number of antimicrobial agents to which isolates were resistant[a]	% of total isolates tested (*n*)[b]	Percent of isolates (*n*) resistant to the indicated antimicrobial agent						
		Penicillin	Clarithromycin	Clindamycin	Doxycycline	Levofloxacin	SXT[c]	Chloramphenicol
0	71.5 (4289)	-	-	-	-	-	-	-
1	17.7 (1064)	0.6 (6)	77.2 (821)	0.3 (3)	8.5 (90)	2.0 (21)	9.6 (102)	2.0 (21)
2	4.6 (276)	6.2 (17)	76.1 (210)	18.5 (51)	47.5 (131)	1.4 (4)	34.1 (94)	16.3 (45)
3[d,e]	3.1 (184)	9.2 (17)	96.7 (178)	84.8 (156)	91.3 (168)	1.1 (2)	12.5 (23)	4.3 (8)
4[d,e]	1.1 (64)	46.9 (30)	100 (64)	79.7 (51)	96.9 (62)	4.7 (3)	51.6 (33)	20.3 (13)
5[d,e]	1.9 (115)	98.3 (113)	100 (115)	99.1 (114)	100 (115)	0.9 (1)	97.4 (112)	4.3 (5)
6[d,e]	0.1 (8)	100 (8)	100 (8)	100 (8)	100 (8)	12.5 (1)	100 (8)	87.5 (7)
7[d,e]	<0.1 (1)	100 (1)	100 (1)	100 (1)	100 (1)	100 (1)	100 (1)	100 (1)

[a] The antimicrobial agents used in this analysis were selected as antimicrobial class markers: penicillin (oral MIC breakpoints), clarithromycin, clindamycin, doxycycline, levofloxacin, trimethoprim/sulfamethoxazole, and chloramphenicol.

[b] A total of 6001 isolates of *S. pneumoniae* were available for antimicrobial susceptibility testing from 2001 to 2015.

[c] SXT, trimethoprim/sulfamethoxazole.

[d] MDR was defined as concurrent resistance to three or more of the seven antimicrobial classes analyzed; 6.2% (372/6001) of all isolates from 2011 to 2015 were MDR.

[e] The percent prevalence of MDR isolates (*n*/total *n*) by year was 8.5% (116/1362) in 2011, 6.8 % (83/1230) in 2012, 5.8% (64/1099) in 2013, 3.9% (45/1159) in 2014, 5.6% (64/1151) in 2015 (P <0.001).

Table 3. Relative associations between resistance to six[a] antimicrobial agents and patient demographic/isolate factors among invasive isolates of *S. pneumoniae* in the SAVE study from 2011 to 2015 (cumulative data)

Patient demographic/isolate factor	*n* of isolates associated with risk factor	Penicillin		Clarithromycin		Doxycycline		SXT[a]		Chloramphenicol		Clindamycin	
		n (%) of resistant isolates	*P*[b]	*n* (%) of resistant isolates	*P*	*n* (%) of resistant isolates	*P*	*n* (%) of resistant isolates	*P*	*n* (%) of resistant isolates	*P*	*n* (%) of resistant isolates	*P*
All isolates	6001	192 (3.2%)	–	1397 (23.3%)	–	575 (9.6%)	–	373 (6.2%)	–	100 (1.7%)	–	384 (6.4%)	–
Patient age, years[c]			0.057		0.307		0.101		0.335		0.026		0.004
<18	851	37 (4.3%)		208 (24.4%)		81 (9.5%)		62 (7.3%)		5 (0.6%)		65 (7.6%)	
18-64	2788	76 (2.7%)		627 (22.5%)		245 (8.8%)		165 (5.9%)		51 (1.8%)		148 (5.3%)	
>64	2231	73 (3.3%)		537 (24.1%)		236 (10.6%)		135 (6.1%)		43 (1.9%)		164 (7.4%)	
Patient gender[d]			0.075		0.041		0.669		0.432		0.627		0.775
Female	2660	97 (3.6%)		660 (24.8%)		262 (9.8%)		158 (5.9%)		47 (1.8%)		175 (6.6%)	
Male	3121	88 (2.8%)		703 (22.5%)		297 (9.5%)		201 (6.4%)		50 (1.6%)		199 (6.4%)	
Invasive specimen source			<0.001		0.154		<0.001		0.004		0.950		<0.001
Blood	5448	155 (2.8%)		1257 (23.1%)		498 (9.1%)		322 (5.9%)		90 (1.7%)		326 (6.0%)	
Cerebrospinal fluid	235	9 (3.8%)		52 (22.1%)		27 (11.5%)		18 (7.7%)		4 (1.7%)		22 (9.4%)	
Other sterile body fluids[e]	318	28 (8.8%)		88 (27.7%)		50 (15.7%)		33 (10.4%)		6 (1.9%)		36 (11.3%)	
Geographic region[f]			<0.001		0.051		<0.001		0.003		<0.001		0.077
Western Canada	1321	53 (4.0%)		336 (25.4%)		89 (6.7%)		102 (7.7%)		12 (0.9%)		67 (5.1%)	
Central Canada	3952	102 (2.6%)		883 (22.3%)		411 (10.4%)		215 (5.4%)		84 (2.1%)		270 (6.8%)	
Eastern Canada	728	37 (5.1%)		178 (24.5%)		75 (10.3%)		56 (7.7%)		4 (0.5%)		47 (6.5%)	
Penicillin MIC interpretative category[g]			–		<0.001		<0.001		<0.001		<0.001		<0.001
Susceptible (≤0.06 mg/L)	5345	–		991 (18.5%)		204 (3.8%)		131 (2.5%)		85 (1.6%)		115 (2.2%)	
Intermediate (0.12-1 mg/L)	464	–		233 (50.2%)		213 (45.9%)		86 (18.5%)		4 (0.9%)		130 (28.0%)	
Resistant (≥2 mg/L)	192	–		173 (90.1%)		158 (82.3%)		156 (81.2%)		11 (5.7%)		139 (72.4%)	
Clarithromycin MIC interpretative category			<0.001		–		<0.001		<0.001		<0.001		<0.001
Susceptible (≤0.25 mg/L)	4512	16 (0.4%)		–		124 (2.7%)		119 (2.6%)		59 (1.3%)		4 (0.1%)	
Intermediate (0.5 mg/L)	92	3 (3.3%)		–		28 (30.4%)		10 (10.9%)		1 (1.1%)		5 (5.4%)	
Resistant (≥1 mg/L)	1397	173 (12.4%)		–		423 (30.3%)		244 (17.5%)		40 (2.9%)		375 (26.8%)	

[a] SXT, trimethoprim/sulfamethoxazole.
[b] The Chi-square test identified significant differences between individual patient demographic/isolate factors within a group of factors but does not specify the identity of the difference within the group of factors.
[c] There were 131 isolates with unknown patient age.
[d] There were 220 isolates with unknown patient gender.
[e] Other sterile body fluids were comprised of pleural fluid (*n*=123), synovial fluid (*n*=38), peritoneal fluid (*n*=12), pericardial fluid (*n*=4), abscess (*n*=3), other sterile site/source not given (*n*=138).
[f] Western 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.
[g] Penicillin (oral penicillin V) MIC breakpoints were used.