

Antimicrobial susceptibility testing of invasive isolates of *Streptococcus pneumoniae* from Canadian patients: the SAVE study, 2011–2020

Morgan A. Alford¹, James A. Karlow^{1,2}, Heather J. Adam^{1,2}, Melanie R. Baxter¹, John Schellenberg¹, Alyssa R. Golden³, Irene Martin³, Walter Demczuk³, Michael R. Mulvey^{1,3}, and George G. Zhanel^{1*}, on behalf of the Canadian Antimicrobial Resistance Alliance (CARA)†

¹Department of Medical Microbiology and Infectious Diseases, Max Rady College of Medicine, University of Manitoba, Room 543-745 Bannatyne Avenue, Winnipeg, Manitoba R3E 0J9, Canada; ²Clinical Microbiology, Shared Health, MS673-820 Sherbrook Street, Winnipeg, Manitoba R3A 1R9, Canada; ³National Microbiology Laboratory, Public Health Agency of Canada, 1015 Arlington Street, Winnipeg, Manitoba R3E 3M4, Canada

*Corresponding author. E-mail: ggzhanel@pcsinternet.ca

†Members are listed in the Acknowledgements section.

Objectives: To assess the antimicrobial susceptibility of 14 138 invasive *Streptococcus pneumoniae* isolates collected in Canada from 2011 to 2020.

Methods: Antimicrobial susceptibility testing was performed using the CLSI M07 broth microdilution reference method. MICs were interpreted using 2022 CLSI M100 breakpoints.

Results: In 2020, 90.1% and 98.6% of invasive pneumococci were penicillin-susceptible when MICs were interpreted using CLSI meningitis or oral and non-meningitis breakpoints, respectively; 96.9% (meningitis breakpoint) and 99.5% (non-meningitis breakpoint) of isolates were ceftriaxone-susceptible, and 99.9% were levofloxacin-susceptible. Numerically small, non-temporal, but statistically significant differences ($P < 0.05$) in the annual percentage of isolates susceptible to four of the 13 agents tested was observed across the 10-year study: chloramphenicol (4.4% difference), trimethoprim-sulfamethoxazole (3.9%), penicillin (non-meningitis breakpoint, 2.7%) and ceftriaxone (meningitis breakpoint, 2.7%; non-meningitis breakpoint, 1.2%). During the same period, annual differences in percent susceptible values for penicillin (meningitis and oral breakpoints) and all other agents did not achieve statistical significance. The percentage of isolates with an MDR phenotype (resistance to ≥ 3 antimicrobial classes) in 2011 and 2020 (8.5% and 9.4%) was not significantly different ($P = 0.109$), although there was a significant interim decrease observed between 2011 and 2015 ($P < 0.001$) followed by a significant increase between 2016 and 2020 ($P < 0.001$). Statistically significant associations were observed between resistance rates to most antimicrobial agents included in the MDR analysis (penicillin, clarithromycin, clindamycin, doxycycline, trimethoprim/sulfamethoxazole and chloramphenicol) and patient age, specimen source, geographic location in Canada or concurrent resistance to penicillin or clarithromycin, but not biological sex of patients. Given the large isolate collection studied, statistical significance did not necessarily imply clinical or public health significance in some analyses.

Conclusions: Invasive pneumococcal isolates collected in Canada from 2011 to 2020 generally exhibited consistent *in vitro* susceptibility to commonly tested antimicrobial agents.

Introduction

Streptococcus pneumoniae is a leading cause of non-invasive infections (e.g. sinusitis, otitis media) and invasive infections of normally sterile sites (e.g. bacteraemia, meningitis).¹ It can produce substantial morbidity and mortality in the very young, elderly and immune-compromised.¹ Invasive pneumococcal disease (IPD) may be preceded by asymptomatic colonization of the respiratory tract. Estimates suggest that up to 60% of healthy children are carriers of pneumococci in their respiratory tracts.² Its

pathogenicity is associated with induction of inflammation through secretion of virulence factors, including cytotoxic pneumolysin, and evasion of phagocytosis conferred by its oligosaccharide capsule.³ So far, >92 antigenically distinct serotypes of pneumococcus have been recognized, of which a small subset are considered major contributors to the global incidence of IPD.³

Early administration of antibacterial agents remains critical for successful management of IPD and reducing its morbid consequences.⁴ Empiric antimicrobial therapy may be administered at clinical presentation, although targeted therapy following

aetiological identification and antimicrobial susceptibility testing is desirable. Since meningitis cannot be ruled out at the onset of suspected IPD, guidelines recommend empiric therapy with vancomycin (15–20 mg/kg every 8–12 h) and ceftriaxone (2 g intravenous every 12 h) or cefotaxime (2 g intravenous every 4–6 h).⁴ Despite its inferior central nervous system penetration, a fluoroquinolone may replace a third-generation cephalosporin when treating β -lactam allergic patients. Once definitive identification of the pathogen and its susceptibility profile are ascertained, vancomycin should be discontinued unless the concern for meningitis remains.⁴ Antimicrobial stewardship strategies are important for subverting the spread of antimicrobial resistance among invasive pneumococcal isolates. *S. pneumoniae* isolates were uniformly susceptible to penicillin prior to 1970 but have since developed resistance to that agent and to several other agents in different antibacterial classes.^{5,6}

In Canada, detection of penicillin-resistant invasive isolates increased astronomically between 1990 and 2000, from <5% to as high as 30% by some measurements.^{7–13} Increased prevalence of resistant isolates was associated with increased antimicrobial consumption coinciding with dissemination of, and selection for, successful clones, particularly those resistant to β -lactams, macrolides and trimethoprim/sulfamethoxazole.^{14–18} There appears to be a propensity for these resistances to associate. Therapeutic use of any one of these antimicrobials may have contributed to the emergence of MDR pneumococcus, defined as isolates simultaneously resistant to three or more antimicrobials from different classes.^{14–16,19}

Non-susceptible pneumococcal serotypes recognized as major causes of IPD have been targeted by vaccination programmes in many countries, including Canada. These programmes have been successful not only in reducing the incidence of IPD but also in reducing transmission of antimicrobial-resistant *S. pneumoniae* isolates in certain populations.^{1,20–24} The seven-valent (comprising capsular serotype antigens 4, 6B, 9 V, 14, 18C, 19F, 23F) pneumococcal conjugate vaccine (PCV7) was licensed for use in Canada in 2001,¹ followed by introduction of infant immunization programmes in all provinces and territories between 2002 and 2006. Accordingly, the number of cases of IPD and rate of antimicrobial resistance were significantly decreased between 2000 and 2007, with the greatest reductions occurring in children <2 years of age.¹ However, increased cases of IPD and antimicrobial resistance due to non-vaccine serotypes (e.g. 19A) was observed concomitantly.^{1,22} Overall, the proportion of penicillin- and cefotaxime/ceftriaxone-resistant isolates remained unchanged in Canada at 17% and 2%.¹ Subsequently, the 13-valent pneumococcal conjugate vaccine (PCV13) targeting additional serotypes (1, 3, 5, 6A, 7F, 19A) representing most of the serotypes in circulation²³ was introduced in Canada in 2010, with rapid uptake by all provinces and territories into their routine immunization schedule by mid-2011. As expected, pneumococcal infection by PCV13 serotypes declined in Canada thereafter, from 55% incidence in 2010/10 to 31% in 2014 with age-associated trends observed.^{24,25} The overall rate (cases per 100 000 population) of IPD decreased from 9.8 to 8.9 between 2009 and 2014, remaining highest in infants <1 year old (16.9), children 1–4 years old (11.0) and patients 60 years and older (21.5).²⁶ Thereafter, increased disease incidence in older patients caused an increase in the overall rate of IPD, with rates as high as

23.4 for patients ≥ 60 years old in 2018. Nonetheless, reasonably high (81%) vaccine coverage (3–4 doses by 2 years of age) was reported across provinces in 2017.²⁷

Despite the availability and use of pneumococcal conjugate vaccines in Canada, invasive infections continue to occur, and antimicrobial failure remains a significant threat to management of infection. Therefore, access to current antimicrobial surveillance data remains important to key stakeholders in medicine, academia and industry. The SAVE study is an annual surveillance programme that collects and characterizes invasive isolates of *S. pneumoniae* submitted by participating provincial public health and hospital laboratories across Canada. In the current study, invasive isolates of *S. pneumoniae* collected from 2011 to 2020, inclusive, were tested for their susceptibilities to a panel of 13 antimicrobial agents using the reference CLSI broth microdilution method.^{28,29} We assessed change in percent susceptibility, MDR rates and whether antimicrobial resistance was associated with factors such as patient age or biological sex, specimen source, geographic location or concurrent resistance to penicillin or clarithromycin. These data demonstrate the epidemiological impact of Canadian immunization programmes on the incidence of IPD and antimicrobial resistance among invasive pneumococcal isolates, and could contribute to the delivery of effective healthcare by reducing use of discordant empiric therapy by knowledge end users.

Materials and methods

Bacterial isolates

From January 2011 to December 2020, *S. pneumoniae* isolates from sterile body sites were forwarded to the Public Health Agency of Canada-National Microbiology Laboratory (PHAC-NML) in Winnipeg, Manitoba, Canada by participating Canadian provincial public health and hospital laboratories. As part of an ongoing collaboration between the Canadian Antimicrobial Resistance Alliance (CARA) and PHAC-NML, PHAC-NML forwarded a collection of 14 138 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.

The annual numbers of isolates that received antimicrobial susceptibility testing were: 1379 isolates from 2011, 1285 from 2012, 1160 from 2013, 1274 from 2014, 1262 from 2015, 1284 from 2016, 1598 from 2017, 1930 from 2018, 1893 from 2019 and 1073 from 2020.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed in the Department of Clinical Microbiology at the Winnipeg Health Sciences Centre using the reference CLSI broth microdilution method^{28,29} with custom-designed, in-house prepared, 96-well microtitre panels containing doubling-dilutions of antimicrobial agents in cation-adjusted Mueller–Hinton broth supplemented to a final concentration of 4% lysed horse blood. Isolates were tested against penicillin, ceftriaxone, cefuroxime, clarithromycin, clindamycin, levofloxacin, moxifloxacin, linezolid, trimethoprim/sulfamethoxazole, doxycycline, tigecycline, chloramphenicol and vancomycin. Isolates were interpreted as susceptible, intermediate or resistant to antimicrobials by interpretation of their MICs using 2022 CLSI breakpoints²⁹ for all antimicrobial agents except tigecycline for which FDA MIC breakpoints were used (susceptible, ≤ 0.06 mg/L).³⁰ MDR was defined as resistance to three or more antimicrobial agents that were selected to

represent different antimicrobial classes (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).²⁹

Of the 14 138 isolates available in the study, 13 712 (97.0%) generated results for all seven agents used to define MDR phenotypes. Of these, patient age and biological sex information was available for 13 475 (98.3%) and 13 062 (95.3%) of the isolates, respectively, distributed across three regions: Western Canada (Saskatchewan and Manitoba, $n = 2989$), Central Canada (Ontario and Quebec, $n = 9291$) and Eastern Canada (New Brunswick, Nova Scotia, Prince Edward Island, and Newfoundland and Labrador, $n = 1432$).

Statistical analysis

Trends in annual antimicrobial agent percent susceptible and percent MDR phenotype values from 2011 to 2020 were assessed for statistically significant differences ($P < 0.05$) using the Cochran–Armitage test for trend. Any antimicrobial agent with low annual non-susceptible isolate counts (i.e. where there were less than five non-susceptible isolates in > 2 years) were excluded from the analysis, consistent with known limitations of the Cochran–Armitage test for trend. Associations between susceptibility, patient demographic or isolate factors and concurrent resistance to other antimicrobials were assessed for statistically significant differences using the χ^2 test.

Results

In 2020, 90.1% and 98.6% of invasive pneumococci were penicillin-susceptible when MICs were interpreted using CLSI meningitis or oral, and non-meningitis breakpoints, respectively; 96.9% (meningitis breakpoint) and 99.5% (non-meningitis breakpoint) of isolates were ceftriaxone-susceptible and 99.9% were levofloxacin-susceptible (Table 1). Only 76.7%, 85.6% and 88.4% of invasive pneumococci collected in 2020 were susceptible to clarithromycin, trimethoprim/sulfamethoxazole and doxycycline, respectively.

Statistically significant differences ($P < 0.05$) in annual percent susceptible values were observed for four of the 13 agents tested across the 10 years of the study (Table 1). However, none of the statistically significant variations observed were associated with increasing or decreasing temporal (year-to-year) trends. Chloramphenicol demonstrated a 4.4% overall difference from 2011 to 2020, with decreasing annual percent susceptible values from 2016 to 2020. Trimethoprim/sulfamethoxazole showed an overall difference of 3.9%, with decreasing annual percent susceptible values from 2014 to 2020. Penicillin, with MICs interpreted by the non-meningitis breakpoint, exhibited an overall 2.7% difference, with initial increasing annual percent susceptible values from 2011 to 2016 followed by decreasing values from 2016 to 2020. Ceftriaxone demonstrated differences for MICs interpreted by both meningitis (a 2.7% overall difference with increasing annual percent susceptible values) and non-meningitis breakpoints (a 1.2% overall difference with increasing annual percent susceptible values). Annual percent susceptible values for penicillin (interpreted by meningitis and oral CLSI breakpoints), cefuroxime and four additional non- β -lactam agents were unchanged ($P > 0.05$) over the same 10-year period, with MIC₉₀ values fluctuating by less than log₂-fold annually, except for doxycycline between 2018 (≤ 0.25 mg/L) and 2019 (1 mg/L). In all years, isolates were 100% vancomycin- or

linezolid-susceptible, and 99.9%–100% tigecycline-susceptible. Uniform susceptibility was only detected in one other instance: for penicillin (non-meningitis breakpoint) in 2016, although near uniform penicillin susceptibility ($\geq 97.3\%$) was observed (using the non-meningitis breakpoint) in all years. Near uniform susceptibility was additionally observed for ceftriaxone (non-meningitis breakpoint) ($\geq 98.6\%$) and for levofloxacin ($\geq 98.9\%$).

Most (70.8%; 9711/13 712) of all invasive pneumococcal isolates tested from 2011 to 2020 were pan-susceptible to the panel of seven antimicrobial agents used in MDR analysis (Table 2). Of the isolates demonstrating resistance to at least one antimicrobial agent, 60.9% (2436/4001) were resistant to a single antimicrobial agent only, which was most frequently clarithromycin (76.7%; 1869/2436). Only 6.6% (902/13 712) of all isolates exhibited an MDR phenotype. The most common MDR phenotype was composed of concurrent resistance to clarithromycin, clindamycin and doxycycline ($n = 329$; 36.5% of all MDR isolates) with or without penicillin (oral breakpoint) and trimethoprim/sulfamethoxazole ($n = 163$; 18.1% of all MDR isolates). The rank order frequency of resistance to specific antimicrobial classes among MDR isolates of invasive *S. pneumoniae* was clarithromycin $>$ doxycycline $>$ clindamycin $>$ penicillin $\approx$ trimethoprim/sulfamethoxazole $>$ chloramphenicol $>$ levofloxacin (Table 2). The rate of MDR among invasive pneumococcal isolates was not significantly different, overall, across the 10-years studied (8.5% in 2011% and 9.4% in 2020) ($P = 0.109$), although there was a significant interim decrease observed between 2011 and 2015 ($P < 0.001$) followed by a significant increase between 2016 and 2020 ($P < 0.001$) (Figure 1). Specifically, rates dipped from 8.5% in 2011% to 5.6% in 2015, with lows of 3.9% in 2014 and 2016, prior to rebounding to 9.4% in 2020.

There was a statistically significant association between patient age and resistance to clarithromycin, clindamycin, doxycycline and chloramphenicol (Table 3). For these antimicrobials, the rate of resistance was generally highest among patients > 64 years of age. The rate of resistance to chloramphenicol and doxycycline was lowest among patients < 18 years of age, although rates of resistance were $\leq 2.7\%$ different across age groups for all antimicrobials tested. There was a statistically significant association between biological sex and resistance to clarithromycin ($P < 0.001$) only, with 2.9% (1481/5904) more isolates from women exhibiting this phenotype. Further, there was a statistically significant association between specimen source and resistance to all antimicrobials included in MDR analysis, except for chloramphenicol (and levofloxacin). Generally, invasive isolates from specimens other than blood or cerebrospinal fluid exhibited greater rates of resistance. The largest differences were observed in rates of resistance to clarithromycin and doxycycline, which were 3.1%–5.8% and 3.7%–5.7% greater. Additionally, there was a statistically significant association between geographic region and antimicrobial resistance to all antimicrobials but trimethoprim/sulfamethoxazole, as well as concurrent resistance to any one of the antimicrobials and penicillin or clarithromycin. For example, 87.3% (352/403) of isolates exhibiting resistance to penicillin (MIC ≥ 2 mg/L) also exhibited resistance to clarithromycin (MIC ≥ 1 mg/L). The percentage of isolates that were resistant to penicillin and other antimicrobials included in MDR analysis ranged from 8.7 (chloramphenicol) to 73.9% (doxycycline) ($n = 35$ –298). In retrospect, given the large isolate collection studied,

Table 1. Annual antimicrobial susceptibility testing results for 13 antimicrobial agents tested against invasive isolates of *S. pneumoniae* as part of the SAVE study from 2011 to 2020

Antimicrobial agent	Year ^a										p ^b
	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	
	Percent susceptible/percent resistant/MIC ₅₀ (mg/L)										
Penicillin (IV, non-meningitis) (n = 13 720)	97.3/0/0.12	98.1/0.1/0.12	99.0/0.1/0.12	99.1/0.1/0.06	99.1/0/0.12	100/0/0.12	99.2/0/0.25	98.7/0.1/0.12	99.6/0/0.12	98.6/0.1/0.06	<0.001
Penicillin (IV, meningitis)	86.4/13.6/0.12	89.0/11.0/0.12	89.9/10.1/0.12	91.0/9.0/0.06	89.5/10.5/0.12	87.9/12.1/0.12	86.0/14.0/0.25	88.6/11.4/0.12	89.2/10.8/0.12	90.1/9.9/0.06	0.463
Penicillin (oral, penicillin V)	86.4/14.2/0.12	89.0/3.1/0.12	89.9/3.4/0.12	91.0/2.0/0.06	89.5/3.1/0.12	87.9/1.0/0.12	86.0/3.8/0.25	88.6/3.4/0.12	89.2/2.2/0.12	90.1/3.1/0.06	0.463
Ceftriaxone (non-meningitis) (n = 13 719)	98.6/0.2/≤0.12	99.2/0.2/≤0.12	99.3/0.2/≤0.12	99.8/0.1/≤0.12	99.8/0/≤0.12	99.8/0/0.25	99.4/0/≤0.12	99.3/0.2/≤0.12	99.8/0.1/≤0.12	99.5/0.2/≤0.12	0.002
Ceftriaxone (meningitis)	95.8/1.4/≤0.12	96.5/0.8/≤0.12	96.6/0.7/≤0.12	97.7/0.2/≤0.12	97.4/0.2/≤0.12	98.5/0.2/0.25	97.8/0.6/≤0.12	97.1/0.7/≤0.12	97.6/0.2/≤0.12	96.9/0.5/≤0.12	0.007
Cefuroxime (parenteral) (n = 10 790)	95.7/14.2/≤0.25	95.7/14.1/≤0.25	94.3/4.8/≤0.25	94.9/4.9/≤0.25	94.1/5.4/≤0.25	94.9/4.9/≤0.25	93.2/6.1/≤0.25	95.4/4.5/≤0.25	Not tested	Not tested	0.114
Cefuroxime (oral)	95.8/3.8/≤0.25	95.9/3.7/≤0.25	95.2/3.7/≤0.25	95.1/3.5/≤0.25	94.6/4.1/≤0.25	95.1/3.8/≤0.25	93.9/5.1/≤0.25	95.5/4.0/≤0.25	Not tested	Not tested	0.119
Clarithromycin (n = 13 718)	76.6/22.6/4	74.1/23.8/4	72.7/25.5/4	76.5/22.2/2	75.6/22.6/2	77.4/21.6/4	78.3/21.1/4	73.5/25.9/8	74.7/24.9/8	76.7/22.9/4	0.747
Clindamycin (n = 13 718)	90.7/8.9/≤0.12	93.5/6.3/≤0.12	93.2/6.6/≤0.12	94.4/5.0/≤0.12	93.8/6.0/≤0.12	95.6/4.2/≤0.12	91.9/7.4/≤0.12	93.0/6.9/≤0.12	92.6/7.3/≤0.12	92.8/7.0/≤0.12	0.660
Levofloxacin (n = 13 719)	99.6/0.4/1	99.3/0.6/1	99.3/0.5/1	98.9/1.0/1	99.7/0.3/1	99.7/0.2/1	99.5/0.4/1	99.7/0.3/1	99.4/0.5/1	99.9/0.1/1	0.063
Moxifloxacin (n = 10 789)	99.5/0.2/0.25	99.3/0.4/0.25	99.5/0/0.25	99.0/0.8/0.25	99.7/0.1/0.12	99.8/0/0.12	99.6/0.3/0.25	99.7/0/0.25	Not tested	Not tested	0.061
Linezolid (n = 13 735)	100/0/2	100/0/2	100/0/2	100/0/1	100/0/1	100/0/2	100/0/2	100/0/2	100/0/2	100/0/2	NA ^d
SXT ^c (n = 13 719)	87.3/5.8/1	88.0/5.8/1	86.2/7.6/1	89.5/5.9/1	86.9/6.6/1	86.1/6.1/1	85.8/6.4/1	86.4/6.3/1	86.7/6.6/1	85.6/7.9/2	0.046
Doxycycline (n = 13 717)	88.5/10.9/2	89.1/10.2/1	89.1/9.9/0.5	90.7/8.3/≤0.25	89.9/9.0/0.5	90.8/8.6/≤0.25	89.8/10.0/0.5	91.2/8.5/≤0.25	89.4/10.4/1	88.4/11.4/2	0.357
Tigecycline (n = 13 736)	100/0/0.03	100/0/0.03	100/0/0.03	100/0/0.03	100/0/0.03	99.9/0.1/0.03	100/0/0.03	100/0/0.03	99.9/0.1/0.03	100/0/0.03	NA ^d
Chloramphenicol (n = 13 719)	98.9/1.1/4	97.7/2.3/4	98.8/1.2/4	96.9/3.1/4	98.9/1.1/4	98.8/1.2/4	97.6/2.4/4	94.5/5.5/4	96.9/3.1/4	96.0/4.0/4	<0.001
Vancomycin (n = 13 735)	100/0/0.5	100/0/0.5	100/0/0.25	100/0/0.25	100/0/0.25	100/0/0.25	100/0/0.25	100/0/0.5	100/0/0.5	100/0/0.5	NA ^d

^aA total of 14 138 isolates of *S. pneumoniae* were available for antimicrobial susceptibility testing from 2011 to 2020. The number of isolates tested per year was 1379 isolates in 2011, 1285 isolates in 2012, 1160 isolates in 2013, 1274 isolates in 2014, 1262 isolates in 2015, 1284 isolates in 2016, 1598 isolates in 2017, 1930 isolates in 2018, 1893 isolates in 2019 and 1073 isolates in 2020.

^bP < 0.05 indicates statistical significance in annual percent susceptible values according to the Cochran–Armitage test for trend.

^cSXT, trimethoprim/sulfamethoxazole.

^dNA, not available; isolate counts of <5 were detected in >2 years, therefore, the Cochran–Armitage test for trend analysis could not be performed.

Table 2. Resistance to one or more antimicrobial agents among invasive isolates of *S. pneumoniae* cumulated during the SAVE study from 2011 to 2020

No. of antimicrobial agents to which isolates were resistant ^a	Percentage of total isolates tested (n) ^b	Percentage of isolates (n) resistant to the indicated antimicrobial agent						
		Penicillin	Clarithromycin	Clindamycin	Doxycycline	Levofloxacin	SXT ^c	Chloramphenicol
0	70.8 (9711)	—	—	—	—	—	—	—
1	17.8 (2436)	0.9 (22)	76.7 (1869)	0.3 (8)	6.9 (167)	1.3 (31)	12.1 (294)	1.8 (45)
2	4.8 (663)	8.3 (55)	72.9 (483)	18.1 (120)	47.2 (313)	1.2 (8)	33.3 (221)	19 (126)
3 ^d	3.3 (452)	9.7 (44)	92.0 (416)	77.4 (350)	89.6 (405)	1.1 (5)	20.6 (93)	9.5 (43)
4 ^d	1.7 (235)	33.2 (78)	99.1 (233)	90.2 (212)	98.3 (231)	2.1 (5)	31.9 (75)	45.1 (106)
5 ^d	1.4 (188)	94.1 (177)	100 (188)	98.9 (186)	99.5 (187)	2.1 (4)	93.6 (176)	11.7 (22)
6 ^d	0.2 (26)	100 (26)	100 (26)	100 (26)	100 (26)	26.9 (7)	100 (26)	73.1 (19)
7 ^d	<0.1 (1)	100 (1)	100 (1)	100 (1)	100 (1)	100 (1)	100 (1)	100 (1)

^aAntimicrobial agents used in this analysis were selected as antimicrobial class markers: penicillin (oral penicillin V MIC breakpoint), clarithromycin, clindamycin, doxycycline, levofloxacin, SXT and chloramphenicol.
^bA total of 13 712 isolates of *S. pneumoniae* were available for MDR analysis from 2011 to 2020.
^cSXT, trimethoprim/sulfamethoxazole.
^dMDR was defined as concurrent resistance to three or more of the seven antimicrobial classes analysed; 6.6% (902/13 712) of all isolates from 2011 to 2020 exhibited an MDR phenotype.

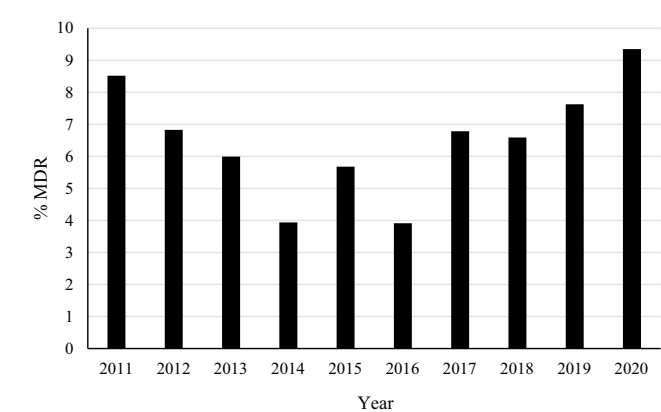


Figure 1. Annual percent prevalence of MDR among invasive isolates of *S. pneumoniae* in the SAVE study from 2011 to 2020. No significant differences were detected between 2011 and 2020 ($P=0.109$), although significant differences were detected between 2011 and 2015 ($P<0.001$) and 2016 and 2020 ($P<0.001$), according to the Cochran–Armitage test for trend.

some of the numerically small but statistically significant findings identified in Tables 1 and 3 may not necessarily imply clinical or public health significance.

Discussion

Historically, increased prevalence of antimicrobial-resistant pneumococci has resulted from the expansion of successful clones and the introduction of new clonal types, coincident with the increased consumption of antimicrobials.^{14–18} Given

that PCV7 and PCV13 included serotypes that were frequently associated with non-susceptibility to penicillin and other antimicrobial agents, their use facilitated changes in the epidemiology of pneumococcal antimicrobial resistance in Canada.^{1,20,22–24} Between 2000 and 2007, the Canadian Immunization Monitoring Program, Active (IMPACT) reported that the overall rate of penicillin resistance in pneumococcal isolates remained steady.¹ Overall stability could be due, at least partially, to the expansion of the generally resistant pneumococcal serotype 19A that was later included in PCV13 but continued to be troublesome due to its propensity to cause breakthrough infections.^{1,27} Thus, between 2011 and 2012, no overall difference in susceptibility to several common antimicrobials was observed among pneumococcal isolates despite introduction of PCV13.²⁴ However, when analysis was focused on susceptibility of PCV13 serotypes, increased resistance to clarithromycin (23.3%) and penicillin (12.4% with MICs interpreted using the CLSI meningitis breakpoint) with concurrent decreased resistance to clindamycin (8%) and trimethoprim/sulfamethoxazole (6%) was noted. A separate surveillance study reported no significant overall changes in fluoroquinolone resistance between 1998 and 2009, although this could be explained by lack of association between fluoroquinolone resistance and clonal spread as well as overall low prevalence (2%).³¹ Further, a significant association between fluoroquinolone resistance and patient age (>65 years old), among other demographic or isolate factors, was observed. Taken together, these data may indicate that detection of small changes in resistance is mitigated by unrestricted analyses. Nonetheless, Canadian vaccination programmes have caused the incidence of IPD in the very young to decline.²⁷ Reflective of this progress, vaccination has slowed the spread of resistant isolates in the USA, where the occurrence of IPD caused by MDR pneumococci has been reduced by up to 84%.³²

Table 3. Relative associations between resistance to six antimicrobial agents and patient demographic or isolate factors among invasive isolates of *S. pneumoniae* cumulated during the SAVE study, 2011 to 2020

Patient demographic/isolate factor	n of isolates associated with risk factor	Penicillin		Clarithromycin		Clindamycin		Doxycycline		SXT ^a		Chloramphenicol	
		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	13 712	403 (2.9%)	—	3216 (23.5%)	—	903 (6.6%)	—	1330 (9.7%)	—	886 (6.5%)	—	362 (2.6%)	—
Patient age, years ^c			0.086		0.002		<0.001		0.038		0.089		<0.001
<18	1670	61 (3.7%)		406 (24.3%)		115 (6.9%)		145 (8.7%)		116 (6.9%)		22 (1.3%)	
18–64	6593	175 (2.7%)		1459 (22.1%)		371 (5.6%)		610 (9.3%)		392 (5.9%)		199 (3.0%)	
>64	5212	156 (3.0%)		1291 (24.8%)		397 (7.6%)		543 (10.4%)		357 (6.8%)		137 (2.6%)	
Patient biological sex ^d			0.566		<0.001		0.286		0.795		0.576		0.105
Female	5904	180 (3.0%)		1481 (25.1%)		407 (6.9%)		571 (9.7%)		375 (6.4%)		139 (2.4%)	
Male	7158	206 (2.9%)		1592 (22.2%)		460 (6.4%)		702 (9.8%)		472 (6.6%)		201 (2.8%)	
Invasive specimen source			<0.001		0.042		<0.001		<0.001		<0.001		0.310
Blood	12 431	334 (2.7%)		2903 (23.4%)		780 (6.3%)		1155 (9.3%)		770 (6.2%)		332 (2.7%)	
Cerebrospinal fluid	459	19 (4.1%)		95 (20.7%)		41 (8.9%)		52 (11.3%)		28 (6.1%)		7 (1.5%)	
Other sterile body fluids ^e	822	50 (6.1%)		218 (26.5%)		82 (10.0%)		123 (15.0%)		88 (10.7%)		23 (2.8%)	
Geographic region ^f			<0.001		0.015		<0.001		<0.001		0.794		<0.001
Western Canada	2989	109 (3.6%)		758 (25.4%)		137 (4.6%)		190 (6.4%)		195 (6.5%)		37 (1.2%)	
Central Canada	9291	235 (2.5%)		2118 (22.8%)		676 (7.3%)		1001 (10.8%)		593 (6.4%)		309 (3.3%)	
Eastern Canada	1432	59 (4.1%)		340 (23.7%)		90 (6.3%)		139 (9.7%)		98 (6.8%)		16 (1.1%)	
Penicillin MIC interpretative category ^g			—		<0.001		<0.001		<0.001		<0.001		<0.001
Susceptible (≤0.06 mg/L)	12 154	—		2273 (18.7%)		310 (2.6%)		499 (4.1%)		400 (3.3%)		287 (2.4%)	
Intermediate (0.12–1 mg/L)	1155	—		591 (51.2%)		333 (28.8%)		533 (46.1%)		220 (19.0%)		40 (3.5%)	
Resistant (≥2 mg/L)	403	—		352 (87.3%)		260 (64.5%)		298 (73.9%)		266 (66.0%)		35 (8.7%)	
Clarithromycin MIC interpretative category			<0.001		—		<0.001		<0.001		<0.001		<0.001
	10 360	45 (0.4%)		—		12 (0.1%)		318 (3.1%)		382 (3.7%)		169 (1.6%)	

Continued

Table 3. Continued

Patient demographic/isolate factor	n of isolates associated with risk factor	Penicillin		Clarithromycin		Clindamycin		Doxycycline		SXT ^a		Chloramphenicol	
		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
Susceptible (≤0.25 mg/L)													
Intermediate (0.5 mg/L)	136	6 (4.4%)	—	—		6 (4.4%)		49 (36.0%)		14 (10.3%)		5 (3.7%)	
Resistant (≥1 mg/L)	3216	352 (10.9%)	—	—		885 (27.5%)		963 (29.9%)		490 (15.2%)		188 (5.8%)	

^aSXT, trimethoprim/sulfamethoxazole.
^bThe χ^2 test identified significant differences between individual patient demographic/isolate factors within a group of factors but does not specify the identity of the difference.
^cThere were 237 isolates with unknown patient age.
^dThere were 650 isolates with unknown patient biological sex.
^eOther sterile body fluids: pleural fluid (n=113), peritoneal fluid (n=35), pericardial fluid (n=6), abscess (n=3) or sterile site/source not given (n=401).
^fWestern Canada: Manitoba, Saskatchewan; Central Canada: Ontario, Quebec; Eastern Canada: Nova Scotia, New Brunswick, Prince Edward Island, Newfoundland and Labrador.
^gPenicillin (oral penicillin V) MIC breakpoints were used.

Clearly, *S. pneumoniae* is a remarkably adaptable human pathogen capable of causing severe disease and undergoing genetic recombination in response to common selective pressures such as vaccination and antimicrobial exposure.^{33,34} Thus, it remains important to monitor resistance phenotypes displayed by circulating serotypes. Here, we show that invasive pneumococcal isolates collected in Canada between 2011 and 2020 trended toward increased susceptibility to ceftriaxone (with MICs interpreted by both the CLSI meningitis and non-meningitis breakpoints) but decreased susceptibility to chloramphenicol and trimethoprim/sulfamethoxazole, although these differences were small in magnitude (<5%) (Table 1). Isolates also exhibited increasing susceptibility to penicillin, with MICs interpreted by the non-meningitis breakpoint, from 2011 to 2016, followed by decreasing susceptibility from 2016 to 2020, but again these changes were small (<3%). Since the primary targets of penicillin are penicillin-binding proteins (PBPs) 2B, and 2X, coincident resistance to ceftriaxone, which primarily targets PBPs 1A and 2X, and penicillin is not expected.³⁵ However, ceftriaxone failure in the context of IPD caused by penicillin-resistant isolates has been reported.^{36,37} Although isolates in these cases were not typed, an independent report detected ceftriaxone resistance as a major risk factor for mortality due to IPD in which most isolates (94.1%) belonged to serotypes covered in PCV13.³⁸ We previously reported small but significant increases in percent susceptibility to clindamycin between 2011 and 2015,³⁹ although this trend did not continue through 2020 (Table 1). Differences between our prior report and now indicate that the epidemiology of pneumococcal resistance in Canada continues to change.

Emergence of MDR serotypes remains a problem of international concern as these isolates are, by definition, difficult to treat. We observed no significant change in the proportion of MDR isolates from 2011 (8.5%) to 2020 (9.4%) despite a statistically significant decrease between 2011 and 2015 (2.5%), followed by a statistically significant increase between 2016 and 2022 (5.5%) (Figure 1). The recent trend upwards could be associated with clonal expansion of MDR lineages consisting of non-PCV-serotypes, as recently observed in France (serotype 24F) and the United States (serotype 35B).^{40,41} Emergence of MDR replacement serotypes highlights a major limitation in the utility of serotype-specific vaccines.

We observed age-associated trends in antimicrobial resistance to therapies including clarithromycin, doxycycline and chloramphenicol, but not penicillin (Table 3). Generally, rates of resistance were highest among patients younger than 18 or older than 64 years of age, similar to prior observations.²⁴⁻²⁷ We also observed geographical trends in rates of antimicrobial resistance; however, this study was limited by under-representation of British Columbia and Alberta, two provinces that do not participate in the SAVE study, possibly affecting the regional analyses included here. Further, trends between resistance to penicillin or clarithromycin and other antimicrobials across classes highlights the propensity for these phenotypes to associate and potentially give rise to strains that are difficult to treat, especially in populations at risk (the young and elderly). However, given the large isolate collection studied, statistical significance may not necessarily imply clinical or public health significance in some analyses with differences of <2% resulting in statistical significance (e.g. percent resistance values for doxycycline stratified by patient age).

In summary, the data presented here show that pneumococcal conjugate vaccines have affected the prevalence of antimicrobial-resistant invasive pneumococcal isolates. The mechanism by which this has occurred may involve reduced occurrence of symptomatic disease and the need for therapeutic (antimicrobial) intervention, thereby reducing selection for resistant pneumococci. Antimicrobial stewardship practices and ongoing surveillance are required to monitor resistance according to MIC results interpreted using standardized breakpoints and MIC distributions themselves. Equally important is careful consideration of factors associated with resistance, so that small or large annual differences in susceptibility are not under- or over-estimated. New formulations of pneumococcal conjugate vaccines should continue to be developed to combat the emergence of antimicrobial-resistant replacement serotypes.

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Disclaimer

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