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Trends in Antimicrobial Susceptibility among Invasive Isolates of *Streptococcus pneumoniae* in Canada from 2011 to 2015: The SAVE Study

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ABSTRACT

Background: Invasive pneumococcal infections continue to occur despite the use of pneumococcal vaccines. Current antimicrobial susceptibility testing surveillance data is important to clinicians, antimicrobial stewardship programs, infection control practitioners, antimicrobial formulary committees, clinical laboratory scientists, academic scientists involved in drug discovery, governments, and the pharmaceutical industry.

Methods: The SAVE study is an annual, national surveillance program that collects and characterizes invasive isolates of *Streptococcus pneumoniae* submitted by selected provincial public health and hospital laboratories across Canada. From 2011 to 2015, the SAVE study collected a total of 6,207 invasive isolates of *S. pneumoniae*; annual isolate counts (per year) were: 1,379 (2011), 1,285 (2012), 1,138 (2013), 1,210 (2014), and 1,195 (2015). Isolates were tested against a panel of antimicrobial agents using the standard CLSI broth microdilution method (M07-A10, 2015). MICs were interpreted using current CLSI M100-S26 (2016) MIC breakpoints. Annual antimicrobial susceptibility rates for each antimicrobial agent were assessed for statistically significant changes (P <0.05) using the Chi Square test.

Results: The annual percent susceptible rates and MIC_{90} results for 14 antimicrobial agents are shown in Table 1. The rate of multidrug resistance (MDR, resistance to ≥3 different antimicrobial agent classes [penicillin resistance defined as MIC ≥2 mg/L]) decreased significantly (P=0.004) from 2011 to 2015: MDR rates were 8.6% in 2011, 6.8% in 2012, 6.0% in 2013, 4.1% in 2014, and 5.6% in 2015.

Conclusion: From 2011 to 2015, small (1.2-4.7%) but significant increases (penicillin, ceftriaxone [non-meningitis MIC breakpoints], and clindamycin) or differences (levofloxacin, and chloramphenicol) (P<0.05) in percent susceptibility for five antimicrobial agents, and in the rate of MDR, were observed for invasive isolates of *S. pneumoniae* in Canada; susceptibility rates for all other antimicrobial agents tested remained unchanged (P>0.05) over this 5-year period.

BACKGROUND

Invasive pneumococcal infections lead to considerable patient morbidity and mortality, particularly among the very young (<5 years), the elderly (≥65 years), and immunocompromised individuals. Vaccination is a primary means of preventing invasive pneumococcal infection. Despite the availability and use of pneumococcal vaccines in Canada, invasive infections continue to occur. Therefore, ongoing surveillance data that reports current antimicrobial agent susceptibilities and trends over time for invasive pneumococcal infections are important to clinicians prescribing empiric therapy for their patients with invasive pneumococcal infections as well as to antimicrobial stewardship programs, infection control practitioners, antimicrobial formulary committees, clinical laboratory scientists, academic scientists involved in drug discovery, governments, and the pharmaceutical industry.

There is strong evidence that the spread of penicillin-, macrolide-, fluoroquinolone-, and multidrug-resistant *Streptococcus pneumoniae* is driven by dissemination of successful clones (1).

The SAVE study is an annual, national surveillance program that collects and characterizes invasive isolates of *S. pneumoniae* submitted by selected provincial public health and hospital laboratories across Canada. In the current study, invasive isolates of *S. pneumoniae* collected from 2011 to 2015 by the SAVE study were tested for their susceptibilities to a panel of 14 antimicrobial agents using the standard CLSI broth microdilution method (2).

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MATERIALS & METHODS

Bacterial isolates. From January 2011 to December 2015, *S. pneumoniae* isolated from sterile body sites by participating Canadian provincial public health and hospital laboratories were forwarded to the Public Health Agency of Canada-National Microbiology Laboratory (PHAC-NML) in Winnipeg, Canada. As part of an ongoing collaboration between the Canadian Antimicrobial Resistance Alliance (CARA) and PHAC-NML, PHAC-NML forwarded their collection of invasive isolates of *S. pneumoniae* isolates from laboratories in eight of the ten Canadian provinces (Saskatchewan, Manitoba, Ontario, Quebec, New Brunswick, Nova Scotia, Prince Edward Island, and Newfoundland and Labrador) to CARA. In total, 6,207 invasive isolates of *S. pneumoniae* collected as part of SAVE study between 2011 and 2015 were forward to the CARA for antimicrobial susceptibility testing. The annual numbers of isolates were: 1,379 isolates from 2011, 1,285 from 2012, 1,138 from 2013, 1,210 from 2014, and 1,195 from 2015.

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was performed at the Winnipeg Health Sciences Centre (Winnipeg, Manitoba, Canada) using the CLSI standard broth microdilution method (2, 3) with custom-designed, in-house prepared, 96-well microtitre panels containing doubling-dilutions of antimicrobial agents in cation-adjusted Mueller-Hinton broth supplemented with 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 (3). Multidrug-resistant (MDR) was defined as resistance to three or more classes of antimicrobial agents (penicillin resistance was defined using the CLSI breakpoint for oral penicillin V, a MIC ≥2 mg/L).

Statistical analysis. Annual antimicrobial susceptibility rates for each antimicrobial agent were assessed for statistically significant differences (P <0.05) using the Chi Square test.

CONCLUSIONS

- From 2011 to 2015, diminutive but significant increases (P<0.05) in percent susceptibility for penicillin (all MIC breakpoints) (1.8-4.7%), clindamycin (3.7%), and ceftriaxone (non-meningitis MIC breakpoints) (1.2%) and differences (P<0.05) in percent susceptibility for chloramphenicol (2.7%) and levofloxacin (1.1%) were observed for invasive isolates of *S. pneumoniae* in Canada; susceptibility rates for all other antimicrobial agents tested (ceftriaxone [meningitis MIC breakpoints], cefuroxime, clarithromycin, telithromycin, moxifloxacin, linezolid, trimethoprim-sulfamethoxazole, doxycycline, tigecycline, and vancomycin) remained unchanged (P>0.05) over that time period (Table 1).
- 71.4% (4,287/6,001) of all isolates of invasive *S. pneumoniae* from 2011 to 2015 were pan-susceptible to the panel of 14 antimicrobial agents tested. 62.1% (1,065/1,714) of isolates demonstrating resistance were resistant to only a single antimicrobial agent. Only 6.3% (379/6,001) of all isolates tested demonstrated a MDR phenotype (Table 2).
- The rate of MDR (resistance to ≥3 antimicrobial classes [penicillin resistance defined as MIC ≥2 mg/L]) among invasive isolates of *S. pneumoniae* decreased significantly (P=0.004) from 2011 to 2015. MDR rates were 8.6% in 2011, 6.8% in 2012, 6.0% in 2013, 4.1% in 2014, and 5.6% in 2015 (Table 2).
- The most common MDR phenotype in invasive isolates of *S. pneumoniae* was concurrent resistance to clarithromycin, doxycycline, and clindamycin (Table 2).
- 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).
- Rates of antimicrobial resistance in invasive isolates of *S. pneumoniae* were not associated with patient age or gender but were associated with geographic location in Canada (Table 3).
- Penicillin resistance among invasive isolates of *S. pneumoniae* was associated with resistance to clarithromycin, doxycycline, trimethoprim-sulfamethoxazole, chloramphenicol, and clindamycin (Table 3).
- We conclude that in vitro susceptibilities of invasive isolates of *S. pneumoniae* in Canada remained constant or increased (penicillin, clindamycin, ceftriaxone [non-meningitis MIC breakpoints]) from 2011 to 2015. Our observations are likely the result of pneumococcal vaccine use in Canada and demonstrate that conjugate vaccine use may serve as one measure to lower antimicrobial resistance among invasive isolates of *S. pneumoniae*.

REFERENCES

1. Nichol KA, Adam HJ, Karlowsky JA, *et al.* Increasing genetic relatedness of ciprofloxacin-resistant *Streptococcus pneumoniae* isolated in Canada from 1997 to 2005. *Antimicrob Agents Chemother* 2008; 52: 1190-4.
2. Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically – Tenth Edition: Approved Standard M07-A10.* Wayne, PA, USA, 2015.
3. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Twenty-sixth Informational Supplement M100-S26.* Wayne, PA, USA, 2016.

RESULTS

Table 1. Annual percent susceptible rates from 2011 to 2015 for 14 antimicrobial agents tested against invasive isolates of *S. pneumoniae* cultured from Canadian patients

	2011	2012	2013	2014	2015	
Antimicrobial agent	% susceptible/MIC_{90} (mg/L)					*P*
Penicillin (IV, nonmeningitis)	97.3/0.12	98.1/0.12	99.0/0.12	99.1/0.12	99.0/0.12	<0.001
Penicillin (IV, meningitis)	86.3/0.12	89.1/0.12	89.8/0.12	91.0/0.06	89.5/0.12	0.003
Penicillin (oral, penicillin V)	86.3/0.12	89.1/0.12	89.8/0.12	91.0/0.06	89.5/0.12	0.003
Ceftriaxone (nonmeningitis)	98.6/≤0.12	99.2/≤0.12	99.3/≤0.12	99.8/≤0.12	99.7/≤0.12	<0.001
Ceftriaxone (meningitis)	95.8/≤0.12	96.5/≤0.12	96.6/≤0.12	97.7/≤0.12	97.3/≤0.12	0.062
Cefuroxime	94.7/≤0.25	95.9/≤0.25	95.2/≤0.25	95.1/≤0.25	94.0/≤0.25	0.281
Clarithromycin	76.9/8	74.2/4	72.5/4	76.5/2	74.9/2	0.078
Clindamycin	90.7/≤0.12	93.5/≤0.12	93.1/≤0.12	94.4/≤0.12	93.8/≤0.12	0.003
Telithromycin	99.9/0.12	100/0.12	100/0.12	100/0.06	100/0.12	0.999
Levofloxacin	99.6/1	99.3/1	100/2	98.9/1	99.7/1	0.012
Moxifloxacin	99.6/0.25	99.4/0.25	99.5/0.25	99.0/0.25	99.7/0.12	0.249
Linezolid	100/1	100/2	100/2	100/1	100/1	1
Trimethoprim-sulfamethoxazole	87.2/1	88.1/1	86.1/1	89.5/1	87.4/1	0.134
Doxycycline	88.5/2	89.1/1	88.9/1	90.7/≤0.25	90.2/≤0.25	0.342
Tigecycline	100/0.03	100/0.03	100/0.03	100/0.03	100/0.03	1
Chloramphenicol	99.0/4	97.6/4	99.0/4	96.3/4	99.0/4	<0.001
Vancomycin	100/0.5	100/0.5	100/0.25	100/0.25	100/0.25	1

Table 2. Resistance to one or more antimicrobial agents among 6,001 invasive isolates of *S. pneumoniae* cultured from Canadian patients from 2011 to 2015 (cumulative data)

		Percent of isolates (n) resistant to the indicated antimicrobial agent						
Number of antimicrobial agents to which isolates were resistant [a,b]	% of total isolates tested (n)	Penicillin	Clarithromycin	Doxycycline	Levofloxacin	SXT[c]	Chloramphenicol	Clindamycin
0	71.4 (4,287)	-	-	-	-	-	-	-
1	17.8 (1,065)	0.6 (6)	77.1 (821)	8.5 (91)	1.9 (20)	9.6 (102)	2.1 (22)	0.3 (3)
2	4.5 (270)	5.9 (16)	75.6 (204)	47.8 (129)	1.9 (5)	34.8 (94)	16.7 (45)	17.4 (47)
3[d]	3.1 (187)	9.1 (17)	96.8 (181)	89.8 (168)	1.1 (2)	12.3 (25)	6.4 (12)	84.5 (158)
4[d,e]	1.1 (66)	45.5 (30)	100 (66)	97.0 (64)	4.5 (3)	50.0 (33)	2.7 (15)	80.3 (53)
5[d,e]	1.9 (116)	98.3 (114)	100 (116)	100 (116)	0.9 (1)	96.6 (112)	5.2 (6)	99.1 (115)
6[d,e]	0.2 (9)	100 (9)	100 (9)	100 (9)	11.1 (1)	100 (9)	88.9 (8)	100 (9)
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 chloramphenicol, clarithromycin, clindamycin, doxycycline, levofloxacin, penicillin (oral MIC breakpoints), and trimethoprim-sulfamethoxazole.
[b] The data in this table included only isolates for which complete antimicrobial susceptibility testing data was available for all 7 antimicrobial agents (n=6,001).
[c] SXT, trimethoprim-sulfamethoxazole.
[d] Multidrug-resistant (MDR) isolates. MDR was defined as resistance to three or more of the seven antimicrobial classes analyzed. 6.3% (379/6,001) of isolates were MDR.
[e] The % prevalence of MDR isolates (n/total n) by year was 8.6% (117/1,362) in 2011, 6.8 % (84/1,230) in 2012, 6.0% (66/1,099) in 2013, 4.1% (48/1,159) in 2014, 5.6% (64/1,151) in 2015 (P=0.0001).

Table 3. Relative associations between resistance to six[a] antimicrobial agents and the patient demographic/isolate factors of age, gender, invasive isolate specimen source, and penicillin MIC interpretative category (cumulative data, 2011-2015) between invasive isolates of *S. pneumoniae*

Patient demographic/isolate factor	n of isolates associated with risk factor	Penicillin		Clarithromycin		Doxycycline		SXT[b]		Chloramphenicol		Clindamycin	
		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*	n (%) of resistant isolates	*P*
All isolates	6,001	193 (3.2%)	–	1398 (23.3%)	–	578 (9.6%)	–	374 (6.2%)	–	109 (1.8%)	–	386 (6.4%)	–
Patient age, years[c]			0.067		0.322		0.088		0.35		0.011		0.004
<18	851	37 (4.3%)		208 (24.4%)		81 (9.5%)		62 (7.3%)		5 (0.6%)		65 (7.6%)	
18-64[a]	2,788	77 (2.8%)		628 (22.5%)		246 (8.8%)		166 (6.0%)		54 (1.9%)		149 (5.3%)	
>64	2,231	73 (3.3%)		537 (24.1%)		238 (10.7%)		135 (6.1%)		49 (2.2%)		165 (7.4%)	
Gender[d]			0.088		0.051		0.695		0.404		0.661		0.831
Female	2,660	97 (3.6%)		659 (24.8%)		263 (9.9%)		158 (5.9%)		51 (1.9%)		175 (6.6%)	
Male	3,121	89 (2.9%)		705 (22.6%)		299 (9.6%)		202 (6.5%)		55 (1.8%)		201 (6.4%)	
Geographic region			0.001		<0.001		<0.001		0.007		<0.001		<0.001
Western Canada[e]	1,321	53 (4.0%)		336 (25.4%)		89 (6.7%)		102 (7.7%)		12 (0.9%)		67 (5.1%)	
Ontario	3,163	81 (2.6%)		700 (22.1%)		313 (9.9%)		169 (5.3%)		73 (2.3%)		170 (5.4%)	
Quebec	789	22 (2.8%)		184 (23.3%)		101 (12.8%)		47 (6.0%)		18 (2.3%)		102 (12.9%)	
Eastern Canada[f]	728	37 (5.1%)		250 (24.5%)		75 (10.3%)		56 (7.7%)		6 (0.8%)		47 (6.5%)	
Penicillin MIC interpretative category[g]			–		<0.001		<0.001		<0.001		<0.001		<0.001
Susceptible (≤0.06 mg/L)	5,344	–		991 (18.5%)		205 (3.8%)		131 (2.5%)		91 (1.7%)		115 (2.2%)	
Intermediate (0.12-1 mg/L)	464	–		233 (50.2%)		213 (45.9%)		86 (18.5%)		5 (1.1%)		130 (28.0%)	
Resistant (≥2 mg/L)	193	–		174 (90.2%)		160 (82.9%)		157 (81.3%)		13 (6.7%)		141 (73.1%)	

[a] Too few isolates (<100) resistant to ceftriaxone, telithromycin, levofloxacin, moxifloxacin, linezolid, tigecycline, or vancomycin were identified and, therefore, these agents were excluded from this analysis.
[b] SXT, trimethoprim-sulfamethoxazole.
[c] 131 isolates with unknown patient age.
[d] 220 isolates with unknown gender.
[e] Western Canada included isolates from Manitoba and Saskatchewan.
[f] 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.

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