

Antimicrobial Resistance among *Streptococcus pneumoniae* Collected from Respiratory Cultures in Canadian Hospitals: CANWARD 2007-2017

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Introduction

Streptococcus pneumoniae is a Gram-positive pathogen responsible for a number of both noninvasive (pneumonia, otitis media) and invasive (meningitis, bacteremia) manifestations of disease (1). Over 90 different capsular types of *S. pneumoniae* have currently been described; these serotypes differ in their ability to invade the bloodstream based on their ability to illicit an immune response, resist phagocytosis and avoid complement (2). For this reason, invasive and noninvasive serotypes of *S. pneumoniae* tend to differ.

Episodes of invasive pneumococcal disease are often transient; however, pneumococcal carriage is frequently long-term. Due to this lengthy occupation of the nasopharynx, these noninvasive serotypes are exposed to prolonged antimicrobial pressure, which can lead to the selection of antimicrobial resistant strains. Studies have shown that there is a correlation between the frequency of serotype isolation from the nasopharynx and the likelihood of antimicrobial resistance².

The purpose of this study was to describe the trends in antimicrobial resistance of *S. pneumoniae* from respiratory cultures in Canada between 2007 and 2017.

Materials and Methods

Isolate Collection

S. pneumoniae isolates from respiratory cultures were collected as a part of the CANWARD study from 2007 to 2017, inclusive. In brief, tertiary-care medical centres were asked to submit clinically significant isolates (consecutive, one per patient per infection site) from both inpatients and outpatients attending hospital clinics, emergency rooms, surgical/medical wards and intensive care units. Centres submitted their first 100 respiratory pathogens of the year. Isolates were shipped to the coordinating laboratory (Health Sciences Centre, Winnipeg, Canada) where they were subcultured onto appropriate media and stocked in skim milk at -80°C.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed on 1782 respiratory isolates of *S. pneumoniae* using custom-designed, in-house produced broth microdilution panels following the methodology and quality control described by the Clinical and Laboratory Standards Institute (CLSI) (3). Minimum inhibitory concentrations were interpreted using CLSI criteria (4).

Multidrug resistance (MDR) was defined as resistance to ≥3 antimicrobial classes (penicillin MIC ≥ 2 µg/mL).

Serotyping

Serotyping was performed using the Quellung reaction using pool, group, type and factor commercial antisera (Statens Serum Institute, Copenhagen, Denmark) and supplementary molecular serotyping was performed with the US Centre for Disease Control's multiplex PCR (<http://www.cdc.gov/ncidod/biotech/strep/pcr.htm>). Isolates for which a serotype was not determined by PCR and a Quellung reaction was not observed were confirmed as *S. pneumoniae* by *spoB* gene sequencing.

Statistical Analysis

Trends in serotype distribution between 2007 and 2017 were assessed for statistical significance using the Cochran-Armitage trend test.

Results

Demographics

Of the 1782 *S. pneumoniae* respiratory isolates collected between 2007 and 2017 from Canadian hospitals, 1104 (62%) were from males and 678 (38%) were from females. By age, 245 (14%), 909 (51%) and 628 (35%) were isolated from individuals ≤17 years, 18-64 years and ≥65 years, respectively.

Table 1. Serotype (ST) Distribution of *S. pneumoniae* Collected from Respiratory Specimens

ST	N	% of Isolates	ST	N	% of Isolates	ST	N	% of Isolates	ST	N	% of Isolates	ST	N	% of Isolates
3	144	8.3%	9N	49	2.8%	34	29	1.7%	12F	12	0.7%	17A	2	0.1%
11A	118	6.8%	16F	48	2.8%	9V	25	1.4%	5	10	0.6%	28F	1	0.1%
19A	115	6.6%	15C	45	2.6%	10A	24	1.4%	38	9	0.5%	11D	1	0.1%
22F	101	5.8%	6A	43	2.5%	20	22	1.3%	28A	8	0.5%	18B	1	0.1%
23A	90	5.2%	35F	41	2.3%	4	22	1.3%	13	7	0.4%	6D	1	0.1%
19F	85	4.9%	33F	40	2.3%	8	22	1.3%	35A	6	0.3%	10F	1	0.1%
6C	74	4.2%	15B	40	2.3%	21	21	1.2%	11B	4	0.2%	33A	1	0.1%
NT	69	4.0%	23F	40	2.3%	7F	19	1.1%	29	3	0.2%	41F	1	0.1%
23B	68	3.9%	31	33	1.9%	7C	18	1.0%	35C	3	0.2%			
15A	66	3.8%	17F	32	1.8%	18C	18	1.0%	37	3	0.2%			
35B	63	3.6%	6B	30	1.7%	14	15	0.9%	22A	2	0.1%			

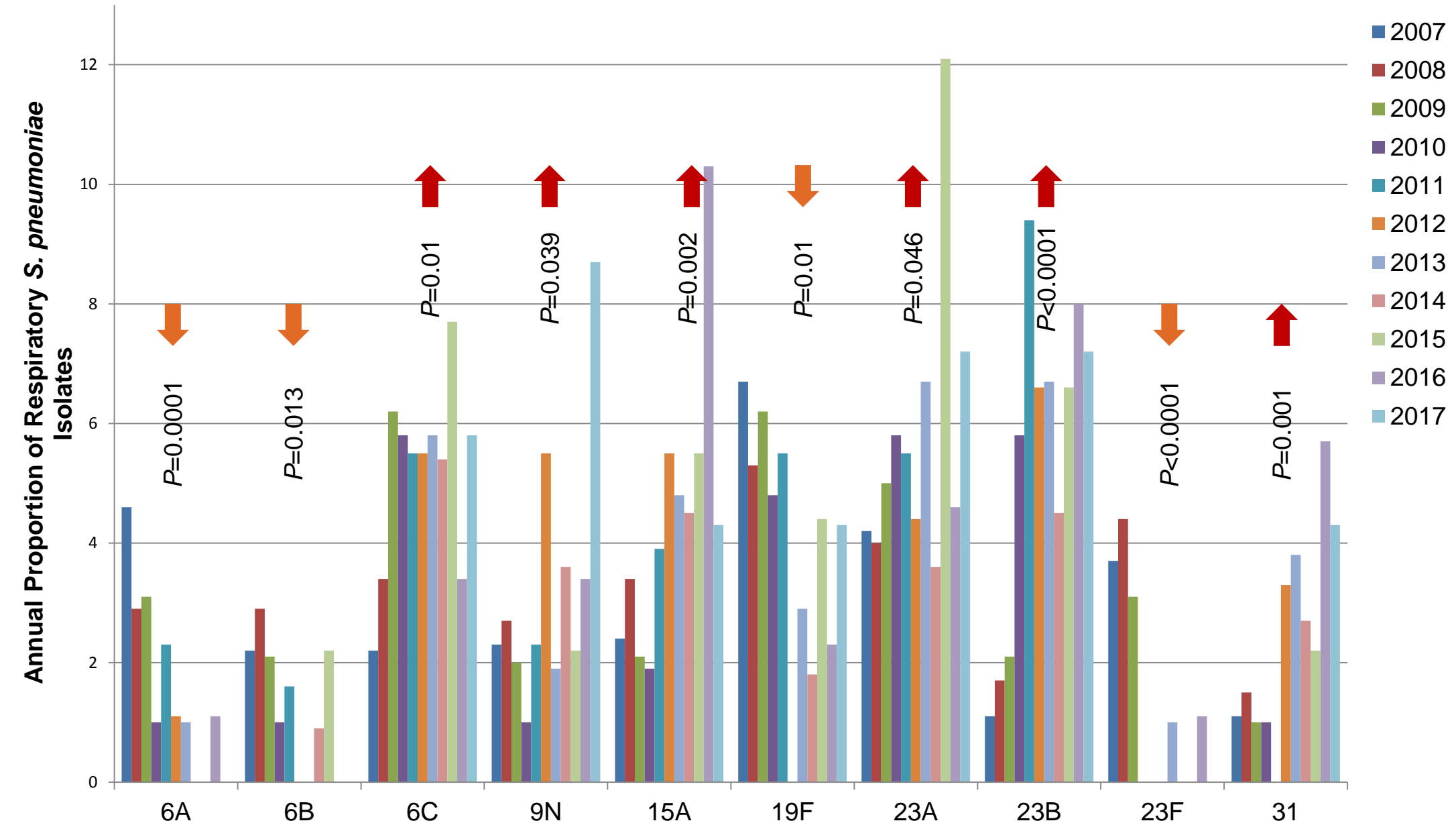


Figure 1. Distribution of Serotypes (Study N≥30) Demonstrating a Statistically Significant Change over Time
↑, increasing trend in the annual proportion of the serotype; ↓, decreasing trend in the annual proportion of the serotype
For serotypes with study N<30, significant increasing trends were observed for serotypes 21 (P=0.02) and 7C (P=0.04) while significant decreasing trends were observed for serotypes 4 (P=0.0004), 5 (P=0.004), 9V (P=0.0005), 13 (P=0.04) and 18C (P=0.002).

Table 2. Antimicrobial Susceptibilities of *S. pneumoniae* Collected from Respiratory Specimens (N*=1684)

Antimicrobial Agent	%S	%I	%R	MIC ₅₀	MIC ₉₀
Ceftriaxone (non-meningitis)	99.2	0.5	0.2	≤0.12	0.12
Ceftriaxone (meningitis)	96.5	2.7	0.8	≤0.12	0.12
Clarithromycin	77.2	3.8	19	≤0.03	4
Clindamycin	91.5	0.6	7.9	≤0.12	≤0.12
Doxycycline	84.85	1.36	13.8	≤0.25	2
Levofloxacin	98.9	0.2	0.9	1	1
Penicillin (non-meningitis)	98.2	1.6	0.2	≤0.03	0.5
Penicillin (meningitis)	79.9	0	20.1	≤0.03	0.5
Penicillin (oral)	79.9	15.1	5	≤0.03	0.5
Trimethoprim-sulfamethoxazole	83.9	6.2	10	≤0.12	2
Vancomycin	100	N/A	N/A	≤0.25	0.25

*, number of isolates which grew in broth for susceptibility testing; N/A, interpretative categories do not exist

Table 3. Annual Rates of MDR in Respiratory Isolates of *S. pneumoniae*

	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	P-value, 07-17
% MDR	7.4%	10.0%	3.1%	7.7%	11.4%	11.3%	10.9%	12.5%	10.0%	11.1%	8.8%	NS

Table 4. MDR in Respiratory Isolates of *S. pneumoniae* by Serotype

Serotype	MDR Rate/Serotype	MDR Patterns	N
6A	2.5%	CLR, CD, DOX	1
6B	16.7%	CLR, CD, DOX CLR, DOX, SXT	4
6C	4.2%	CLR, CD, DOX	3
9V	17.6%	CLR, CD, DOX, PEN, SXT CLR, PEN, SXT	1
10A	4.3%	CLR, CD, DOX	1
11A	0.9%	CLR, CD, DOX	1
14	26.7%	CLR, CD, DOX, LEV CLR, CD, DOX, PEN CLR, CD, DOX, SXT	1
15A	53.2%	CLR, CD, DOX CLR, CD, DOX, PEN CLR, DOX, PEN CLR, DOX, SXT	28
19A	38.3%	CLR, CD, DOX CLR, CD, DOX, LEV, PEN, SXT CLR, CD, DOX, PEN, SXT CLR, CD, DOX, SXT CLR, CD, PEN, SXT CLR, DOX, PEN CLR, DOX, PEN, SXT	6
19F	24.7%	CLR, CD, DOX CLR, CD, DOX, PEN, SXT CLR, CD, DOX, SXT CLR, CD, PEN, SXT CLR, DOX, PEN CLR, DOX, SXT CLR, DOX, SXT	3
20	9.1%	CLR, CD, DOX CLR, CD, DOX, PEN	1
22F	2.0%	CLR, CD, DOX, SXT CLR, PEN, SXT	1
23A	1.1%	CLR, CD, DOX	1
23F	15.0%	CLR, CD, DOX, PEN, SXT CLR, DOX, PEN, SXT DOX, PEN, SXT	1
33F	5.0%	CLR, CD, DOX	2
35A	83.0%	CLR, DOX, SXT	5
35B	1.6%	CLR, CD, DOX, SXT	1
35C	66.7%	CLR, DOX, SXT	2
NT	28.6%	CD, DOX, SXT CLR, CD, DOX CLR, CD, DOX, LEV, SXT CLR, CD, DOX, SXT CLR, DOX, LEV, SXT CLR, DOX, SXT CLR, PEN, SXT	1
Total	9.1%		154

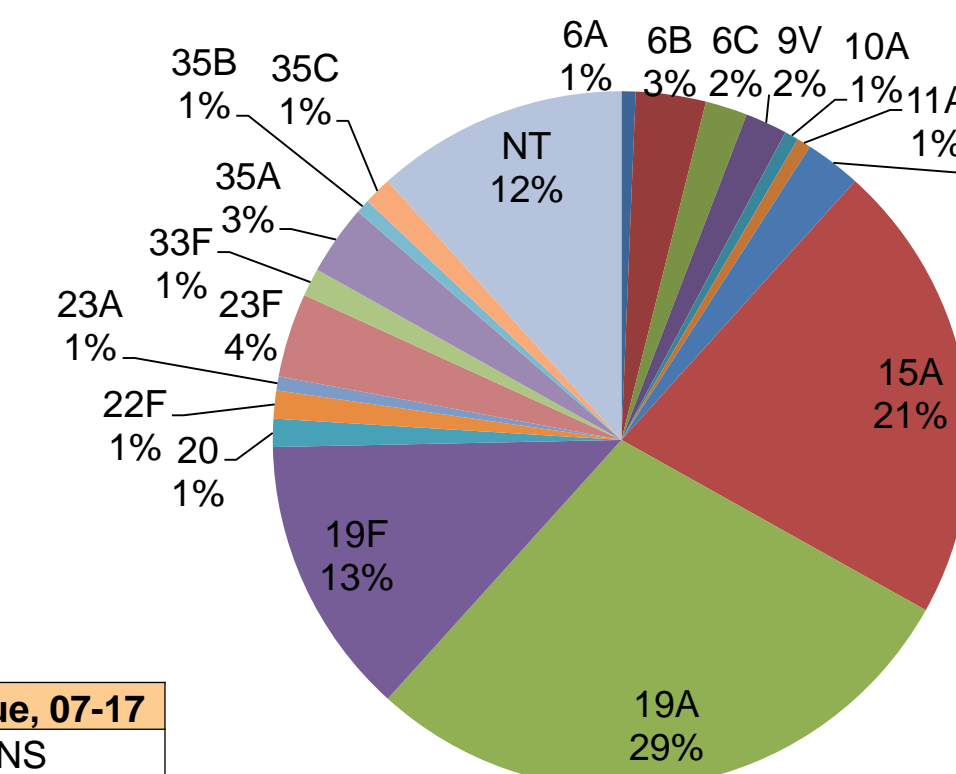


Figure 2. Distribution of MDR Serotypes in Respiratory Isolates of *S. pneumoniae* in Canada

Conclusions

1. Respiratory *S. pneumoniae* isolates were most frequently collected from adult males.
2. The top 10 most common serotypes of respiratory isolates of *S. pneumoniae* were: 3, 11A, 19A, 22F, 23A, 19F, 6C, NT, 23B and 15A.
3. Between 2007 and 2017, significant increases were observed in the annual proportion of serotypes 6C, 7C, 9N, 15A, 21, 23A, 23B and 31 while significant decreases were observed for serotypes 4, 5, 6A, 6B, 9V, 13, 18C, 19F and 23F.
4. The rate of MDR in respiratory isolates of *S. pneumoniae* in Canada between 2007 and 2017 was 9.1%. MDR was observed in 19 serotypes, with 19A, 15A, 19F and non-typeable strains predominating. No statistically significant change in the rate of MDR was noted over the course of the study.
5. The most common MDR pattern overall was resistance to clarithromycin, clindamycin and doxycycline. The most common serotype-MDR patterns were 15A resistant to clarithromycin, clindamycin and doxycycline and 19A resistant to clarithromycin, clindamycin, doxycycline, penicillin and trimethoprim-sulfamethoxazole.

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