

Trends in Antimicrobial Resistance Over Time among *Streptococcus pneumoniae* Collected from Blood Cultures in Canadian Hospitals: CANWARD 2007-2017

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Introduction

Streptococcus pneumoniae is a clinically relevant Gram-positive pathogen, frequently identified as the causative agent of a number of human diseases with varying clinical manifestations¹. Pneumococcal diseases commonly present as local respiratory tract infections such as otitis media, sinusitis and pneumonia¹. However, severe systemic manifestations of disease (bacteremia, meningitis) occur less commonly following bacterial penetration of the respiratory epithelium^{1,2,3}. Isolation of *S. pneumoniae* from a normally sterile clinical site is termed invasive pneumococcal disease (IPD)¹. Despite the widespread availability of pneumococcal vaccines, severe pneumococcal diseases remain a significant cause of global morbidity and mortality^{1,4}.

The key determinant in the development of IPD is the pneumococcal polysaccharide capsule, where increased capsular thickness is associated with increased systemic virulence². Encapsulation is essential for host immune evasion by shielding the pneumococcal cell from opsonophagocytosis^{1,2}. The pneumococcal capsule demonstrates vast serological diversity; to date, greater than 90 serotypes have been identified¹. Of the total, a subset of serotypes demonstrate increased invasive potential and are associated with the majority of IPD¹.

The purpose of the present study was to assess the trends in antimicrobial resistance in *S. pneumoniae* blood culture isolates collected in Canadian hospitals between 2007 and 2017.

Materials and Methods

Isolate Collection: From 2007-2017, inclusive, a total of 941 *S. pneumoniae* blood culture isolates were collected as part of the annual CANWARD surveillance study. In brief, tertiary-care hospitals from participating Canadian provinces were asked to submit the first 10 blood culture isolates collected per month for ten consecutive months. All *S. pneumoniae* isolates were identified at the originating center using established criteria and subsequently shipped to the coordinating laboratory (Health Sciences Center, Winnipeg, Canada) where they were subcultured onto appropriate media and stocked at -80°C.

Antimicrobial Susceptibility Testing: Antimicrobial susceptibility testing was performed on all isolates using in-house made broth microdilution panels, designed and tested in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines and quality control measures⁵. Minimum inhibitory concentration (MIC) values were interpreted using CLSI breakpoint criteria⁶. Multidrug (MDR) was defined as resistance to ≥3 antimicrobial classes (penicillin MIC ≥2 µg/mL).

Serotyping: Serotyping was performed by 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 PCR multiplex method (<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 rpoB gene sequencing.

Statistical Analysis: The Cochran-Armitage test was used to assess linear trends in serotype prevalence and MDR rates over time.

Results

Table 1. Serotype (ST) Distribution of *S. pneumoniae* Isolates Obtained from Blood Cultures, 2007 - 2017

ST	N	% of Isolates	ST	N	% of Isolates	ST	N	% of Isolates	ST	N	% of Isolates	ST	N	% of Isolates
19A	118	12.5	9N	32	3.4	15C	13	1.4	18C	7	0.7	7C	2	0.2
3	84	8.9	15A	30	3.2	17F	13	1.4	34	5	0.5	11B	1	0.1
22F	74	7.9	6C	27	2.9	6A	13	1.4	23F	5	0.5	11F	1	0.1
7F	55	5.8	23A	24	2.6	35B	12	1.3	33A	4	0.4	22A	1	0.1
12F	45	4.8	15B	21	2.2	35F	12	1.3	13	4	0.4	25F	1	0.1
4	43	4.6	19F	20	2.1	16F	11	1.2	NT	4	0.4	9A	1	0.1
11A	39	4.1	23B	19	2.0	38	10	1.1	28A	3	0.3	9L	1	0.1
5	36	3.8	9V	19	2.0	31	9	1.0	21	2	0.2	Total	941	
8	33	3.5	14	17	1.8	10A	8	0.9	29	2	0.2			
33F	33	3.5	20	17	1.8	6B	8	0.9	35A	2	0.2			

Demographics:

Of the 941 *S. pneumoniae* blood culture isolates for which gender demographic data was available, 416 (44.2%) were collected from females and 525 (55.8%) from males. By age, 174 (18.5%), 476 (50.6%) and 291 (30.9%) were isolated from individuals ≤17 years, 18-64 years and ≥65 years, respectively. The largest proportion of isolates were collected in Canadian emergency rooms (n=539, 57.3%), as compared to other specified inpatient and outpatient locations

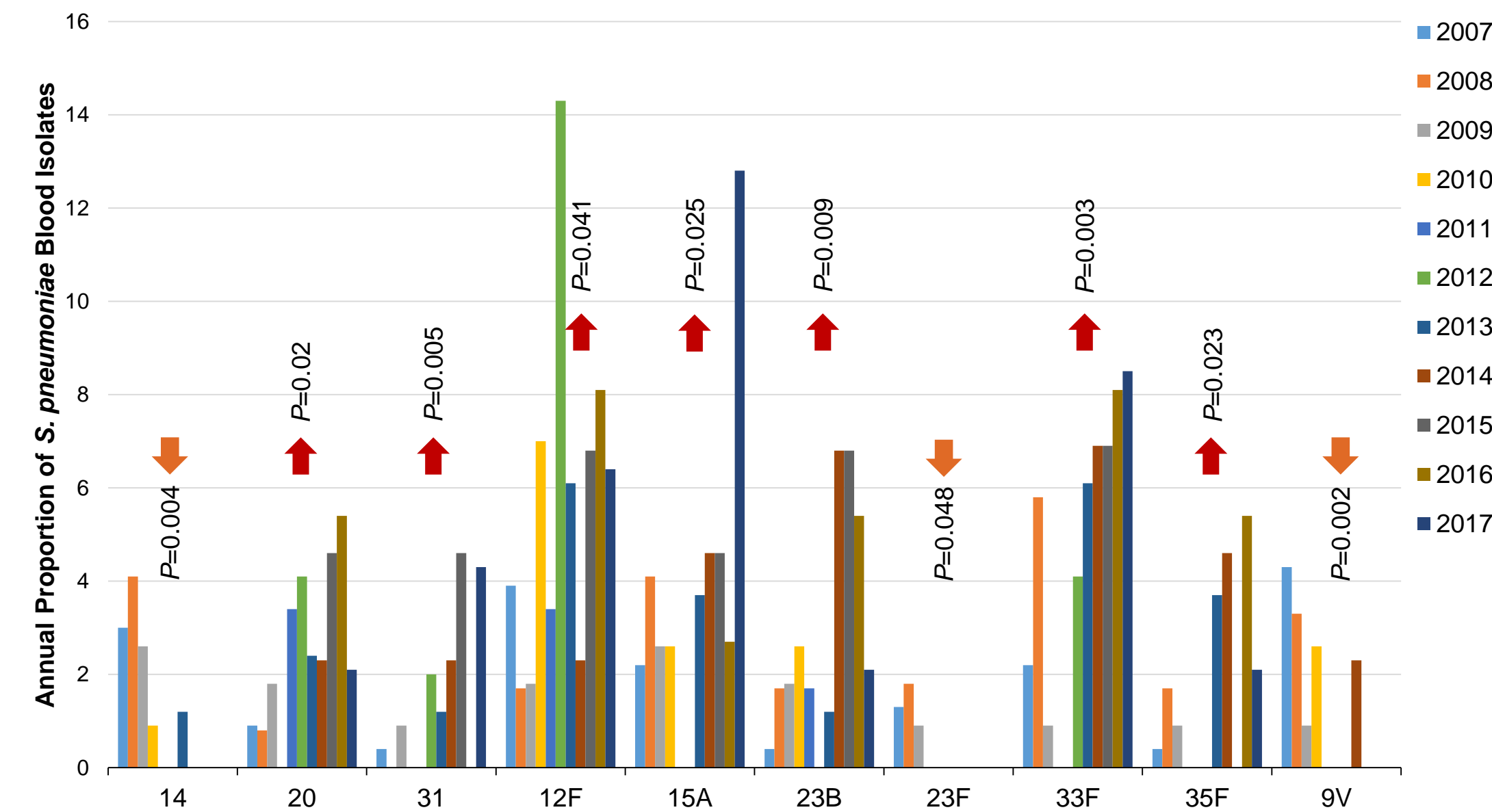


Figure 1. Distribution of *S. pneumoniae* Serotypes Obtained from Blood Cultures that Demonstrate a Statistically Significant Change Over Time, 2007-2017

↑, increasing trend in the annual proportion of the serotype; ↓, decreasing trend in the annual proportion of the serotype

Table 2. Antimicrobial Susceptibilities of *S. pneumoniae* Blood Culture Isolates, 2007 - 2017 (*N=895)

Antimicrobial Agent	%S	%I	%R	MIC ₅₀ (µg/mL)	MIC ₉₀ (µg/mL)
Ceftriaxone (non-meningitis)	99.8	0.2	0	≤0.12	≤0.12
Ceftriaxone (meningitis)	98.6	1.2	0.2	≤0.12	≤0.12
Clarithromycin	80.7	2.9	16.4	≤0.03	2
Clindamycin	95	0.4	4.6	≤0.12	≤0.12
Doxycycline	90.8	0.9	8.3	≤0.25	≤0.25
Levofloxacin	99.4	0.1	0.5	1	1
Penicillin (non-meningitis)	99.1	0.9	0	≤0.03	0.12
Penicillin (meningitis)	88.1	N/A	11.9	≤0.03	0.12
Penicillin (oral)	88.1	8.9	3	≤0.03	0.12
Trimethoprim-sulfamethoxazole	87	6.5	6.5	≤0.12	1
Vancomycin	100	N/A	N/A	0.25	0.25

*. Number of isolates for which antimicrobial susceptibility data is available; N/A, interpretive criteria does not exist

Multidrug Resistance:

Throughout the study period (2007-2017), no significant trends were observed in the MDR rate of *S. pneumoniae* blood culture isolates. MDR was noted in twelve pneumococcal serotypes, with 19A and 15A being most common (Table 3). The annual rates of MDR were 3.2%, 7.0%, 2.8%, 5.6%, 10.2%, 6.3%, 1.3%, 4.8%, 7.0%, 2.8% and 8.7% in 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016 and 2017, respectively.

Table 3. Multidrug Resistance in *S. pneumoniae* Blood Culture Isolates by Serotype, 2007 - 2017

Serotype	MDR		N
	Rate/Serotype	MDR Patterns	
19A	20.3%	CLR, CD, DOX	7
		CLR, CD, DOX, LEV, PEN, SXT	1
		CLR, CD, DOX, PEN, SXT	10
		CLR, CD, DOX, SXT	1
		CLR, DOX, PEN	2
		CLR, DOX, PEN, SXT	1
15A	33.3%	CLR, CD, DOX, SXT	1
		CLD, CD, DOX	8
		CLR, CD, DOX, PEN	1
14	5.9%	CLR, PEN, SXT	1
11A	2.6%	CLR, CD, DOX	1
15B	4.8%	CLR, CD, DOX	1
15C	7.7%	CLR, CD, DOX	1
22F	1.4%	CLR, CD, DOX, PEN, SXT	1
23F	20.0%	CLR, DOX, PEN, SXT	1
35A	50.0%	CLR, DOX, SXT	1
6C	3.7%	CLR, CD, DOX	1
9N	3.1%	CLR, DOX, SXT	1
9V	5.3%	CLR, CLD, DOX, SXT	1
Total	4.7%		44

CLR, clarithromycin; CD, clindamycin; DOX, doxycycline; LEV, levofloxacin; PEN, penicillin; SXT, trimethoprim-sulfamethoxazole

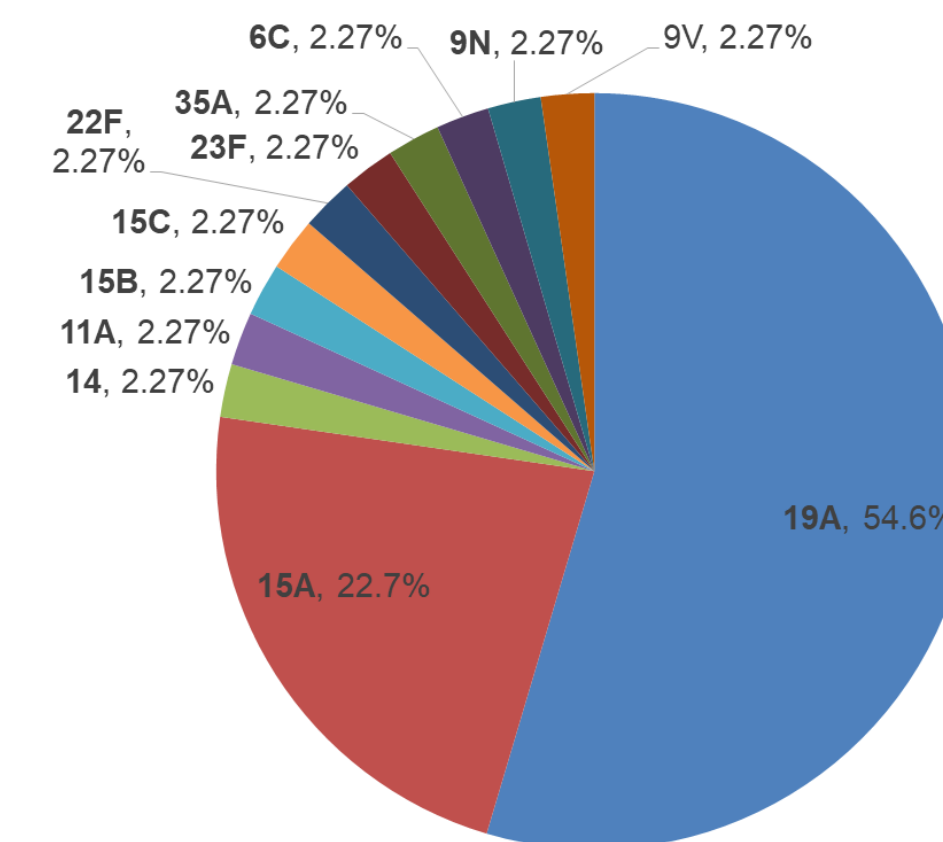


Figure 2. Distribution of MDR *S. pneumoniae* Serotypes Obtained from Blood Cultures, 2007-2017

Conclusions

- Blood culture isolates of *S. pneumoniae* were most frequently obtained from males and from individuals 18-64 years of age.
- The 10 most common *S. pneumoniae* serotypes were: 19A, 3, 22F, 7F, 12F, 4, 11A, 5, 8 and 33F.
- By trend analysis, statistically significant increases in the annual proportions were observed in serotypes 20, 31, 12F, 15A, 23B, 33F and 35F. Conversely, the annual proportions of serotypes 14, 23F and 9V decreased significantly over the course of the study.
- Between 2007 and 2017, the MDR rate in blood culture isolates of *S. pneumoniae* was 4.9%, with MDR being noted in twelve serotypes. Serotypes 19A and 15A most commonly demonstrated MDR, with rates of 20.3% and 33.3%, respectively. For all isolates, no significant trends in MDR were noted over time.
- Overall, the most frequently observed MDR phenotype in *S. pneumoniae* blood culture isolates was resistance to CLR, CD and DOX. Of the top two MDR serotypes, the most common MDR patterns were: serotype 19A resistant to CLR, CD, DOX, PEN and SXT and serotype 15A resistant to CLR, CD and DOX.

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