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Characterization of the 10 Most Common Serotypes Causing Invasive Pneumococcal Disease in Canada, 2011-2014

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ABSTRACT

Background: A significant proportion of invasive pneumococcal disease (IPD) in Canada is caused by a small number of *Streptococcus pneumoniae* serotypes. The goal of this study was to characterize the ten most common serotypes causing IPD in Canada in the years 2011-2014.

Methods: In collaboration between CARA and the National Microbiology Laboratory, 5,012 *S. pneumoniae* isolates causing IPD were collected from across Canada from January 2011 to December 2014, inclusive. Serotyping was performed by the Quellung reaction using commercial antisera (Statens Serum Institute, Copenhagen, DK). Antimicrobial susceptibility testing was performed using CLSI methods. All top ten serotype isolates were characterized for putative virulence by PCR to detect pili. Ten isolates of each serotype per year (40 of each serotype, 400 total isolates) were characterized for genetic relatedness by PFGE and MLST. MLST sequence types (STs) were compared to the Pneumococcal Molecular Epidemiology Network (PMEN) database.

Results: The top ten *S. pneumoniae* serotypes (listed in the table below) accounted for 63% of the total isolates collected in 2011-14.

Serotype (n, %)	Clonality	Antimicrobial Resistance Patterns	Piliation
7F (655, 13.1%)	PMEN clone ST191 (Netherlands^{7F}-39)	Susceptible	PI-2
19A (508, 10.1%)	Variants of PMEN clone ST236 (Taiwain^{19F}-14)	CLR, CLD, DOX, PEN, SXT	PI-1, PI-2
	PMEN clone ST199 (Netherlands^{15B}-37)	Susceptible	-
	ST695	CLR	PI-1
22F (492, 9.8%)	ST433	Susceptible	-
3 (395, 7.9%)	PMEN clone ST180 (Netherlands^{3}-31)	Susceptible	-
12F (224, 4.5%)	PMEN clone ST218 (Denmark^{12F}-34)	CLR	-
11A (219, 4.4%)	ST62	CLR	PI-2
8 (181, 3.6%)	PMEN clone ST53 (Netherlands^{8}-33)	Susceptible	-
	ST1268/ST1480	Susceptible	-
15A (177, 3.5%)	PMEN clone ST63 (Sweden^{15A}-25)	CLR, CLD, DOX	-
	ST58 and variants	Susceptible	PI-1
9N (176, 3.5%)	Variants of PMEN clone ST67 (Tennessee^{14}-18)	Susceptible	-
6C (165, 3.3%)	ST5241	CLR, CLD, DOX	-
	13 Unique STs	-	-

CLR, clarithromycin; CLD, clindamycin; DOX, doxycycline; PEN, penicillin; SXT, trimethoprim-sulfamethoxazole.

Conclusion: The ten most common serotypes causing IPD in Canada account for 63% of all isolates collected in 2011-14. Many of the ten most common serotypes demonstrate clonal spread, however they are susceptible to antimicrobial treatments. Multi-drug resistance was observed in particular clones of serotypes 19A, 15A and 6C, while piliation was detected in serotypes 7F, 19A, 11A and 15A. Only serotype 19A related to PMEN clone Taiwan^{19F}-14 demonstrated both piliation and multi-drug resistance.

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BACKGROUND

Although there are over 90 currently identified pneumococcal serotypes, it is widely acknowledged that a small number of them account for the vast majority of invasive pneumococcal disease (IPD) [1]. Serotypes have different invasive capacities, based on their ability to illicit an immune response, resist phagocytosis and avoid complement [2]. The serotypes most commonly associated with IPD in the early 2000s were originally developed into the PCV-7 vaccine (serotypes 4, 6B, 9V, 14, 18C, 19F and 23F). However, serotype prevalence shifted after years of use of this vaccine; once prevalent PCV-7 serotypes drastically decreased as causes of IPD while other non-PCV-7 serotypes (such as 3 and 19A) increased. The introduction of this conjugate vaccine, and its successor PCV-13 in 2010, have resulted in large variations in the serotype distribution for IPD in Canada over the past number of years, including which serotypes are responsible for the majority of IPD cases.

The goal of this study was to characterize the antimicrobial resistance and molecular characteristics of the ten most common serotypes causing IPD in Canada in the years 2011-2014.

MATERIALS & METHODS

Isolate Collection

Invasive *S. pneumoniae* isolated from sterile sites were forwarded from Canadian Public Health Laboratories to the Public Health Agency of Canada – National Microbiology Laboratory. Serotyping of these isolates was performed using pool, group, type and factor specific commercial antisera (Statens Serum Institute, Copenhagen, Denmark). Through a collaboration between the Canadian Antimicrobial Resistance Alliance (CARA) and the Public Health Agency of Canada – National Microbiology Laboratory, these *S. pneumoniae* isolates were forwarded to CARA for further testing. A total of 5,012 isolates were sent to CARA from January 2011 to December 2014, inclusive.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using custom-designed, in-house prepared broth microdilution panels, in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines [3]. Quality control was performed using *S. pneumoniae* ATCC 49619. Minimum inhibitory concentrations (MICs) were interpreted using CLSI breakpoints [4], and multi-drug resistance (MDR) was defined as resistance to ≥3 antimicrobial classes (penicillin resistance MIC ≥2 μg/mL). Isolates resistant to ≥5 antimicrobial classes were defined as extensively drug-resistant (XDR).

Characterization of Top 10 Serotypes

Ten isolates of each of the ten most common serotypes per year (40 of each serotype, 400 total isolates) were characterized for genetic relatedness by pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST). PFGE was performed as previously described [5]; gels were analyzed using BioNumerics Software v3.5 (Applied Maths Inc, Austin, TX). MLST was performed using methods and primers previously described at http://pubmlst.org/spneumoniae. Resulting PFGE fingerprints and MLST sequence types (STs) were compared to the Pneumococcal Molecular Epidemiology Network (PMEN) clone database. STs were assigned to clonal complexes (CCs) where possible using eBURST software available on the MLST website. To assess putative virulence, PCR to determine the presence of pneumococcal pili was performed using previously described primers [6].

CONCLUSIONS

1. The ten most common serotypes isolated from Canadian hospitals in 2011-14 were 7F, 19A, 22F, 3, 12F, 11A, 8, 15A, 9N and 6C, accounting for 63% of all isolates collected in this time.
2. Serotypes 7F, 22F, 3, 8, 12F, 11A and 15A demonstrated clonal spread, generally displaying a smaller number of STs within a clonal complex; the presence of several international strains in Canada indicates the success of these clones. Serotypes 19A, 9N and 6C demonstrated increased ST diversity; this could result in the emergence of less common serotypes through diversification as opposed to clonal spread.
3. MDR was demonstrated by isolates of serotype 15A related to Sweden^{15A}-25, 19A related to Taiwan^{19F}-14 and 6C (ST5241).
4. PI-2 piliation was identified in all serotype 7F isolates (ST191) and roughly half of serotype 11A (ST62) isolates. Serotype 15A isolates unrelated to the MDR international strain demonstrated the presence of PI-1. Only serotype 19A isolates demonstrating MDR and relation to Taiwan^{19F}-14 demonstrated the presence of both PI-1 and PI-2 simultaneously.

RESULTS

Table 1. Antimicrobial susceptibilities and percent MDR demonstrated by the ten most common *S. pneumoniae* serotypes collected in Canada in 2011-2014.

Serotype (n)	Antimicrobial Susceptibilities (%)									
	CLR	CLD	CRO (M)	CRO (NM)	DOX	LEV	PEN (M)	PEN (NM)	SXT	% MDR
7F (645)	97.1	99.5	100	100	96.6	99.7	99.2	100	99.4	0.5
19A (505)	38.8	74.7	93.7	98.4	69.5	99.6	67.5	99.8	69.7	25.6
22F (490)	74.1	98.4	99.8	100	99.4	98.6	99.4	99.8	98.8	0.8
3 (364)	95.9	97.5	100	100	88.7	100	99.7	100	98.6	2.4
12F (224)	33.5	98.2	100	100	97.8	100	100	100	98.2	0.9
11A (218)	72.5	97.7	100	100	99.1	99.5	98.6	100	81.7	1.4
8 (180)	98.3	98.9	100	100	93.9	100	98.3	100	97.8	1.2
15A (143)	22.4	39.2	100	100	24.5	100	40.6	100	93.0	47.5
9N (176)	91.5	100	100	100	98.3	100	98.9	100	97.7	0
6C (164)	76.2	93.9	100	100	92.7	100	78.0	100	82.3	6.1

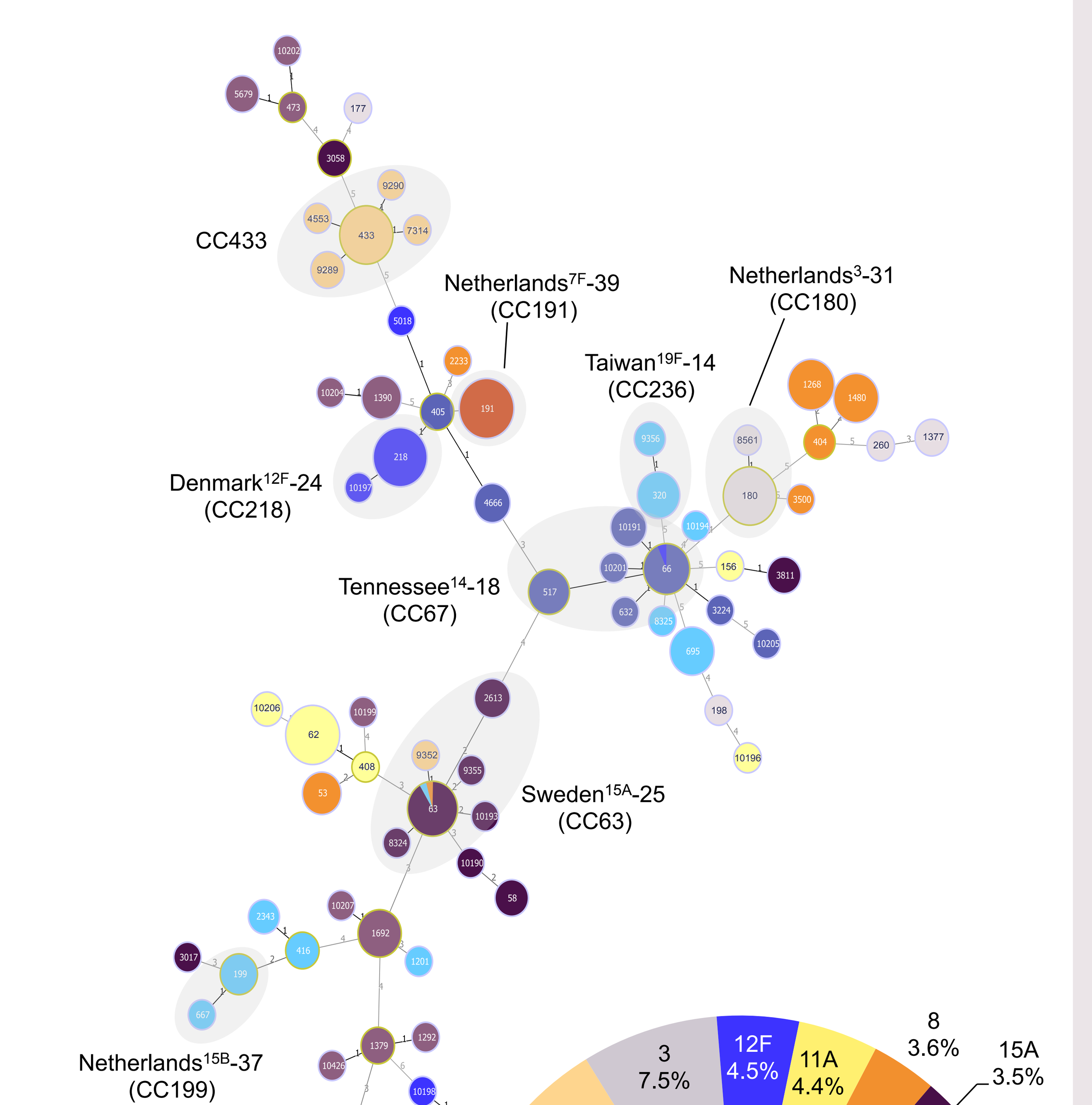
*, n for which complete susceptibility data available; CLR, clarithromycin; CLD, clindamycin; CRO, ceftriaxone; M, meningitis breakpoints; NM, nonmeningitis breakpoints; DOX, doxycycline; LEV, levofloxacin; PEN, penicillin; SXT, trimethoprim-sulfamethoxazole.

Table 2. MDR and XDR patterns demonstrated by the ten most common *S. pneumoniae* serotypes.

MDR/XDR Phenotype	Serotype (n)								
	3	6C	7F	8	11A	12F	15A	19A	22F
3 Classes:									
Chloramphenicol, Clarithromycin, Clindamycin					1				1
Chloramphenicol, Clarithromycin, Doxycycline							3		
Chloramphenicol, Clindamycin, Doxycycline	1			1					
Clarithromycin, Clindamycin, Doxycycline	2	9	2			2	66	18	1
Clarithromycin, Doxycycline, Penicillin							1	1	
Clarithromycin, Doxycycline, Trimethoprim-sulfamethoxazole			1					1	
Clarithromycin, Penicillin, Trimethoprim-sulfamethoxazole		1						4	
Doxycycline, Penicillin, Trimethoprim-sulfamethoxazole								3	
4 Classes:									
Chloramphenicol, Clarithromycin, Clindamycin, Doxycycline	6						3		
Clarithromycin, Clindamycin, Doxycycline, Levofloxacin								1	2
Clarithromycin, Clindamycin, Doxycycline, Penicillin							7	1	
Clarithromycin, Clindamycin, Doxycycline, Trimethoprim-sulfamethoxazole					2		1	10	
Clarithromycin, Clindamycin, Penicillin, Trimethoprim-sulfamethoxazole								1	
Clarithromycin, Doxycycline, Penicillin, Trimethoprim-sulfamethoxazole								4	
5 Classes:									
Chloramphenicol, Clarithromycin, Clindamycin, Doxycycline, Penicillin							3		
Chloramphenicol, Clarithromycin, Doxycycline, Penicillin, Trimethoprim-sulfamethoxazole								1	
Clarithromycin, Clindamycin, Doxycycline, Penicillin, Trimethoprim-sulfamethoxazole				1				77	
6 Classes:									
Chloramphenicol, Clarithromycin, Clindamycin, Doxycycline, Penicillin, Trimethoprim-sulfamethoxazole								5	
Clarithromycin, Clindamycin, Doxycycline, Levofloxacin, Penicillin, Trimethoprim-sulfamethoxazole								1	
Total Isolates	9	10	3	2	3	2	84	130	4
Percentage	2.4	6.1	0.5	1.2	1.4	0.9	47.5	25.6	0.8

Only particular clones of *S. pneumoniae* demonstrated the presence of a pilus. Variants related to ST58 (15A) and ST695 (19A) possessed PI-1. All isolates of ST191 (7F) and some ST62 (11A) displayed PI-2. Only isolates related to international clone ST236 (19A) demonstrated the presence of both pilus types.

Figure 1. Minimum spanning tree (generated by PHYLOViZ) of MLST sequence types demonstrated by the ten most common *S. pneumoniae* serotypes collected in Canada in 2011-2014. Serotype colours are identical to those listed in the pie chart in Figure 2.



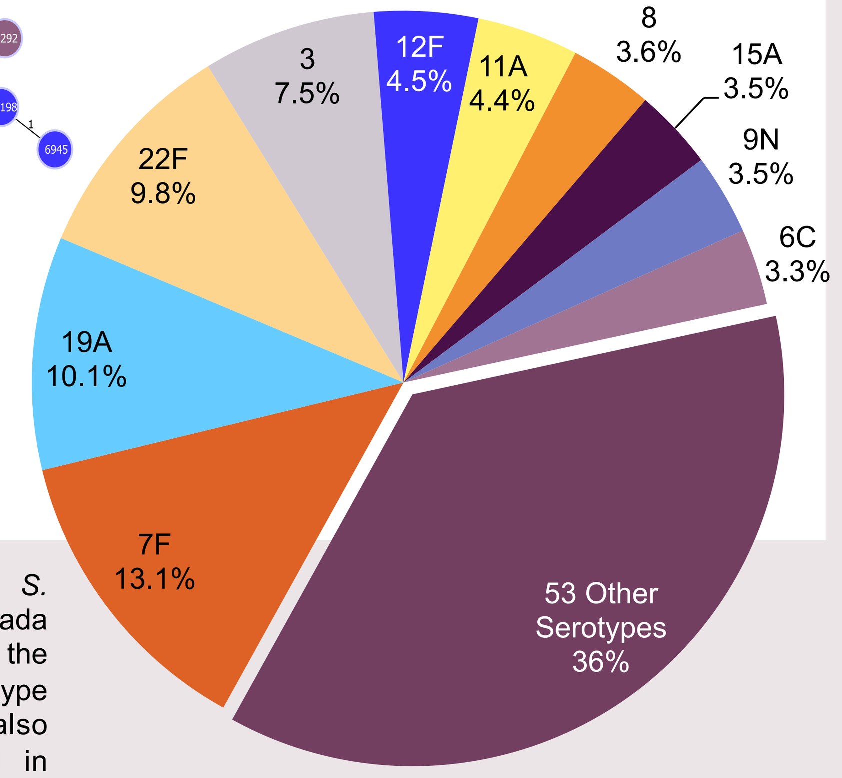


Figure 2. Proportion of the 5,012 *S. pneumoniae* isolates collected in Canada in 2011-2014 represented by each of the ten most common serotypes. Serotype colours used in the pie chart also represent the serotypes depicted in Figure 1.