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Characterization of Potentially Emerging *Streptococcus pneumoniae* (SPN) Non-Vaccine Serotypes (NVS) 15A, 22F, 33F and 35B in Canada, 2011-13

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REVISED ABSTRACT

Background: The proportion of SPN NVS circulating in Canada increased after the introduction of PCV-7 (2002-2005). A similar shift in serotypes is expected to occur post-PCV-13 introduction (2010); these potentially emerging NVS include 15A, 22F, 33F and 35B. The purpose of this study was to assess the antimicrobial susceptibility, virulence and genetic relatedness of emerging NVS isolated from Canada in 2011-13.

Methods: As part of a collaboration between CARA and the National Microbiology Laboratory, 3723 SPN isolates causing invasive pneumococcal disease (IPD) were collected from across Canada from January 2011 to December 2013, 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 serotype 15A, 22F, 33F and 35B isolates were characterized for putative virulence by PCR to detect pili. One third of each were tested for genetic relatedness by PFGE/MLST. MLST sequence types (STs) were compared to the Pneumococcal Molecular Epidemiology Network (PMEN) database.

Results: Serotypes 15A (135, 3.6%), 22F (373, 10.0%), 33F (109, 2.9%) and 35B (67, 1.8%) together accounted for 18.3% (684/3723) of the IPD isolates tested in 2011-13. Multi-drug resistance (MDR) was observed in 59.8% (70/117) of serotype 15A isolates, most commonly to clindamycin, clarithromycin and doxycycline, and found to be PMEN clone ST63, or closely related. Serotype 22F isolates shown to be similar by PFGE were demonstrated to be a common clone, ST433. Several novel STs of MDR serotype 33F were identified. Penicillin-nonsusceptible and piliated ST558 was common in serotype 35B. Potential capsular switch variants were identified (ST63 to ST9352 [MDR 22F] and PMEN clone ST156 [MDR 9V] to ST9346 [MDR 35B]).

Conclusion: From 2011-2013, NVS 15A, 22F, 33F and 35B accounted for 18.3% of IPD in Canada. These isolates were frequently MDR, piliated and related to international clones. Ongoing surveillance is necessary to assess the evolution of potentially emerging NVS.

BACKGROUND

The introduction of the 7-valent pneumococcal conjugate vaccine (PCV-7, serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F) in Canada (2002-2005) dramatically reduced the prevalence of *Streptococcus pneumoniae* vaccine serotypes through active vaccination and herd protection [1]. However, the introduction of this vaccine was associated with a subsequent increase in non-PCV-7 serotypes, notably 19A [2]. New conjugate vaccine formulations were developed to expand serotype coverage, including PCV-13 (PCV-7 serotypes plus 1, 3, 5, 6A, 7F and 19A). However, with continued use of PCV-13, it is hypothesized that certain individual non-PCV-13 serotypes will again increase as a cause of invasive pneumococcal disease (IPD). Further, we hypothesize that a successful emerging non-PCV-13 serotype would be antimicrobial resistant, clonal and have the potential to increase overall rates of IPD in Canada.

Potentially emerging non-PCV-13 serotypes of interest include serotypes 22F and 33F. Isolated frequently in Canada, these serotypes are also included in a 15-valent pneumococcal conjugate vaccine currently undergoing clinical trials in the United States [3]. Other serotypes of interest include 15A, which is increasingly antimicrobial and multi-drug resistant [4,5], and 35B, which like serotype 19A, has recently been found to harbor pneumococcal pili and penicillin-nonsusceptibility [6,7].

The purpose of this study was to characterize specific non-PCV-13 serotypes 15A, 22F, 33F and 35B, collected from across Canada in 2011-13.

ACKNOWLEDGMENTS

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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 3,623 isolates were sent to CARA from January 2011 to December 2013, 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 [8]. Quality control was performed using *S. pneumoniae* ATCC 49619. Minimum inhibitory concentrations (MICs) were interpreted using CLSI breakpoints [9], 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 Serotypes 15A, 22F, 33F and 35B

To determine genetic relatedness, pulsed-field gel electrophoresis (PFGE) was performed as previously described[10] for 236 serotype 15A, 2F, 33F and 35B isolates. Gels were analyzed using BioNumerics Software v3.5 (Applied Maths Inc, Austin, TX). In addition, multi-locus sequence typing (MLST) was performed on the same 236 isolates using methods and primers previously described at http://spneumoniae.mlst.net. 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. Minimum spanning trees were generated with PubMLST. To assess putative virulence, PCR to determine the presence of pneumococcal pili was performed using previously described primers [11].

CONCLUSIONS

1. Serotypes 15A, 22F, 33F and 35B accounted for 18.3% of IPD isolates collected in Canada in 2011-13
2. Multi-drug resistance was demonstrated by all four serotypes. Serotype 15A demonstrated 59.8% MDR, with two isolates being XDR.
3. Predominant clones included PMEN clone ST63 (MDR 15A), common clone ST433 (22F) and penicillin-nonsusceptible and piliated ST558 (35B).
4. Two potential capsular switch variants were identified, including ST63 to ST9352 (MDR 22F), as well as ST156 (MDR 9V) to ST9346 (MDR, piliated 35B).

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RESULTS

Figure 1. PFGE dendrogram of a selection of serotype 15A, 22F, 33F and 35B isolates. Bar on the top indicates percent similarity when digested with SmaI. Generated using BioNumerics.

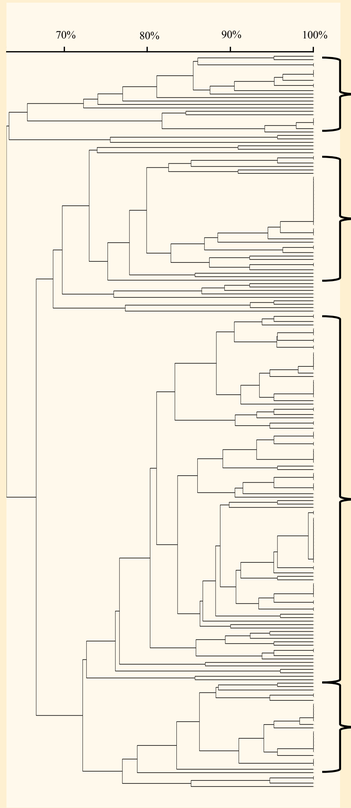
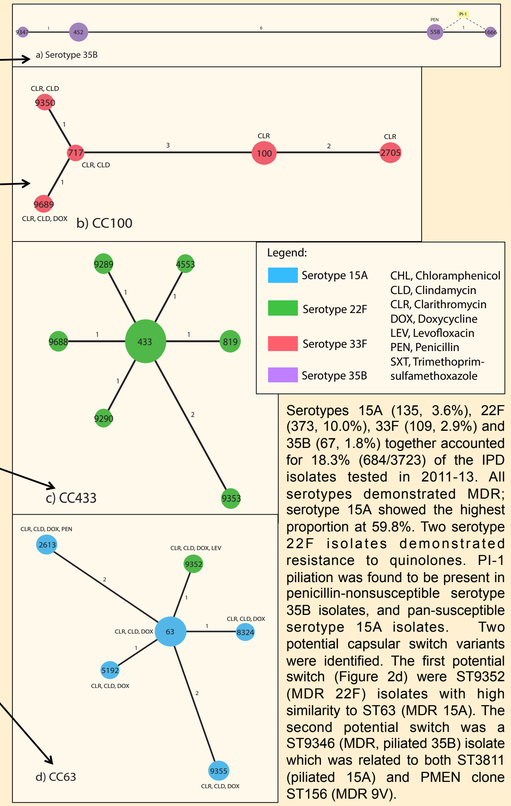


Figure 2. Minimum spanning trees of commonly identified MLST clonal clusters (CCs) and sequence types (STs). ST indicated in middle of node; lines infer relatedness by stating the number of differing alleles between each ST; node size proportional to number of isolates tested; resistance to antimicrobials and presence of pneumococcal pili (yellow) listed next to nodes. Generated using PubMLST. a) serotype 35B; b) CC100; c) CC433; d) CC63.



Serotypes 15A (135, 3.6%), 22F (373, 10.0%), 33F (109, 2.9%) and 35B (67, 1.8%) together accounted for 18.3% (684/3723) of the IPD isolates tested in 2011-13. All serotypes demonstrated MDR; serotype 15A showed the highest proportion at 59.8%. Two serotype 22F isolates demonstrated resistance to quinolones. PI-1 piliation was found to be present in penicillin-nonsusceptible serotype 35B isolates, and pan-susceptible serotype 15A isolates. Two potential capsular switch variants were identified. The first potential switch (Figure 2d) were ST9352 (MDR 22F) isolates with high similarity to ST63 (MDR 15A). The second potential switch was a ST9346 (MDR, piliated 35B) isolate which was related to both ST3811 (piliated 15A) and PMEN clone ST156 (MDR 9V).

Table 1. Demographics of 15A, 22F, 33F and 35B collected in Canada in 2011-13.

	Percent by Serotype (n)			
	15A (135)	22F (373)	33F (109)	35B (67)
Region				
West	13.3	14.5	23.9	23.9
Central	80.7	75.6	64.2	61.2
East	3.4	9.9	11.9	14.9
Age				
0 to less than 2	8.1	8.6	9.2	1.5
2 – 4 years	1.5	4.0	4.6	-
5 – 14 years	-	1.6	2.8	1.5
15 – 64 years	36.3	37.3	39.4	34.3
65 years or greater	52.3	44.5	39.4	61.2
Gender				
Male	45.2	54.2	47.8	46.3
Female	54.8	42.6	47.8	47.8

Table 2. MDR and XDR phenotypes demonstrated by serotypes 15A, 22F, 33F and 35B collected in Canada in 2011-13.

MDR Phenotype	Serotype (n*)			
	15A (117)	22F (373)	33F (109)	35B (67)
3 Classes:				
Chloramphenicol, Clarithromycin, Clindamycin			1	
Chloramphenicol, Clarithromycin, Doxycycline	3			
Clarithromycin, Clindamycin, Doxycycline	57	1	9	
Clarithromycin, Clindamycin, Trimethoprim-sulfamethoxazole	1			
Clarithromycin, Doxycycline, Penicillin				1
Clarithromycin, Penicillin, Trimethoprim-sulfamethoxazole				1
4 Classes:				
Chloramphenicol, Clarithromycin, Clindamycin, Doxycycline	1		1	
Clarithromycin, Clindamycin, Doxycycline, Penicillin	6			
Clarithromycin, Clindamycin, Doxycycline, Levofloxacin		2		
5 Classes:				
Chloramphenicol, Clarithromycin, Clindamycin, Doxycycline, Penicillin	2			
Total Isolates (%)	70 (59.8)	3 (0.8)	11 (10.1)	2 (3.0)

*, n for which complete susceptibility data available.