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Characterization of Emerging Non-PCV-13 *Streptococcus pneumoniae* (SPN) Serotypes 22F and 33F Causing Invasive Infections in Canada, 2011-2014

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ABSTRACT

Background: Emerging non-PCV-13 SPN serotypes 22F and 33F are included in a new 15-valent pneumococcal conjugate vaccine currently undergoing clinical trials in the United States. The goal of this study was to characterize the antimicrobial resistance, virulence and genetic relatedness of these two emerging serotypes.

Methods: In collaboration between CARA and NML, 5,012 SPN isolates causing invasive pneumococcal disease (IPD) were collected from across Canada from 2011-14. Serotyping was performed by the Quellung reaction and susceptibility testing was performed using CLSI methods. Multi-drug resistance (MDR) was defined as resistance to ≥3 classes of antimicrobials. All serotype 22F and 33F isolates were characterized by PCR to detect pili and genetic relatedness by PFGE; a subset of isolates were further characterized by MLST.

Results: Serotype 22F accounted for 9.8% (492/5012) of IPD isolates collected from 2011-14, ranking consistently in the top 4 most common serotypes collected each year. These isolates demonstrated ≥95% susceptibility to all antimicrobials except clarithromycin (CLR, 74%), and few were MDR (0.8%, 4/492). Serotype 22F isolates were highly clonal (ST433), with two isolates showing high relatedness to international clone Sweden^{15A}-25. Serotype 33F isolates made up 3.2% (158/5012) of the isolates collected during 2011-14 and increased significantly in prevalence during this time period (p=0.005). Isolates demonstrated decreased susceptibility to CLR (21%) and trimethoprim-sulfamethoxazole (33%). Serotype 33F isolates demonstrated 8.9% (14/158) MDR and included 9 different sequence types. No serotype 22F or 33F isolates demonstrated piliation.

Conclusion: Despite its high prevalence in Canada, serotype 22F demonstrated limited antimicrobial resistance and a very low MDR rate. Serotype 33F is increasingly prevalent in Canada, with a high MDR rate of 8.9%. While serotype 33F was demonstrated to be genetically diverse, serotype 22F was highly clonal across Canada.

BACKGROUND

Routine use of pneumococcal conjugate vaccine PCV-7 (providing protection against *S. pneumoniae* serotypes 4, 6B, 9V, 14, 18C, 19F and 23F) has been instrumental in the reduction of invasive pneumococcal disease (IPD), specifically due to vaccine and vaccine-related serotypes [1]. This decrease in vaccine types led to the phenomenon known as serotype replacement, where non-vaccine serotypes expanded to fill the niche vacated by PCV-7 serotypes. Such non-PCV-7 serotypes included 3 and 19A, which were subsequently included in PCV-13 (PCV-7 serotypes plus 1, 3, 5, 6A, 7F and 19A) in response to this shift [2].

Since the release of PCV-13 in Canada in 2010, similar serotype replacement trends are already manifesting. A new 15-valent formulation of the pneumococcal conjugate vaccine is currently in clinical trials in the United States, encompassing the PCV-13 serotypes plus emerging serotypes 22F and 33F [3]. Studies in Canada, the United Kingdom and the United States have demonstrated trends of increasing prevalence of these two serotypes [2, 4-6]. In addition, serotypes 22F and 33F both possess high invasive capacity compared to other non-PCV-13 serotypes[7], making them important inclusions in the 15-valent pneumococcal vaccine formulation.

The purpose of this study was to perform antimicrobial susceptibility testing and molecular analyses on PCV-15 *S. pneumoniae* serotypes 22F and 33F, that were collected in Canada in 2011-14.

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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 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 [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).

Characterization of Serotypes 22F and 33F

To determine genetic relatedness, pulsed-field gel electrophoresis (PFGE) was performed as previously described[10] for all serotype 22F and 33F isolates. Gels were analyzed using BioNumerics Software v3.5 (Applied Maths Inc, Austin, TX). In addition, multi-locus sequence typing (MLST) was performed on approximately 40% of the isolate cohort for both serotypes 22F and 33F, 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 [11].

CONCLUSION

1. Serotype 22F isolates demonstrated high prevalence in Canada during 2011-14. These isolates displayed limited antimicrobial resistance and little multi-drug resistance. Serotype 22F isolates were largely comprised within the highly prevalent CC433.
2. Serotype 33F isolates increased in prevalence in Canada from 2011 to 2014. These isolates demonstrated lower susceptibility to a greater number of antimicrobial classes, higher multi-drug resistance (8.9%) and more genetic diversity in comparison to serotype 22F.

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RESULTS

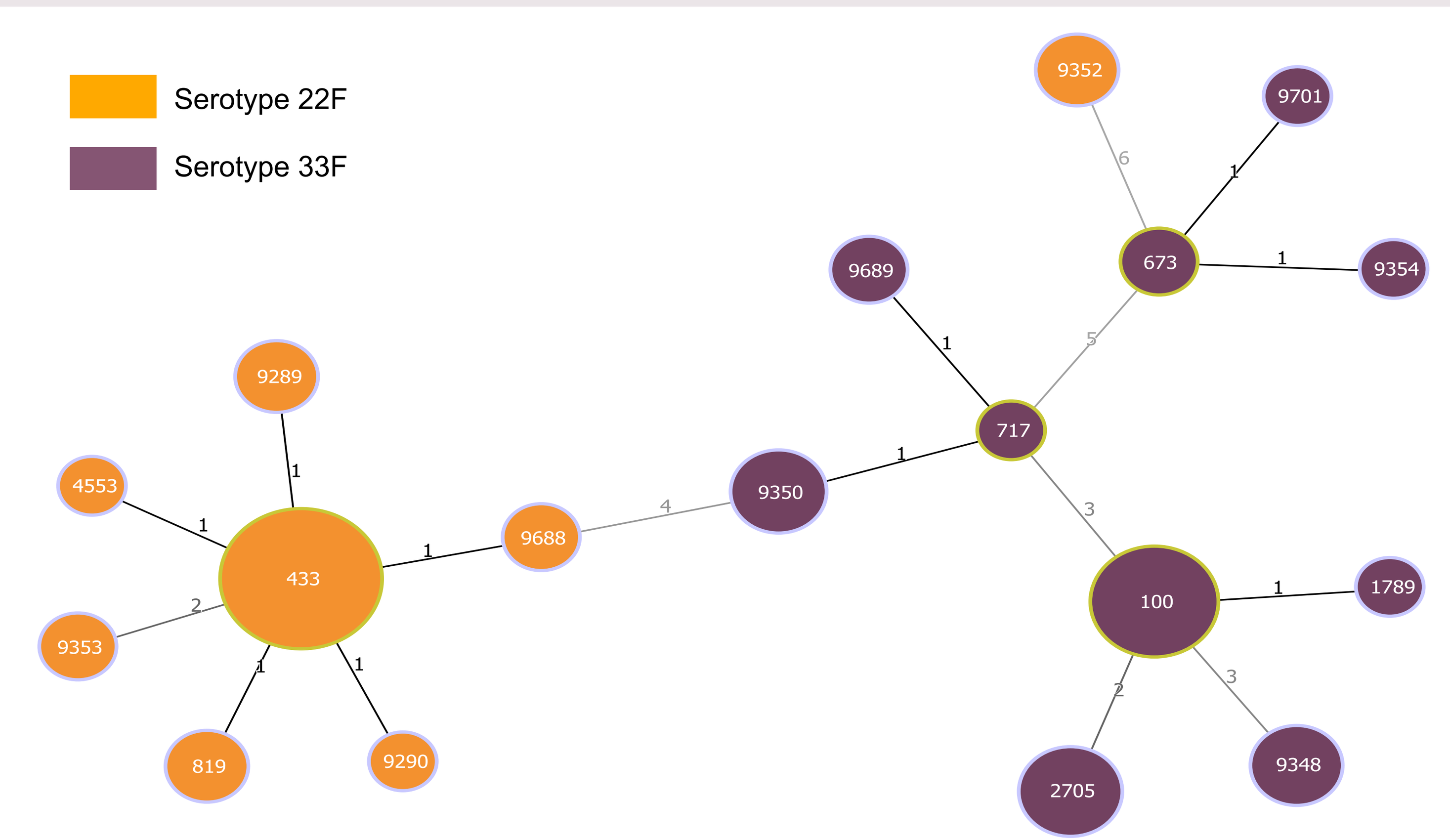
Table 1. Antimicrobial susceptibilities of *S. pneumoniae* serotypes 22F and 33F collected from Canadian provinces in 2011-14.

Serotype (n*)	Antimicrobial Susceptibilities (%)									
	CLR	CLD	CRO (M)	CRO (NM)	DOX	LEV	PEN (M)	PEN (NM)	SXT	% MDR
22F (490)	74.1	98.4	99.8	100	99.4	98.6	99.4	99.8	98.8	0.8
33F (158)	20.9	79.7	100	100	82.3	100	99.4	100	32.9	8.9

*, 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.

Overall, serotype 22F isolates accounted for 9.8% (492/5,012) of IPD isolates collected in 2011-14. Isolates demonstrated >98% susceptibility to all antimicrobial agents tested except clarithromycin. Serotype 22F isolates were highly clonal, falling primarily within CC433. Two isolates (ST9352) demonstrated relatedness to international clone Sweden^{15A}-25 (ST63), with an MDR pattern of clarithromycin, clindamycin, doxycycline and levofloxacin. Serotype 33F accounted for 3.2% (158/5,012) of IPD isolates collected in 2011-14, and increased significantly over this time period (p=0.005). Isolates demonstrated decreased susceptibility to clindamycin and doxycycline, and extremely low susceptibility to clarithromycin and trimethoprim-sulfamethoxazole. 8.9% (14/158) of serotype 33F isolates were MDR, most frequently to clarithromycin, clindamycin and doxycycline. All MDR serotype 33F isolates were newly identified sequence types, specifically STs 9348, 9350 and 9689. This serotype also demonstrated higher diversity, breaking down into three clusters in comparison to one cluster of serotype 22F. No serotype 22F or 33F isolates demonstrated piliation with PI-1 or PI-2.

Figure 1. Minimum spanning tree demonstrating the genetic relatedness of *S. pneumoniae* serotypes 22F and 33F collected from Canadian provinces in 2011-14. Green outlines indicate a group founder; light blue outlines indicate relatedness to founder; numbers indicate the number of differences between the MLST profiles of the two connected nodes.



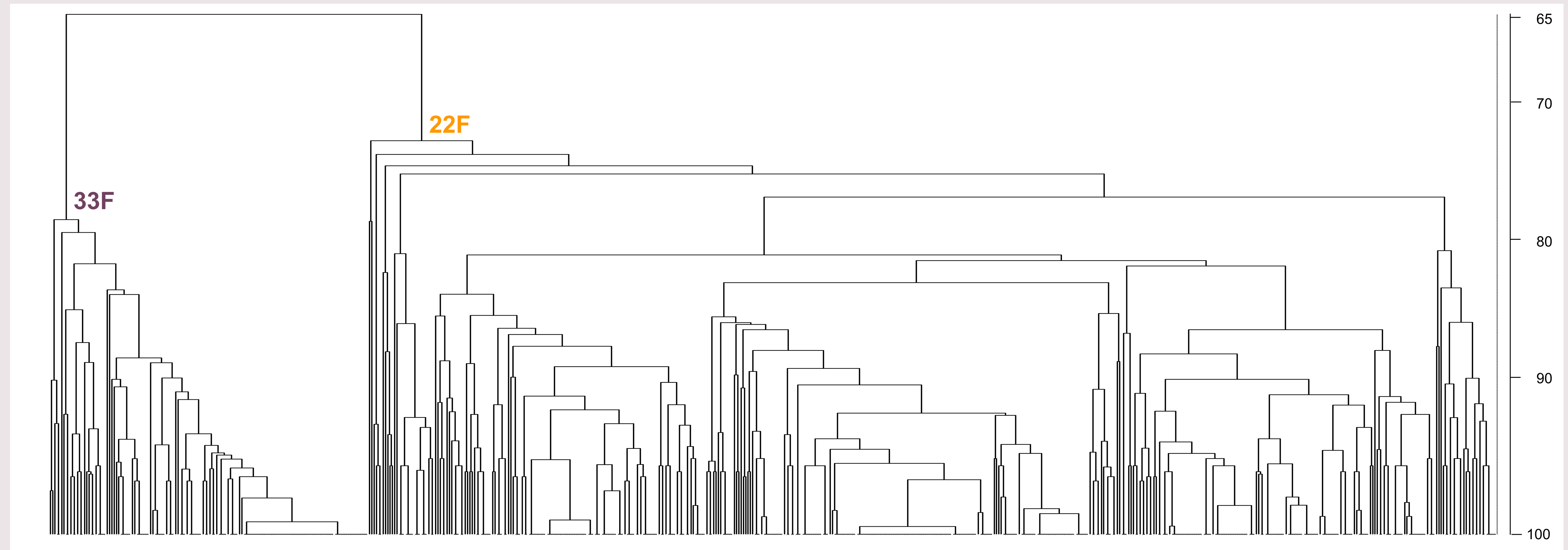


Figure 2. PFGE dendrogram demonstrating the genetic relatedness of *S. pneumoniae* serotypes 22F and 33F collected from Canadian provinces in 2011-14.