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Characterization of *Streptococcus pneumoniae* Serotypes 22F and 33F Causing Invasive Pneumococcal Disease in Canada: SAVE Study 2011-2020

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Introduction

Streptococcus pneumoniae remains a significant source of morbidity and mortality due to respiratory/invasive infections worldwide [1]. Prevnar® is a protein conjugate vaccine introduced in 2000, highly effective in reducing invasive pneumococcal disease (IPD) and carriage of vaccine serotypes. However, a significant shift in IPD serotypes was subsequently observed, including multidrug-resistant (MDR) serotype 19A [2].

Due to the inclusion of more serotypes, including 19A, PCV-13 is today the recommended vaccine for protection of Canadian children and a subset of adults against invasive *S. pneumoniae* infection [3]. PCV-15 and PCV-20, which include serotypes 22F and 33F, have been approved in the U.S. and Canada for use in adults 18 years of age or older, due to increasing concern over high representation of these isolates among invasive isolates [4, 5]. PCV-24, and non-serotype specific vaccines, are also in development [6].

Designed to assess invasive *S. pneumoniae* serotypes and their antimicrobial susceptibility patterns in Canada after the introduction of the PCV-13 vaccine, CARA and PHAC-NML have collaborated on the ongoing national SAVE (*S. pneumoniae* Serotyping and Antimicrobial Susceptibility: Assessment for Vaccine Effectiveness in Canada After the Introduction of PCV-13) study annually since 2011 [3]. Combining phenotypic and genotypic tools, we characterized serotypes 22F and 33F causing IPD in Canada from 2011-20.

Materials and Methods

Isolate Collection

S. pneumoniae isolated from sterile sites are forwarded from the Canadian public health laboratories [Canadian Public Health Laboratory Network (CPHLN)] to the Public Health Agency of Canada – National Microbiology Laboratory (PHAC-NML). Through a collaboration between the Canadian Antimicrobial Resistance Alliance (CARA) and PHAC-NML, and subsequent to the permission of the select submitting CPHLN sites (as detailed in the acknowledgements), the *S. pneumoniae* isolates were forwarded to CARA. A total of 14,138 invasive *S. pneumoniae* isolates from across Canada were included in the SAVE study as part of this collaboration (Jan. 1, 2011 – Dec. 31, 2020). Serotyping was performed using the Quellung reaction using commercial antisera (SSI Diagnostica, Denmark). From 2011 to 2020, serotypes 22F and 33F accounted for 1282 and 539 isolates, respectively.

AST and MLST

Antimicrobial susceptibility testing was performed using custom designed in-house manufactured antimicrobial susceptibility panels using the CLSI brothmicrodilution method [7]. MICs were interpreted using CLSI criteria [8]. MDR isolates were defined as resistant to ≥3 antimicrobial classes (penicillin MIC ≥ 2 µg/mL). In collaboration with PHAC-NML, traditional PCR-based MLST or short-read whole genome sequencing was performed on a subset of serotype 22F and 33F isolates. From whole genomes, MLST sequence types and clonal clusters (ST/CC) were identified using ResFinder 2.1.

The Cochran-Armitage test was used to identify significant trends over time.

Acknowledgements

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References

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Results

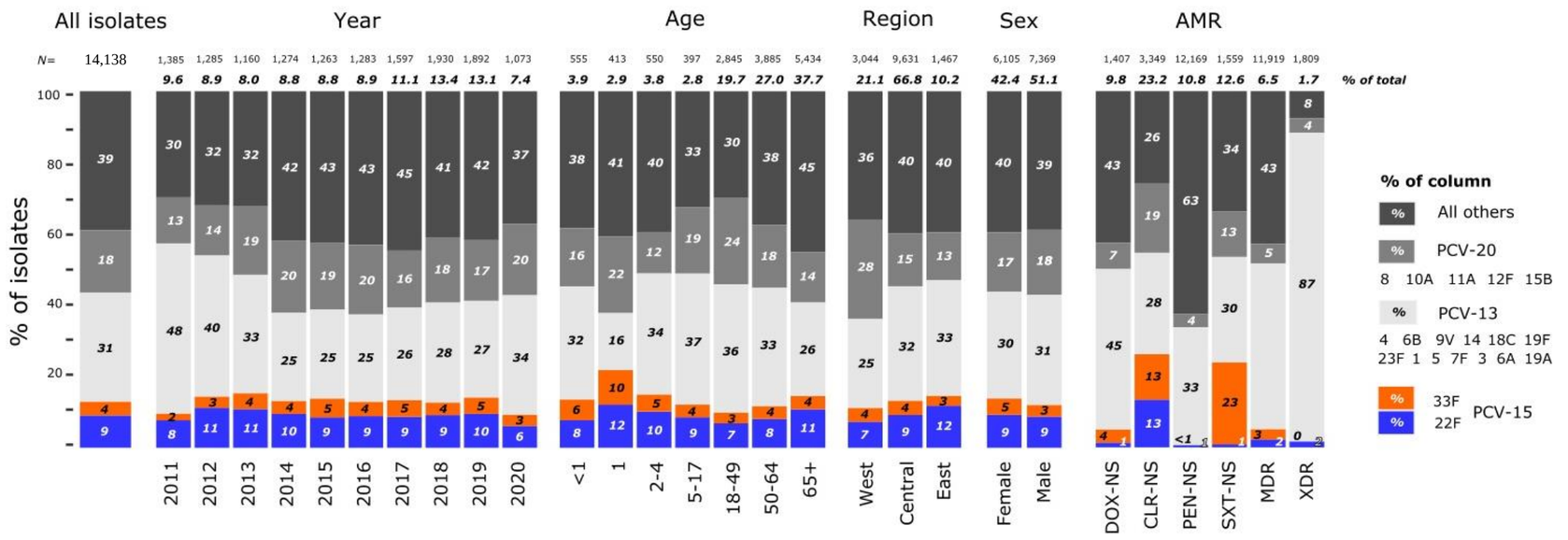

Figure 1: *Distribution of PCV-15 serotypes 22F (blue) and 33F (orange) isolates, as well as PCV-13, PCV-20 and other serotypes, by year, age group, region, sex and antimicrobial resistance (AMR). Numbers on bars indicate percentages.*

DOX, doxycycline; CLR, clarithromycin; PEN, penicillin (oral penicillin V breakpoints - nonsusceptible MIC ≥0.12 mg/L); SXT, trimethoprim-sulfamethoxazole; NS, non-susceptible; MDR, multi-drug resistant (resistant to 3 or more antibiotics); XDR, extensively drug resistant (5 or more antibiotics).

From 2011-2020, serotypes 22F and 33F accounted for 9.1% (n=1282) and 3.8% (n=539) of isolates, respectively, making up a greater proportion of the isolates in the youngest and oldest age groups **(Fig. 1)**. Both serotypes are well-represented among clarithromycin non-susceptible isolates, while only 33F was well-represented among trimethoprim-sulfamethoxazole (SXT) non-susceptible isolates **(Fig. 1)**. The proportion of 22F/33F and PCV-13 isolates from each age group has fluctuated considerably over the study period **(Fig. 2).**

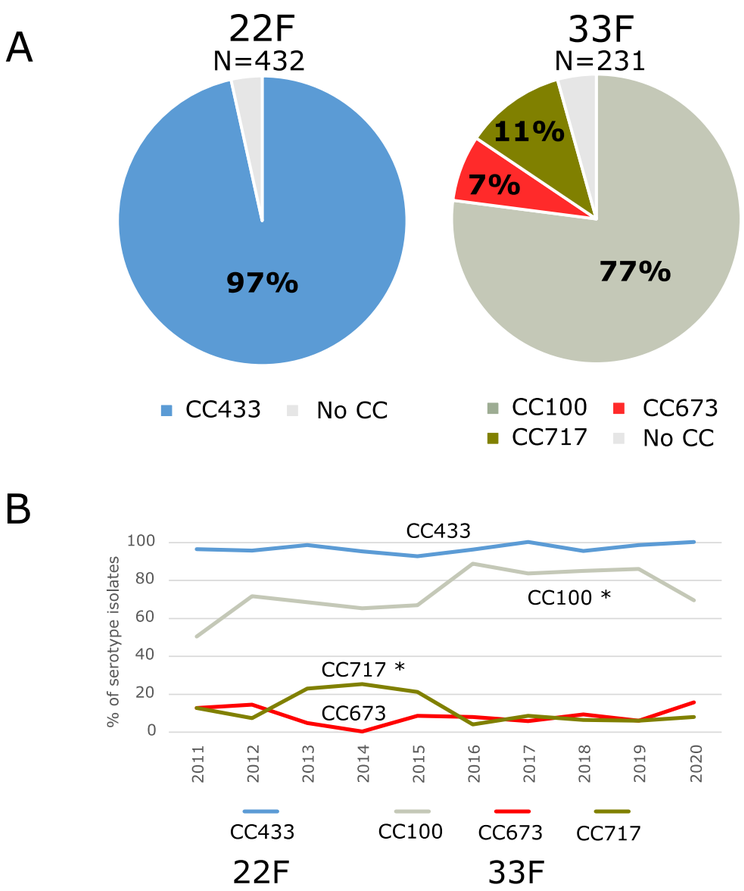

Figure 3: A. *Clonal clusters identified in a subset of 22F and 33F isolates.* ***B.*** *Proportion of isolates from each clonal cluster over time, * p < 0.05*

Isolates from serotype 33F - CC100 increased in prevalence over time (50.0% [n=4] in 2011 to 69.2% [n=9] in 2020, *P*<0.012) **(Fig. 3B)**. Serotype 33F demonstrated low susceptibilities to clarithromycin and SXT, but only SXT susceptibility decreased significantly over time (44.0% in 2011 to 18.2% in 2020, *P*<0.0001) **[Fig. 4]**. MDR serotype 33F isolates were predominantly CC717, with 48.4% (13/27) MDR isolates falling within this cluster. Of CC717 isolates, 50% were MDR (13/26) with the predominant pattern of clarithromycin, clindamycin and doxycycline.

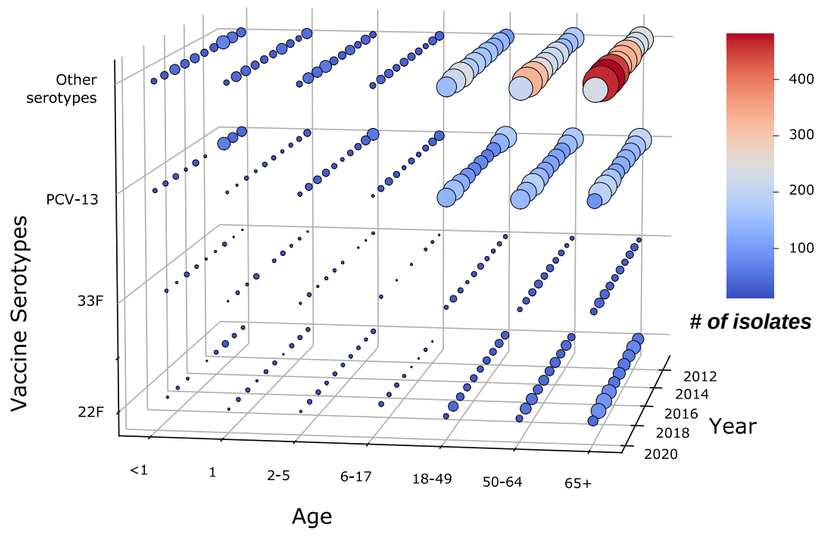

Figure 2: *Distribution of PCV-15 serotype 22F and 33F isolates, as well as PCV-13 and other serotypes, by year and age group. Circle size and colour reflects the number of isolates in each category (N-14,142).*

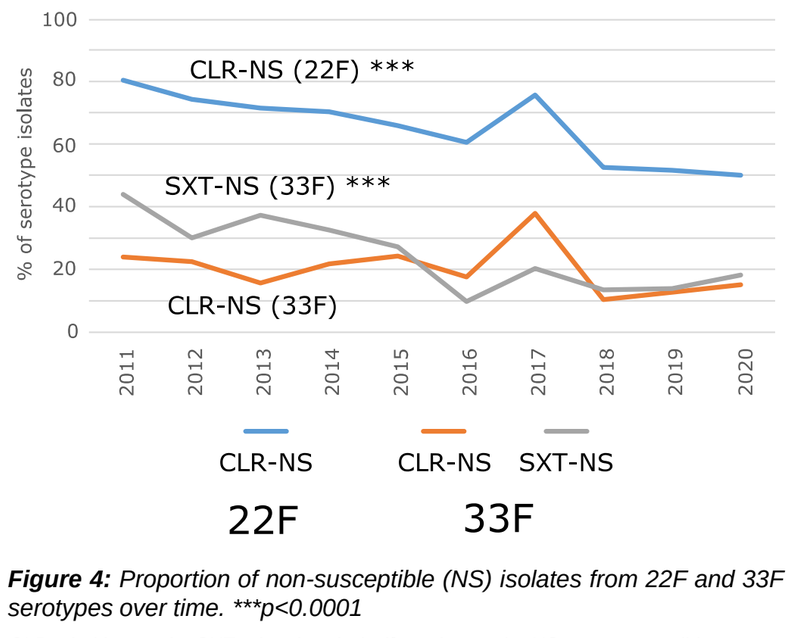

Figure 4: *Proportion of non-susceptible (NS) isolates from 22F and 33F serotypes over time. ***p<0.0001*

CLR, clarithromycin; SXT, trimethoprim/sulfamethoxazole; NS, non-susceptible.

Serotype 22F was highly clonal, where 96.5% (n=417) of sequenced strains were CC433 **(Fig. 3A)**. While serotype 22F demonstrated high susceptibilities to antimicrobials, the exception was clarithromycin where susceptibility decreased significantly from 2011 (80.3%) to 2020 (50.0%, *P*<0.0001) **[Fig. 4]**.

Conclusions

1. From 2011-2020, serotypes 22F and 33F accounted for 9.1% and 3.8% of isolates, respectively.
2. Serotype 22F was highly clonal and generally susceptible to antimicrobials (except clarithromycin) with little MDR.
3. Serotype 33F demonstrated low susceptibility to clarithromycin and SXT, MDR within CC717 and an increasing prevalence of CC100.
4. Further surveillance is necessary to determine clonal spread or diversification of these serotypes in Canada.