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Characterization of *Streptococcus pneumoniae* Serotype 3 Causing Invasive Pneumococcal Disease in Canada: SAVE Study 2011-2020

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Introduction

Streptococcus pneumoniae is a leading cause of respiratory (eg. community acquired pneumonia - CAP) and invasive (eg. bacteremia and sepsis) infections, and a significant source of morbidity and mortality worldwide [1-7]. As of 2020, ~100 biochemically and serologically distinct versions of *S. pneumoniae* capsule have been identified [8]. Serotype 3 infections are characterized as having severe clinical manifestations including empyema, bacteremia, cardiotoxicity, and meningitis [8]. Serotype 3 capsule synthesis and presentation on the bacterial surface is distinct from other serotypes, and the biochemical and physiological properties of this capsule facilitate its ability to cause disease. In addition, the large amount of capsule produced by serotype 3 overwhelms the antibody threshold provided by PCV-13 [8]. Thus, despite being covered by PCV-13, serotype 3 remains one of the most common serotypes causing invasive pneumococcal disease (IPD) in Canada and remains an important unmet medical need. We characterized serotype 3 causing IPD in Canada from 2011 to 2020.

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 National Microbiology Laboratory - Public Health Agency of Canada (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 acknowledgments), 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).

Antimicrobial Susceptibility Testing: Antimicrobial susceptibility testing was performed using custom designed in-house manufactured antimicrobial susceptibility panels using CLSI methods [5,7]. MICs were determined by the broth microdilution method, which was performed in adherence to all CLSI practices and quality control measures and interpreted utilizing CLSI criteria [5,7]. Multidrug resistance was defined as resistance to ≥3 antimicrobial classes (penicillin MIC ≥ 2 µg/mL) [5].

Serotyping: Serotyping was performed using 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 *rpo*B gene sequencing [6,9].

Whole Genome Sequencing: In collaboration with PHAC-NML, short-read whole genome sequencing was performed on 17 serotype 3 isolates. MLST sequence type (ST) and acquired resistance genes were identified using ResFinder 2.1. Core genome single nucleotide variants (SNVs) were identified, and phylogenetic trees generated using the PHAC-NML built SNVPhyl pipeline [10] using *S. pneumoniae* OXC141 (NC_017592) as a reference.

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

Acknowledgements

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References

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Results

A total of 14,138 invasive *S. pneumoniae* isolates from across Canada were included in the SAVE study (Jan. 1, 2011 – Dec. 31, 2020).

- From 2011-2020, serotype 3 accounted for 9.6% (1354/14138) of all isolates, increasing in prevalence from 7.6% (105/1385) in 2011 to 10.7% (115/1073) in 2020 (*P*<0.0001) [Figure 1].
- Serotype 3 represented 5.4% (44/820), 11.7% (52/446), 8.2% (41/501), 8.4% (255/3042), 11.1% (409/3688) and 9.7% (528/5434) of isolates in 0-<2, 2-4, 5-17, 18-50, 51-64 and ≥65-year age groups, respectively [Figure 2].
- Serotype 3 was common in all geographic areas of Canada and in both males and females [Figures 3 and 4].

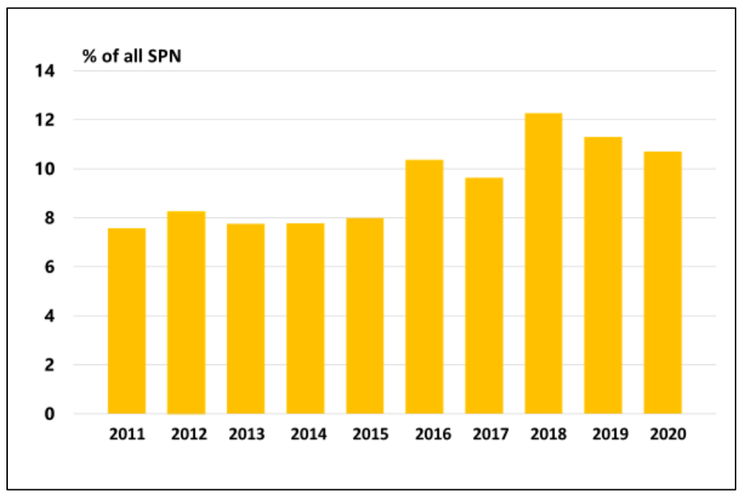

Figure 1. Prevalence of *Streptococcus pneumoniae* Serotype 3 Causing Invasive Infections in Canada (By Year)

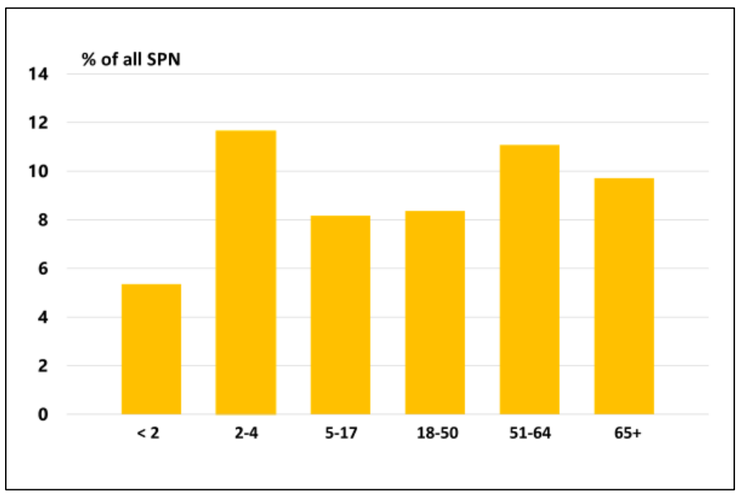

Figure 2. Prevalence of *Streptococcus pneumoniae* Serotype 3 Causing Invasive Infections in Canada (By Age, Years)

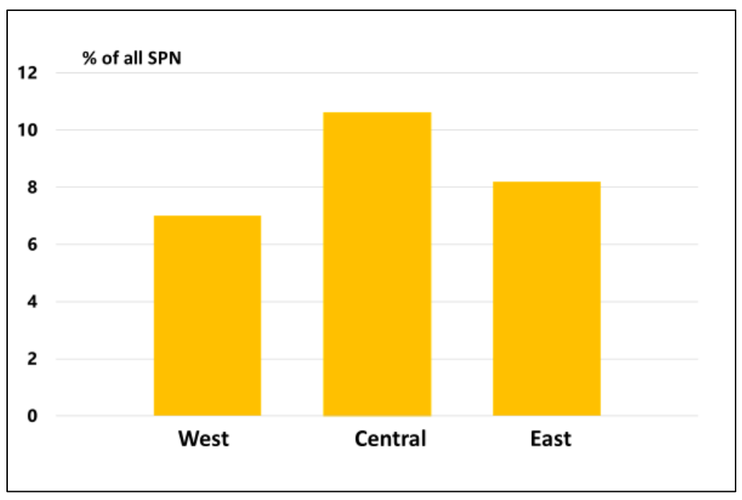

Figure 3. Prevalence of *Streptococcus pneumoniae* Serotype 3 Causing Invasive Infections in Canada (By Geographic Region)

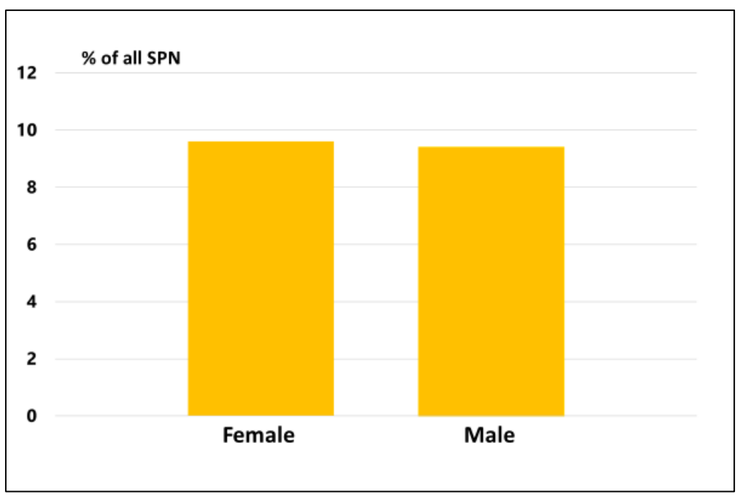

Figure 4. Prevalence of *Streptococcus pneumoniae* Serotype 3 Causing Invasive Infections in Canada (By Gender)

Serotype 3 demonstrated slightly decreased susceptibilities to doxycycline and clarithromycin, though there were no trends over time. MDR in serotype 3 was 4.0% over the 2011-2020 period, with a high of 7.2% in 2017 [Table 1].

Table 1. Antimicrobial susceptibilities of Serotype 3 *Streptococcus pneumoniae*

Year (N)	% Susceptible								% MDR
	PEN (IV, M)	PEN (IV, NM)	CRO (M)	CRO (NM)	CLR	LVX	SXT	DOX	
All (1354)	**99.5**	**100**	**99.9**	**100**	**94.1**	**100**	**98.6**	**87.7**	**4.0**
2011 (105)	100	100	100	100	97.1	100	97.1	92.2	1.0
2012 (106)	100	100	100	100	94.4	100	100	86.7	3.3
2013 (90)	98.8	100	100	100	96.4	100	97.6	89.3	2.4
2014 (99)	100	100	100	100	95.7	100	100	85.9	3.3
2015 (101)	100	100	100	100	97	100	100	90.9	3.0
2016 (133)	99.2	100	100	100	94.6	100	98.5	86.9	3.1
2017 (154)	97.4	100	99.4	100	91.5	100	97.4	85	7.2
2018 (237)	100	100	100	100	92.4	100	99.2	86.5	5.9
2019 (214)	100	100	100	100	94.8	100	100	87.2	3.8
2020 (115)	100	100	100	100	90.4	100	94.8	89.6	3.5

N, number of serotype 3 isolates; M, meningitis; NM, nonmeningitis; PEN, penicillin; CRO, ceftriaxone; CLR, clarithromycin; LVX, levofloxacin; SXT, trimethoprim-sulfamethoxazole; DOX, doxycycline; MDR, multidrug resistance [resistance to ≥3 antimicrobial classes (penicillin resistance defined as MIC ≥2 µg/ml)]

Of 17 sequenced isolates, 14 were ST180 or related [Figure 5]. There were two distinct phylogenetic clades of ST180. Clade I was generally susceptible to all antimicrobials, while clade II was MDR, demonstrating resistance to clarithromycin, doxycycline and often clindamycin (*ermB*/*tetM*), as well as chloramphenicol (*cat*).

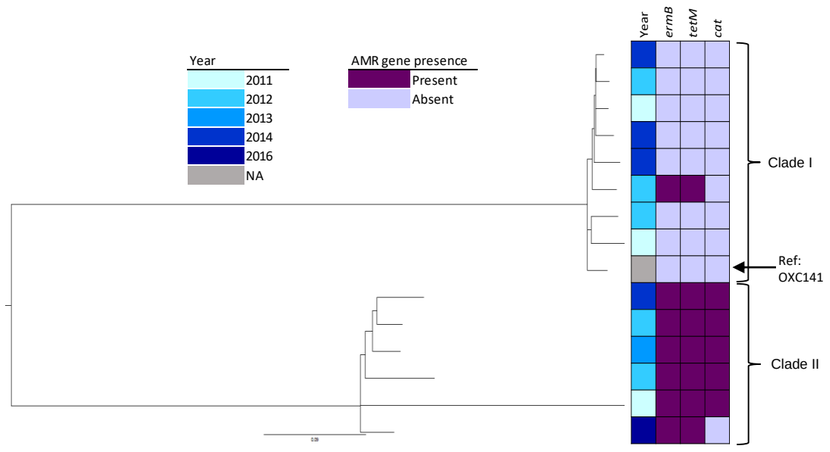

Figure 5. Maximum likelihood core SNV phylogenetic tree of *S. pneumoniae* serotype 3 – ST180 isolates collected in Canada from 2011 – 2016 (n=14). A total of 1539 sites were used in the phylogeny using 90.3% of the core genome. OXC141 (NCBI: NC_017592.1) used as a mapping reference; midpoint rooted.

Conclusions

1. *Streptococcus pneumoniae* serotype 3 isolates represent 9.6% of all isolates and increased from 7.6% in 2011 to 10.7% in 2020 (P<0.0001).
2. *Streptococcus pneumoniae* serotype 3 isolates cause invasive pneumococcal disease in all groups and in both genders.
3. *Streptococcus pneumoniae* serotype 3 isolates cause invasive pneumococcal disease in all geographic areas of Canada.
4. Serotype 3 demonstrated slightly decreased susceptibilities to doxycycline and clarithromycin, though there were no resistance trends over time.
5. MDR in serotype 3 ranged 4.0 – 7.2% and did not significantly change from 2011-2020.
6. Most tested serotype 3 isolates were ST180, comprising two distinct phylogenetic clades: Clade I were generally susceptible to all antimicrobials and Clade II which were MDR.
7. Further surveillance and sequencing is required to determine if MDR ST180-clade II is increasing in Canada over time.