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Comparison of *Streptococcus pneumoniae* Serotype 3-ST180 Isolates Collected from Canada and the United States Pre- and Post-PCV-13 Introduction

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Introduction

Streptococcus pneumoniae is a significant source of morbidity and mortality worldwide. Use of pneumococcal conjugate vaccines has drastically decreased the incidence of invasive disease due to serotypes included in the vaccine,[1] with the exception of serotype 3. Serotype 3 prevalence has remained stable in numerous countries despite it's inclusion in PCV-13;[2-4] it also has a high invasive capacity,[5] making it an important serotype for continued study. In Canada and abroad, serotype 3 isolates most commonly belong to international clone ST180, originally isolated in the Netherlands.[6]

Previous analysis of invasive *S. pneumoniae* serotype 3 isolated in Canada revealed two clades of ST180 with different characteristics. The purpose of this study was to compare the genomes of serotype 3-ST180 isolates collected in Canada to a subset of publicly available ST180 genomes collected in the USA.

Materials and Methods

Bacterial Isolates and Sequencing

Canadian ST180 isolates examined in this study comprised three invasive *S. pneumoniae* strains collected from 2008-2009 by the CANWARD study, and seven acquired from the collaborative SAVE 2011-2014 study between CARA and NML. Sequencing was performed using the Illumina MiSeq platform. Invasive ST180 strains from the USA were obtained as draft genomes from NCBI (Bioproject PRJEB3084/PRJNA284954), and included 15 strains collected from 2008-2013.

Data Analysis

Single nucleotide variant (SNV) phylogeny was performed using the NML-built bioinformatics pipeline SNVPhyl. Recombination analysis was performed using Gubbins and visualized with Phandango. Isolates were screened for antimicrobial resistance determinants, *pspA* family and typed using a penicillin-binding protein (PBP) transpeptidase domain scheme.[7]

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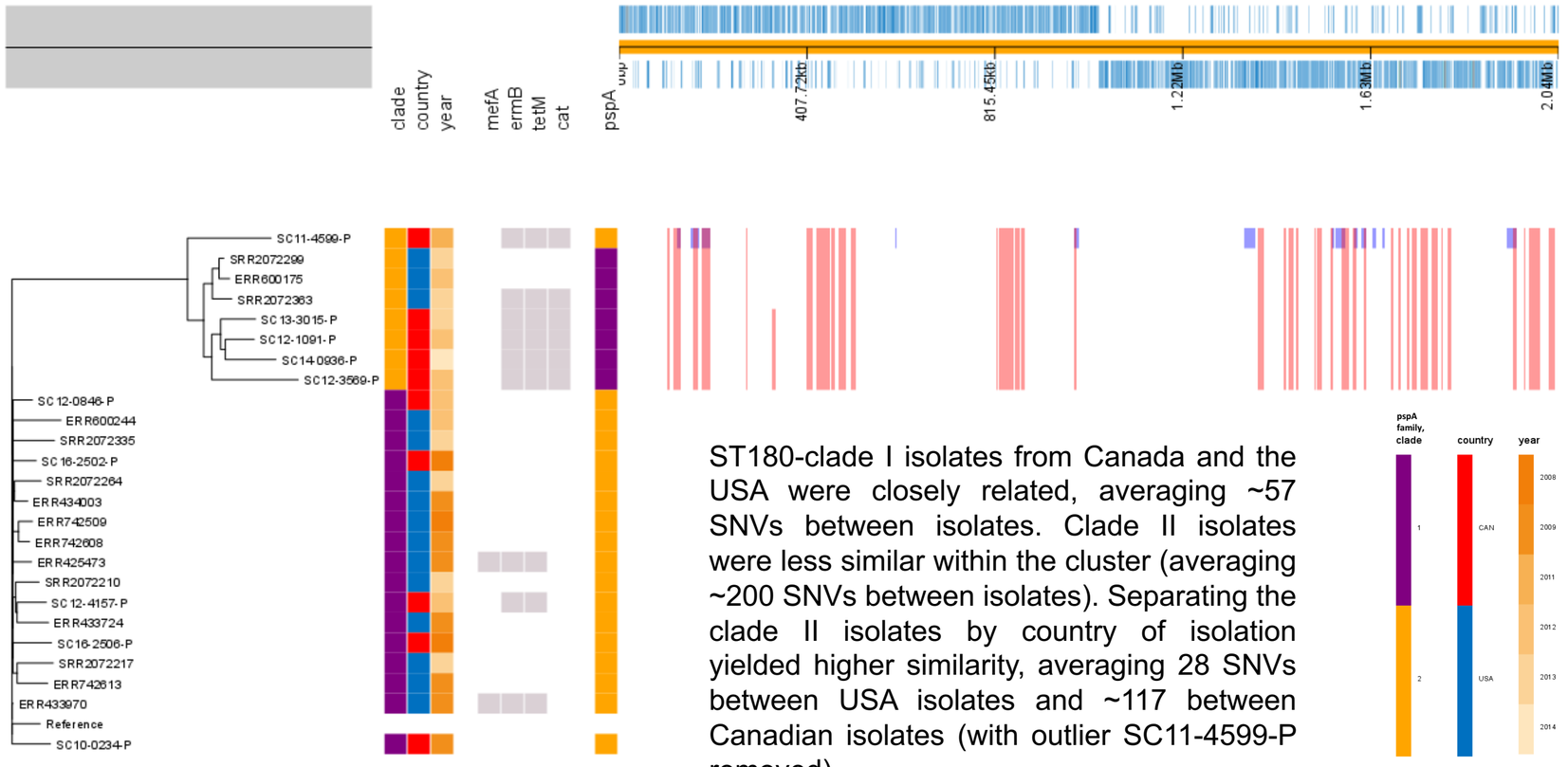
Results

Figure 1. Phylogeny, recombination events and associated metadata for *S. pneumoniae* serotype 3-ST180 isolates from Canada and the USA, predicted by Gubbins and visualized using Phandango and *S. pneumoniae* R6 reference genome. Red blocks indicate areas of recombination that are shared by multiple isolates through common descent. Blue blocks indicate areas of recombination that are unique to that isolate.



ST180-clade I isolates from Canada and the USA were closely related, averaging ~57 SNVs between isolates. Clade II isolates were less similar within the cluster (averaging ~200 SNVs between isolates). Separating the clade II isolates by country of isolation yielded higher similarity, averaging 28 SNVs between USA isolates and ~117 between Canadian isolates (with outlier SC11-4599-P removed).

Table 1. Penicillin-binding protein profiles for *S. pneumoniae* clade I and II ST180 isolates from Canada and the United States.

Clade	Country	PBP Active Site Alterations									PBP TP-typing pattern
		PBP1A			PBP2B			PBP2X			
		STMK	SRNVP	KTG	SVVK	SSNT	KTGTA	STMK	AHSSNV	LKSGT	(1A-2B-2X)
I	Canada and USA	----	-----	---	----	----	-----	----	------	-----	2-3-2
II	Canada	----	-----	---	----	----	-----	-A--	------	-----	2-0-111
II	USA	----	-----	---	----	----	-----	----	------	-----	2-0-2

Gubbins analysis demonstrated that the accumulation of genetic variation within ST180 has been in clade II. The only isolate to demonstrate unique recombination areas was SC11-4599-P, indicating it may be further diversifying into a new clade. Regardless of country of origin, all ST180-clade I isolates demonstrated a PBP-pattern of 1A-2, 2B-3, 2X-2 corresponding to no active site alterations. In comparison, clade II isolates from Canada demonstrated a more significantly altered pattern (2-0-111) than clade II isolates from the US (2-0-2).

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Conclusions

1. ST180-clade I *S. pneumoniae* demonstrated high similarity regardless of country of origin (an average ~57 SNVs between isolates). Isolates possessed *pspA* family 2 variants and few antimicrobial resistance determinants.
2. ST180-clade II isolates demonstrated increased genetic variation (averaging ~200 SNVs between isolates). Isolates commonly possessed *ermB*, *tetM* and *pspA* family 1 variants.
3. ST180-clade II isolates from Canada invariably possessed *cat*-mediated chloramphenicol resistance.
4. Regardless of country of origin, clade I isolates had the same PBP typing pattern and were not associated with active site mutations; clade II isolates from the USA were similar. Clade II isolates from Canada possessed an altered PBP typing pattern corresponding to an alteration in PBP2X. This indicates that clade II isolates from Canada may be closer to acquiring resistance to another antimicrobial class.