Genomic dissection of emergent multi-drug resistant (MDR) serotype 19A *Streptococcus pneumoniae* (SPN)

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Background:

Emergence of MDR serotype 19A SPN is well-documented. A similar increase has been observed in Canada. Several hypotheses exist in order explain the increased prevalence of MDR 19A. A plausible mechanism is that MDR 19A emerged from MDR 19F following a capsule switch event.

Methods:

Representative isolates of 19A and 19F identified in the Canadian Bacterial Diseases Surveillance Network (CBSN) before and after the introduction the heptavalent polysaccharide capsule vaccine (PCV 7) were used in this study. Isolates were subjected to whole genome sequencing (WGS), multi-locus sequence typing (MLST), serotyping, and *in vitro* susceptibility testing.

Results:

Serotypic surveillance of SPN as part of the CBSN demonstrated that MDR 19A is increasing in the post-PCV 7 introduction era in Canada, while 19F and 14 were decreasing. MLST of MDR 19A (n =97) revealed that sequence type (ST) 320 predominated amongst MDR 19A but not susceptible 19A controls. ST 320 was identified as the major contributor to MDR 19F in the pre-PC7 era. ST320 was unique amongst MDR 19A in that MIC values for penicillin, amoxicillin, ceftriaxone, and erythromycin were higher than for other ST present amongst post-PCV7 MDR 19A. Sequencing revealed that alleles at key drug resistance loci *pbp2a*, *pbp2x*, *pbp2b*, *ermB*, *mefA/E*, and *tetM* were conserved between pre-PCV7 ST 320 19F and post-PCV7 ST 320 19A. Sequence analysis of the transposon *Tn2010* insertion site showed that this event was heterogeneous amongst ST320 MDR 19A but common between early Korean MDR 19A and recent Canadian MDR 19A isolates. The capsule sequence revealed that recombination event(s) may explain the evolution of post-PCV7 MDR 19A from pre-PCV7 MDR 19F. WGS data of MDR 19A ST320 identified over 15,000 unique mutations some of which reside in key virulence and colonization factors.

Conclusions:

Our study builds on 10 years of SPN surveillance conducted in Canada which revealed the emergence of MDR 19A. Surveillance trends in relation to PCV7 introduction, WGS, serotype, and susceptibility data suggest that recombination events may explain the evolution of post-PCV7 ST320 MDR 19A from pre-PCV7 MDR 19F. The time and place of this event is hard to decipher as importation of MDR 19A is possible as demonstrated by analysis of Korean isolates in the pre-PCV7 era. Taken together, the apparent success of MDR 19A is likely a combination of high-level resistance alleles, resistance acquired through transposon insertion, propensity for genetic recombination, mutations in key virulence and colonization factors, and the lack of coverage of the 19A serotype by PCV7.