Vaccine Escape of *Streptococcus pneumoniae* is Coupled to Amoxicillin Resistance in Canada

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ABSTRACT

Background: The Canadian Bacterial Surveillance Network (CBSN) is a network of Canadian clinical laboratories across Canada with a central repository in Toronto. Since amoxicillin remains essential in the treatment of SPN infections, we looked at the impact of amoxicillin usage and pneumococcal conjugate vaccine (PCV7) introduction on the rapid emergence of AMOXR (MIC≥8µg/mL) isolates in Canada.

Methods: SPN isolates submitted to CBSN from 1988-2007 were reviewed (n=25,584). IMS Health Canada provided the total number of antimicrobial prescriptions dispensed in Canadian retail pharmacies and data on use of the pneumococcal conjugate in clinics. SPN MICs were obtained using standard broth microdilution methods per CLSI. Serotypes were determined using pneumococcal antisera (Statens Serum Institut, Copenhagen, DK)). Pre-PCV7 era (1995-2001), PCV7 era (2002-5), and post-PCV7 era (2006-2007) AMOXR SPN isolates were compared by multi-locus sequence typing (MLST) (http://www.mlst.net/).

Results: Between 1988 and 2007 we noted a significant increase in AMOXR SPN isolates (0 to 1.9%, p < 0.0001, n=174). β-lactam usage pre-PCV7 remained above 300 prescriptions per 1000 persons. Serotyping demonstrated that while the majority of post-PCV7 AMOXR SPN continue to be 19F (pre-PCV7 55.6%, post-PCV7 48%), serotype 19A (pre-PCV 0%, post-PCV7 42%) is an emerging contributor to rising AMOXR. MLST of a subset AMOXR 19A post-PCV7 identified clonal complex (CC) 320 as dominant serotype (100%, n=17). Pre-PCV7 AMOXR 19A were not present. Instead pre-PCV7 AMOXR SPN CC320 was found amongst serotype 19F (100%, n=10). We are characterizing the genetic event that led to putative capsular switch from CC320 19F to 19A.

Conclusions: We hypothesize that drug selection pressure exerted on AMOXR CC320 19F pre-PCV7 sustained its presence in Canada. During the PCV7 era immune selection pressure likely drove a capsular switch resulting in AMOXR CC320 19A emergence post-PCV7. The propensity for vaccine escape of genetically labile organisms like SPN should be taken into account when designing vaccine strategies.

BACKGROUND

- ***S. pneumoniae*** is the leading cause of acute otitis media, sinusitis, and bacterial pneumoniae in children. Invasive pneumococcal disease (IPD) leads to sepsis, bacteraemia and meningitis and is most common in infants (39.8 cases per 100,000 population per year), elderly and immunocompromised (13.3 cases/year)[1].
- The heptavalent pneumococcal conjugate vaccine Prevnar (PCV7, Wyeth-Ayerst) was introduced to provincial universal vaccination programs between 2002-2005. PCV7 protects against 7 of the 91 pneumococcal serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F).
- Following PCV7 introduction in Canada the incidence of pneumococcal disease caused by PCV7 serotypes decreased significantly. However, disease caused by non-vaccine serotypes such as 19A significantly increased in the post-PCV7 era[2].
- Taiwan 19F-14 is a penicillin-resistant pandemic strain that spread globally in the pre-PCV7 era, and belongs to a family of closely related *S. pneumoniae* genotypes called clonal complex 320 (CC320).

OBJECTIVE: To characterize the epidemiological and genetic events that contributed to the increase in amoxicillin-resistant *S. pneumoniae* serotype 19A following introduction of the heptavalent pneumococcal vaccine, PCV7.

METHODS

A total of 25,584 *Streptococcus pneumoniae* isolates from the Canadian Bacterial Surveillance Network database (1988-2007) were available for study, from which multi-drug resistant serotype 19A and amoxicillin-resistant 19F isolates from pre-PCV7 era (1995-2001), PCV7 era (2002-2005), and post-PCV7 era (2006-2007) were taken. MICs were obtained by standard CLSI broth microdilution methods and serotypes were determined using pneumococcal antisera. Genotyping was performed by multi-locus sequence typing (MLST) as previously described[3]. IMS Health Canada provided total number of antimicrobial prescriptions from retail pharmacies and data on PCV7 use in clinics.

RESULTS

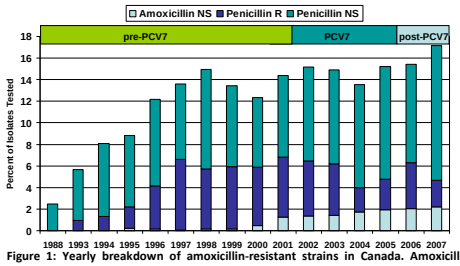

Figure 1: Yearly breakdown of amoxicillin-resistant strains in Canada. Amoxicillin resistance increased between 1995 and 2007 from 0 to 3.2% of total yearly isolates (p<0.0001, n=174). Dashed line shows that β-lactam usage remained above 300 prescriptions per 100 persons in this time period. Pneumococcal conjugate vaccine PCV7 increased from 73 doses per 1000 children in 2001 to 2,436 doses/1000 children in 2005, which equates to 60.9% of Canadian infants.

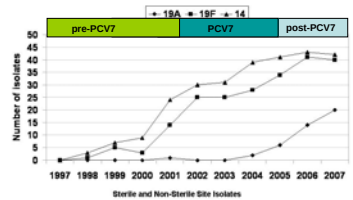

Figure 2: Serotype distribution changed dramatically between pre- and post-PCV7 eras with serotype 19A emerging as a significant contributor to amoxicillin resistance (pre-PCV7 0%, post-PCV7 36% averaged between 2006-2007). Serotype 19F continued to be the dominant amoxicillin-resistant serotype (pre-PCV7 55.6%, post-PCV7 54% averaged between 2006-2007).

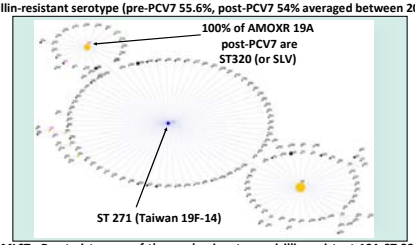

Figure 3: MLST eBurst pictogram of the predominant amoxicillin resistant 19A ST 320/CC320 in the post-PCV7 era. ST320 is a single locus variant (SLV) of ST 271/CC320 which is found commonly amongst pre-PCV7 amoxillin resistant 19F serotype. The first such clonal complex amongst 19A serotype was found in 2005. All 19A CC320 isolates were resistant to amoxicillin at either an intermediate (MIC≥4µg/mL) or high-level (MIC≥8µg/mL) as well as two other antibiotics. Pre-PCV7 amoxicillin-resistant 19A were not present. MLST of a subset of pre-PCV7 amoxicillin-resistant 19F identified CC320/ST271 OR ST320 as the dominant genotype (100%, n=10). Whole genome sequencing of pre-PCV7 19F and post-PCV7 19A are ongoing to identify the genetic switch event and other factors which have made this strain a globally dominant pathogen.

Table 1: Typical MIC/MLST profile of predominant ST320 19A post-PCV7 and ST271 19F pre-PCV7

Era	Serotype	ciprmic	penmic	tetmic	txmic	erymic	amxmic	aro	gdh	gki	rec	spi	xpt	sbp	ST	CC
pre-PCV7	19F	1.00	4.00	32.00	8.00	64.00	8	4	16	19	15	6	20	26	271	320
post-PCV7	19A	1.00	4.00	16.00	8.00	64.00	8.00	4	16	19	15	6	20	1	320	320

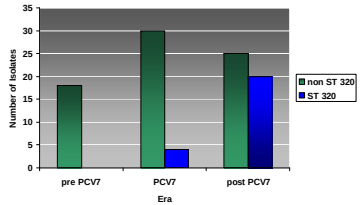

Figure 4: Frequency of ST 320 MLST amongst MDR 19A in relation to PCV7 roll out. ST320 is now the single dominant genotype for MDR 19A in Canada and harbours resistance to PEN, TS, ERYTHRO and AMOX.

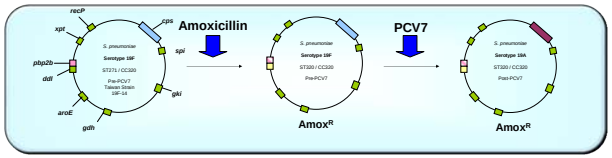

Figure 5: Amoxicillin resistance is mediated primarily by alterations in penicillin-binding protein 2b ***(pbp2b),*** which is genetically linked to MLST locus ***ddl.*** We hypothesize that drug pressure selected for ***pbp2b*** alterations in a CC320/ST271 19F progenitor strain pre-PCV7, which was followed by immune selection pressure in the PCV7 era to drive a capsular switch between a 19A donor and 19F recipient, resulting in amoxicillin-resistant CC320/ST320 19A emergence post-PCV7. The elucidation of the precise mechanism is ongoing.

SUMMARY & CONCLUSIONS

Using isolates from the Canadian Bacterial Surveillance Network we have accumulated preliminary evidence of drug and vaccine pressure driving the evolution of amoxicillin-resistant *Streptococcus pneumoniae* following introduction of PCV7 to provincial universal vaccination programs. Amoxicillin resistance increased between 1988-2007 from 0% to 3.2% while overall β-lactam usage remained over 300Rx/1000 persons. The heptavalent pneumococcal vaccine PCV7 was introduced between 2002-2005, after which there was a marked increase in amoxicillin-resistant serotype 19A (0% pre-PCV7 to 36% of amoxicillin-resistant isolates post-PCV7). Multi-locus sequence typing suggests that these strains originated from the most common amoxicillin-resistant genotype, 19F CC320 which includes the worldwide pandemic clone Taiwan 19F-14, which under vaccine pressure in the PCV7 era switched capsule with a 19A donor. It is hypothesized that capsular switch has contributed to the increase in 19A disease in the United States following PCV7 introduction[4]. Sequence analysis of the major amoxicillin resistance determinant ***pbp2b*** is ongoing. This work suggests that the propensity for vaccine escape of genetically labile organisms like *Streptococcus pneumoniae* should be taken into account when designing vaccine strategies.

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