



Evolution of Serotypes among Macrolide Resistant *Streptococcus pneumoniae* (SPN) and Coverage of the 7-Valent SPN conjugate vaccine (PCV7) in Canada from 1998 to 2008

A.K. Wierzbowski, P.Lagace-Wiens, N. Laing, D.J. Hoban, G.G.Zhanel
and The Canadian Antimicrobial Resistance Alliance (CARA)
University of Manitoba and Health Sciences Centre



Abstract

Background: The purpose of this study was to examine the evolution of serotypes among macrolide resistant SPN obtained during 1998 to 2008 and to assess ongoing coverage of the 7-valent SPN conjugate vaccine, Prevnar (PCV 7), which was introduced in Canada from 2002-2005 depending on the province.

Methods: All respiratory SPN isolates submitted from 1997 to 2008 were studied. MICs were determined by broth microdilution according to CLSI guidelines. Macrolide resistant (ERY MIC $\geq 1\mu\text{g/ml}$) isolates were serotyped by Quellung reaction at the central laboratory. Isolates were grouped into PCV7-serotypes, PCV7-related serotypes and non-PCV7 serotypes. In total, 1031 macrolide resistant SPN have been serotyped. Results were analyzed for statistical significance using linear regression analysis. A p value of <0.05 was considered statistically significant.

Results: PCV7 serotypes ranged from 18% (2007) to 74% (2001) and overall decreased 36%, from 67% to 31%. PCV7-related serotypes ranged from 0% (1998) to 17% (2007). Overall these serotypes increased from 0% to 12% in ten years. Non-PCV7 serotypes ranged from 20% (1999) to 65% (2007), an overall increase of 24%.

Conclusion: PCV7 serotypes decreased 2.4% per year ($p=0.0072$), while non-PCV7 serotypes increased 2.4% per year ($p=0.0152$). No significant change in PCV7-related serotypes was observed during the study ($p=0.785$).

Background

Streptococcus pneumoniae is an important human pathogen associated with respiratory tract infections and invasive diseases such as bacteremia and meningitis. It mostly affects young children, particularly those under the age of two, and older adults. Infections by this pathogen are least likely to resolve without treatment. The extensive use of antibiotics has led to marked reduction in mortality and morbidity due to pneumococcal infections; however non-susceptibility to antibiotics has become an unwanted consequence. Beta-lactam and macrolide antibiotics are among the recommended treatment options for *S. pneumoniae* infections. Antibiotic resistance in *S. pneumoniae* is widespread, affecting treatment options and outcomes. In Canada, macrolide resistance has more than doubled since the late 1990s (8%) and surpassed 20% in 2009. Macrolide resistance occurs when either the ribosomal target site is modified by *ermB* methylase or by mutations, or by *mefE* efflux pump. In light of increasing resistance and limited new drug options, the use of vaccines to prevent pneumococcal infections and to reduce antibiotic resistance has become a necessity.

To date, 91 serotypes of *S. pneumoniae* have been identified; 23 of these are included in a polysaccharide-based vaccine, PPV23, recommend for adults and at-risk children. Before the introduction of the pneumococcal vaccine for children aged <2 , seven serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F) were responsible for most of the invasive pneumococcal disease and most of the antibiotic resistant isolates and were therefore included in a protein-conjugate (PCV7) vaccine suitable for use in children in this age group. PCV7 has been shown to be very successful at reducing the incidence of invasive pneumococcal disease caused by the serotypes covered by the vaccine and also at decreasing the prevalence of vaccine type antibiotic resistant isolates. As the PCV7 vaccine program expanded, new disease patterns were revealed. Invasive infections and resistance caused by vaccine type *S. pneumoniae* receded. An increase in the incidence of invasive pneumococcal disease due to non-vaccine serotypes, particularly serotypes 1, 3, 5 and 7F, has been observed. Structurally similar serotypes 6A and 19A were shown to have limited cross-reaction with antibodies elicited against 6B and 19F, as previously thought, and diseases caused by *S. pneumoniae* with these serotypes increased since the introduction of the vaccine. Serotype 19A is particularly concerning as it has been shown to be predominant among penicillin- and multi-drug resistant isolates and has led to increases in penicillin and multi-drug resistance due to clonal expansion.

In the present study we examined the evolution of serotypes and assessed PCV7 vaccine coverage among macrolide-resistant *S. pneumoniae* isolates in Canada in the last 10 years.

Materials and Methods

Bacterial Isolates:

Between 1998 and 2006, *S. pneumoniae* isolates for this study were collected as part of the Canadian Respiratory Organism Susceptibility Study (CROSS) and between 2007 and 2008 as part of the Canadian Ward (CANWARD) study. Both studies were conducted at the central laboratory (Health Sciences Centre, Winnipeg, Manitoba, Canada). More information and details of the study methodologies can be found on the www.can-r.com website. Briefly, 25 medical centres in 9 of 10 Canadian provinces participated in the CROSS study. The goal of the CROSS study was to assess the prevalence of resistances to commonly used antimicrobials in lower respiratory tract *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* isolates. In addition, the study included *S. pyogenes* from throat and nasal swabs and *S. pneumoniae* from blood. Isolates were collected from all age groups and from in- and out-patients. In terms of *S. pneumoniae*, each centre was asked to submit the first 100 consecutive, one patient, significant respiratory tract isolates and all blood isolates to the surveillance study. Ten to twelve medical centres from all regions of Canada participated in the CANWARD study. Each centre collected and submitted clinical isolates from patients attending hospital clinics (IH), emergency rooms (ER), medical (MED) and surgical (SUR) wards, and intensive care units (ICUs). During 2007 and 2008, respectively, each centre was asked to submit clinical isolates, consecutive, one organism/infection site per patient from blood ($n=30/\text{month}$, $n=20/\text{month}$), respiratory ($n=200$, $n=150$), urine ($n=100$, $n=100$), and wound/intravenous infections ($n=50$, $n=50$). Among the respiratory isolates, each centre was asked to collect a minimum of 50 *S. pneumoniae*.

Antimicrobial Susceptibilities:

Susceptibility testing was carried out by microbroth dilution in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. Antimicrobial agents were obtained as laboratory grade powders from their respective manufacturers. Stock solutions were prepared and dilutions made as described by CLSI guidelines. The MICs of the antimicrobial agents were determined using a custom designed 96-well microtitre plate containing double antimicrobial dilutions in 100 μl well Catlon Adjusted Mueller-Hinton Broth (CAMHB). Laked Horse Blood (LHB), inoculated to a final concentration of approximately 5×10^6 CFU/ml and incubated at 37°C in 5% CO_2 for 20-24hr. Colony counts were performed periodically to confirm inocula counts. Quality control was performed using ATCC QC *S. pneumoniae* 49619.

Molecular Characterization of Macrolide Resistance:

Macrolide resistance (erythromycin MIC $\geq 1\mu\text{g/ml}$) was detected in 1461 out of 12,759 respiratory SPN isolates. Detection of *ermB* and *mefE* macrolide resistance genes in all macrolide-resistant isolates was performed by multiplex PCR. ATCC 49619 was used as a negative control.

Serotyping:

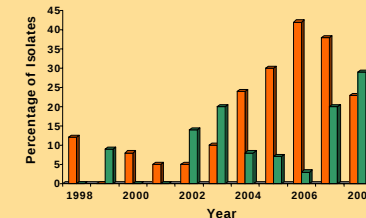
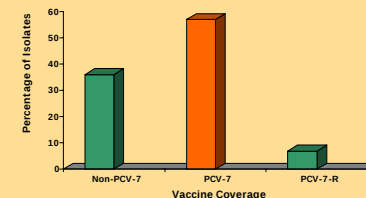
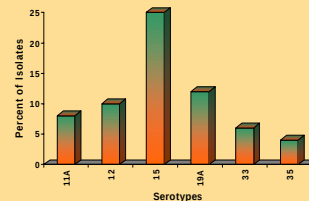
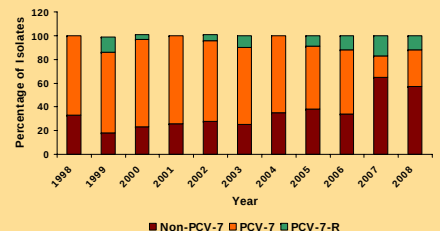
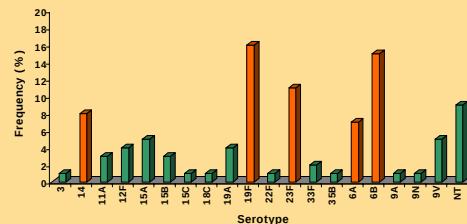
Serotyping was performed by capsular swelling (Quellung reaction) in antisera from the Statens Serum Institut (Copenhagen, Denmark) according to the manufacturer's instructions on 1031 of the 1461 macrolide-resistant respiratory *S. pneumoniae*. The goal was to have 50% or greater of macrolide-resistant isolates per year serotyped to well represent each genotype and geographic region.

Vaccine Coverage:

Vaccine coverage was analyzed by grouping serotypes into three groups: PCV7 serotypes - 4, 6B, 9V, 14, 18C, 19F and 23F; PCV7-related - 6A, 9L, 9L, 16A, 18F, 19B, 19C, 23A and 23B; Non-PCV7 - all other serotypes, plus 19A. Results were analyzed statistically and a p value of <0.05 was considered statistically significant.

Results

Serotype Distribution and Vaccine Coverage among Macrolide-Resistant Respiratory *S. pneumoniae* (1998-2008)



Conclusions

An average decrease of 2.4% was observed for PCV7 serotypes while an average increase of 3.2% was observed for the non-PCV7 serotypes.

Serotype 19A increased from 0% to 29% between 1998 and 2008.

Serogroup 15 increased from 12% to 23% between 1998 and 2008.

Serotype 11A, 12 and serogroups 33 and 35 are the other emerging serotypes in Canada.