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Macrolide-Resistant *Streptococcus pneumoniae* (Mac-R SPN) in Canada: 1998 to 2008 Experience

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ABSTRACT

Background: Macrolide resistance among SPN in Canada has increased from 8% (1998) to 21% (2008) compromising the use of these agents for community-acquired infections. Macrolide resistance is primarily mediated by *ermB* or by *mefE* genes. The use of vaccines has proven effective in reducing both antibiotic resistance and the incidence of disease, but revealed other disease processes. This study summarises ten years of experience with respiratory Mac-R SPN in Canada; it examines susceptibility, mechanisms of resistance, serotype distribution and vaccine coverage, as well as the genetic relatedness in this collection of isolates.
Methods: 1438 Mac-R (ERY MIC ≥1µg/mL) respiratory SPN isolates submitted to the Health Sciences Centre, Winnipeg, Canada from 1998 to 2008 were studied. Detection of *ermB* and *mefA* genes was performed by PCR, serotyping by Quellung reaction. Isolates were subjected to PFGE to determine their genetic relationships.
Results: The incidence of *mefE* changed from 55% (1998) to 49% (2008) while *ermB* decreased from 40% to 29%. *mefE/ermB*-positive and *mefE/ermB*-negative isolates increased from 4% to 19% and from 1% to 5% in ten years. 45 different serotypes were found; the most common were: 19F (16%), 6B (15%), 23F (11%), 14 (8%), 6A (7%), 9V (5%) and 15A (5%). Most serotype diversity was found among *mefE*-positive (35/45 serotypes) and least serotype diversity among *mefE/ermB*-positive (10/45 serotypes) SPN. Serotypes 6A (11%) and 19F (11%) were the most common among *mefE*-positive while 6B (25%) and 23F (18%) were most common among *ermB* positive isolates. 71% of *mefE/ermB*-positive isolates were of 19F (59%) or 19A (12%) serotype. Serogroup 15 increased from 12% (1998) to 23% (2008) as did serotype 19A from 0% (1998) to 29% (2008).
Conclusion: In addition to serotype 19A in Canada, serogroups 15, 33, and 35 have emerged since the introduction of PCV-7 vaccination. As new vaccines (PCV-13) become available ongoing monitoring of serotype distribution is warranted to ensure its efficacy and limit the spread of resistance. Study of the genetic relationship of SPN with the emerging serotypes is important to monitor capsular switching.

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 option 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 either 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 the 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 the protein-conjugate (PCV7) vaccine suitable for use in children in this age group. PCV7 has 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, 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 increase in penicillin and multi-drug resistance due to clonal expansion.
In the present study we examined the mechanisms of resistance, serotypes distribution and PCV7 vaccine as well as genetic relatedness among macrolide-resistant *S. pneumoniae* isolates in Canada in the last 10 years.

MATERIALS & METHODS

Bacterial Isolates:
Between 1998-2006 *S. pneumoniae* isolates for this study were collected as part of Canadian Respiratory Organism Susceptibility Study (CROSS) and between 2007 and 2008 as part of the Canadian Ward (CANWARD) study. Both national studies were conducted at the central laboratory (Health Sciences Centre, Winnipeg, Manitoba, Canada). More information and details of the studies methodologies can be found on the www.can-r.ca website.

Antimicrobial Susceptibilities:
Susceptibility testing was carried out using microbroth dilution in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (M07-A8). Antimicrobial agents were obtained as laboratory grade powders from their respective manufactures. 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 plates containing doubling antimicrobial dilutions in 100µl/well Cation Adjusted Mueller-Hinton Broth (CAMHB) with 2.5-5% Laked Horse Blood (LHB), inoculated to a final concentration of approximately 5 x105 CFU/ml and incubated at 37°C in 5% CO2 for 20-24hr. Colony counts were performed periodically to confirm inocula counts. Quality control was performed using ATCC QC *S. pneumoniae* 49619. MICs were interpreted using CLIS (M100-S20) guidelines.

Molecular Characterization of Macrolide Resistance:
Macrolide resistance (erythromycin MIC ≥1µg/mL) was detected in 1438 out of 12,759 respiratory SPN isolates. Detection of *ermB* and *mefE/mefA* macrolide resistance genes in all macrolide-resistant isolates was performed by multiplex PCR (Monaco, M. *et al.* 2005. JAC 55, 256-259 Pantosti, A. et al. 2001. CMI 7, 503-6). The ATCC 49619 strain was used as a negative control.

Serotyping:
Serotyping was performed by the capsular swelling in antisera (Quellung reaction) from the Statens Serum Institut (Copenhagen, Denmark) according to 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, 9A, 9L, 9N, 18A, 18F, 19B, 19C, 23A, and 23B; **Non-PCV7**- all other serotypes plus 19A. P value of <0.05 was considered statistically significant.

Genetic Relatedness:
Genetic relationships of the macrolide-resistant SPN were assessed by pulsed-field gel electrophoresis (PFGE) following digestion with *Sma*I as previously described. PFGE profiles were digitized for analysis with BioNumerics™ software, and strain relatedness was determined following established criteria (Tenover et al. 1995. J. Clin. Microbiol. 33:2233-2239).

CONCLUSIONS

Mac-R SPN increased from 8% to 21% in ten years.

***mefA*-mediated and *ermB*-mediated resistance were more the most common forms of macrolide resistance in SPN.**

More serotype diversity was observed for *mefE* mediated resistance.

Dual *mefE+/ermB+* 19F serotype SPN increased gradually each year and were replaced by the 19A serotype starting in 2007.

Serogroups 15, 33 and 35 have emerged since 2001 in Canada.

RESULTS

Table 1. Erythromycin and clindamycin MICs for various Mac-R genotypes

SPN Isolates (n/%)	All Ery-R (1438/100)	*mefE+* (748/52)	*ermB+* (545/38)	*mefE+/ermB+* (84/6)	*mefE-/ermB-* (61/4)	Ery-S (100)
Erythromycin						
MIC_{90} (µg/mL)	>64	4	>64	>64	64	0.06
Range (µg/mL)	1->64	1-16	1->64	1->64	1->64	0.03-025
Clindamycin						
MIC_{90} (µg/mL)	16	0.25	16	16	8	0.25
Range (µg/mL)	0.12->16	0.12-16	0.12->16	1->16	0.12-8	0.12-0.25

Fig. 2 Incidence of *mefE* and *ermB* mediated resistance over ten years (1998-2008) in Canada.

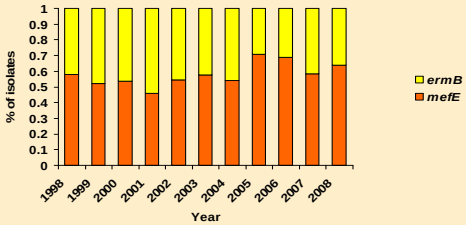


Fig. 4 Emergence of serogroups 15, 33, and 35 during 1998 and 2008 in Canada.

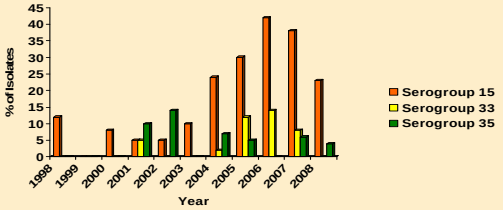


Fig. 1 Increase in macrolide resistance in SPN over 10 years (1998-2008) in Canada.

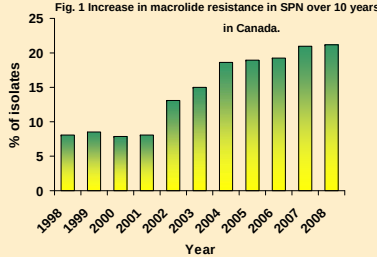


Fig. 3 Serotype distribution among *mefE*+ SPN and *ermB*+ SPN.

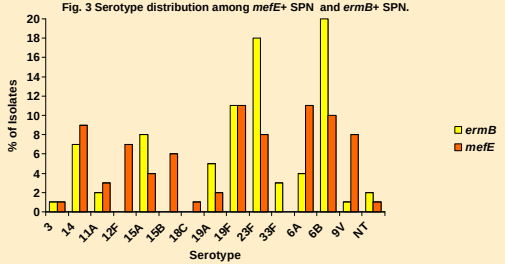
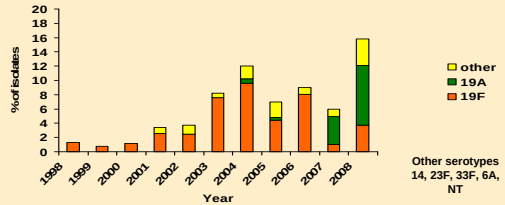


Fig. 5 Increase in dual *mefE+/ermB+* SPN and emergence of 19A Serotype



Other serotypes 14, 23F, 33F, 6A, NT