ASM Microbe 2018
SATURDAY - 743

CARA
www.can-r.ca

Pneumococcal Conjugate Vaccines: Potential Serotypes for Future Formulations

A.R. GOLDEN[1], H.J. ADAM[1,2], M. BAXTER[1], I. MARTIN[3], W. DEMCZUK[3], K. NICHOL[2], J.A. KARLOWSKY[1,2], M.R. MULVEY[1,3], G.G. ZHANEL[1], and the CANADIAN ANTIMICROBIAL RESISTANCE ALLIANCE (CARA)

[1]University of Manitoba, [2]Shared Health and [3]National Microbiology Laboratory, Winnipeg, Manitoba, Canada

Public Health Agency of Canada | Agence de santé publique du Canada

University of Manitoba

Alyssa Golden
Microbiology, Health Sciences Centre
MS673-820 Sherbrook St.
Winnipeg, MB R3A 1R9
Email: alyssa-golden@hotmail.com

Abstract

Background: The serotype distribution of *Streptococcus pneumoniae* (SPN) is constantly shifting due to vaccine pressure. Despite the addition of key serotypes (3, 19A) to PCV-13 in 2010, serotype replacement is already evident in the pneumococcal population. The purpose of this study was to identify additional SPN serotypes that warrant consideration for inclusion in future conjugate vaccine formulations. **Methods:** In a collaboration between CARA and NML, invasive SPN isolates were collected from across Canada from 2011-14 as part of the SAVE study. All isolates were serotyped by the Quellung reaction and tested for antimicrobial susceptibilities using CLSI methods. A subset of commonly collected non-PCV-13 serotypes (6C, 8, 9N, 11A, 12F, 15A, 22F, 33F, 35B) were further characterized using MLST and WGS analyses. **Results:** Serotypes 22F and 33F (already included in a 15-valent vaccine undergoing development in the US) appear to be good choices for inclusion based on our Canadian data; serotype 22F was highly clonal, with almost all isolates sharing relatedness to ST433. This characteristic may contribute to vaccine success for serotype 22F, as similarly clonal vaccine types (e.g., 7F) have rapidly decreased in prevalence following conjugate vaccine use. Serotype 33F demonstrated increasing genetic diversity and MDR, characteristics that are often possessed by successful serotypes. Other serotypes of interest included 35B and 15A. Approximately 40% of 35B were penicillin (PEN)-nonsusceptible, attributed to rapidly expanding ST558. These isolates possessed PBP1A alterations most commonly seen in 19A-ST320, indicating that, like serotype 19A, 35B may have the capacity to acquire full PEN resistance in the future. Additionally, capsular switch variant 35B-ST156 was identified in Canada; this persistent and successful international clone is associated with PEN resistance and MDR, and has recently increased in prevalence in the US. Serotype 15A was associated with the highest rate of MDR in Canada, attributable to international clone ST63. However, serotype 15A demonstrated an overall decreasing trend from 2011-2014, necessitating future monitoring to determine if prevalence continues to decrease. **Conclusion:** Based on analysis of Canadian SPN isolates, there are many serotypes that warrant consideration for inclusion in future conjugate vaccines, including 35B and 15A. Continued surveillance is necessary to identify trends moving forward, both within these serotypes and others that may become prevalent.

Introduction

The serotype distribution of *Streptococcus pneumoniae* is constantly shifting due to vaccine pressure. PCV-7, utilized in Canada beginning in 2002, was associated with significant decreases in the overall incidence of invasive pneumococcal disease (IPD), particularly due to vaccine serotypes (1). As the prevalence of IPD caused by vaccine serotypes decreased with PCV-7 use, non-vaccine serotypes expanded to fill the niche, thus necessitating the formulation of vaccines with enhanced serotype coverage (1). Despite the addition of common and antimicrobial-resistant serotypes such as 3 and 19A to PCV-13 in 2010, serotype replacement is already evident in the Canadian pneumococcal population (2,3).

The purpose of this study was to describe additional *S. pneumoniae* serotypes that may warrant consideration for inclusion in future conjugate vaccine formulations.

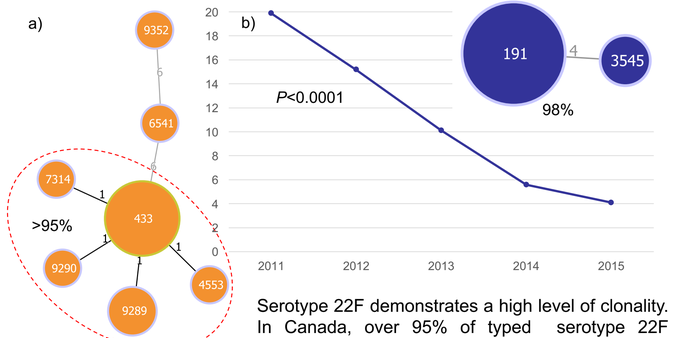
Materials and Methods

The opinions given in this work were drawn from isolates characterized as part of the SAVE Study, a collaborative effort between the Canadian Antimicrobial Resistance Alliance (CARA) and the Public Health Agency of Canada - National Microbiology Laboratory (PHAC-NML). Invasive *S. pneumoniae* isolates were collected from across Canada from 2011-2015 as part of the SAVE study and serotyped by the Quellung reaction at PHAC-NML. Antimicrobial susceptibility testing was performed by CARA using CLSI methods (4,5). Commonly collected non-PCV-13 serotypes (6C, 8, 9N, 11A, 12F, 15A, 22F, 33F, 35B) were characterized using MLST (50 isolates of each serotype) to determine sequence type (ST), and a small subset of these isolates were further characterized with WGS analyses as previously described (6).

Results

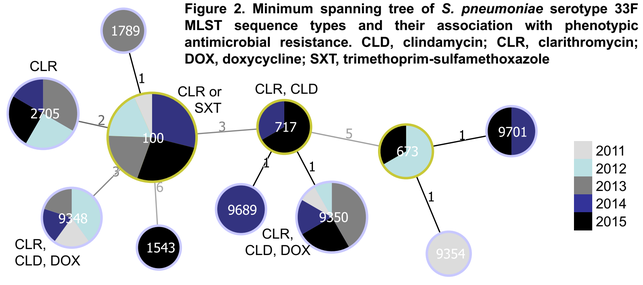
Serotypes 22F and 33F: included in 15-valent conjugate vaccine undergoing clinical trials in the United States

Figure 1. a) Minimum spanning tree of *S. pneumoniae* serotype 22F MLST sequence types; b) Comparison minimum spanning tree of clonal serotype 7F, and its decline in prevalence as a cause of IPD in Canada



Serotype 22F demonstrates a high level of clonality. In Canada, over 95% of typed serotype 22F isolates were related to ST433. This characteristic clonality may contribute to vaccine success for serotype 22F. A similar serotype, 7F, was included in PCV-13; almost all test isolates were ST191. Despite being a significant source of invasive disease at the time of PCV-13 release, serotype 7F has rapidly decreased in prevalence from 19.9% of IPD isolates collected in 2011 to 4.1% of isolates in 2015 (P<0.0001). Studies have estimated the specific PCV-13 vaccine effectiveness for this clonal serotype to be over 90% (7). It is possible that the similarly clonal serotype 22F will demonstrate comparable vaccine success upon the release and widespread use of PCV-15.

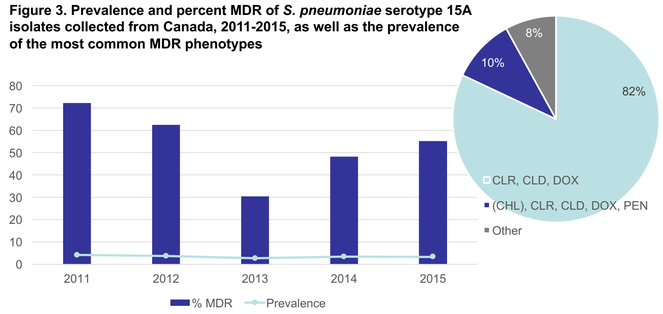
Figure 2. Minimum spanning tree of *S. pneumoniae* serotype 33F MLST sequence types and their association with phenotypic antimicrobial resistance. CLD, clindamycin; CLR, clarithromycin; DOX, doxycycline; SXT, trimethoprim-sulfamethoxazole



The prevalence of serotype 33F from invasive infections increased significantly from 2011 to 2015 (P<0.0001). Many STs identified in later study years were newly assigned and associated with antimicrobial resistance; in Canada, these new STs were commonly related to ST717 and possessed *ermB*/*tetM*-mediated resistance. In comparison, a recent US study identified newly assigned STs as related to ST100, possessing resistance mediated by *mef*A and *fol* mutations (8). This suggests that serotype 33F is diversifying from two lineages possessing different resistance mechanisms. Serotype 33F also demonstrated increasing genetic diversity (data not shown), a characteristic often possessed by successful serotypes such as 19A.

Serotypes 15A and 35B: not included in any known conjugate vaccine formulations

Figure 3. Prevalence and percent MDR of *S. pneumoniae* serotype 15A isolates collected from Canada, 2011-2015, as well as the prevalence of the most common MDR phenotypes



Though not particularly prevalent, serotype 15A was associated with high rates of MDR. These strains were most commonly resistant to clarithromycin, clindamycin and doxycycline and were related to international clone Sweden^{15A}-25 (ST63). ST2613 strains (an uncommon double-locus variant of ST63) possessed additional penicillin resistance, likely obtained through a past recombination event with multidrug resistant international clone Spain^{6B}-2 (ST90) resulting in an altered *pbp2B*. These strains are particularly concerning as a reservoir for the spread of antimicrobial resistance to other strains; serotype 15A-ST63 genomes were associated with a number of capsular switch events conferring resistance to other normally susceptible donor serotypes, such as 7F, 8 and 22F.

Table 1. Features and prevalence of key *S. pneumoniae* serotype 35B clones in Canada, in comparison to recent American data

Pneumococcal Clone • Features	Canadian Data	USA Comparator [a]
ST452 • Generally antimicrobial susceptible	**Common** ~40% of isolates [b]	**Decreasing** 5%
ST558 • SLV of PMEN24 • Penicillin-nonsusceptible	**Common** ~40% of isolates were penicillin-nonsusceptible [c]	**Increasing** 84%
ST156 • PMEN3 – persistent clone • Penicillin-resistant • Multi-drug resistant • Capsular switch variant	**Uncommon** 1 confirmed and 1 putative (over 5 years)	**Increasing** 11% (in 2015)

[a] (9) Chochua S et al. *Emerg Infect Dis.* 2017; **23**: 922-30. [b] of isolates with a MLST sequence type. [c] of all serotype 35B isolates with antimicrobial susceptibility data.

ST558 isolates possessed alterations in all three penicillin-binding proteins, conferring either intermediate or full penicillin resistance. Interestingly, the mutation identified in *pbp1A*, Thr371Ser in the STMK motif, was only seen in one other isolate group: extensively studied and known penicillin-resistant serotype 19A-ST320. ST320 isolates possessed an additional Pro432Thr alteration in the SRNVP motif of this gene, resulting in the highest penicillin MICs (4 µg/mL) observed within the cohort. As point mutations in PBPs occur in a stepwise manner, it is possible that in the future, a ST558 strain may acquire the additional *pbp1A* mutation resulting in higher level penicillin-resistance. If successful expansion of this clone occurred, serotype 35B-ST558 could become as big of a treatment issue as serotype 19A was in the post-PCV-7 era.

Conclusions

1. Based on analysis of Canadian *S. pneumoniae* isolates, there are a number of serotypes that warrant consideration for inclusion in future conjugate vaccines.
2. Serotypes 22F and 33F, already included in a 15-valent formulation, appear to be good selections. Serotype 22F demonstrated clonality, a characteristic possessed by recent vaccine-success serotype 7F. Serotype 33F appears to possess increasing multidrug resistance and diversity, making it an important serotype to gain control of in the population.
3. Serotype 15A demonstrated high levels of multidrug resistance; an uncommon variant of ST63 (ST2613) is concerning due to its unusual resistance pattern, which includes penicillin. It would be useful for this type to be vaccine preventable to reduce antibiotic use. However, this serotype demonstrated relatively low prevalence in Canada.
4. The expansion of penicillin-nonsusceptible (ST558) and multidrug resistant (ST156) clones of serotype 35B is concerning, though most strains in Canada were an antimicrobial susceptible clone. It will be important to monitor the clonal expansion of ST156, as it is a particularly resilient international clone. The potential development of higher-level penicillin resistance within ST558 (similar to serotype 19A) is also of concern.
5. Continued surveillance is necessary to identify trends moving forward, both within these serotypes and others that may become prevalent.

Acknowledgements

The authors would like to thank the participating Canadian Public Health Laboratory Network (CPHLN) sites: Saskatchewan Disease Control Laboratory (Regina, SK), Cadham Provincial Laboratory (Winnipeg, MB), Ontario Provincial Laboratory (Etobicoke, ON), Quebec Public Health Laboratory (Ste-Anne-de-Bellevue, QC), Queen Elizabeth Hospital Laboratory Medicine (Charlottetown, PEI), Horizon and Vitalité Health Networks (Various cities, NB), IWK Health Center (Halifax, NS) and Newfoundland Public Health Laboratory (St. John's, Newfoundland). We would also like to thank the Streptococcus and STI Unit at the Public Health Agency of Canada – National Microbiology Laboratory for their continued efforts on this project.

Support for this project was provided in part by the University of Manitoba, Health Sciences Centre and the National Microbiology Laboratory in Winnipeg, Manitoba, Canada, and Merck Canada.

The opinions expressed in this work are those of the authors, and do not necessarily represent those of Merck Canada.

References

1. Bettinger JA *et al. Vaccine*. 2010; 28: 2130-6.
2. Demczuk WHB *et al. Can J Microbiol.* 2013; 59: 778-88.
3. Golden AR *et al. J Antimicrob Chemother.* 2015; 70: 1960-4.
4. CLSI. *Methods for dilution and antimicrobial susceptibility tests for bacteria that grow aerobically; Approved Standard – 10th edition. M07-10.* Wayne, PA. CLSI 2015.
5. CLSI. *Performance standards for antimicrobial susceptibility testing; 27th informational supplement. M100-S27.* Wayne, PA. CLSI 2017.
6. Golden AR *et al. J Antimicrob Chemother.* 2018, in press.
7. Andrews NJ *et al. Lancet Infect Dis.* 2014; 14: 839-46.
8. Metcalf BJ *et al. Clin Microbiol Infect.* 2016; 22: 60.e9-60.e29.
9. Chochua S et al. *Emerg Infect Dis.* 2017; **23**: 922-30.