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Characterization of Non-PCV-13 *Streptococcus pneumoniae* Serotypes 22F and 33F Collected from Invasive Infections in Canada: The SAVE Study 2011-2016

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Introduction

Streptococcus pneumoniae is a clinically important Gram-positive pathogen responsible for significant global morbidity and mortality[1,2]. The clinical presentation of pneumococcal disease is highly variable, ranging from mild upper respiratory tract infections (otitis media, sinusitis) to severe diseases such as pneumonia and meningitis[1,2]. Isolation of *S. pneumoniae* from a sterile clinical site is termed invasive pneumococcal disease (IPD)[1].

Vaccination with pneumococcal conjugate vaccines (PCVs) has proven to be an effective strategy in combating pneumococcal disease. In Canada, routine vaccination with Prevnar® (PCV-7), a seven-valent formulation targeting pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F and 23F, both directly and indirectly reduced the incidence of IPD caused by vaccine serotypes in immunized and unimmunized children, respectively[3]. However, in response to vaccine pressure, multiple non-PCV-7 *S. pneumoniae* serotypes increased in prevalence through serotype replacement[3,4]. Subsequently, Prevnar®13 (PCV-13) was developed, offering expanded coverage against PCV-7 serotypes, plus 1, 3, 5, 6A, 7F and 19A[4,5].

Following introduction of PCV-13 in Canada, pneumococcal serotype distributions have continued to evolve over time; important replacement serotypes include 22F and 33F[5]. The increased invasiveness of serotypes 22F and 33F has led to their inclusion in a new, 15-valent candidate PCV formulation (PCV-15) currently undergoing clinical trials[5,6].

In the present study we assessed genetic relatedness, antimicrobial resistance and prevalence of *mef*(A/E), *erm*(B) and *tet*(M) resistance genes among serotype 22F and 33F isolates collected in Canada from 2011-2016.

Materials and Methods

Bacterial Isolates: A total of 7415 invasive *S. pneumoniae* isolates were collected from Canadian public health laboratories [Canadian Public Health Laboratory Network (CPHLN)] and forwarded to the Public Health Agency of Canada – National Microbiology Laboratory (PHAC-NML) from 2011-2016, inclusive. As part of the SAVE (Serotyping and Antimicrobial Susceptibility: Assessment for Vaccine Efficacy in Canada) study, carried out in collaboration with the Canadian Antimicrobial Resistance Alliance (CARA) and PHAC-NML, submitted isolates were subsequently forwarded to CARA with permission from participating CPHLN sites.

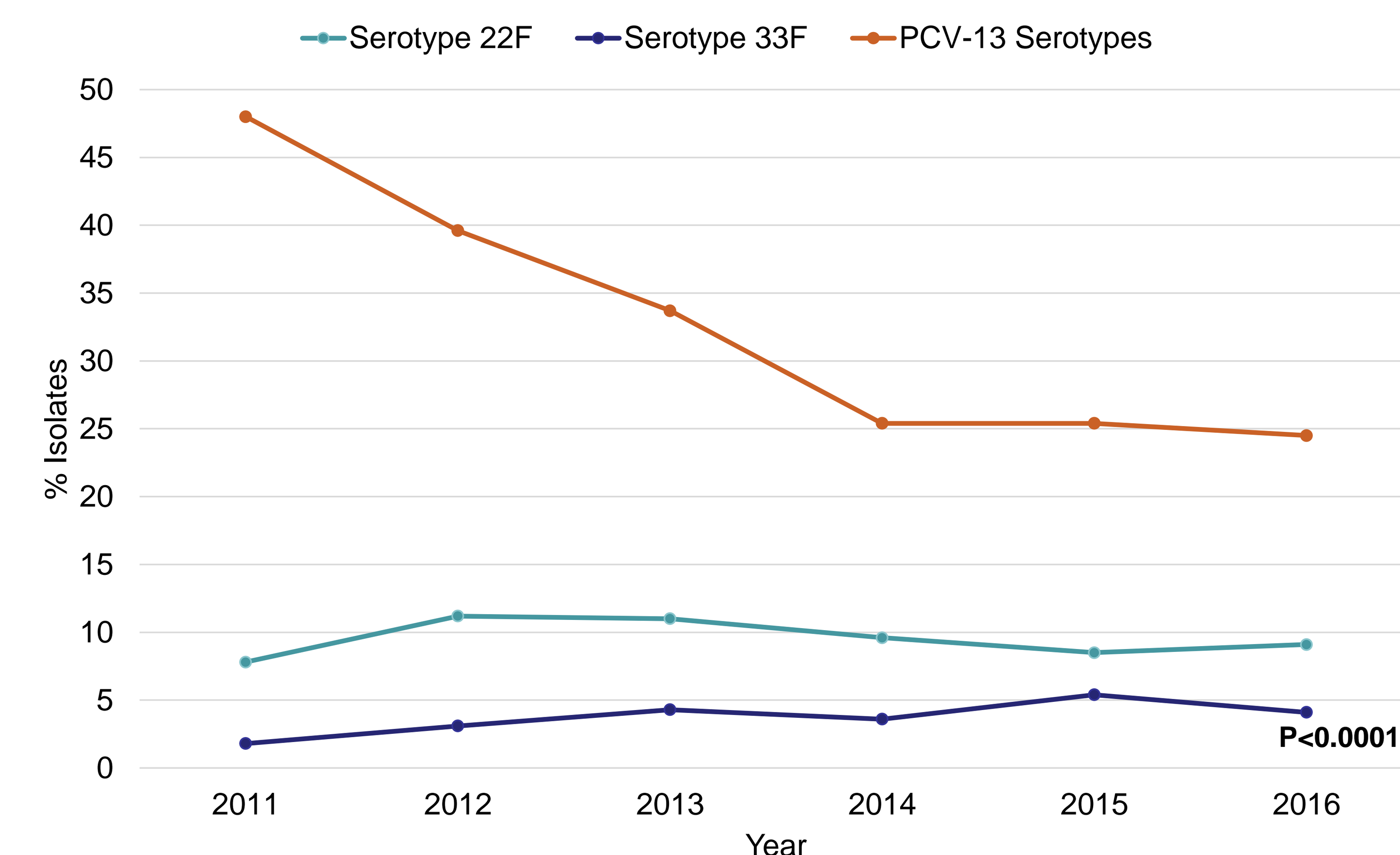
Phenotypic Characterization: Antimicrobial susceptibility testing was performed using in-house made broth microdilution panels, designed and tested in accordance with CLSI guidelines and quality control measures[7]. MIC interpretive criteria were defined according to CLSI breakpoints[8]. Multidrug resistance (MDR) was defined as resistance to ≥3 chemically unique antimicrobial classes (penicillin MIC ≥2 μg/mL). All isolates were serotyped by the Quellung reaction using pool, group, type and factor specific antisera (Statens Serum Institute, Copenhagen, Denmark).

Genotypic Characterization: To determine genetic relatedness, pulsed-field gel electrophoresis (PFGE) was performed on all isolates following digestion with *SmaI* as previously described[9]. Further genetic characterization of select isolates by multi-locus sequence typing (MLST) was performed using primers and methods as previously described[5]. Detection of *mef*(A/E), *erm*(B) and *tet*(M) resistance genes in all isolates was performed by PCR using primers and parameters as previously described[10,11].

Statistical Analysis: The Cochran-Armitage test was used to assess linear trends in serotype and genotype prevalence over time (p≤0.05).

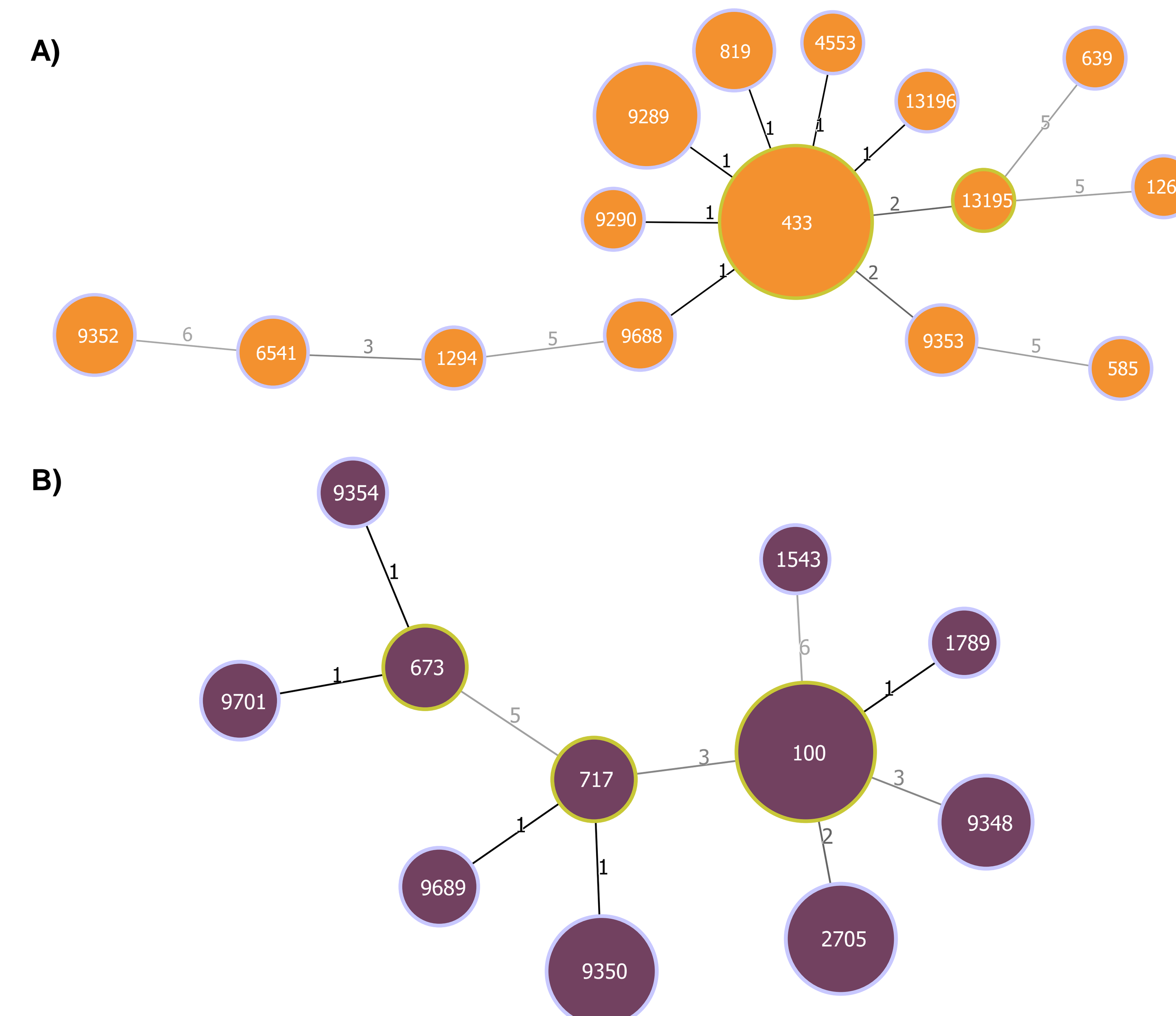
Results

Figure 1. Annual prevalence of invasive *S. pneumoniae* serotype 22F and 33F isolates compared to PCV-13 serotypes (2011-2016)



Multidrug Resistance:
From 2011-2016, serotype 22F demonstrated a MDR rate of 0.6% (4/701), with two MDR phenotypes being observed in equal proportion: CLR, CLD, DOX and CLR, CLD, DOX, LEV. Conversely, serotype 33F demonstrated a MDR rate of 5.2% (14/271), with a predominant CLR, CLD, DOX MDR phenotype (12/14).

Figure 2. Minimum spanning trees of *S. pneumoniae* serotypes A) 22F and B) 33F MLST sequence types. Green outlines indicate a group founder; numbers indicate the number of allele differences between the MLST profiles of the two connected nodes



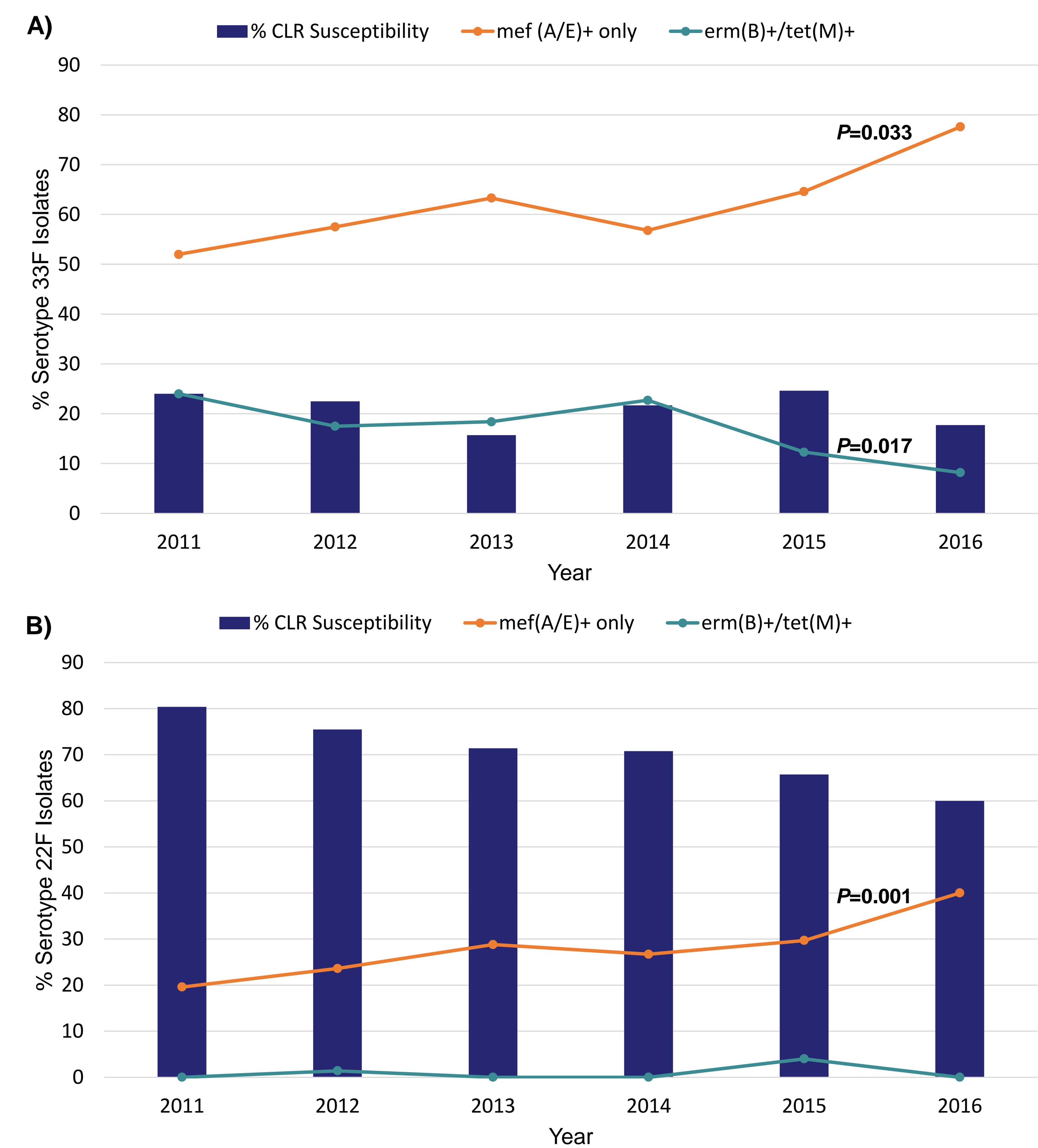
Demographics:
Of the 671 serotype 22F isolates for which gender was available, 353 (52.6%) were collected from males and 318 (47.4%) females. Conversely, of the 261 serotype 33F isolates for which gender was available, 121 (46.4%) were collected from males and 140 (53.6%) females. By age, 91 (13.2%), 286 (41.4%), and 314 (45.4%) of age-defined serotype 22F isolates (691) were collected from individuals ≤17 years, 18-64 years and ≥65 years, respectively. Of the 266 age-defined serotype 33F isolates, 52 (19.5%), 117 (44.0%), and 97 (36.5%) were collected from individuals ≤17 years, 18-64 years and ≥65 years, respectively. For both serotype 22F (490, 96.6%) and 33F (187, 68.8%), the largest proportion of isolates were collected from Central Canada (Ontario and Quebec).

Table 1. Antimicrobial susceptibility of *S. pneumoniae* serotype 22F and 33F isolates collected in Canada from 2011-2016

Serotype (N)	% Susceptibility								
	CLR	CLD	CRO (M)	CRO (NM)	DOX	LEV	PEN (M)	PEN (NM)	SXT
22F (701)	71.9	98.3	99.9	99.9	98.9	99	99.6	99.9	98.7
33F (271)	20.9	83.8	100	100	86.7	100	99.6	100	28.8

CLR, clarithromycin; CLD, clindamycin; CRO, ceftriaxone; M, meningitis breakpoints; NM, non-meningitis breakpoints; DOX, doxycycline; LEV, levofloxacin; PEN, penicillin; SXT, trimethoprim-sulfamethoxazole

Figure 3. Annual clarithromycin susceptibility and prevalence of macrolide resistant genotypes among *S. pneumoniae* serotype A) 33F and B) 22F isolates (2011-2016)



Conclusions

1. Of the 7415 invasive *S. pneumoniae* isolates collected from 2011-2016, serotypes 22F and 33F accounted for 9.5% (n=704) and 3.7% (n=272), respectively.
2. By trend analysis, it was determined that the prevalence of serotype 22F remained stable throughout the study period. Conversely, serotype 33F demonstrated a statistically significant increase in prevalence from 2011-2016 (*P*<0.0001).
3. Serotype 22F demonstrated >98% susceptibility to all antimicrobials except CLR, with a MDR rate of 0.6%. Serotype 33F demonstrated decreased susceptibility to common antimicrobials (CLR, CLD, DOX and SXT), with a MDR rate of 5.2%.
4. Efflux, encoded by *mef*(A/E), was identified as the predominant mechanism of macrolide resistance, where the *mef*(A/E)+ only genotype of serotypes 22F and 33F increased significantly from 2011-2016 (*P*=0.001 and *P*=0.033, respectively).
5. The *erm*(B)+/*tet*(M)+ genotype, conferring high-level macrolide resistance and tetracycline resistance, was more commonly identified in serotype 33F isolates; however, there was a statistically significant decrease from 2011-2016 (*P*=0.017). This genotype was negligible in serotype 22F isolates (n=6, 0.9%).
6. Both 22F and 33F serotypes were highly clonal by PFGE (data not shown), with >85% of isolates falling within a single cluster (isolates with ≥80% relatedness were considered a cluster). Serotype 22F was similarly clonal by MLST (~95% of isolates related to ST433); however, serotype 33F demonstrated higher diversity by MLST in comparison to PFGE.

Acknowledgements

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