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Population structure of invasive *Streptococcus pneumoniae* isolates among Alaskan children in the conjugate vaccine era, 2001 to 2013☆,☆☆

Karen M. Miernyk *, Lisa R. Bulkow, Samantha L. Case [1], Tammy Zulz, Michael G. Bruce, Marcella Harker-Jones, Debby A. Hurlburt, Thomas W. Hennessy, Karen M. Rudolph

Arctic Investigations Program, Division of Preparedness and Emerging Infections, National Center for Emerging and Zoonotic Infections Diseases, Centers for Disease Control and Prevention, 4055 Tudor Centre, Dr., Anchorage, AK, 99508, USA

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ABSTRACT

Here we describe the relationships between serotypes, genotypes, and antimicrobial susceptibility among isolates causing invasive pneumococcal disease in Alaskan children during the pneumococcal conjugate vaccine (PCV) era. From 2001 to 2013 we received 271 isolates representing 33 serotypes. The most common serotypes were 19A (29.5%, $n=80$), 7F (12.5%, $n=34$), 15B/C (6.3%, $n=17$), and 22F (4.8%, $n=13$). Multilocus sequence typing identified 11 clonal complexes (CC) and 45 singletons. Five CCs accounted for 52% (141/271) of the total: CC199 (21% [$n=57$], serotypes 19A, 15B/C), CC191 (12.2% [$n=33$], serotype 7F), CC172 (10.3% [$n=28$], serotypes 19A, 23A, 23B), CC433 (4.4% [$n=12$], serotype 22F), and CC100 (4.4% [$n=12$], serotype 33F). The proportion of isolates nonsusceptible to erythromycin and tetracycline increased after 13-valent PCV use (14% [$n=30$] versus 29% [$n=14$]; $P=0.010$) and (4% [$n=9$] versus 22% [$n=11$]; $P<0.001$), respectively. The genetic diversity also increased after 13-valent PCV use (Simpson's diversity index =0.95 versus 0.91; $P=0.022$).

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1. Introduction

Worldwide each year, there are over 14 million serious *Streptococcus pneumoniae* (*S. pneumoniae*) infections in children <5 years of age leading to over 800,000 deaths (O'Brien et al., 2009). There are at least 95 capsular serotypes, but only a few cause the majority of disease. In 2001, the United States began 7-valent pneumococcal conjugate vaccine (PCV7) use in children <2 years of age. This vaccine contains polysaccharides from serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F. These serotypes caused approximately 80% of invasive pneumococcal disease (IPD) in US infants at that time (Robinson et al., 2001). After PCV7 use, many countries documented a decrease in PCV7-serotype IPD rates; however, increases in non–PCV7-serotype IPD were also documented in many of these same countries (Whitney et al., 2003; Hicks et al., 2007; Tyrrell et al., 2009; Oftadeh et al., 2013; Maraki et al., 2010; Isaacman et al., 2010; Ben-Shimol et al., 2014). This serotype shift led to the 2010 US implementation of a 13-valent pneumococcal conjugate vaccine (PCV13) for children <2 years of age. PCV13 includes PCV7 serotypes as well as 6 additional (PCV6; serotypes 1, 3, 5, 6A, 7F, and 19A). Data from many parts of the world, including the United States, show that PCV13 is decreasing IPD caused by these additional serotypes (Ben-Shimol et al., 2014; Richter et al., 2014; Waight et al., 2015; Moore et al., 2015; Cohen et al., 2016).

Alaska IPD rates have historically been among the highest in the world (Davidson et al., 1994). Alaska began using PCV7 in 2001 with vaccine rollout being rapid and fairly consistent throughout the state. As was seen elsewhere, PCV7 use in Alaska resulted in a change in the serotypes causing IPD. In children <2 years of age, the PCV7-serotype IPD rate decreased 96% and the non–PCV7-serotype IPD rate increased 140% (Singleton et al., 2007). PCV13 replaced PCV7 in the Alaska vaccination schedule in April 2010. PCV13 rollout was more rapid in one region as compared with the rest of the state due to an existing PCV13 clinical trial in that region (Singleton et al., 2013). In children <5 years of age, the overall IPD rate in Alaska decreased 59% from 2010 to 2013 as compared with 2005 to 2008 (Bruce et al., 2015).

Pneumococcal conjugate vaccines (PCVs) target the serotype-specific capsular genes; however, it is known that pneumococci include different clones that can express the same capsular gene (Henriques-Normark et al., 2008). Therefore, relying on serotype data alone gives only partial information about the pneumococcal population structure. Multilocus sequence typing (MLST) is now commonly used to better understand pneumococcal epidemiology (Enright & Spratt, 1998). In this study, we used MLST to characterize pneumococci causing IPD in

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* Corresponding author: Tel.: +1-907-729-3453; fax: +1-907-729-3429.
E-mail address: kmiernyk@cdc.gov (K.M. Miernyk).

[1] Present address: Alaska Office, Western States Division, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Anchorage, Alaska USA.

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young Alaskan children and to describe relationships between serotypes, antimicrobial nonsusceptibility, and MLST clonal complexes (CC) and sequence types (ST). We also investigated changes in the pneumococcal diversity.

2. Materials and methods

2.1. Bacterial isolates

Since 1986, *S. pneumoniae* isolated from a normally sterile site in an Alaska resident are sent from laboratories around the state to the US Centers for Disease Control and Prevention's Arctic Investigations Program (AIP) in Anchorage (Bruce et al., 2015). The AIP laboratory confirms all pneumococci using standard methods (Ruoff et al., 1999). Isolates are serotyped using latex agglutination and the Quellung reaction with group-specific and type-specific antisera (Staten Serum Institute, Copenhagen, Denmark) (Austrian, 1976).

2.2. Antimicrobial susceptibility testing

The AIP laboratory performs all susceptibility testing. Broth microdilution is used to determine minimum inhibitory concentrations for penicillin (PEN), erythromycin (ERY), trimethoprim-sulfamethoxazole (SXT), tetracycline (TET), and cephalosporins (cefotaxime/ceftriaxone [CTX/CRO]) (Clinical and Laboratory Standards Institute, 2007). Per Gertz et al., nonsusceptibility interpretation is made using the 2007 interpretive Clinical and Laboratory Standards Institute guidelines and includes isolates that are intermediate or fully resistant to a particular antibiotic (Clinical and Laboratory Standards Institute, 2007; Gertz et al., 2010). An isolate is considered multidrug resistant (MDR) if it has intermediate or full resistance to 3 or more antibiotic classes.

2.3. Multilocus sequence typing

For this study, we completed MLST on pneumococcal isolates received from children <5 years of age collected from 2001 to 2013. To extract DNA, we transferred *S. pneumoniae* cells to 100 μL nuclease-free water. The suspensions were vortexed for 10 to 15 seconds, heated at 100 °C for 10 minutes, and centrifuged at 13,000 rpm for 5 minutes. The supernatant was removed and stored at −30 °C until use. We performed MLST as previously described (Enright & Spratt, 1998) determining the STs by comparing our sequences with those from the *S. pneumoniae* MLST database (http://pubmlst.org/spneumoniae). For alleles or allelic profiles not in the MLST database, we submitted the trace files or allelic profiles to the MLST database curator for new ST assignments. We assigned CCs using the eBURST algorithm (http://eburst.mlst.net) and the stringent definition of 6/7 identical loci.

2.4. Statistical analysis

We compared isolate proportions by serotype, ST, and additional factors using chi-squared or Fisher's exact test as appropriate. We defined time periods to correspond with Alaska's vaccine introduction: 2001 to 3/2010 "PCV7 era", and 4/2010 to 2013 "PCV13 era". Incidence rates were calculated using population estimates obtained from the State of Alaska, Department of Labor (http://laborstats.alaska.gov/pop/popest.htm) and were compared using the statistics described in Rothman (Rothman, 1986). We measured diversity using the Gini-Simpson Index which estimates the probability that 2 randomly drawn isolates are different types (Heip & Engels, 1974) and compared diversity indices using a Student's *t* test as described in Lande (Lande, 1996). *P* values are two-sided and a *P* value <0.05 is considered statistically significant. Analyses were conducted in Stata Ver10.

3. Results

3.1. Bacterial isolates

From 2001 to 2013, the AIP received 271 pneumococcal isolates from Alaska residents <5 years of age with IPD. Isolates came from blood (n= 252), cerebrospinal fluid (n= 22), pleural fluid (n= 9), and other sterile sites (n= 8). Twenty cases had 2 isolates (blood/cerebrospinal fluid, n= 18; blood/pleural fluid, n= 2), but they were identical to each other so they were considered as one for this analysis.

3.2. Serotype distribution

Overall, we identified 33 serotypes among the 271 IPD isolates (Fig. 1). There were no serotype differences between the isolate sources and for serotypes for which there was a large enough sample size, serotypes were distributed across the state. Four serotypes accounted for over 53% (144/271) of the isolates: 19A (29.5%, n= 80), 7F (12.5%, n= 34), 15B/C (6.3%, n= 17), and 22F (4.8%, n= 13). In the PCV7 era, 3 serotypes covered over 51% (114/222) of the isolates: 19A (32.4%, n= 72), 7F (14.4%, n= 32), and 15B/C (4.5%, n= 10). In the PCV13 era, 5 serotypes covered over 55% (27/49) of isolates: 19A (16.3%, n= 8), 15B/C (14.3%, n= 7), 12F (8.2%, n= 4), 15A (8.2%, n= 4), and 22F (8.2%, n= 4).

Despite only 1 PCV7 serotype (19F) identified in the PCV13 era, the proportion of PCV7 serotypes did not change from the PCV7 era to the PCV13 era (10.4% [23/222] versus 4.1% [2/49]; P= 0.27; Fig. 2). The proportion of PCV6 serotypes decreased from 54% (120/222) in the PCV7 era to 20% (10/49) in the PCV13 era (P< 0.001). All PCV6 isolates in the PCV13 era were serotypes 19A (n= 8) and 7F (n= 2). The PCV6-IPD rate also decreased during this time period (25.5 versus 4.85 per 100,000; P< 0.001).

We identified 22 nonvaccine type (NVT) serotypes. Five serotypes accounted for 53% (62/116) of the NVT isolates: 15B/C (14.7%, n= 17), 22F (11.2%, n= 13), 12F (10.3%, n= 12), 33F (10.3%, n= 12), and 15A (6.9%, n= 8). Although the NVT-IPD rate did not change (16.79 versus 17.93 per 100,000; P= 0.733), the proportion of NVT serotypes increased from 36% (79/222) in the PCV7 era to 76% (37/49) in the PCV13 era (P< 0.001). NVT serotypes contributing to this increase were serotypes 15B/C (4.5% [10/222] versus 14.3% [7/49]; P= 0.019), 15A (1.8% [4/222] versus 8.2% [4/49]; P= 0.038), and 35B (0.9% [2/222] versus 6.1% [3/49]; P= 0.043). IPD rates did not change for any NVT serotype.

3.3. MLST

Overall, we identified 83 STs; 18 were new to the MLST database. ST199 (serotypes 19A [n= 32] and 15B/C [n= 8]) was the most common; 44 STs had only 1 isolate. Two STs, ST644 and ST3934, were associated with serotype 15B/C which had not previously been documented in the MLST database. Of the 83 STs, 52 were found only during the PCV7 era (Fig. 3; black), 16 were found only during the PCV13 era (green), and 15 were found in both eras (pink). A majority of isolates from the PCV13 era (63% [31/49]), were associated with STs that were circulating in the PCV7 era.

The 83 STs resolved into 11 CCs and 45 singletons (Table 1). PCV7 serotypes were represented by 14 CCs/STs, PCV6 serotypes by 12 CCs/STs, and NVT serotypes by 33 CCs/STs. The genetic diversity within PCV7 (Simpson's diversity index [SDI]= 0.94) and NVT serotypes (SDI = 0.95) was significantly higher than the genetic diversity within PCV6 serotypes (SDI = 0.77; P< 0.001 for both PCV7 versus PCV6 and NVT versus PCV6). Five CCs accounted for 52% (142/271) of the isolates: CC199 (21% [n= 57]; serotypes 19A, 15B/C), CC191 (12.2% [n= 33]; serotype 7F), CC172 (10.3% [n= 28]; serotypes 19A, 23A, 23B), CC433 (4.4% [n= 12]; serotype 22F), and CC100 (4.4% [n= 12]; serotype 33F). CC199 was the most common CC/ST in both the PCV7 (22.1%, n= 49) and PCV13 (16.3%, n= 8) eras. In the PCV13 era 50% (4/8) of CC199