International Journal of Medical Microbiology 307 (2017) 415–421

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

journal homepage: www.elsevier.com/locate/ijmm

Key features of invasive pneumococcal isolates recovered in Lima, Peru determined through whole genome sequencing

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ARTICLE INFO

Keywords:
S. pneumoniae
Whole genome sequencing
Antimicrobial resistance

ABSTRACT

Before PCV7 introduction, invasive pneumococcal disease (IPD) was responsible for approximately 12,000–18,000 deaths annually among children < 5 years in Latin America. In Peru, PCV7 was introduced in 2009. We used whole genome sequencing to deduce key features of invasive strains collected in Lima, Peru from 2006 to 2011. We sequenced 212 IPD isolates from 16 hospitals in Lima pre (2006–2009; n = 133) and post (2010–2011; n = 79) PCV7 introduction; 130 (61.3%) isolates were from children ≤ 5 years old. CDC's *Streptococcus* lab bioinformatics pipeline revealed serotypes, sequence types (STs), pilus genes, PBP types and other resistance determinants. During the pre-PCV7 period, serotype 14 was the most common serotype (24.8%), followed by 6 B (20.3%), 19F (10.5%), and 23F (6.8%). Post-PCV7, the proportion of PCV7 serotype 6 B decreased significantly (to 6.3%), while 19F (16.3%), 14 (15.0%), 23F (7.5%), and 19A (7.5%) were the most common serotypes; only serotypes 3 and 10A increased significantly. Overall, 82% (n = 173) of all isolates carried at least one resistance determinant, including 72 (34%) isolates that carried resistance determinants against 3 or more antimicrobial classes; of these 72 isolates, 56 (78%) belonged to a PCV7 serotype. Eighty-two STs were identified, with 53 of them organized in 14 clonal complexes. ST frequencies were distributed differently pre and post-PCV7 introduction, with only 18 of the 57 STs identified in years 2006–2009 isolates also observed in years 2010–2011 isolates. The apparent expansion of a 19F/ST1421 lineage with predicted β-lactam resistance (PBP type 13:16:20) and carrying resistance determinants against four additional antimicrobial classes was observed.

1. Introduction

Infections caused by *Streptococcus pneumoniae* include serious conditions such as meningitis, bacteremia, and pneumonia as well as less severe conditions such as sinusitis and otitis media. The World Health Organization (WHO) estimated that pneumococcal infections caused 476,000 (5%) deaths among HIV-negative children under five years of age during 2008 (WHO, 2008). *S. pneumoniae* includes > 90 serotypes. Prior to introduction of pneumococcal conjugate vaccines, only 11 of these serotypes accounted for the majority of invasive pneumococcal disease (IPD) in children worldwide (Johnson et al., 2010).

The first pneumococcal conjugate vaccine covered 7 serotypes (PCV7: 14, 6B, 19F, 23F, 4, 9 V, 18C) and was licensed in 2000, followed by PCV10 (PCV7 serotypes plus 1, 5, and 7F) in 2009, and PCV13 (PCV10 serotypes plus 3, 6A, and 19A) in 2010. These vaccines have been shown to be highly effective for protecting infants and young children against IPD caused by vaccine serotypes and to diminish acquisition of carriage by serotypes included in the vaccine (WHO, 2012).

In Latin America and the Caribbean, pneumococcal infections were estimated to account for 12,000–18,000 deaths, 327,000 cases of pneumonia, 4000 cases of meningitis and 1229 cases of sepsis each year in children aged under five years before vaccine introduction

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http://dx.doi.org/10.1016/j.ijmm.2017.07.008
Received 17 April 2017; Received in revised form 20 June 2017; Accepted 18 July 2017

(Constenla et al., 2007). In Peru, PCV7 was introduced into the national immunization program in 2009 as a 3 dose schedule at 3, 5, and 12 months of age; it was replaced by PCV10 in late 2011, given in three doses at 2, 4, and 12 months of age. A catch-up campaign included two doses for unvaccinated children between 12 and 24 months of age and a single dose for children 2–5 years old with a comorbidity. In 2007, it was estimated that PCV7 would cover 62% of the circulating isolates recovered from children under 6 years of age in Peru, while PCV10 would cover 71% (Ochoa et al., 2007).

The aim of this study was to determine serotypes, genotypes, and resistance determinants of IPD isolates relevant to current conjugate vaccine evaluation and prospective prevention efforts in Lima, Peru. A secondary aim was to evaluate the performance of the automated whole genome sequence (WGS) bioinformatics pipeline developed by the *Streptococcus* lab at CDC in deducing these key features among these isolates, since it had only been previously employed with U.S. IPD isolates (Metcalf et al., 2016a,b; Li et al., 2016).

2. Materials and methods

We extracted DNA from 212 IPD isolates from children and adults in 16 hospitals in Lima, pre (2006–2009; n = 133) and post (2010–2011; n = 79) PCV7 introduction, as part of a passive surveillance study conducted by the Peruvian Group on Pneumococcal Research (Grupo Peruano de Investigación en Neumococo, GPIN). All isolates were serotyped by latex agglutination and the Quellung reaction employing CDC antisera. E-tests (Biomérieux, Marcy l'Etoile, France) were performed on select isolates.

Whole genome sequencing was performed at the Sanger Institute using the Illumina HiSeq 2500 system, as part of the Global Pneumococcal Sequencing project (www.pneumogen.net), and data submitted to the European Nucleotide Archive (accession numbers in Table S1). Sequences were analyzed using the CDC's *Streptococcus* lab pneumococcal typing pipeline to identify serotypes, sequence types (STs), pilus genes, transpeptidase domain amino acid sequences from penicillin-binding proteins (PBPs) 1a, 2b, and 2x, and other resistance features (https://github.com/BenJamesMetcalf/Spn_Scripts_Reference) (Metcalf et al., 2016a,b). Non-susceptibility to 6 different β-lactams was predicted by assigning a PBP type as previously described (Metcalf et al., 2016a,b; Li et al., 2016), and correlating this PBP type with phenotypically measured MIC values for isolates with the same type (http://www.cdc.gov/streplab/mic-tables.html), based on current CLSI guidelines (CLSI, 2015). Penicillin susceptibility, intermediate resistance, and resistance were defined as MIC of $\leq$0.06, 0.12–1.0, and $\geq$2.0 mg/L, respectively. For cefotaxime and ceftriaxone, susceptibility, intermediate resistance, and resistance were defined as MIC of $\leq$1.0, 2.0, and $\geq$4.0 mg/L, respectively. Cefuroxime susceptibility, intermediate resistance, and resistance were defined as MIC of $\leq$0.5, 1.0, and $\geq$2.0 mg/L, respectively. Amoxicillin susceptibility, intermediate resistance, and resistance were defined as MIC of $\leq$2.0, 4.0, and $\geq$8.0 mg/L, respectively. Meropenem susceptibility, intermediate resistance, and resistance were defined as MIC of $\leq$0.25, 0.5, and $\geq$1.0, respectively. For previously unreported PBP types, MIC values against penicillin and cefotaxime were determined using E-tests (Biomérieux, Marcy l'Etoile, France). Contingency tables and a chi-squared test (or a Fisher's exact test) were used to determine significance of associations (at $\alpha = 0.05$).

3. Results and discussion

3.1. Serotype and sequence type distribution

The majority of the samples were isolated from blood (57%), CSF (24%), or pleural fluid (10%); 130 (61.3%) were isolated from children age 5 and under (101 pre-PCV7 and 29 post-PCV7). The most common clinical manifestations were pneumonia (45.3%) and meningitis (29.2%); the proportions did not differ significantly pre and post-PCV7 introduction. There was a significant association between serotype 14 and pneumonia (p = 0.01), as well as between serotype 5 and pneumonia, where 86% of isolates (6/7) were obtained from patients with pneumonia (five of those patients were age 2 or younger). Thirty-four serotypes were identified among the 212 isolates, with serotype 14 being the most common (24.8%), followed by 6 B (20.3%), 19F (10.5%), and 23F (6.8%) pre-PCV7 introduction; 19F (16.3%), 14 (15.0%), 23F (7.5%), and 19A (7.5%) were the most common serotypes post-PCV7. We observed almost perfect concordance between conventional and WGS-based serotype determination. The only exception was an isolate that was non-typable by quellung, but was determined to be serotype 13 by WGS-based serotyping.

The proportion of PCV7 serotype 6 B decreased significantly (to 6.3%, p = 0.003) after vaccine introduction, as did that of serotype 14 (to 15.2%, p = 0.05). The proportion of serotypes 23F, 4, and 9 V changed very little. On the other hand, the proportion of 19F increased slightly by 5.9% (p = 0.11), possibly due to serotype-specific PCV7 effectiveness (lowest for 19F at 87%) and the short time period between PCV7 introduction and our observations; it has been shown that serotype 19F isolates can still persist several years after vaccine introduction (Metcalf et al., 2016a). The proportion of non-PCV7 serotypes 3 and 10A increased significantly (p = 0.05 and p = 0.02, respectively) (Fig. 1).

Among children age 5 and under, 14 (29.7%), 6 B (23.8%), and 19F (6.9%) were the most common serotypes recovered pre-PCV7; this distribution is consistent with previous reports (Constenla et al., 2007), including a study on a subset of these isolates (Ochoa et al., 2010) and the PAHO's SIREVA II program findings in Peru for 2008 (OPS, 2009). The overall proportion of PCV7 serotypes (69.3%) was in concordance with previous estimates (Ochoa et al., 2007). After PCV7 was introduced, serotype 14 decreased to 24.1% (p = 0.28) and 6 B decreased to 6.9% (p = 0.02), while the proportions of 19F and 19A isolates increased, but not significantly (p = 0.09 and p = 0.17, respectively), and the proportion of non-PCV7 serotypes 3 and 10A increased significantly (p = 0.05 and p = 0.01, respectively) (Fig. 2). In contrast, the SIREVA II 2012 report showed that 19A was the most common serotype among isolates recovered from children under 5 years old in Peru (OPS, 2013a). Replacement by non-vaccine serotypes, such as 19A, was also observed in the United States (Hicks et al., 2007; Moore et al., 2008) and other countries (WHO, 2010) after PCV7 introduction.

Eighty-two STs were identified among all isolates, with 53 organized in fourteen clonal complexes (CC) (Table 1). The most prevalent CCs were CC156 pre-PCV7 and CC1421 post-PCV7 introduction. Of the 57 STs identified among 2006–2009 isolates, only eighteen remained after PCV7 introduction, along with 26 unique STs from 2010 to 2011 isolates. Pre-PCV7, CC156 was most commonly associated with serotype 14 and thus expectedly declined along with this serotype after PCV7 introduction; this was also the case for ST15 and ST25. ST1121, ST90 and ST135 declined along with serotype 6 B after PCV7 introduction, while CC1421 increased alongside serotype 19F. No significant capsular switching or clonal shift was observed among these isolates, probably due to the limited post-PCV7 period covered by this study.

There were several PMEN clones (www.pneumogen.net/pmen/) represented among these isolates: PMEN1, PMEN2, PMEN3, PMEN14, PMEN15, PMEN19, PMEN20, and PMEN26. Most notably, PMEN3 (Spain^{9V}-ST156) was represented by 38 ST156 isolates (36 serotype 14, one 9 V, and one 23F); while PMEN14 (Taiwan^{19F}-ST236) was represented by 14 19F/ST1421 (double locus variant) isolates, in addition to the previously reported 'vaccine escape' 19A/ST320 isolates (Metcalf et al., 2016a; Moore et al., 2008; Beall et al., 2011).

3.2. Antimicrobial resistance

The capability of a WGS-based approach to accurately and reliably