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Population genetic structure, antibiotic resistance, capsule switching and evolution of invasive pneumococci before conjugate vaccination in Malawi

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ABSTRACT

Introduction: Pneumococcal infections cause a high death toll in Sub Saharan Africa (SSA) but the recently rolled out pneumococcal conjugate vaccines (PCV) will reduce the disease burden. To better understand the population impact of these vaccines, comprehensive analysis of large collections of pneumococcal isolates sampled prior to vaccination is required. Here we present a population genomic study of the invasive pneumococcal isolates sampled before the implementation of PCV13 in Malawi.
Materials and methods: We retrospectively sampled and whole genome sequenced 585 invasive isolates from 2004 to 2010. We determine the pneumococcal population genetic structure and assessed serotype prevalence, antibiotic resistance rates, and the occurrence of serotype switching.
Results: Population structure analysis revealed 22 genetically distinct sequence clusters (SCs), which consisted of closely related isolates. Serotype 1 (ST217), a vaccine-associated serotype in clade SC2, showed highest prevalence (19.3%), and was associated with the highest MDR rate (81.9%) followed by serotype 12F, a non-vaccine serotype in clade SC10 with an MDR rate of 57.9%. Prevalence of serotypes was stable prior to vaccination although there was an increase in the PMEN19 clone, serotype 5 ST289, in clade SC1 in 2010 suggesting a potential undetected local outbreak. Coalescent analysis revealed recent emergence of the SCs and there was evidence of natural capsule switching in the absence of vaccine induced selection pressure. Furthermore, majority of the highly prevalent capsule-switched isolates were associated with acquisition of vaccine-targeted capsules.
Conclusions: This study provides descriptions of capsule-switched serotypes and serotypes with potential to cause serotype replacement post-vaccination such as 12F. Continued surveillance is critical to monitor these serotypes and antibiotic resistance in order to design better infection prevention and control measures such as inclusion of emerging replacement serotypes in future conjugate vaccines.

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1. Introduction

With over one million deaths and approximately fifteen million disease episodes annually, *Streptococcus pneumoniae* (the pneumococcus), is one of the most significant global causes of serious human infections including pneumonia, bacteremia and meningitis [1]. The highest burden and mortality due to invasive pneumococcal disease (IPD) occurs in resource poor settings such as Sub-Saharan Africa (SSA). In Malawi, it is the highest cause of bacterial meningitis [2,3] and the second highest cause of bacteremia [4]. The incidence of adult IPD is estimated at 58 per 100,000 with the highest rates (108 per 100,000) recorded in adults aged between 35 and 40 due to high HIV prevalence [5] and rates in children substantially higher than this based on hospitalization data [6]. Nasopharyngeal carriage rates have been reported as 20% in adults [7] and 42% in children [8] often involving simultaneous carriage with multiple serotypes [9].

The heptavalent pneumococcal conjugate vaccine (PCV7), licensed in 2000 (www.gavi.org) targeted the seven most prevalent serotypes in the US out of nearly 100 serotypes characterized globally [10] and is highly effective against vaccine type IPD [11]. In contrast with the US, there was low theoretical serotype coverage in SSA (e.g. 40% in Malawi) due to dominance of non-PCV7 targeted serotypes particularly serotype 1 [8,12]. Despite the high efficacy of PCV7 [13], following vaccination non-vaccine serotypes became more common in carriage and IPD, a phenomenon termed serotype replacement [14]. To guard against the emerging replacement serotypes such as 19A [14] and expand serotype coverage, higher valency PCVs (PCV10 and PCV13) were licensed. PCV13 was introduced in Malawi in 2011 [15], which targets serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F.

Given that changes in the pneumococcal genome particularly the capsule biosynthesis genes could impact effectiveness of pneumococcal conjugate vaccine formulations, a crucial component of ensuring sustained prevention of pneumococcal disease will be monitoring pneumococcal genomic and phenotypic evolution over time. In Malawi, previous work investigated the genetic structure of the invasive isolates [16] but due to limitations including a smaller dataset (n = 134), it was not adequate to effectively resolve the genetic structure and temporal evolution of the pneumococcal lineages. Here we extend this analysis to conduct a population genomic analysis of whole genome sequenced invasive isolates (n = 585) sampled over a seven-year period (2004–2010) before the implementation of PCV13 vaccine in November 2011 in Malawi. Due to the fact that pneumococci frequently switch their serotype by swapping genes between different serotypes involved in capsule biosynthesis, we analysed the serotype and lineage distribution, antibiotic resistance, temporal evolution and capsule switching in context of genetic structure of the isolates.

2. Materials and methods

We retrospectively sampled 585 invasive pneumococcal isolates from blood and cerebral spinal fluid (CSF) from the bacterial isolate archive at the Malawi-Liverpool-Wellcome Trust Clinical Research Programme for whole genome sequencing (Supplemental Table S1). The isolates were sampled blindly of the serotype in order to represent their prevalence in IPD and not based on inclusion based on their serotypes. The isolates in the archive were collected from patients at the Queen Elizabeth Central Hospital in Blantyre, the largest referral hospital in Southern Malawi. We extracted DNA using QIAamp DNA mini kit, QIAgen Biorobot (Qiagen, Hilden, Germany), and Wizard® DNA Genomic DNA Purification Kit (Promega, WI, USA). DNA sequencing was done at the Wellcome Trust Sanger Institute using Illumina Genome Analyzer II and HiSeq platforms (Illumina, CA, USA). Whole genome alignment, sequence assembly, phylogeny construction, recombination detection, detection of antibiotic resistance genes, coalescent, and statistical analyses were done as described in Supplemental Materials and Methods. Sequence typing and serotyping were done using multilocus sequence typing (MLST) [17,18], and PCR [19] and genomic approach respectively [20]. The sequence reads for the isolates were deposited in the European Nucleotide Archive (www.ebi.ac.uk/ena) and their accession numbers are provided in Supplemental Table S1. We used disc diffusion for antibiotic susceptibility testing and interpreted the findings using the British Society Antimicrobial Chemotherapy (BSAC) guidelines. The study was approved by the University of Malawi's College of Medicine Research and Ethics Committee (approval number: P08/14/1614).

3. Results

3.1. Characteristics of pneumococcal isolates before vaccination

Pneumococcal isolates from blood and CSF were collected from adults and children through routine pathogen surveillance at the Queen Elizabeth Central Hospital, the largest referral hospital in Blantyre, Malawi. We sequenced a randomly sample of 585 isolates from collection of >5000 pneumococcal isolates from 2004 to 2010 for whole genome sequencing in order to determine the pneumococcal genomic epidemiology and evolution pre-PCV13 implementation in 2011 (Fig. 1A and B and Table S1). Of these samples, 65.5% and 38.5% of the isolates were from blood and cerebrospinal fluid (CSF) respectively. By vaccine status, 68.7% of the study isolates contained a vaccine type (VT) serotype targeted by the PCV13 vaccine formulation. Although the number of isolates collected were higher in children <5 years old and adults above 30 years old, the prevalence of vaccine type (VT) serotypes decreased consistently with increasing age (Fig. S1A–C). The prevalence of serotypes also varied by these age groups, with some serotypes common in the under fives.

3.2. Genetic population structure analysis reveals high population diversity

To determine the pneumococcal population structure and diversity, we did comparative genomic analysis of the isolates by clustering the isolates into sequence clusters (SC) using an unsupervised Bayesian hierarchical clustering approach [21]. Such SCs defines the unique subpopulations of genetically similar isolates, which are predominantly of the same serotype but some SCs contained multiple serotypes because of serotype switching due to recombination-mediated swapping of genes between isolates of different capsule types (Fig. 1C). Overall 22SCs were identified and of these 22 SCs, SCs 1–21 were monophyletic with a single common ancestor while SC22 had multiple common ancestors and thus it was polyphyletic (Fig. 1D). Due to the inclusion of more isolates in this study, the number of SCs identified were identified than in a previous study [22]. Because of the high sequence diversity in SC22, our analysis of SCs focuses largely on SCs 1–21. Overall, the sequenced samples were comprised of 46 serotypes and 134 sequence types (ST).

3.3. Prevalence of pneumococcal serotypes and SCs – high dominance of serotype 1 lineage

The most dominant monophyletic SC was SC2 and was comprised of only serotype 1 isolates (19.3%) which are mostly (84.96%) of multi locus sequences type 217 (ST217, also known as Sweden[1]-27 or PMEN27) [23,24] (Figs. 1C, 2A). The prevalence of