ORIGINAL ARTICLE

Characterization of 19A-like 19F pneumococcal isolates from Papua New Guinea and Fiji

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Abstract

Molecular identification of *Streptococcus pneumoniae* serotype 19F is routinely performed by PCR targeting the *wzy* gene of the capsular biosynthetic locus. However, 19F isolates with genetic similarity to 19A have been reported in the United States and Brazil. We screened 78 pneumococcal carriage isolates and found six 19F *wzy* variants that originated from children in Papua New Guinea and Fiji. Isolates were characterized using multilocus sequence typing and opsonophagocytic assays. The 19F *wzy* variants displayed similar susceptibility to anti-19F IgG antibodies compared to standard 19F isolates. Our findings indicate that these 19F variants may be more common than previously believed.

Keywords: Antibodies, Capsule, Pneumococcus, Serotyping, *Streptococcus pneumoniae*, Surveillance
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Introduction

Streptococcus pneumoniae (the pneumococcus) is a major cause of childhood morbidity and mortality, with the highest burden of disease occurring in resource-limited countries [1]. Pneumococcal serotyping is important for disease surveillance and monitoring changes in serotype distributions associated with pneumococcal conjugate vaccine (PCV) use. The antibody-based Quellung reaction is the current reference standard, whereas serotyping by multiplex PCR is successfully used as an alternative to antibody-based methods in many laboratories worldwide [2]. However, problems differentiating the closely related serotypes 19F and 19A by PCR have been reported [3]. These serotypes are common in invasive disease, but pneumococcal vaccine coverage differs: 19F is included in both PCV10 and PCV13, whereas 19A is included only in PCV13 [4].

In 2009, the United States Centers for Disease Control and Prevention (CDC) identified a 19F isolate with genetic similarity to serotype 19A (multilocus sequence type 3040) and revised the serotype 19A primers, which target *wzy*, to avoid false-positive results [5]. More recently, similar 19F *wzy* variants were found in Brazil [6], prompting speculation that these strains are more common than previously believed.

The objectives of this study were to identify and characterize 19F *wzy* variant isolates present in nasopharyngeal swabs collected from young children aged 0 to 24 months in low- and middle-income countries (as categorized by the World Bank, http://data.worldbank.org/about/country-and-lending-groups) and to examine the susceptibility of 19F *wzy* variants to antibody-mediated killing.

Methods

A total of 78 pneumococcal carriage isolates were screened using a PCR designed to target 19F *wzy* variants (BUGS-19E F:

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TABLE 1. Multilocus sequence typing results for 19F *wzy* variants

Isolate	Origin	*aroE*	*gdh*	*gki*	*recA*	*spi*	*xpt*	*ddl*	ST
PMP1107	Fiji	10	8	312	131	6	7	29	10233[a]
PMP1108	PNG	7	8	312	131	5	7	11	6904
PMP1109	PNG	7	8	312	131	5	7	11	6904
PMP1110	PNG	7	8	312	131	5	7	11	6904
PMP1111	PNG	7	13	12	6	6	6	8	10234[a]
PMP1112	PNG	12	10	9	15	17	3	8	10235[a]
2584-08 [5]	USA	5	5	1	3	8	14	180	3040

PNG, Papua New Guinea; ST, sequence type.
[a]Novel ST identified in this study.

TGACAATTCTGGTTGACTTGTTG, R: AGTACGGGTACCAAGGATTCAC). These primers were designed *in silico* using publically available 19F, 19A and 19F variant *wzy* gene sequences to target regions of sequence divergence and ensure specificity. These primers, plus the current CDC 19F and 19A primers, were validated using a panel of reference pneumococcal isolates of serotype 19F, 19A and 19F variants that confirmed the specificity of each primer pair (data not shown). The 78 isolates screened included 33 isolates from Kenya, Papua New Guinea (PNG), South Africa, The Gambia, Fiji and Bangladesh (n = 13, 6, 5, 4, 3 and 2, respectively) previously typed as 19F by Quellung reaction as part of the PneuCarriage project (Satzke et al., paper presented at the 9th International Symposium on Pneumococci and Pneumococcal Diseases, 2014), and an additional 45 isolates from Fiji collected during pneumococcal vaccine studies and typed as 19F, 19A or nontypeable by reverse-line blot testing or Quellung reaction [7]. Informed consent and approval by local human ethics committees were obtained before sample collection as part of the original studies.

Results and Discussion

Six isolates (five from PNG and one from Fiji) were found to be 19F *wzy* variants. Results for the 78 isolates were 100% concordant when PCR was performed using the CDC 19Fvar primers [6], which target a similar region of the *wzy* gene. These six isolates were characterized by multilocus sequence typing (MLST) [8] and sequencing of the 607 bp BUGS-19E PCR product (partial *wzy* gene). MLST results are shown in Table 1. Three of the PNG isolates were the same sequence type (ST) and the other two were novel STs not previously found in the MLST database. The Fiji isolate was a novel ST (ST10233) with 5 of 7 alleles identical to ST6904. The six isolates in our study had identical partial *wzy* sequences (GenBank accession no. KR002679). Sequence alignment was performed by ClustalW [9] to compare this sequence with the *wzy* sequences from original ST3040 isolate from CDC [5] and the more recently identified *wzy* 19F variants from Brazil [6]. The isolates from our study had four single nucleotide variations compared to the ST3040 *wzy* sequence, three of which were shared with the Brazil *wzy* sequence.

To investigate whether the 19F *wzy* variants had altered susceptibility to killing by anti-19F IgG (which could potentially affect vaccine effectiveness), opsonophagocytic assays (OPAs) were performed using sera from vaccinated children in Fiji who participated in a pneumococcal vaccine trial [10]. A reference 19F laboratory strain (SPEC19F), a standard 19F carriage isolate from Fiji (PMP1113) and two 19F *wzy* variant isolates (PMP1107 from Fiji and PMP1108 from PNG) were examined in OPAs using sera from six children (all isolates tested with each sera). OPA titres were similar for all four isolates: for sera with high levels of 19F IgG (> 50 μg/mL), the geometric mean was 2435 (95% confidence interval 370, 16 015) for standard 19F isolates and 2048 (95% confidence interval 832, 5039) for 19F *wzy* variants (p 0.46, unpaired t test). For sera with lower levels of 19F IgG, titres were low, and no differences were observed (data not shown). These results indicate that 19F *wzy* variants do not have a different susceptibility to anti-19F IgG–mediated killing than standard 19F isolates.

19F *wzy* variants may be more common than previously believed, especially in certain geographical regions such as Brazil and Oceania. It is important that appropriate serotyping methods are used to avoid falsely identifying them as serotype 19A. Data from OPAs suggest that these 19F isolates are genetic variants without functional differences in their capsule.

Conflict of Interest

None declared.

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