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Nasopharyngeal Carriage of *Streptococcus pneumoniae* in Children in Coastal Kenya

Claire J. Heath,[1]* Monica Nayakwadi-Singer,[2] Charles H. King,[3] Indu Malhotra,[3] Francis Mutuku,[4] Dunstan Mukoko,[5] and A. Desiree LaBeaud[1]

[1]*Division of Pediatric Infectious Diseases, Stanford University School of Medicine, Palo Alto, California;* [2]*Division of Pediatric Infectious Diseases, UCSF Benioff Children's Hospital Oakland and Children's Hospital Oakland Research Institute, Oakland, California;* [3]*Center for Global Health and Diseases, Case Western Reserve University, Cleveland, Ohio;* [4]*Technical University of Mombasa, Mombasa, Kenya;* [5]*Vector Borne Disease Control Unit, Nairobi, Kenya*

Abstract. *Streptococcus pneumoniae* (SP) is a leading cause of child mortality globally, killing around half a million children aged 5 years and less per year. Nasopharyngeal carriage of SP is a prerequisite to disease, and the prevalence of colonization reaches 100% within the first few years of life. Serotype prevalence varies geographically, impacting the serotype coverage of pneumococcal vaccines, and serotype prevalence data are limited from large regions of the world, including sub-Saharan Africa. We enrolled 323 unvaccinated children, aged 4–7 years from coastal Kenya and obtained nasopharyngeal swab samples before and after vaccination with the 10-valent pneumococcal vaccine. Vaccination did not reduce the overall prevalence of pneumococcal carriage in our cohort, with 65 (20%) children colonized before vaccination and 63 (19.4%) colonized postvaccination. However, the prevalence of vaccine-included serotypes (vaccine strains) declined from 43% to 19% of positive swabs, whereas non-vaccine serotypes increased from 46% to 73%. This study contributes to the few data available regarding pneumococcal carriage and serotype prevalence in Kenya and is in concordance with reports of dynamic serotype replacement, driven by vaccine pressure.

INTRODUCTION

Streptococcus pneumoniae (SP or the pneumococcus) is a common cause of invasive (meningitis, sepsis, and pneumonia) and respiratory tract infections. Recent estimates report that SP kills around 541,000 children less than 5 years of age per year, of which approximately 309,000 occur in Africa,[1] making it one of the leading causes of child mortality globally.[2] Of all deaths attributable to pneumococcal disease, more than 98% occur in developing countries and just 10 countries in Asia and sub-Saharan Africa account for more than 60% of all pneumococcal deaths worldwide.[3]

Streptococcus pneumoniae is commonly carried asymptomatically as a commensal organism in the nasopharynx of healthy children. It is believed that colonization prevalence ranges from < 10% during the first few weeks of life to 100% within the first few years of life and nearly all children have at least one episode of pneumococcal carriage during their early childhood.[4] Nasopharyngeal carriage of SP is a prerequisite to invasive disease,[5] and the serotype prevalence varies geographically. Because pneumococcal vaccines have been designed largely based on seroprevalence studies in North America and Europe,[6] the serotype coverage of these vaccines is often reduced in other regions of the world.[7–9]

In 2011, Kenya became one of the first countries in Africa to introduce the 10-valent pneumococcal conjugate vaccine (PCV10) (Synflorix; GSK, Brussels, Belgium) into the routine infant vaccination schedule.[10] Comprehensive data on the prevalence of nasopharyngeal carriage and the circulating pneumococcal serotypes in Kenya are limited.

Here, we describe the prevalence of pneumococcal carriage, and the serotypes observed in a child cohort in coastal Kenya, and the effects on serotype colonization due to PCV10. Understanding the localized serotype epidemiology is necessary for the optimization of vaccine development and implementation of public health policies.

METHODS

Ethics statement. Children who had participated in a 2006–2010 maternal–infant cohort study at the Msambweni District Hospital on the southern coast of Kenya[11,12] were eligible for the present study of pneumococcal carriage. The data sharing and nasopharyngeal swabbing were performed after written re-consent under a newly approved study protocol supervised by Kenyatta National Hospital Ethical Review Committee (protocol P85/03/2013) and the Institutional Review Board for Human Studies at University Hospitals of Cleveland Case Medical Center (protocol 01-13-13).

Participant selection. We located, re-consented, and enrolled eligible children from a maternal–infant study cohort that we had developed in 2006–2010 in Msambweni, Kwale County, Kenya.[12] These children had been enrolled at birth and followed until 3 years of age.[11,12] However, SP vaccination with PCV began in Kenya in 2011. The children in this previous study, were all born before 2011 and therefore missed this vaccine as infants, so a new study funded by the Gates foundation (PI King OPP1066865) to examine the effects of parasitism on SP vaccine response was undertaken.[13] In January 2014, all available children from the original birth cohort, now aged 4–7 years, were re-enrolled in the present study to receive catch-up SP vaccination. Children were excluded from the study if they had moved from the study area, if re-consent was not provided, or if the child or family would be unable to return for the 4- to 6-week follow-up visit for post-vaccination swabs.

Using Kenyan census data[14] and UNICEF estimates of PCV10 coverage,[15] we have estimated that at the time of our study, a maximum of 10% of the local population (these being mostly children born after 2011 vaccine rollout) were immunized. Among children born after 2011, estimates of vaccine coverage in Kwale County were that 96.7% had received at least one dose of the vaccine.[16]

*Address correspondence to Claire J. Heath, Stanford University School of Medicine, 300 Pasteur Drive, Palo Alto, CA 94305. E-mail: claire.j.heath@gmail.com

Nasopharyngeal swab collection. In January 2014, unvaccinated children aged 4–7 years in the Msambweni district of coastal Kenya were identified and recruited from a previous child cohort.[11,12] Informed parental consent was obtained according to protocols approved by the institutional review boards of Case Western Reserve University and Kenyatta National Hospital. Nasopharyngeal swabs were performed using calcium alginate flexible aluminum shaft swabs (Harmony Business Supplies, Inc., Garden Grove, CA). The swabs were stored and transported in a skim milk, tryptone, glucose, and glycerin (STGG) medium and stored at –80°C until processed.[17] Children then received a single dose of the PCV10. A repeat nasopharyngeal swab was then collected 4–6 weeks postvaccination.

Bacterial culture. A 200-μL sample of STGG media for each sample was used to inoculate 6-mL vials of LIM broth (BD Biosciences, Franklin Lakes, NJ) for enrichment of streptococcal species.[18] Cultures were incubated at 37°C and 5% CO_2 for 6 hours. The cultures were then streaked onto 5% sheep's blood agar plates (BAP) using a 10-μL inoculating loop and optochin discs were used to enable identification of SP. The plates were incubated at 37°C and 5% CO_2 overnight. Positivity for SP was defined as alpha-hemolysis of BAP and optochin sensitivity and confirmation by subculture was performed where required.

DNA extraction. *Streptococcus pneumoniae* DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Venlo, The Netherlands) according to the manufacturer's protocol for bacterial DNA extraction. In summary, single SP colonies were picked from BAP with 20-μL inoculation loops and resuspended in 200 μL of phosphate buffered saline. The suspension was then treated with 20 μL of Proteinase K and 200 μL of the proprietary lysis buffer and incubated in a water bath at 56°C for 10 minutes. Ethanol (100%, 200 μL) was then added to the lysate and the mixture passed through an extraction column by centrifugation at 8,000 rpm for 1 minute. The column was washed by the addition of 500 μL of two sequential wash buffers, which were passed through the column, again by centrifugation at 8,000 rpm for 1 minute. Purified DNA was then eluted from the column into 100 μL of elution buffer. DNA was then stored at –20°C until analysis by polymerase chain reaction (PCR).

Serotyping by PCR. *Streptococcus pneumoniae* serotyping was performed according to the Centers for Disease Control and Prevention's "PCR deduction of pneumococcal serotype" protocol, using the pool matrix optimized for African clinical samples.[19]

Electrophoresis. To visualize DNA bands, 2 μL of 10,000× SYBR Green solution (ThermoFisher, Waltham, MA) was added to 10 μL of the PCR product and electrophoresis was performed on a 2% agarose gel at 200 V for 20 minutes. Serotype was assigned according to DNA band size.[19]

Descriptive analysis. The PCR serotyping results were recorded in an Excel database (Microsoft Corporation, Redmond, WA), and this software was used to calculate the total number and relative proportion of samples positive for each pneumococcal serotype.

RESULTS AND DISCUSSION

Sample pairs (pre- and postvaccine) were obtained from a total of 323 children. We have previously described the characteristics of this cohort in detail.[13] Of relevance to the present study, the median age of participants was 6.1 years and the median time to sampling postvaccine was 5.3 weeks.

Sample pairs (pre- and postvaccine) were tested for the presence of SP. The numbers of each serotype observed are detailed in Table 1. The overall rate of SP carriage in the prevaccine samples was 65/323 (20%) and postvaccine was 63/323 (19.4%). Two hundred and eleven (65%) sample pairs were negative for pneumococci both before and after vaccination (Neg/Neg). Forty-nine (15.2%) of the sample pairs were positive for pneumococci prevaccine but negative postvaccine (Pos/Neg) (Table 2). Forty-seven (14.6%) of the sample pairs were negative prevaccination and positive postvaccination (Neg/Pos) (Table 3) and 16 (5.0%) of the sample pairs were SP positive both before and after vaccination (Table 4).

Of the 65 positive prevaccine samples, 28 (43%) were typed as vaccine serotypes (VS), 30 (46%) were non-vaccine serotypes (NVS), and 7 (10.7%) were non-typeable (NT). Of the 63 positive postvaccination samples, 12 (19%) were VS, 46 (73%) were NVS, and 5 (7.9%) were NT.

Of the 10 pneumococcal serotypes included in the vaccine, five were detected in the prevaccination samples; 19F (12), 23F (9), 14 (4) 7F (2), and 1 (1) (Table 1). With the exception of serotype 1, which was not detected in any postvaccine samples, these serotypes were the same VS that were also detected in the postvaccine samples, though each at a reduced frequency; 19F (6), 23F (3), 14 (3), and 7F (1) (Table 1). No VS "emerged" in the postvaccine serotypes that had not been detected in samples before vaccination.

Sixteen different NVS were found in the prevaccine swabs and 17 in the postvaccine swabs (Table 1). Only three of the NVS detected were decreased in frequency after vaccine, namely serotypes 21, 35A, and 23B, whereas 11 NVS serotypes increased in frequency and four remained the same (Table 1).

TABLE 1

Distribution of vaccine-included and non-vaccine–included serotypes in pre- and postvaccine nasopharyngeal swab samples

	Number of isolates	
Serotype	Prevaccine	Postvaccine
Non-vaccine serotypes		
35F/47F	0 (0%)	1 (1.6%)
23A	0 (0%)	2 (3.2%)
19A	0 (0%)	3 (4.8%)
21	1 (1.5%)	0 (0%)
7C/B/40	1 (1.5%)	2 (3.2%)
35B	1 (1.5%)	4 (6.3%)
35A	1 (1.5%)	0 (0%)
17F	1 (1.5%)	4 (6.3%)
13A	1 (1.5%)	4 (6.3%)
10F/C/33	1 (1.5%)	1 (1.6%)
31	1 (1.5%)	2 (3.2%)
16F	2 (3.1%)	4 (6.3%)
34	2 (3.1%)	4 (6.3%)
3	2 (3.1%)	2 (3.2%)
24F/A/B	3 (4.6%)	7 (11.1%)
22F/A	3 (4.6%)	4 (6.3%)
15B/C	3 (4.6%)	3 (4.8%)
10A	3 (4.6%)	3 (4.8%)
23B	6 (9.2%)	2 (3.2%)
Vaccine serotypes*		
7F	2 (3.1%)	1 (1.6%)
1	4 (6.2%)	0 (0%)
14	4 (6.2%)	3 (4.8%)
23F	6 (9.2%)	3 (4.8%)
19F	12 (18.5%)	6 (9.5%)

* The vaccine-included serotypes 4, 5, 6B, 9V, and 18C were not observed in our sample.

TABLE 2

Serotypes observed in participants who were positive for pneumococci in their prevaccine swab but were not subsequently colonized with pneumococci in their postvaccine swab

Serotype observed	Number of participants colonized (% Pos/Neg participants)
19F	8 (16.3%)
Non-typeable	7 (14.3%)
1	4 (8.2%)
14	4 (8.2%)
15B/15C	3 (6.1%)
22F/A	3 (6.1%)
23B	3 (6.1%)
24F/24A/24B	3 (6.1%)
3	2 (4.1%)
34	2 (4.1%)
23F	2 (4.1%)
21	1 (2.0%)
31	1 (2.0%)
13A	1 (2.0%)
16F + 10A + 23B	1 (2.0%)
17F	1 (2.0%)
23B and 19F	1 (2.0%)
35B	1 (2.0%)
7F	1 (2.0%)

Pos/Neg = positive for pneumococci prevaccine but negative postvaccine. Vaccine serotypes appear in bold type.

Of the 49 Pos/Neg participants, 20 (41%) were colonized by VS and eight of these (16%) were serotype 19F (Table 2). In the 47 Neg/Pos participants, only 11 (23%) were colonized by a VS. Interestingly, this included one subject who acquired both VS 14 and 19F (Table 3). Co-colonization by two or more pneumococcal serotypes was only detected in eight samples, of which six were prevaccine and two were postvaccine samples (Tables 2 and 3).

TABLE 3

Serotypes observed in participants who were negative for pneumococci in their prevaccine swab but were subsequently colonized with pneumococci in their postvaccine swab (Neg/Pos)

Serotype observed	Number of participants colonized (% of Neg/Pos participants)
34	4 (8.5%)
19F	4 (8.5%)
24F/24A	4 (8.5%)
13	3 (6.4%)
16F	3 (6.4%)
17F	3 (6.4%)
22F/A	3 (6.4%)
23F	3 (6.4%)
10A	2 (4.3%)
15B/15C	2 (4.3%)
35B + 24F/A/B	2 (4.3%)
3	1 (2.1%)
14	1 (2.1%)
10F	1 (2.1%)
14 + 19F	1 (2.1%)
14 + 3	1 (2.1%)
15B	1 (2.1%)
19A	1 (2.1%)
23B	1 (2.1%)
3 + 22F/A or 23A	1 (2.1%)
35B	1 (2.1%)
35F/47F	1 (2.1%)
7C/7B/40 and 35B	1 (2.1%)
7F or 15B/C	1 (2.1%)
Non-typeable	1 (2.1%)

Neg/Pos = negative prevaccination and positive postvaccination. Vaccine serotypes appear in bold type.

TABLE 4

Pneumococcal serotype carriage in participants who were positive for pneumococcal carriage in both their pre- and postvaccine samples (Pos/Pos)

	Prevaccine	Postvaccine
1	**19F**	13
2	**19F**	**19F**
3	**7F**	NT
4	35A	NT
5	**23F**	10A
6	10A	16F
7	10F/C/33	15B/C
8	**23F**	24F/A/B
9	10A	17F
10	**19F**	24F/A/B
11	25F/A/B +**23F**	3
12	16F	NT
13	7C	NT
14	23B	7C/B/40 + 35B
15	NT	31
16	23F	19A

NT = non-typeable; Pos/Pos = positive both before and after vaccination. Vaccine serotypes are shown in bold type.

Only 16 participants were positive for pneumococcal carriage both before and after vaccination (Table 4). Eight of these 16 participants (50%) were colonized with a VS prevaccine. With the exception of one participant, who retained carriage of the same serotype in both samples, despite it being the VS 19F, only NVS and NT pneumococci were detected in the postvaccine swabs from these subjects.

In this study, only about 20% of the children aged 4–7 were colonized with SP in both the pre- and postvaccine samples. This rate of colonization is comparable to findings reported in previous studies of pneumococcal carriage in children of this age group in Kenya. One study, conducted by Hammitt et al.[20] in the 2 years preceding introduction of the pneumococcal vaccine in Kenya, reported pneumococcal colonization in approximately 30% children aged less than 5 years, but a prevalence more comparable to those reported herein (20–25%) in older children and adults. Because our study included children up to 7 years of age, and was conducted 3 years after routine vaccine rollout, the slightly lower rate of colonization is not surprising as colonization in the community as a whole would likely be reduced via herd immunity mechanisms. In addition, it is a limitation of our study that we did not give a second dose of the vaccine and that we sampled at only a single time point postvaccine. Given the dynamic and rapid nature of natural pneumococcal acquisition and clearance, alongside vaccine pressure, it is likely that further changes in nasopharyngeal colonization have evolved over time. Immunization with the PCV10 did not affect the overall nasopharyngeal colonization rate in this cohort; however, vaccination did reduce overall colonization with vaccine serotypes. With the exception of one participant, who retained carriage of the same serotype in both samples, only NVS and NT pneumococci were detected in the postvaccine swabs from these subjects. Carriage of non-vaccine serotypes increased and no VS "emerged" between pre- and postvaccine sampling to suggest possible serotype replacement due to vaccine pressure. However, although this may be indicative of serotype replacement due to vaccine pressure, it should also be noted that changes to carriage serotypes were also observed in patients who were colonized with NVS in their prevaccine sample, emphasizing the dynamic nature of the

nasopharyngeal microbiome. In addition, older children who missed the opportunity to receive the vaccine when it was introduced are likely a reservoir of continued circulation of VS. Catch-up vaccine initiatives, such as the one conducted in Kilifi County, Kenya,[20] therefore may be an effective strategy for improving the overall impact of PCV10 on the population.

For non-vaccine serotypes that were detected in this study, the frequency was increased in postvaccine samples, with the exception of serotype 23B. These data also provide further evidence that pneumococcal serotype replacement occurs rapidly postvaccination in sub-Saharan Africa.

In a comprehensive meta-analysis of pneumococcal carriage throughout sub-Saharan Africa, Usuf et al.[21] addressed the variability of epidemiological data from this region but concluded that although vaccination did reduce NP colonization with VS, overall prevalence remained stable because of concomitant increase in NVS, as observed in this study. Furthermore, in agreement with our data, this meta-analysis also found that serotypes 19F, 14, and 23F were among the most common throughout sub-Saharan Africa.

Notwithstanding these observations of changes in pneumococcal carriage, when considering the impact of the vaccine, it is critical to examine the effect on the incidence of invasive pneumococcal disease (IPD). In coastal Kenya, since the introduction of PCV10, the decrease in carriage of VS has been concurrent with a decline not only in VS IPD but also in all IPD,[22] demonstrating that these "replacement" strains are seemingly noninvasive in this setting. However, the effect of serotype replacement on IPD has been variable, whereas, in most settings, the incidence of both VS and NVS IPD has been reduced, in a few cases, replacement strain IPD has minimized the impact of vaccines in some countries.[23]

Of the children who were colonized before immunization, only 41% were colonized with vaccine strains that are present in the PCV administered in Kenya, suggesting that serotype concordance of this vaccine is low in our cohort. However, the numbers of each serotype observed in our cohort were too few to allow meaningful statistical modeling, posing a challenge in assessing the concordance of our data with other studies of vaccine impact in the region. Our findings do, however, lend support to evidence that pneumococcal serotype replacement with NVS after immunization is common,[24,25] and for this reason, until an antigen-based vaccine common to all pneumococci can be developed, continued detailed and localized surveillance and reporting will be required if serotype-based vaccine specificity is to be maximized.

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Authors' addresses: Claire J. Heath and A. Desiree LaBeaud, Division of Pediatric Infectious Diseases, Stanford University School of Medicine, Palo Alto, CA, E-mails: cjheath@stanford.edu and dlabeaud@stanford.edu. Monica Nayakwadi-Singer, Division of Pediatric Infectious Diseases, UCSF Benioff Children's Hospital Oakland, Children's Hospital Oakland Research Institute, Oakland, CA, E-mail: msinger@mail.cho.org. Charles H. King and Indu Malhotra, Center for Global Health and Diseases, Case Western Reserve University, Cleveland, OH, E-mails: chk@case.edu and indu.malhotra@case.edu. Francis Mutuku, Technical University of Mombasa, Mombasa, Kenya, E-mail: fmutuku73@gmail.com. Dunstan Mukoko, Vector Borne Disease Control Unit, Nairobi, Kenya, E-mail: dunstan.mukoko29@gmail.com.

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