RESEARCH ARTICLE

*Streptococcus pneumoniae* serotype epidemiology among PCV-10 vaccinated and unvaccinated children at Gertrude’s Children’s Hospital, Nairobi County: a cross-sectional study [version 1; peer review: 1 approved, 1 approved with reservations, 1 not approved]

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Abstract

**Background:** *Streptococcus pneumoniae* (SPn) serotype replacement and emergence of multidrug resistant SPn has exacerbated the need for continuous regional serotype surveillance. We investigated SPn serotypes circulating among children ≤5 years in Nairobi County.

**Methods:** *Streptococcus pneumoniae* stocks stored at −70°C in brain heart infusion medium were thawed at room temperature for 30 minutes. In total, 10 µl of the stored SPn cells were suspended in 50 µl PBS and gently vortexed. About 10 µl of the suspended cells were added on to a glass slide and mixed with 10 µl pooled antisera. The glass slide was swirled gently while observing for any reaction. The process was repeated with individual groups under various antisera pools. Those serotypes that did not belong to any pool were typed directly until a positive agglutination reaction was observed. The cells/PBS/serotype-specific antisera mixture on the glass slide were covered with a coverslip and observed under a phase contrast microscope at ×100 objective lens with oil emulsion.

**Results:** Out of the 206 subjects sampled, 20.39% (n=42) were found to be carriers of SPn. About 52% (n=22) of the SPn carriers had received the recommended dose of PCV-10, while 48% (n=20) of the carriers had not. Almost all (n=41; 19.90% of subjects) isolates contained non-vaccine type SPn serotypes, while n=1 of the serotypes (in 0.49% of subjects) were untypeable. Serotypes 28F, 6A, 11A, 3 and 7C were prevalent in both vaccinated and unvaccinated children, whereas serotypes 23A, 17F, 35F, 48, 13 and 35B, and 23B, 20, 19B, 21, untypeable, 15B and 39 were found among unvaccinated and vaccinated groups, respectively.

Any reports and responses or comments on the article can be found at the end of the article.
Conclusions: All SPn serotypes isolated from the subjects sampled were non PCV-10 vaccine type. Therefore Kenyan children receiving PCV-10 vaccine are not protected.

Keywords
Streptococcus pneumoniae, serotypes, Nairobi, Quellung reaction, Optochin test, Bile solubility

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Competing interests: No competing interests were disclosed.

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Introduction

*Streptococcus pneumoniae* (SPn) is a highly invasive, Gram-positive, extracellular bacterial pathogen (Mitchell & Mitchell, 2010). It is a major cause of morbidity and mortality globally, causing more deaths than any other infectious disease (Jones et al., 2010). SPn is classified into serogroups (denoted by numbers and letters, e.g. 18c, 23f) (Kellogg et al., 2001). There are over 90 known serotypes and the prevalence of different serotypes varies by regions of the world (Hackel et al., 2013). Different serotypes exhibit differing potentials to cause disease and may cause different syndromes in different age groups (Harboe et al., 2009).

Some strains also have a greater potential to develop antibiotic resistance (Song et al., 2012). The 13 most common serotypes of SPn pneumonia cause 80–93% of serious pneumococcal disease in children (Johnson et al., 2010). According to the World Health Organization (WHO) and UNICEF Global Action Plan for the Prevention and Control of Pneumonia, pneumonia kills more children than any other illness in the world (WHO & UNICEF, 2009). Given the high burden of under-five mortality associated with pneumonia, control efforts are critical to achieving Sustainable Development Goal 3 (Colglazier, 2015).

WHO and UNICEF estimates indicate that over 800,000 children under 5 years of age die from pneumococcal disease each year (O’Brien et al., 2009). In Kenya, an estimated one in every five children under 5 years of age dies from this disease every year (WHO, 2013).

SPn vaccines protect against several severe forms of pneumococcal disease, such as meningitis, pneumonia and bacteremia (Feldman & Anderson, 2014). These vaccines will not protect against these conditions if they are caused by agents other than SPn or from other strains of SPn that are not contained in the vaccine (Moffitt & Malley, 2011). The 10-valent pneumococcal conjugate vaccine (PCV10) was introduced into the Kenya Expanded Program on Immunization (KEPI) in February 2011 with a 2+1 schedule (at 6, 10, 14 weeks) without catch-up vaccinations (Hammitt et al., 2014). The vaccine covers 1, 4, 5, 6b, 7f, 9v, 14, 18c, 19f and 23f SPn serotypes.

Currently over 90 different serotypes have been identified, six of them very recently (Weinberger et al., 2011). Various SPn serotypes with antigenic similarities are classified under the same groups (9A, 9L, 9N and 9V) while those lacking antigenic similarities are given numbers only (1, 2, 3, 4 and 5). The degree of interaction (cross-reactivity) between various SPn groups may vary. For instance, serotypes 6A and 6B have identical chemical composition except for one of the bonds between two sugars yet they are highly cross-reactive but serotypes 19F and 19A are less reactive.

Pneumococcal conjugate (PCVs) and polysaccharide (PPVs) vaccines are designed according their virulence mechanisms and how they generally interact with the human immune system (Castañeda-Orjuela et al., 2012). The WHO has advised that all children ≤5 years should be immunized against pneumococcal disease and continuous surveillance done to keep out the disease especially in the developing world (Vandenbos et al., 2013). The need for continuous surveillance has been exacerbated by the acute emergence of multi-drug resistant SPn strains and escalated child mortality and morbidity due to pneumococcal disease, despite the availability of PCVs and PPVs. (Väkeväinen et al., 2010). This study therefore sought to establish the SPn serotypes among vaccinated and unvaccinated children ≤5 years of age in Nairobi County, Kenya.

Methods

Study location

This study was conducted among children ≤5 years attending the outpatient department of Gertrude’s Children’s Hospital, Muthaiga, and its satellite clinic in Githongoro, Nairobi County between May 2017 and February 2018. Subjects were clinically assessed by a physician and those who presented with pneumococcal disease symptoms recommended to the study nurse for recruitment. Gertrude’s Children’s Hospital is the largest standalone health care facility specializing in pediatric care in East and Central Africa. The hospital is accredited by the Joint Commission on International Accreditation (JCIA). SPn isolation and stocking was done at Gertrude’s Children’s Hospital Main Laboratory and capsular serotyping done at KEMRI Wellcome Trust, Kilifi, Kenya.

Study design

This was a descriptive cross-sectional study. *Streptococcus pneumoniae* serotype epidemiology among PCV-10 vaccinated and unvaccinated children between 6 months and 5 years of age was measured. Children who had no history of any chronic disease and whose parents or legal guardians consented to the study were systematically recruited. Children whose parents or legal guardians declined to give consent and those with any known immunosuppressive conditions were excluded from the study.

Sample size determination

To determine the minimum sample size, the formula developed by Chow et al. (2007) was used, with a prevalence rate of 16% (Agweyu et al., 2014).

\[
\hat{n} = \frac{z^2 \hat{p}(1 - \hat{p})}{m^2}
\]

Where \(n\) = desired minimal sample size; \(z\) = standard normal deviation (1.96, from the tailed normal table); \(\hat{p}\) = prevalence rate; and \(m\) = the desired degree of accuracy at a 95% confidence level of 0.05. This gave a sample size of 206.

Identification of SPn

Nasopharyngeal swabs were per nasally collected using Copan flocked swabs and temporarily suspended in Armies medium for transportation to the main laboratory. Each swab was inoculated onto a selective gentamicin with 5% sheep blood agar (BA) plate. All swabs were plated within 24 h of collection. The plates were incubated at 37°C in a 5% CO₂ atmosphere and examined at 16–24 h and then again at 40–48 h for growth of SPn. Isolates were identified as SPn by colony morphology (Mucoid,
draughtsmen appearance, \( \alpha \)-haemolysis) and susceptibility to optochin (positive, \( \geq 14 \) mm zone of inhibition; negative, \(< 14 \) mm zone of inhibition). Plates with colonies akin to SPn morphological features but with optochin clearance zones below \( 14 \) mm were further subjected to solubility in bile salts (positive, bile soluble; negative, bile insoluble).

The isolation of a single colony indicated carriage. Single colonies were picked using sterile inoculating loops and evenly plated on BA. After 24–48 h, enough inoculum was stocked in brain heart infusion (BHI) agar with 5% sheep blood (Ultralab East Africa, Ltd), gently vortexed and stored at \(-70^\circ\)C for serotyping.

**Serotyping of SPn**

Capsular serotyping was done using the Quellung reaction test. Frozen vials containing SPn stocks stored at \(-70^\circ\)C were thawed at room temperature for about 30 min. Next, 10 µl of the stored SPn cells were suspended in 50 µl PBS and gently vortexed. Subsequently, 10 µl of the suspended cells were added on to a glass slide and mixed with 10 µl pooled antisera (Statens Serum Institute, cat. No. 16744). The glass slide was swirled gently while observing for any agglutination reaction until a positive reaction was observed with various pooled antisera. The process was repeated with individual groups under various antisera pools.

After that, 10 µl of the suspended cells in PBS were added to a glass slide and mixed with various SPn serotype-specific antisera included in the antisera pools that gave a positive reaction. This was done until a positive reaction with the particular serotype specific antisera was observed. Those serotypes that did not belong to any pool were typed directly until a positive agglutination reaction was observed. The cells/PBS/serotype-specific antisera mixture on the glass slide were covered with a cover slip and observed under a phase contrast microscope with a \( \times 100 \) objective lens with oil emulsion.

**Results**

Out of \( n=206 \) (100%) of the subjects sampled, \( n=97 \) (47.1%) were male and \( n=109 \) (52.9%) were female. In total, \( 68 \) (33.0%) of the children studied were within the age bracket of 6–12 months, \( 47 \) (22.8%) were between the ages of 13–24 months, \( 46 \) (22.3%) were between the ages of 25–36 months, \( 17 \) (8.3%) were between the ages of 37 and 48 months and \( 28 \) (13.6%) were between the ages of 49 and 60 months. Out of the total number of subjects (\( n=206 \)) sampled, \( 20.39\% \) (\( n=42 \)) were found to be carriers of SPn; 52% (\( n=22 \)) of the SPn carriers had received the recommended dose of PCV-10 immunization, while 48% (\( n=20 \)) had not. All isolates (\( n=42 \); 20% of subjects) contained non-vaccine-type SPn serotypes, while \( n=1 \) (0.49% of the subjects) of the serotypes were untypeable (Table 1). In total, 18 different SPn serotypes were found in this population. They include: 28F (8 instances), 6A (5 instances), 3 (4 instances), 23B (3 instances), 20 (3 instances), 23A (3 instances), 19B (2 instances), 17F (2 instances), 7C (2 instances), 11A (2 instances), 35F (1 instance), 15B (1 instance), untypeable (1 instance), 48 (1 instance), 35B (1 instance), 21 (1 instance), 39 (1 instance) and 13 (1 instance).

Various serotypes were found to be prevalent in different age groups. For instance, out of the 42 serotypes found, 9 (23.53%) were prevalent among children at 6–12 months of age (\( n=16 \)). They include: 28F (4 instances), 11A (2 instances), 23A (2 instances), 3 (2 instances), 6A (2 instances), 17F (1 instance), 35F (1 instance), 7C (1 instance) and untypeable (1 instance). There were 7 (16.67%) serotypes prevalent among children at 13–24 months (\( n=8 \)), including: 20 (2 instances), 21 (1 instance), 39 (1 instance), 28F (1 instance), 35B (1 instance), 17F (1 instance) and 13 (1 instance). There were 8 (19%) serotypes found among children of 25–36 months of age (\( n=12 \)), including: 23B (3 instances), 19B (2 instances), 3 (2 instances), 20 (1 instance), 28F (1 instance), 7C (1 instance), 23A (1 instance) and 48 (1 instance). There were 3 (7%) serotypes prevalent among children at 37–48 months old (\( n=4 \)), including: 6A (2 instances), 15B (1 instance) and 28F (1 instance). There were 2 (4.76%) of the total serotypes prevalent among children at 49–60 months (\( n=2 \)); 6A (1 instance) and 28F (1 instance) (Table 2). Out of the 42 isolates (found in 20.39% of subjects), serotype 28F was the most prevalent (3.88% of the total), followed by 6A (2.43%), 3 (1.94%) and 20, 23A and 23B at all 1.46% (\( n=3 \)). Each of the serotypes 7C, 11A, 17F and 19B represented 0.97% (\( n=2 \)) of the total serotypes, while serotypes: 13, 21, 39, untypeable, 48, 15B, 35B and 35F represented 0.49% (\( n=1 \)) each of the total serotypes found (Figure 1 and Figure 2). In total 51% (\( n=106 \)) of the total sampled subjects were confirmed to have received a full dose of the PCV-10

**Table 1. Overall Streptococcus pneumoniae (SPn) carriage of vaccine type and non-vaccine type serotypes.** The percentage of SPn carriage status among PCV-10 vaccinated and unvaccinated children is shown.

<table>
<thead>
<tr>
<th></th>
<th>All children</th>
<th>Vaccinated children</th>
<th>Unvaccinated children</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( n )</td>
<td>%</td>
<td>( n )</td>
</tr>
<tr>
<td>Overall SPn carriage</td>
<td>42</td>
<td>20.39</td>
<td>22</td>
</tr>
<tr>
<td>Proportion of SPn serotypes</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
</tr>
<tr>
<td>PCV-10</td>
<td>41</td>
<td>19.90</td>
<td>41</td>
</tr>
<tr>
<td>Non-PCV-10 serotypes</td>
<td>1</td>
<td>0.49</td>
<td>1</td>
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</tbody>
</table>
Table 2. *Streptococcus pneumoniae* (SPn) Serotype distribution by age. The SPn serotypes as found among PCV-10 vaccinated and unvaccinated children of varying age groups is shown.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Subjects</th>
<th>All subjects</th>
<th>6–12 months</th>
<th>13–24 months</th>
<th>25–36 months</th>
<th>37–48 months</th>
<th>49–60 months</th>
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<tbody>
<tr>
<td>Numbers with carriage, n</td>
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<tr>
<td></td>
<td>42</td>
<td>16</td>
<td>8.00</td>
<td>12</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Carriage, %</td>
<td>20.39</td>
<td>23.53</td>
<td>17.02</td>
<td>26.09</td>
<td>23.53</td>
<td>7.14</td>
<td></td>
</tr>
<tr>
<td>Total different serotypes, n</td>
<td>18</td>
<td>9</td>
<td>7</td>
<td>8</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Serotypes seen (n)</td>
<td>28F (8)</td>
<td>28F (4)</td>
<td>20 (2)</td>
<td>23B (3)</td>
<td>6A (2)</td>
<td>6A (1)</td>
<td></td>
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<tr>
<td></td>
<td>6A (5)</td>
<td>11A (2)</td>
<td>21 (1)</td>
<td>19B (2)</td>
<td>15B (1)</td>
<td>28F (1)</td>
<td></td>
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<tr>
<td></td>
<td>3 (4)</td>
<td>23A (2)</td>
<td>39 (1)</td>
<td>3 (2)</td>
<td>28F (1)</td>
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<tr>
<td></td>
<td>23B (3)</td>
<td>3 (2)</td>
<td>28F (1)</td>
<td>20 (1)</td>
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<td></td>
<td>20 (3)</td>
<td>6A (2)</td>
<td>35B (1)</td>
<td>28F (1)</td>
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<td></td>
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<tr>
<td></td>
<td>23A (3)</td>
<td>17F (1)</td>
<td>17F (1)</td>
<td>7C (1)</td>
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<td></td>
<td>19B (2)</td>
<td>35F (1)</td>
<td>13 (1)</td>
<td>23A (1)</td>
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<td></td>
<td>17F (2)</td>
<td>7C (1)</td>
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<td>48 (1)</td>
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<td></td>
<td>7C (2)</td>
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<td>Untypeable (1)</td>
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<td></td>
<td>11A (2)</td>
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<td>35F (1)</td>
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<td>15B (1)</td>
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<td>Untypeable (1)</td>
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<td>35B (1)</td>
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<tr>
<td></td>
<td>21 (1)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>39 (1)</td>
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<td></td>
<td>13 (1)</td>
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</tbody>
</table>

Figure 1. Serotype distribution in the *Streptococcus pneumoniae* (SPn) isolates. This figure shows the prevalence of various SPn serotypes among PCV-10-vaccinated and –unvaccinated children.
vaccination as per the recommended schedule of immunization at 6, 10 and 14 weeks. Approximately 11% (n=12) of the immunized children were carriers of SPn in their nasopharyngeal region; 10% (n=10) of the non-immunized group were also carriers (Table 3). Serotypes 28F (5 instances), 23A (3 instances), 6A (3 instances), 17F (2 instances), 11A (1 instance), 3 (1 instance), 35F (1 instance), 48 (1 instance), 13 (1 instance), 35 (1 instance) and 7C (1 instance) were prevalent among the 9.71% (n=20) of the total sample group that had not received PCV-10 immunization. Serotypes 3 (3 instances), 28F (3 instances), 23B (3 instances), 20 (3 instances), 19B (2 instances), 6A (2 instances), 21 (1 instance), 11A (1 instance), 7C (1 instance), untypeable (1 instance), 15B (1 instance) and 39 (1 instance) were prevalent among the 10.68% (n=22) of the total sample group that received immunization (Table 4).

Discussion
This study found that 20.39% of all children studied, from both the PCV-10 vaccinated and unvaccinated groups, were carriers of SPn. While this is a significant reduction from the pre-vaccine era, it is still high compared to malaria, diarrhea and HIV/AIDS (Feikin et al., 2010). In total, n=41 of the serotypes found were non-vaccine type (in 19.90% of the subjects), with one additional untypeable serotype. This is a very important finding as it explains the high level of child morbidity and mortality due to pneumococcal disease despite the availability of PCV-10.

While these findings agree partially with those of Jacobs et al. (2008), where a significant decrease in the vaccine type SPn serotypes found in isolates was observed, a 97.6% (n=41) decrease is, at the very least, surprising. This trend may be attributed to the increased level of antimicrobial misuse by a greater percentage of the study population (Domenech de Cellès et al., 2011). 10-valent pneumococcal conjugate vaccine contains 10 different serotypes, which include: 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F (Slotved et al., 2016). None of these 10 serotypes was found in the study population yet this is the vaccine currently included in KEPI, targeting the same population.

Streptococcus pneumoniae carriage decreased with age as 11.65% (n=24) were obtained from children aged between 6–24 months and 8.74% (n=18) from children >24 months. The study results demonstrated a linear relationship between child age and SPn carriage. A similar study done elsewhere reported findings that partly agree with this and partly disagree (Hill et al., 2008). The former being attributable to development of SPn-specific IgG antibodies due to vaccination and during that window before most children start attending school (Corscadden et al., 2013). Unlike findings from a study by de Paz et al. (2015), serotype 28F was the most prevalent and was present in all five age groups profiled. This is a likely scenario of serotype replacement as SPn attempts to evade the action of the immune system and eventually shares the resistant genes within the microbial community, especially in the nasopharyngeal region (Donati et al., 2010).

Serotypes 28F, 6A, 11A, 3 and 7C were prevalent in both vaccinated and unvaccinated children, whereas serotypes 23A, 17F, 35F, 48, 13, 35B and 23B, 20, 19B, 21, untypeable, 15B, 39 were found among unvaccinated and vaccinated groups respectively.
There exists different antigenic features between and within various strains of SPn (Song et al., 2012). While the majority, if not all, pneumococcal serotypes are capable of causing disease, the frequency with which they are isolated varies (Kalin, 1998). In this case, vaccination would only be partially effective and, if so, due to inter-strain antigenic characteristics.

While trying to evade the action of the immune system, SPn has a tendency to exchange resistant genes and other antigenic correlates at the nasopharyngeal region (Johnston et al., 2014). Resistance to antimicrobial agents is occasioned by among other factors, misuse of antibiotics (Dinsbach, 2012). This is largely due to a lack of properly enforced antibiotic use regulations by the authorities.

References
Published Abstract | Publisher Full Text | Free Full Text
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Published Abstract | Publisher Full Text | Free Full Text
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Table 3. Streptococcus pneumoniae (SPn) serotype distribution by PCV-10 vaccination status. The percentage prevalence of SPn serotypes among PCV-10 vaccinated and unvaccinated children is shown.

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Unvaccinated (n=100)</th>
<th>Vaccinated (n=106)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serotype n %</td>
<td>Serotype n %</td>
</tr>
<tr>
<td>2BF</td>
<td>5 5 3 2.83</td>
<td>3 3 2.83</td>
</tr>
<tr>
<td>23A</td>
<td>3 3 28F 2.83</td>
<td>3 3 2.83</td>
</tr>
<tr>
<td>6A</td>
<td>3 3 23B 2.83</td>
<td>3 3 2.83</td>
</tr>
<tr>
<td>17F</td>
<td>2 2 20 1.89</td>
<td>3 3 2.83</td>
</tr>
<tr>
<td>11A</td>
<td>1 1 19B 1.89</td>
<td>2 1.89</td>
</tr>
<tr>
<td>3</td>
<td>1 1 6A 1.89</td>
<td>2 1.89</td>
</tr>
<tr>
<td>35F</td>
<td>1 1 21 0.94</td>
<td>1 0.94</td>
</tr>
<tr>
<td>4B</td>
<td>1 1 11A 0.94</td>
<td>1 0.94</td>
</tr>
<tr>
<td>13</td>
<td>1 1 7C 0.94</td>
<td>1 0.94</td>
</tr>
<tr>
<td>35B</td>
<td>1 1 Untypeable 0.94</td>
<td>1 0.94</td>
</tr>
<tr>
<td>7C</td>
<td>1 1 35B 0.94</td>
<td>1 0.94</td>
</tr>
<tr>
<td></td>
<td>39 1 0.94</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Pneumococcal carriage by vaccination status.

<table>
<thead>
<tr>
<th>Child immunization status</th>
<th>SPn</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunized</td>
<td>NGR</td>
<td>84</td>
</tr>
<tr>
<td>Not immunized</td>
<td></td>
<td>10.68</td>
</tr>
<tr>
<td>Total</td>
<td>N/A</td>
<td>206</td>
</tr>
</tbody>
</table>

NGR, no growth observed; SPn, Streptococcus pneumoniae; NA, not applicable.

Data availability
Dataset 1. List of basic demographic information for each subject, with the size of the optochin clearance zone and serotype of Streptococcus pneumoniae, if found. DOI: http://doi.org/10.5256/f1000research.14387.d207458 (Walekhwa et al., 2018).

Competing interests
No competing interests were disclosed.

Grant information
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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements
Special thanks to Prof. Anthony Scott, Dr. Claire Gordon and Ms. Angela Karani of Kemri Wellcome Trust, Kilifi for their professional input. The laboratory and technical team: Ann Karanu, Elizabeth Mbiite, Shadrack Mutua and Alfred Too. Your technical input was amazing! The Gertrude’s Hospital clinical and laboratory team (Linet Okose, Barasa Kasili, Charles Muia, Lilive Njagi, Carolyn Thumi, and Peninah Chege), you made this possible. Thanks to Japheth Wambani Rapando for the review of my work.

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Felix Dube
Division of Medical Microbiology, Department of Pathology, University of Cape Town, Cape Town, South Africa

The study reports pneumococcal carriage in a cohort of Kenyan children. This has significant implication when it comes to the evaluation of the impact of PCV10 on the population structure of pneumococcus.

Specific comments:

1. Pneumococcus is a normal commensal, not "highly invasive" as is reported in intro.

2. The authors must avoid the use of non-standard nomenclature such as SPn. Further, there is inconsistent use of S. pneumoniae and pneumococcus throughout the text. These cannot be used interchangeably.

3. The introduction needs to be reworked and repetitions avoided, i.e. in paragraph 1 and 5, the authors talk about 90 serotypes. The last sentence of paragraph 5 does not include references.

4. The study was conducted between 2017 and 2018, it would really have benefited from the WHO working group report on pneumococcal carriage studies. Most importantly, 79% (164/206) being non-viable seriously indicates problems in the experimental design. The authors don't say anything about broth enrichment in order to improve recovery of the pneumococcus. Amies media is not ideal for pneumococcus compared to STGG. A 10ul inoculum is very little, did the authors attempt to use bigger volumes especially for the samples with no growth? A 2% BA plate is used as primary culture then selective media, if possible, do this in parallel.

5. Need to be more consistent in reporting proportions.

6. What was the PCV10 vaccine coverage at each timepoint? Also authors need to report non-pcv as non-PCV10 because some of the serotypes the report as "non-vaccine" such as serotype 3 are included in PCV13.
7. Repetition of results. The authors do not report any metadata looking at risk factors for carriage, hence the "epi" in title must fall out.

8. The authors repeatedly report "decrease" in carriage post PCV, but they really can't say this without data on Pre-PCV10.

9. Avoid sweeping statement to infer serotype replacement if they actually don't show this evidence.

10. "While this is a significant reduction from the pre-vaccine era, it is still high compared to malaria, diarrhea and HIV/AIDS" doesn't make sense, what are the authors referring to?

11. "This is a very important finding as it explains the high level of child morbidity and mortality due to pneumococcal disease despite the availability of PCV-10." How do you arrive at this if working with carriage and not invasive disease isolates. This and other strong conclusions need to be avoided, you surely can't with 42 isolates.

References

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Medical microbiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.
Jackie K. Obey
School of Health Sciences, Department of Medical Laboratory Sciences, University of Eastern Africa, Baraton, Eldoret, Kenya

The study carried out by the authors on Streptococcus pneumoniae is extremely important for Kenya. It addresses a major health problem that has been addressed by other authors in the past and for which a lasting solution is currently being sought. The study is detailed and was able to employ modern techniques to assess the carriage state of children at The Gertrude's Children Hospital, Nairobi, Kenya. The methods used were appropriate and in line with the study's objectives. The sample size was appropriately determined and descriptive statistics have been used appropriately to describe the results obtained by the authors.

The title of the study however includes the word ‘epidemiology' and this may lead the reader to think that the authors would have tried to determine factors that influenced the establishment of the research problem at the study site. The authors have not determined those factors or risks that lead to Kenyan children being vaccinated, yet not being protected by the PCV-10 vaccine. Those findings would then have given an idea of the risk of expose of children to Streptococcus pneumoniae disease and given the hospital and government the strategies to employ for preventive measures against the disease. The conclusion is brief and does not give recommendations to the Government of Kenya or The Gertrude's Children Hospital on what to do after the findings of the study.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly
**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Medicinal parasitology (Malariology), immunoparasitology and medical entomology, antimicrobial activity of plant extracts

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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Bartholomew N. Ondigo  
Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya

Title: To include Kenya

Corresponding author contacts need to be indicated

Abstract:

**Background:**
It needs to be indicated that circulating SPn serotypes are being investigated after the introduction of 10-valent pneumococcal conjugate vaccine (PCV10) in 2011.

The methods need to be a summary of the techniques used in data collection. As it is written it appears to be copy pasted from the method section. Indicate number of children assessed. For instance:

**Materials:**
Two hundred and six children attending and not-attending in …..20-2009 were studied. Materials for study were pharyngeal swabs and sputum. Identification was performed using optochin disks, Quellung reaction,……agglutination on the glass, viewed under a phase contrast microscope and the sent to KEMRI- Kilifi for further confirmative identification tests.

The conclusion seem alarming and need to suggest or indicate. Therefore Kenyan children receiving PCV-10 vaccine are not protected – Revise to something like:-
This study highlights the importance of monitoring and evaluation to provide epidemiological information to determine the effectiveness of PCV10 in Kenya’s Public health services.

Repeating ideas should be deleted - causing more deaths than any other infectious disease vs. kills more children than any other illness in the world.

Introduction need to be shortened, preferably to three paragraphs.

**Methods:**
Were the children admitted or outpatient? All the source of equipment used and consumables need to be indicated, for instance incubator etc. The Research Ethics Committee that approved the study need to be indicated.

Software used for calculation of %s need to be indicated?

Results:
Headings need to be introduced that are in agreement with the content. This will help the reader to when reading. Suggested possible headings include:

Demography of the Study Participants
Prevalence of SPn carriage status among PCV-10 vaccinated and unvaccinated children
Prevalence of SPn carriage status by age – You probably have several age groups on this for instance <1, 2-4 years, 4–5 years.

Authors need to clarify on the following:

How many pneumococcus serotypes were identified? Which serotype was most frequent? Can you please arrange them in a descending order?

Discussion:
Adequate in content.
Consider serotype replacement to enrich your discussion.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
No

Are the conclusions drawn adequately supported by the results?
Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Immunoparasitology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.
Dear Dr. Ondigo,

Many thanks for your review of this article.

I have read through and keenly updated the areas you highlighted during your review. Kindly revisit.

Many thanks

**Competing Interests:** No competing interests were disclosed.