Microbial ecology of the infant nasopharynx: Impact of the PCV-7 vaccination

Martin Antonio PhD
MRC Laboratories, The Gambia

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The Gambia
MRC Labs and Field Sites

Facilities for research in a rural setting with a high disease burden
Nearly 70% of child pneumonia deaths occur in Africa & South Asia.

Pneumococcus is the leading cause of child pneumonia deaths (~40%).

Each dot representing 1000 deaths

(Williams BG et al Lancet 2002)
Pneumococcal serotype (%) distribution from pneumoWAR sites (2010)

S. pneumoniae serotypes/groups

Source: Dr Martin Antonio, pneumoWAR
PCV13, PCV10 & PCV 7 coverage (%) in West Africa (2010)

S. pneumoniae serotypes/groups

Source: Dr Martin Antonio, pneumoWAR
Streptococcus pneumoniae

- The available licensed pneumococcal conjugate vaccines (Prevenar®) contain either 7 or 13 of the 94 pneumococcal serotypes

Concerns:
- Replacement colonisation by non-vaccine serotypes and a significant increase in IPD caused by these bacteria
- An occurrence of species replacement could be of public health concern
- The ecological events that occur after elimination of vaccine serotypes are unclear
Transmission of *Streptococcus pneumoniae* in rural Gambian villages: a longitudinal study

1. The duration of carriage of *S. pneumoniae* varies significantly by serotype.
2. Intra-household transmission is more important than community transmission.
3. Serotype and sequence type specific analyses suggest children tend to bring *S. pneumoniae* into the household with subsequent spread among other children and adult members.
AIMS

• Investigate the impact of vaccination with a polysaccharide conjugate vaccine of limited valency on the nasopharyngeal microbiome

• Characterization of the development and composition of the nasopharyngeal microbiome in rural Gambian infants in the first twelve months of life
Brenda Kwambana
Graduate student
Studying the Nasopharyngeal Microbiota

- Sterile Calcium alginate swabs with aluminium shafts used (WHO recommended)
- Nasopharyngeal swabs are collected by sterile technique and stored in STGG
- NPS swabs are stored at -70 degrees Celsius

Trained field nurse prepares study infant for nasopharyngeal (NP) swabbing

The nurse collects the NP swab by carefully inserting the swab in the nasopharynx, waiting 5 seconds and then rotating it 360 degrees before careful removal

Mother consoles baby while the trained field worker collects metadata including antibiotic use, respiratory tract infections, ear infections and travel and dietary information.
Environmental Factors that may influence the infant nasopharyngeal microbiota

- Overcrowding and exposure to many children
- Proximity to domestic animals and livestock
- Malnutrition
- Seasonal effects
- Breastfeeding
- Diet
One NP swab collected from each of 12 infants on the same day

Duplicate DNA extractions of each of the 12 Fresh NP swabs

T-RFLP analysis on the DNA extracts (n=24)

T-RFs generated for 96% of the NP swabs (n=24)

NP swabs frozen at -70°C for 30 days in STGG

Duplicate DNA extractions of each of the 12 NP swabs

T-RFLP analysis on the DNA extracts

T-RFs generated for 75% of the NP swabs (n=24)

Effect of frozen-storage (-70°C) on the detection of bacterial taxa

Relative distribution of the bacterial OTUs detected before and after frozen storage of NP swabs at -70°C amongst male and female infants

Bacterial OTU richness before and after freezing dichotomized by gender. Red lines represent females, and dotted lines show the mean change.

The difference in composition pre and post freezing was significant for female (p = 0.0014) but not for male infants (p=0.56).

T-RFLP Comparative community analysis

483 NP swabs collected from 29 infants at regular intervals during infancy

DNA extracted from NP swabs

16S based rRNA-based Terminal Restriction Analysis (V3 – V9) (108 OTUs found)

Clone library analysis (242 clones)
The infant nasopharyngeal microbiome (T-RFLP Analysis)
There was a significant difference in the number of OTUs detected between infants older and younger than one month of age (p=0.016) where older infants had on average 0.73 (95% CI: 0.13-1.32) more OTUs detected after allowing for gender.
T-RFLP useful for community analysis but there are major limitations of T-RFLP

- From T-RFLP analysis it emerges that a few OTUs form the backbone of the microbiome
- Several low abundance OTUS are transient colonizers
- It is difficult to identify all the OTUs, even with parallel clone library analysis
- A single OTU may represent a wide spectrum of organisms
- T-RFLP-based analysis may misrepresent taxonomic diversity and richness
Co-detection of bacterial pathogens with pneumococci

Species specific PCR-based detection of three respiratory pathogens
Factors influencing co-carriage of respiratory pathogens with *S. pneumoniae*

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>NP swabs</th>
<th>Co-occurrence (%)</th>
<th>Unadjusted</th>
<th>Adjusted</th>
<th>Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>OR</td>
<td>p Value</td>
<td>95% CI</td>
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<tr>
<td><strong>Age (weeks)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>&lt; 5</td>
<td>84</td>
<td>32 (38%)</td>
<td>4.26</td>
<td>&lt;0.01</td>
<td>2.08, 8.73</td>
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<tr>
<td>&lt; 12</td>
<td>92</td>
<td>61 (66%)</td>
<td>12.94</td>
<td></td>
<td>5.96, 28.11</td>
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<tr>
<td>&lt; 20</td>
<td>112</td>
<td>93 (83%)</td>
<td>18.12</td>
<td></td>
<td>8.07, 40.72</td>
</tr>
<tr>
<td>&lt; 28</td>
<td>115</td>
<td>99 (86%)</td>
<td>13.26</td>
<td></td>
<td>5.90, 29.80</td>
</tr>
<tr>
<td>&gt; 28</td>
<td>95</td>
<td>79 (83%)</td>
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<tr>
<td><strong>Ethnic Group</strong></td>
<td></td>
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<tr>
<td>Mandinka</td>
<td>182</td>
<td>13 (78%)</td>
<td>0.86</td>
<td>0.07</td>
<td>0.41, 1.84</td>
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<tr>
<td>Jola</td>
<td>232</td>
<td>174 (75%)</td>
<td>0.63</td>
<td>0.21</td>
<td>0.16, 2.50</td>
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<tr>
<td>Fula</td>
<td>34</td>
<td>24 (70%)</td>
<td>0.21</td>
<td></td>
<td>0.07, 0.65</td>
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<tr>
<td>Other</td>
<td>50</td>
<td>23 (46%)</td>
<td></td>
<td></td>
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<tr>
<td><strong>Sex (male)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Male</td>
<td>313</td>
<td>220 (70%)</td>
<td>1.51</td>
<td>0.13</td>
<td>0.69, 3.31</td>
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<tr>
<td>Female</td>
<td>185</td>
<td>144 (78%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Antibiotic Course</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Not Administered</td>
<td>410</td>
<td>319 (78%)</td>
<td>0.35</td>
<td>0.01</td>
<td>0.16, 0.75</td>
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<tr>
<td>Administered</td>
<td>39</td>
<td>23 (59%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>NA</td>
<td>49</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Type of feeding</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Exclusive</td>
<td>312</td>
<td>225 (72%)</td>
<td>2.66</td>
<td>&lt;0.01</td>
<td>1.47, 4.79</td>
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<tr>
<td>Mixed</td>
<td>138</td>
<td>118 (85%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>NA</td>
<td>48</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Weight Kg</strong></td>
<td></td>
<td></td>
<td>1.41</td>
<td>&lt;0.01</td>
<td>1.19, 1.67</td>
</tr>
</tbody>
</table>

*Logistic regression modelling was used, both unadjusted and adjusted presented. Adjusted model included age, ethnic group, sex, antibiotic course, type of feeding and weight. The baseline category is shown in bold italics for each factor. NA means that the metadata for the samples was missing. Red indicates significant data.*
102 Infants Recruited with Parental Informed Consent

**SNM STUDY DESIGN**

**GROUP I**
- 33 Infants Recruited
- CONTROL
  - Non-Vaccinated Infants
  - 9 Non-Vaccinated Villages

**GROUP II**
- 30 Infants Recruited
- DIRECT IMPACT OF VACCINATION
  - Vaccinated Infants
  - 9 Non-Vaccinated Villages

**Group III**
- 39 Infants Recruited
- DIRECT & INDIRECT IMPACT OF VACCINATION
  - Vaccinated infants
  - 9 Vaccinated villages

### SAMPLE COLLECTION SCHEDULE

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPS Sample A</td>
<td>&lt;7 days after birth</td>
<td>&lt;7 days after birth</td>
<td>&lt;7 days after birth</td>
</tr>
<tr>
<td>NPS Samples B to N (First 6 months)</td>
<td>Bi-weekly (14 times)</td>
<td>Bi-weekly (14 times)</td>
<td>Bi-weekly (14 times)</td>
</tr>
<tr>
<td>NPS Samples O to Q (Last 6 months)</td>
<td>Bi-monthly (3 times)</td>
<td>Bi-monthly (3 times)</td>
<td>Bi-monthly (3 times)</td>
</tr>
</tbody>
</table>

### VACCINATION SCHEDULE

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV7 Dose 1</td>
<td>NONE</td>
<td>8 Weeks (sample E)</td>
<td>8 Weeks (sample E)</td>
</tr>
<tr>
<td>PCV7 Dose 2</td>
<td>NONE</td>
<td>12 Weeks (sample G)</td>
<td>12 Weeks (sample G)</td>
</tr>
<tr>
<td>PCV7 Dose 3</td>
<td>NONE</td>
<td>16 Weeks (sample I)</td>
<td>16 Weeks (sample I)</td>
</tr>
</tbody>
</table>
454-pyrosequencing of the nasopharyngeal microbiome

- For the V3-V5 region, 7 - 203 (Avg. 43) unique genera detected per sample
- Microbes represent at least 31 phyla
- For both regions, the number of unique genera present in a single sample ranged from 19 to 311 (median 65).
- The number of unique genera present in a single week (any subject) ranged from 292 to 498
- Each subject had between 2 and 10 bacteria that were present at every time point.
Effect of vaccination on bacterial loads: Individual analysis

Figure 8a. Bacteria loads for the 5 most prevalent Families found in the nasopharynx of a PCV-7 naïve infant in the first year of life based on 454-pyrosequencing

Figure 8.b Bacteria loads for the 5 most prevalent Families found in the nasopharynx of a PCV-7 vaccinated infant in the first year of life based on 454-pyrosequencing. Dotted lines represent vaccination points
Streptococcus, Moraxella, Haemophilus, Corynebacterium and Shewenella make up at least 80% of the microbiome amongst the infants.
Major shift in relative distribution occurs in the between 1 and 3 weeks of birth.
Composition of the infant nasopharyngeal microbiome

V1-V3 Analysis

- Firmicutes
- Bacteroidetes
- Actinobacteria
- Proteobacteria
- Others

Mouth (56)

Skin (48)

Colon (195)

V3-V5 Analysis

- Firmicutes
- Bacteroidetes
- Actinobacteria
- Proteobacteria
- Others

Oesophagus (43)

Stomach (25)

Vagina (5)

Dethlefsen et al. 2007
Development of the microbiome in vaccinated and unvaccinated infants

Significant changes in vaccinated children

Significant change in unvaccinated children
Effects of vaccination on microbial ecology

Shannon alpha Diversity

Diversity increases significantly in unvaccinated infants but not vaccinated infants – this is interesting!
Summary

- The infant nasopharyngeal microbiome is dynamic
  - High rates of acquisition and loss
- Acquisition of bacteria, including pathogenic microbes occurs rapidly after birth
- The infant nasopharyngeal microbiome is diverse but,
  - A few taxonomic groups make up the bulk of the microbiome including pathogens *S. pneumoniae, H. influenzae* and *M. catarrhalis* which display high carriage
  - There are numerous low abundance transient taxonomic groups
- There is preliminary evidence of a vaccine non-effect on other taxa though there may be large variations between individual subjects
Villagers came out to Welcome George
Acknowledgements

Many thanks to the infants participating in the study, their mothers and village leaders
MRC Hosts First Genomics Symposium in The Gambia

Classical and emerging infectious diseases still represent the single most important threat to human health on a global scale and infectious diseases are seldom out of the news. However, advances in genetics and genomic technologies promise to provide new approaches to understanding and combating these diseases.