The nasopharyngeal microbiome and respiratory disease in African children

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Important bacterial constituents of the NP flora

Kwambana et al. BMC Infect Dis 2011
Figure 2: Pneumococcal mortality rate

Pneumococcal deaths in children aged 1–59 months per 100,000 children younger than 5 years (HIV-negative pneumococcal deaths only). The boundaries shown and the designations used on this map do not imply the expression of any opinion by WHO concerning the legal status of any country, territory, city, or area, or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.
Nearly 70% of child pneumonia deaths occur in Africa & South Asia

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

Each dot represents 1000 deaths (Williams BG et al Lancet 2002)

Slide courtesy Martin Antonio
Drakenstein Child Lung Health Study

• A birth cohort of 500 mother-infant pairs followed for 2 (5) years

• Longitudinal data on incident pneumonia and its aetiology

• Investigate antecedent risk factors for pneumonia and for pneumonia progression
Key points in our strategic approach

• Drakenstein sub-district – peri-urban, high prevalence of risk factors, births at a single hospital

• Birth Cohort – temporality, repeated measures of risk factors

• Outcomes: pneumonia and wheezing illness

• Very detailed phenotyping (including repeated lung function testing) and extensive biological and environmental sample collection

• Diverse risk factors in 7 areas – microbiological, immunological, environmental, maternal, nutritional, genetic & psychosocial
Study measures

- Enrolment
  - Demographic/Clinical Info
  - ANC2
  - Maternal Health
  - Environmental Home Visit
  - Psychosocial
  - Paternal Health

- Birth
  - Microbiological/Infectious
  - Immunological
  - Genetic

- 6-10 Weeks
  - Infant Lung Function
  - Microbiological/Infectious
  - Nutrition
  - Immunological
  - Genetic

- 10 Weeks
  - Microbiological/Infectious
  - Psychosocial
  - Nutrition

- 14 Weeks
  - Microbiological/Infectious
  - Psychosocial
  - Environmental Home Visit

- 6 Mo
  - Microbiological/Infectious
  - Psychosocial

- 9 Mo
  - Microbiological/Infectious
  - Psychosocial
  - Nutrition

- 12 Mo
  - Infant Lung Function
  - Microbiological/Infectious
  - Nutritional
  - Immunological
  - Genetic
  - Psychosocial
  - Environmental CRF

- 18 Mo
  - Microbiological/Infectious
  - Psychosocial
  - Nutrition

- 24 Mo
  - Infant Lung Function
  - Microbiological/Infectious
  - Nutritional
  - Immunological
  - Genetic
  - Psychosocial
  - Environmental CRF

Pregnant women enrolled at 20-28 weeks gestation.

NP (2 weekly) & Stool (monthly) Cohort
Key H3Africa Project Aims

• Describe, in detail, patterns of nasopharyngeal colonization in healthy infants from birth to two years (bacterial and viral)

• Determine the role of the nasopharyngeal microbiome in pneumonia and wheezing illness in African children by:
  – Identifying patterns of nasopharyngeal colonization that are associated with the development of pneumonia/wheezing.
  – Investigating interactions between the nasopharyngeal microbiome and other risk factors

• Development of capacity for full pipeline microbiome analysis
Nested H3Africa studies: NP sampling

Enroll 500 mother-infant pairs

200 infants with NP sampling at birth, 6w, 6m, 12m, 18m, 24m

Pneumonia cases and controls

Wheezing cases and controls
Nested H3Africa studies: experimental approach

• Routine bacterial culture, with focus on respiratory pathogens
  – Sequetyping of pneumococci
  – Genotyping of *S. aureus*

• 33-plex real-time PCR assay (Fasttrack Diagnostics FTD33) for common bacterial and viral pathogens
  – Including quantitative assessment

• Microbiome analysis - sequencing 16S rRNA gene amplicons (MiSeq, Cape Town)

• Association analysis (incorporating time-series approaches) for pneumonia and wheezing outcomes
Cumulative enrolments by month through 22 Sept 2013
2-weekly NP Cohort: cohort retention

83% 88% 75% 86% 91% 90% 84% 94% 87% 97% 88% 89% 82% 79% 92% 94% 91% 80% 80%
Pneumonia Cases

- Total WHO-defined pneumonia cases: 118
  - <28 days: 26 cases (19 congenital)
  - >28 days: 92 cases (58 ambulatory, 34 hospitalised)
Preparatory studies: Flocked NP swabs are superior to Dacron NP swabs

Flocked (3.0 x 10^5 copies/mL) vs. Dacron (9.3 x 10^4 copies/mL)

Dube et al. PLoS One In press
Early data: NP bacterial co-colonization

Multiple correspondence analysis: 321 NP samples from Drakenstein cohort
Early data: NP co-colonization viruses/bacteria
Early data: NP co-colonization viruses/bacteria
Capacity development

• 1-week ‘wet’ workshop on 16S microbiome sequencing (with JCVI)

• Exchange programme with JCVI

• Postgraduate students
  – Current: MSc 3, PhD 3, postdoc 3
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