The Bacteriology of Bronchiectasis in Australian Indigenous children

Kim Hare, Amanda Leach, Peter Morris, Heidi Smith-Vaughan, Anne Chang

discovery for a healthy tomorrow

Presentation outline

- What is bronchiectasis?
- Our research at Menzies
- Other respiratory infections and the bacteria responsible
- Studies and laboratory methods
- **Aim 1**: bacteriology of upper and lower airways
- **Aim 2**: impact of azithromycin on carriage, lower airway infection, and antibiotic resistance
- Summary
Acknowledgements

- My **supervisors and co-investigators**
  - **Menzies:** Heidi Smith-Vaughan
  - Anne Chang
  - Amanda Leach
  - Peter Morris
  - **Melbourne:** Allen Cheng
  - **Brisbane:** Keith Grimwood

- **NHMRC** – project grant 2009-11 and PhD scholarship 2012-13

- **Menzies clinical staff**, especially Gabrielle McCallum (Australian coordinator of the Multicentre Bronchiectasis Study)

- **Menzies laboratory staff**, especially Vanya Hampton

- **Staff of Royal Darwin Hospital**, especially Dr Paul Bauert

- **Clinic staff** in participating communities and all the **children and their carers** who have participated in these studies

What is bronchiectasis?

- Chronic suppurative lung disease (CSLD)

- Irreversible, abnormal dilation of the bronchial tree leading to poor clearance and pooling of mucus

- Symptoms frequently date back to infancy

- Chronic wet cough is the main symptom

**Diagnosis**

- History taking
- Clinical exam
- High resolution computed tomography (HRCT) = **gold standard**
Background

- NT Indigenous children have the world’s highest recorded rate of non-cystic fibrosis bronchiectasis (1 in 68 in Central Australia: Chang et al, 2003)
- Pneumonia is a risk factor, especially severe and recurrent pneumonia (Valery et al, 2004)
- The aetiology of non-CF bronchiectasis is not well understood, especially in Indigenous children
Aetiology of bronchiectasis

Various causes including bacterial infections by:

- *Streptococcus pneumoniae* (the pneumococcus)
- *Haemophilus influenzae* (mostly nontypeable, not Hib)
- *Moraxella catarrhalis*
- *Staphylococcus aureus* and *Pseudomonas aeruginosa* and are more important in children with cystic fibrosis (CF) bronchiectasis

Acute and chronic lower respiratory disease
Bronchiectasis studies

High rates of bronchiectasis are also recorded in Indigenous children in Alaska and New Zealand -> Multicentre Bronchiectasis Study, consisting of:

- **Bronchiectasis Observation Study (BOS)** in Aust & Alaska – to follow the clinical course of children with CSLD
- **Bronchiectasis Intervention Study (BIS)** in Aust & NZ – randomised controlled trial (RCT) to examine the efficacy of azithromycin in preventing exacerbations
- In addition, the **Bronchoscopy Study** is being conducted at RDH (with comparison specimens from RCH, Brisbane)

Laboratory methods

- Specimens collected = Nasopharyngeal (NP) swabs, plus throat (OP) swabs and broncho-alveolar lavage (BAL) fluid (from children undergoing bronchoscopy)
- Specimens plated on selective agar for recovery of:
  - *Streptococcus pneumoniae* (Spn)
  - *Haemophilus influenzae* (NTHi)
  - *Moraxella catarrhalis* (Mcat), *Staph aureus* (Sa), *Pseudomonas*
- Semi-quantitative growth scores recorded
- Lower airway infection defined as >10⁴ CFU/mL BAL (to exclude possible contamination during the procedure)
Aim 1
Bacteriology of the upper and lower airways of Indigenous children with bronchiectasis

Bronchoscopy study – BAL micro

<table>
<thead>
<tr>
<th></th>
<th>BAL fluid (any growth)</th>
<th>BAL fluid (&gt; 10^4 CFU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=114</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>33%</td>
<td>15%</td>
</tr>
<tr>
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<td><strong>77%</strong></td>
<td><strong>30%</strong></td>
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</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>11%</td>
<td>3.5%</td>
</tr>
<tr>
<td><em>Pseudomonas</em> species</td>
<td>3%</td>
<td>0%</td>
</tr>
<tr>
<td><em>Haemophilus parainfluenzae</em></td>
<td>23%</td>
<td>3.5%</td>
</tr>
<tr>
<td>Alpha-haemolytic Strep.</td>
<td><strong>98%</strong></td>
<td><strong>53%</strong></td>
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### Bronchoscopy study – BAL & NP micro

<table>
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<tr>
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<td>30%</td>
<td>48%</td>
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<tr>
<td><em>Moraxella catarrhalis</em></td>
<td>25%</td>
<td>11%</td>
<td>30%</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>11%</td>
<td>3.5%</td>
<td>18%</td>
</tr>
<tr>
<td><em>Pseudomonas species</em></td>
<td>3%</td>
<td>0%</td>
<td>3%</td>
</tr>
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*Apparent NTHi

### Bronchoscopy study – BAL & NP & OP micro

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<tr>
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<th>BAL fluid (any growth)</th>
<th>BAL fluid (&gt; 10^4 CFU/mL)</th>
<th>NP swab</th>
<th>OP swab</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=114 (OP=86)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>33%</td>
<td>15%</td>
<td>35%</td>
<td>6%</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td>77%</td>
<td>30%</td>
<td>48%</td>
<td>52%</td>
</tr>
<tr>
<td><em>Moraxella catarrhalis</em></td>
<td>25%</td>
<td>11%</td>
<td>30%</td>
<td>3%</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>11%</td>
<td>3.5%</td>
<td>18%</td>
<td>10%</td>
</tr>
<tr>
<td><em>Pseudomonas species</em></td>
<td>3%</td>
<td>0%</td>
<td>3%</td>
<td>1%</td>
</tr>
<tr>
<td><em>Haemophilus parainfluenzae</em></td>
<td>23%</td>
<td>3.5%</td>
<td>3%</td>
<td>56%</td>
</tr>
<tr>
<td><em>Alpha-haemolytic Strep.</em></td>
<td>98%</td>
<td>53%</td>
<td>34%</td>
<td>100%</td>
</tr>
</tbody>
</table>

*Apparent NTHi
How do the pathogen strains match up?

**Strains** defined by:
- Serotype and antibiotype (Spn)
- PCR-ribotype and antibiotype (NTHi)
- Beta-lactamase status (Mcat)

**Multiple strains** detected by isolating 4 colonies each of Spn and NTHi and 2 colonies of Mcat from each positive specimen

**Concordance** defined as the same bacterial strain identified in paired NP swab and BAL cultures when a lower airway infection is identified

### Bronchoscopy study

<table>
<thead>
<tr>
<th></th>
<th>NP swab</th>
<th>BAL fluid (&gt; 10^4 CFU/mL)</th>
<th>NP &amp; BAL (&gt; 10^4 CFU/mL)</th>
<th>Concordance (same strain)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N=45</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Streptococcus pneumoniae</strong></td>
<td>42%</td>
<td>18%</td>
<td>13%</td>
<td>6/6 (100%)</td>
</tr>
<tr>
<td><strong>Haemophilus influenzae</strong></td>
<td>51%</td>
<td>47%</td>
<td>36%</td>
<td>15/16 (94%)</td>
</tr>
<tr>
<td><strong>Moraxella catarrhalis</strong></td>
<td>42%</td>
<td>20%</td>
<td>20%</td>
<td>9/9 (100%)</td>
</tr>
<tr>
<td><strong>Any of the 3 pathogens</strong></td>
<td>73%</td>
<td>53%</td>
<td>47%</td>
<td>nd</td>
</tr>
<tr>
<td><strong>All 3 pathogens</strong></td>
<td>24%</td>
<td>7%</td>
<td>7%</td>
<td>nd</td>
</tr>
</tbody>
</table>
**Bronchoscopy study: summary (1)**

In 45 Indigenous children with bronchiectasis we found:

- High density and diversity of respiratory bacteria (multiple strains in lung = Mcat 22%, Spn 25%, NTHi 67%)
- Strain concordance between upper and lower airways
- Possible pathogenic role of recurrent aspiration of NP secretions
- Possible accumulation of strains in the lower airways

Respiratory Bacterial Pathogens in the Nasopharynx and Lower Airways of Australian Indigenous Children with Bronchiectasis.

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**Bacteria found in adult bronchiectasis**

<table>
<thead>
<tr>
<th></th>
<th>Steinfort study* (57 patients)</th>
<th>King study (89 patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td>67%</td>
<td>47%</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>21%</td>
<td>12%</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>16%</td>
<td>7%</td>
</tr>
<tr>
<td><em>Moraxella catarrhalis</em></td>
<td>5%</td>
<td>8%</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>7%</td>
<td>3%</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>7%</td>
<td>0%</td>
</tr>
</tbody>
</table>

*97% were Indigenous; 70% had past histories notable for recurrent childhood respiratory infections

*Bronchiectasis in Central Australia: A young face to an old disease.* Respiratory Medicine 2008; 102:574–578. Steinfort et al. Mean age 42 ± 15 years, male 52%

*Microbiologic follow-up study in adult bronchiectasis.* Respiratory Medicine 2007; 101:1633–1638. King et al. Mean age 57 ± 14 years, male 30%
Is it really *Haemophilus influenzae*?

*Haemophilus haemolyticus*: A Human Respiratory Tract Commensal to Be Distinguished from *Haemophilus influenzae*

JID 2007:195 • TF Murphy et al.

- Standard methods do not reliably distinguish nonhaemolytic strains of *H. haemolyticus* from nontypeable *H. influenzae*
- 12 to 27% of phenotypic NTHi isolates from the NP of children were actually *H. haemolyticus*

Nasopharyngeal Carriage of *Haemophilus haemolyticus* in Otitis-Prone and Healthy Children


- 11.7% of NTHi-like isolates from the NP were *H. haemolyticus*
  and 9.4% gave equivocal results
- *H. haemolyticus* has not been identified in middle ear effusion

“Fuzzy” species

![Diagram showing relationships between different *Haemophilus* species.](image-url)
Differentiating *H. influenzae* from *H. haemolyticus*

- hpd#3 real time PCR assay the superior method to discriminate NTHi from closely related Haemophilus species
- Added potential for quantification of *H. influenzae* directly from specimens

Molecular surveillance of true nontypeable *Haemophilus influenzae*: an evaluation of PCR screening assays
PLoS ONE 2012: 7 • MJ Binks et al.

### H. influenzae & H. haemolyticus in BAL, NP & OP

<table>
<thead>
<tr>
<th></th>
<th>NP swabs (n=84)</th>
<th>OP swabs (n=56)</th>
<th>BAL fluid* (n=84)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenotypic NTHi</td>
<td>50%</td>
<td>64%</td>
<td>36%</td>
</tr>
<tr>
<td><em>H. influenzae</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>confirmed by hpd#3 PCR</td>
<td>45% (90%)</td>
<td>25% (39%)</td>
<td>36% (100%)</td>
</tr>
<tr>
<td><em>H. haemolyticus</em></td>
<td>10%</td>
<td>50%</td>
<td>10%</td>
</tr>
<tr>
<td>Concurrent NTHi &amp; Hh</td>
<td>5%</td>
<td>11%</td>
<td>10%</td>
</tr>
</tbody>
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*Lower airway infection = >10^4 cfu/mL BAL fluid*
Conclusions from Hi_Hh differentiation

- The nasopharynx (not the oropharynx) is the preferred site for NTHi colonization studies
- NTHi is confirmed as an important lower-airway pathogen

Culture and PCR Detection of *H. influenzae* and *H. haemolyticus* in Australian Indigenous Children with Bronchiectasis.

Aim 2
Impact of azithromycin on airway bacteriology and antibiotic resistance
Role of azithromycin

- Can be given weekly (vs. 2x/day for amoxicillin) and doesn’t require refrigeration
- Many children are prescribed long-term weekly azithromycin to reduce respiratory exacerbations
- There is no high level evidence of clinical benefit or harm (development of antibiotic resistance)
- Also has anti-inflammatory properties

Nasopharyngeal carriage – antibiotics & resistance

<table>
<thead>
<tr>
<th>Antibiotics in 2 weeks preceding bronchoscopy</th>
<th>None (n=39)</th>
<th>Macrolides (n=39)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>64%</td>
<td>28%</td>
</tr>
<tr>
<td>Azithromycin resistant</td>
<td>12%</td>
<td>82%</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td>59%</td>
<td>41%</td>
</tr>
<tr>
<td><em>Moraxella catarrhalis</em></td>
<td>44%</td>
<td>21%</td>
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Lower airway infection – antibiotics & resistance

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<td><strong>Moraxella catarrhalis</strong></td>
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Bronchoscopy study – *H. influenzae* resistance

- A study of >6000 clinical isolates (Peric et al, 2003) found that most (97%) *H. influenzae* strains have an intrinsic macrolide efflux mechanism => intermediate resistance (EUCAST MICs 0.25-4 mg/L)
- Only 1.3% had high-level resistance (due to ribosomal and other mutations), and 1.8% were truly susceptible to macrolides such as azithromycin
- In our study 6% of NP carriage-positive children and 13% of children with *H. influenzae* lower airway infection had strains with high-level resistance
- There was an association with azithromycin use but this was not statistically significant (small numbers)
Bronchoscopy study: summary (2)

In 104 Indigenous children with bronchiectasis we found:
- Recent azithromycin use was associated with reduced carriage
- However lower airway infection was not reduced (except Mcat in children given azithromycin)
- Children who received azithromycin were significantly more likely to carry or be infected by azithromycin-resistant *Streptococcus pneumoniae*, which is a concern


Bronchiectasis Observational Study (BOS)

- Observational study with children examined and NP swabs taken at 3-monthly visits
- 38 children from Central Australia, enrolled from 2004 to 2008, average 4.2 visits per child
- 41 children from Top End, enrolled 2006 to 2009, average 6.9 visits per child
- Top End children received more azithromycin
BOS groups for analysis

NOT a RCT (drug vs placebo), therefore:

**Group 1:** no record of azithromycin use during study period

**Group 2:** children received azithromycin at 1% to 50% of study visits

**Group 3:** children received azithromycin at 51% to 100% of study visits

**Group 1:** 26 children – 111 NP swabs collected
**Group 2:** 26 children – 151 NP swabs collected
**Group 3:** 27 children – 181 NP swabs collected

Impact of azithromycin use on NP carriage in 79 Indigenous children with bronchiectasis

![Graph showing the impact of azithromycin use on NP carriage](image)

Group 1 (n=26): azithromycin use not recorded during study period
Group 2 (n=26): azithromycin use recorded at 1-50% of visits
Group 3 (n=27): azithromycin use recorded at 51-100% of visits

Error bars = 95% confidence interval (CI) adjusted for repeated sampling
Impact of azithromycin use on azithromycin resistance in 79 Indigenous children with bronchiectasis

Summary of BOS findings

**Azithromycin use associated with:**
- Reduced carriage of the three main respiratory pathogens
- Increased carriage of *Staphylococcus aureus*
- Increased azithromycin resistance

**In conclusion:**
- Results from BIS are needed to assess the clinical benefits of azithromycin alongside the potential harms of antibiotic resistance
Bronchiectasis Interventional Study

Study completed but not yet unblinded

- 42 children enrolled in NT and TSI
- Randomised to receive azithromycin or placebo
- Followed for 1-2 years with swabs taken 3-monthly
- Total of 210 swabs collected
- 51% Spn positive (26% swabs AziR = 54/108 isolates)
- 31% Hi and 15% Mcat positive
- 23% swabs Sa positive  (22% swabs EryR = 46/49 isolates)
  (3% swabs MRSA = 7/49 isolates)
### Summary - bacteriology

- Bronchiectasis is an important and prevalent condition in Indigenous children contributing to a high burden of respiratory disease.
- Bacterial respiratory pathogens are frequently found in the lower airways and match strains found in the upper airways, suggesting aspiration of secretions is important.
- NTHi is the most important pathogen found in these children, and in adults with bronchiectasis.

### Summary – azithromycin

- Azithromycin is associated with reduced NP carriage of *S. pneumoniae*, NTHi and *M. catarrhalis* but not reduced lower airway infection.
- Azithromycin is associated with increased NP carriage of *S. aureus* and increased macrolide resistance.
- Results from the BIS RCT are needed to determine if azithromycin has a clinical benefit in these children.