Alloiococcus otitidis: a neglected bacterium in otitis media

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The team ... in alphabetical order

- Chris Ashhurst-Smith
- Caroline Blackwell
- Christine Burns
- Rod Givney
- Stephen Graves
- Sharron Hall
- John Stuart
Not the usual suspects

- Stuart *et al.*, 2003 – the three classical pathogens were not the principal isolates from Indigenous children

- Ashhurst-Smith *et al.*, 2007 - Study no. 1 Hunter area *Alloiococcus otitidis* (AO) was the main isolate in first study of both Indigenous and non-Indigenous children – altered direction of our work

- Study no. 2 – New England Area *A. otitidis* 60% isolation rate; 90% of children sampled positive by PCR.
A. *otitidis*: the myths

- Fastidious and difficult to grow
- Difficult to identify
- No information on antibiotic susceptibility
- Contaminant / commensal of outer ear
- Non-pathogenic
Isolation of bacteria from middle ear samples of children with otitis media with effusion (OME)

<table>
<thead>
<tr>
<th>Species</th>
<th>No.</th>
<th>(%)</th>
<th>Indigenous %</th>
<th>Non-indigenous %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. otitidis.</td>
<td>36</td>
<td>(46)</td>
<td>21 (55)</td>
<td>15 (38)</td>
</tr>
<tr>
<td>Corynebacterium spp</td>
<td>21</td>
<td>(27)</td>
<td>13 (34)</td>
<td>8 (20)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>5</td>
<td>(6 )</td>
<td>1 (3)</td>
<td>4 (10)</td>
</tr>
<tr>
<td>H. influenzae</td>
<td>2</td>
<td>(2.6)</td>
<td>0</td>
<td>2 (5)</td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>1</td>
<td>(1.3)</td>
<td>0</td>
<td>1 (2.5)</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>1</td>
<td>(1.3)</td>
<td>1 (2.6)</td>
<td>0</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>1</td>
<td>(1.3)</td>
<td>1 (2.6)</td>
<td>0</td>
</tr>
<tr>
<td>S. mutans</td>
<td>1</td>
<td>(1.3)</td>
<td>1 (2.6)</td>
<td>0</td>
</tr>
<tr>
<td>Sphingomonas sp</td>
<td>1</td>
<td>(1.3)</td>
<td>1 (2.6)</td>
<td>0</td>
</tr>
</tbody>
</table>
Identification of *A. otitidis* by current diagnostic tools

<table>
<thead>
<tr>
<th>System</th>
<th>No. (%) correct identifications</th>
<th>Confidence level</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBL Crystal</td>
<td>36 (90)</td>
<td>94.3%</td>
</tr>
<tr>
<td>API Strep</td>
<td>38 (97)</td>
<td>95.2%</td>
</tr>
<tr>
<td>Vitek2 GP card</td>
<td>39 (100)</td>
<td>98.3%</td>
</tr>
<tr>
<td>Maldi-tof</td>
<td>39 (100)</td>
<td>99%+</td>
</tr>
</tbody>
</table>
## Susceptibility of *A. otitidis* to macrolide antibiotics

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th><em>S. pneumoniae</em></th>
<th>staphylococcus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E-Test® guidelines n (%)</td>
<td>guidelines n (%)</td>
</tr>
<tr>
<td><strong>erythromycin</strong></td>
<td>19 (49)</td>
<td>12 (31)</td>
</tr>
<tr>
<td></td>
<td>11 (28)</td>
<td>7 (18)</td>
</tr>
<tr>
<td></td>
<td>9 (23)</td>
<td>20 (51)</td>
</tr>
<tr>
<td><strong>clarithromycin</strong></td>
<td>17 (44)</td>
<td>12 (31)</td>
</tr>
<tr>
<td></td>
<td>6 (15)</td>
<td>1 (2)</td>
</tr>
<tr>
<td></td>
<td>16 (41)</td>
<td>26 (67)</td>
</tr>
<tr>
<td><strong>azithromycin</strong></td>
<td>14 (36)</td>
<td>13 (33)</td>
</tr>
<tr>
<td></td>
<td>5 (13)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>20 (51)</td>
<td>26 (67)</td>
</tr>
</tbody>
</table>
Co-infections and susceptibility to penicillins

- Alloioococcus is often detected with β-lactamase producers, *H. influenzae* or *M. catarrhalis*

- Incubation of AO with *M. catarrhalis* increased MBC of *A. otitidis* from 0.05 to 8 μg ml\(^{-1}\)
Persistence, pathogenesis and antibiotic resistance

- Persistent bacteria first noted in 1944

- Joseph Bigger - penicillin could not completely kill a culture of *S. aureus*

- Allows bacteria to cope with harsh environments – low O$_2$, lack of nutrients, adverse temperatures
Activity in broth medium; otopathogens compared to *A. otitidis*

-10
0
10
20
30
40
1 10 100 1000

Incubation time (hours)

**CO2** production

*Otopathogens* compared to *A. otitidis*:

- *A. otitidis*
- *M. catarrhalis*
- *H. influenzae*
- *S. pneumoniae*

![Graph showing CO2 production over incubation time for different otopathogens compared to A. otitidis.](image-url)
A. *otitidis* – a persistent organism?

- Slow growth on agar or in liquid medium
- Long term storage – 2 years at 4°C in BHI, 34/36 (94%) grew on HBA
- Anaerobic – at 14 days, 8/36 (22%) grew
A. *otitidis*: an unlikely contaminant of the outer ear?

- Spanish study of ear samples, 26/1119 (2.3%) [Gómez-Hernando *et al.* 1998]
- Australian study 17/800 (2.1%) [W. Pederick, personal communication]
Inflammatory responses: first line of defence

- **LOW**
  - Infection: +
  - Inflammation: invasive
  - Disease: no harm

- **BALANCED**
  - Infection: +
  - Inflammation: control
  - Disease: but no harm

- **HIGH**
  - Infection: +
  - Inflammation: tissue
  - Disease: damage
Inflammatory responses to infection

Infection

LEUKOCYTES

TNFα

IFNγ

IL-1β

IL-6

IL-8

CYTOKINES
Does A. otitidis induce inflammatory responses?

- Previous studies assessed only 1 type culture isolate.
- Current studies tested *A. otitidis* (n=39) and two *S. pneumoniae*, ATCC 49619 and a recent blood culture isolate (SP2).
- Human monocytic THP-1 cells used for uniform genetic background of cytokine responses.
- Interferon-γ used as surrogate for virus infections that often precede AOM.
- Cytokine responses quantified by BioRad bead assay and the Luminex 200.
Cytokine responses induced by *S. pneumoniae* or *A. otitidis* from THP-1 cell primed with IFN-γ (10 ng ml⁻¹)

<table>
<thead>
<tr>
<th></th>
<th>IL-1β (pg m⁻¹ mean (SD))</th>
<th>IL-6 (pg m⁻¹ mean (SD))</th>
<th>IL-8 (pg m⁻¹ mean (SD))</th>
<th>TNF-α (pg m⁻¹ mean (SD))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S. pneumoniae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATCC 49619</td>
<td>63 (16)</td>
<td>846 (95)</td>
<td>5981 (1373)</td>
<td>29 (19)</td>
</tr>
<tr>
<td>“wild” SP2</td>
<td>320 (15)</td>
<td>5288 (422)</td>
<td>&gt;26,000</td>
<td>536 (54)</td>
</tr>
<tr>
<td><strong>A. otitidis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White type</td>
<td>627 (46)</td>
<td>5643 (371)</td>
<td>&gt;26,000</td>
<td>1605 (137)</td>
</tr>
<tr>
<td>Green type</td>
<td>283 (11)</td>
<td>4161 (110)</td>
<td>&gt;26,000</td>
<td>17 (30)</td>
</tr>
</tbody>
</table>
Differences in cytokines elicited by colony type

- Initial studies indicated large white colony type elicited higher levels of cytokines than the small green type.

- Analysis of the green type and white type found green induced higher levels of: IL-8 ($P < 0.05$); IL-1β ($P < 0.05$).
Soluble “virulence factors” and cytokine induction

- Inflammatory responses NOT reduced by treatment of AO filtrates with lysozyme
- Responses significantly reduced by treatment with proteinase K
- Two factors potentially associated with induction of inflammation: β-haemolysin; 70-75 kD extracellular protein.
Future studies

- Eradication of AO by antibiotics unlikely due to resistance to macrolides and “persister” populations

- Need to develop vaccines against AO – so far identified 2 potential virulence factors that cover 100% of clinical isolates tested (patent pending)

- Need to consider immunisation route – mucosal route might be more efficient than parenteral
Support

- University of Newcastle Pilot Studies
- Hunter Area Pathology Service
- John Hunter Children’s Hospital Research Fund
- Hunter Medical Research Institute