Otitis Media

Are probiotics an answer?

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Outline

• Background

• Study Design

• *in vitro* Assays

• Future Directions
Otitis Media (OM)

OM – one of the most common infection in children  
(Rovers et al 2004)

Aboriginal children – one of the highest prevalence of OM  
(Gunasekara et al 2008)

Starts as URI   >   acute OM   >   chronic OM  
(Kong et al 2009)
Recurrent ear discharge > hearing loss > poor speech development (Williams et al 2009)

*Streptococcus pneumoniae* is one of the main bacteria causing OM (Stoodley et al 2008)

Colonise the nasopharynx > travel to middle ear > OM (Wiertsema et al 2009)
How to tackle OM?

• Various strategies

• *S. pneumoniae*
  • Early colonisation (3 m) - increased risk of OM (Faden et al 1997)
  • Indigenous children earlier (3 wks) (Smith et al 2008)
  • Increased bacterial load - increased risk of OM (Smith et al 2006)

• *Can inhibition of colonisation be an alternative method in OM prevention?*
Probiotics

• Live micro organisms- confer health benefits to the host

• Possible mechanisms of action
  - Competitive inhibition of microbes
  - Immuno-modulation
  - Bacteriocin production (Iannitti et al 2010)

• Alpha haemolytic streptococci - inhibitory effect against OM pathogens
  (Tano et al 1999)
Streptococcus salivarius

• Potential pharyngeal probiotic
  (Guglielmetti et al 2010)
• Safe, commercially available
• Produces bacteriocins, BLIS
• Harbours megaplasmids
  (Tagg et al 2009)
Does *S. salivarius* inhibit the colonisation of *S. pneumoniae*?
Study Design

• *in vitro* lab assays

• *in vivo* animal experiments

• Clinical trial
in vitro Lab Assays

• Administration of *S. salivarius* will inhibit *S. pneumoniae* colonisation of HEp-2 cells in an in vitro laboratory setting

• Probiotic – *S. salivarius* (Ss)

• Bacteria – *S. pneumoniae* (Pnc)

• Epithelial cell- HEp-2cell (Human Epidermoid laryngeal carcinoma cell)
The Assay

24-well tray

Pneumococci
HEp-2 cells

[Diagram showing a 24-well tray with cells and a culture containing Pneumococci and HEp-2 cells]
Various testing conditions

• cells & Pnc (standard)
• cells, Pnc & heparin (control)
• cells, Pnc & Ss (high)
• cells, Pnc & Ss (medium)
• cells, Pnc & Ss (low)

• Quantification of Pnc - qPCR
Addition of *S. salivarius*

- **Pre-administration** – one hour before Pnc
- **Co-administration** – along with Pnc
- **Post-administration** – one hour after Pnc
Results of *in vitro* assays
Pre-administration

% adherence

Pneumo alone  +heparin  + Salivarius (high)  + Salivarius (med)  + Salivarius (low)

* * *

n=4; * indicates p<0.05 when compared to pneumo alone which was normalised to 100% for each assay (Students t test)
Co-administration

% adherence

Pneumo alone  +heparin  + Salivarius (high)  + Salivarius (med)  + Salivarius (low)

0  50  100  150

* * *

n=3; * indicates p <0.05 when compared to pneumo alone which was normalised to 100% for each assay (Students t test)
Conclusion

• Pre-administration and co-administration of *S. salivarius* show a dose dependent inhibition of *S. pneumonie* colonisation

• Pre-administration was more effective than co-administration

• Post administration – no effect

• On going experiments with 2 other serotypes
Future directions

• *in vivo* mouse model experiment
  - Does *S. salivarius* colonise the mouse?
  - Does *S. salivarius* inhibit the colonisation of *S. pneumoniae*?
  - Does *S. salivarius* prevent OM in a mouse model?

• Design a clinical trail
Thank you...

• My supervisors – Prof Stephen O’Leary, Prof Roy Robins Browne, Dr Catherine Satzke, Dr Odilia Wijburg, Dr Paul Licciardi

• Dr Eileen Dunne, Zheng Quan Toh
• Prof John Tagg and his colleagues
• Graeme Clark Foundation
• Hugh Noel Puckel Scholarship
• The University of Melbourne
Clinical trial

• Does *S. salivarius* inhibit the colonisation of *S. pneumoniae* in the nasophaynx?
• Does *S. salivarius* prevent OM?
• If *S. salivarius* is effective in OM, then
  - mode of administration
  - dose of administration
  - time of administration
Statistical Analysis

- Results were compared to pneumococcal adherence in wells containing *S. pneumoniae* alone, normalised to 100%. The student’s t-test was used to evaluate differences % adherence between test conditions and *S. pneumoniae* alone. P values < 0.05 were considered statistically significant and are indicated with an asterisk.
qPCR

- Lyta gene specific for pneumococcus used
Figure 1. Growth of *S. pneumoniae* and *S. salivarius* over time as measured by optical density (OD$_{600}$).
### Comparing qPCR to serial dilution and plating

<table>
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<th></th>
<th>qPCR</th>
<th>Serial dilution &amp; plating</th>
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<tbody>
<tr>
<td></td>
<td>CFU/ml PMP843</td>
<td>CFU/ml PMP 843</td>
</tr>
<tr>
<td><strong>Pnc</strong></td>
<td>1.1 x 10^7</td>
<td>9.1 x 10^6</td>
</tr>
<tr>
<td><strong>Pnc + Heparin</strong></td>
<td>1.5 x 10^6</td>
<td>7.6 x 10^5</td>
</tr>
<tr>
<td><strong>Pnc + Ss (high)</strong></td>
<td>6.1 x 10^5</td>
<td>3.2 x 10^5</td>
</tr>
<tr>
<td><strong>Pnc + Ss (medium)</strong></td>
<td>3.6 x 10^6</td>
<td>2.7 x 10^6</td>
</tr>
<tr>
<td><strong>Pnc + Ss (low)</strong></td>
<td>9.8 x 10^6</td>
<td>8.2 x 10^6</td>
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<table>
<thead>
<tr>
<th></th>
<th>% colonisation</th>
<th>% colonisation</th>
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<tbody>
<tr>
<td><strong>Pnc</strong></td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><strong>Pnc + Heparin</strong></td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td><strong>Pnc + Ss (high)</strong></td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td><strong>Pnc + Ss (medium)</strong></td>
<td>33</td>
<td>29</td>
</tr>
<tr>
<td><strong>Pnc + Ss (low)</strong></td>
<td>92</td>
<td>90</td>
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In vitro colonisation of *S. pneumoniae* (pre)

<table>
<thead>
<tr>
<th></th>
<th>CFU/mL</th>
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<tbody>
<tr>
<td>Pneumo alone</td>
<td>10^4</td>
</tr>
<tr>
<td>+heparin</td>
<td>10^5</td>
</tr>
<tr>
<td>+ Salivarius (high)</td>
<td>10^6</td>
</tr>
<tr>
<td>+ Salivarius (med)</td>
<td>10^7</td>
</tr>
<tr>
<td>+ Salivarius (low)</td>
<td>10^8</td>
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* indicates p < 0.05 when compared to *S. pneumoniae* alone (Mann Whitney test).

**Figure 3.** Colonisation of *S. pneumoniae* (CFU/mL) on Hep-2 cells when incubated alone, with heparin, or following pre-administration of high (5-10 *S. salivarius*: 1 *S. pneumoniae*), medium (1 *S. salivarius*: 1 *S. pneumoniae*), or low (1 *S. salivarius*: 5-10 *S. pneumoniae*) *S. salivarius*. * indicates p < 0.05 when compared to *S. pneumoniae* alone (Mann Whitney test).
Figure 5. Colonisation of *S. pneumoniae* (CFU/mL) on Hep-2 cells when incubated alone, with heparin, or following co-administration of high (5-10 *S. salivarius*: 1 *S. pneumoniae*), medium (1 *S. salivarius*: 1 *S. pneumoniae*), or low (1 *S. salivarius*: 5-10 *S. pneumoniae*) *S. salivarius*. 