The Role of *Alloiococcus otitidis* in Otitis Media

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This thesis is presented for the degree of Doctor of Philosophy

Department of Biomedical Sciences and Pharmacy
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Declaration

The thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis, when deposited in the University library, being made available for loan and photocopying subject to the provisions of the copyright Act 1968.

______________________________________________
Christopher Ian James Ashhurst-Smith
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Abstract

Ear infections are a major problem worldwide. In 1995, the annual cost of medical and surgical treatment of otitis media (OM) in the United States was estimated between US$3-4 billion. These infections are a particular problem among Indigenous Australians and lead to problems of hearing loss, impairment of learning, development of speech, and social skills.

An epidemiological study of microorganisms present in middle ear effusions of Indigenous and non-Indigenous children with otitis media with effusion (OME) found the major isolate was a rarely isolated species, *Alloiococcus otitidis*. This collection of isolates (n = 39) provided a unique opportunity to: characterise this “new” pathogen; determine if current routine diagnostic techniques were sufficient to identify *A. otitidis*; assess antibiotic susceptibilities; determine if it really is “fastidious” and difficult to isolate; assess potential virulence in a model system employing the human monocytic cell line THP-1.

This is the first report of *A. otitidis* in an Australian population. It is the first description of different phenotypes of this species. It refuted the dogma that the organism is fastidious. It assessed the largest number of isolates to date for antibiotic susceptibilities and found a significant proportion (>33%) resistant to macrolide antibiotics. Slow growth of the organism and presence of β-lactamase producing otopathogens (with which it is often identified in ear effusions by molecular methods) might allow it to survive routine antibiotic treatment for ear infections. In contrast to previous reports using type culture collection isolates, the study provided the only assessment of induction of pro-inflammatory cytokines using recent clinical isolates. The findings have implications for future research on the role of *A. otitidis* in the aetiology of both acute and chronic otitis media; it also has implications for diagnostic microbiology, appropriate treatment of these infections, and development of vaccines against this species.
## Abbreviations and definitions

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<tr>
<td>AOM</td>
<td>acute otitis media</td>
</tr>
<tr>
<td>ATCC</td>
<td>American Type Culture Collection</td>
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<tr>
<td>COM</td>
<td>chronic otitis media</td>
</tr>
<tr>
<td>CSOM</td>
<td>chronic suppurative otitis media</td>
</tr>
<tr>
<td>CD69</td>
<td>proliferation-associated surface marker</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>interferon-gamma</td>
</tr>
<tr>
<td>LPS</td>
<td>lipopolysaccharide</td>
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<tr>
<td>MEE</td>
<td>middle ear effusion</td>
</tr>
<tr>
<td>OM</td>
<td>otitis media (inflammation of the middle ear)</td>
</tr>
<tr>
<td>OME</td>
<td>otitis media with effusion</td>
</tr>
<tr>
<td>PBMC</td>
<td>peripheral blood moncytic cells</td>
</tr>
<tr>
<td>PMT</td>
<td>photomultiplier</td>
</tr>
<tr>
<td>RFLP</td>
<td>restriction fragment length polymorphism</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like Receptor</td>
</tr>
<tr>
<td>VitD3</td>
<td>1α, 25-dihydroxyvitaminD₃</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
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<tr>
<td>Acute otitis media</td>
<td>Presence of fluid in the middle ear, without perforation, but with clinical signs of acute illness</td>
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<tr>
<td>Antibiogram</td>
<td>The antibiotic susceptibility pattern of an organism</td>
</tr>
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<td>Chronic suppurative otitis media</td>
<td>A persistent discharge from the middle ear through a perforated ear drum for more than 6 weeks</td>
</tr>
<tr>
<td>Filtrate</td>
<td>Material that has been passed through a filter</td>
</tr>
<tr>
<td>Lysate</td>
<td>A fluid containing the contents of lysed cells</td>
</tr>
<tr>
<td>Myringotomy</td>
<td>Surgical procedure on the ear drum</td>
</tr>
<tr>
<td>Otitis media with effusion</td>
<td>Presence of fluid in the middle ear without signs or symptoms of acute infection</td>
</tr>
<tr>
<td>Persistent AOM</td>
<td>Lack of improvement in symptoms for 48-72 hrs after starting antibiotics</td>
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<tr>
<td>Recurrent AOM</td>
<td>Three or more episodes of AOM in 6 months or four to five episodes in 12 months</td>
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<tr>
<td>Recurrent infections</td>
<td>Three or more episodes</td>
</tr>
<tr>
<td>Sonicate</td>
<td>Bacterial cells disrupted by exposure to high frequency sound waves</td>
</tr>
<tr>
<td>Tympanic membrane</td>
<td>Ear drum</td>
</tr>
<tr>
<td>Tympanostomy tube</td>
<td>Ventilation tube or “grommet”</td>
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Publications and abstracts

During the development of this thesis the following publications and conference abstracts were prepared:


Ashhurst-Smith, C., ST Hall, JE Stuart, E Liet, PJ Walker, R Dorrington, R Eisenberg, M Robilliard, CC Blackwell. *Alloiococcus otitidis*: the major isolate from both urban and rural/remote children with chronic otitis media with effusion (glue ear) Third Conference of Aboriginal Health Research, Sydney, Australia 2011


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Figure 2.2  Use of antibiotic discs to inhibit non-\textit{A. otitidis} flora from BHI broth subcultures (2).

Figure 3.1  Comparison of \textit{A. otitidis} colony types; large white colony variant (isolate 27) with small green variant (isolate 33) grown on HBA (bioMérieux) for 7 days at 35°C.

Figure 3.2  Non-haemolytic colonies of \textit{A. otitidis} (isolate 10) grown on HBA for 5 days at 35°C.

Figure 3.3  Colonies of \textit{A. otitidis} with beta-haemolysis (isolate 30) grown on HBA for 10 days at 35°C.

Figure 3.4  \textit{CO}_2 production detected for \textit{S. pneumoniae}, \textit{H. influenzae}, \textit{M. catarrhalis} and \textit{A. otitidis} in standard conditions for blood culture.

Figure 4.1  \textbf{Log}_{10} of MIC (by agar dilution) vs diameter of zone of inhibition (mm).

Figure 6.1  SDS-PAGE: filtrates of \textit{A. otitidis}.

Figure 6.2  SDS-PAGE: filtrates of \textit{A. otitidis} before and after treatment with lysozyme or proteinase K.