

***Alloiococcus otitidis*: a neglected bacterium in otitis media**



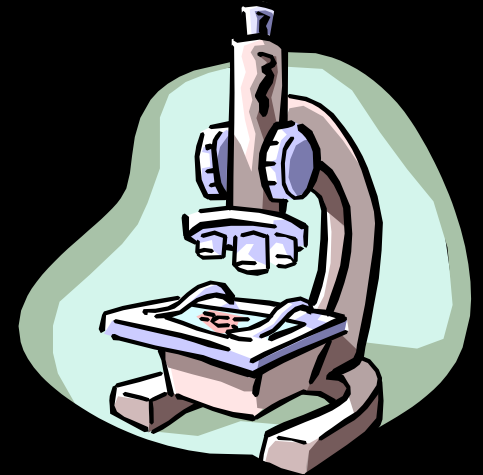
**School of Health Sciences
University of Newcastle
HAPS Immunology and Genetics
Newcastle
Australia**



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)

Species	No. (%)	Indigenous %		Non-indigenous %	
		N=40		N=38	
<i>A. otitidis.</i>	36 (46)	21 (55)	15 (38)		
<i>Corynebacterium spp</i>	21 (27)	13 (34)	8 (20)		
<i>S. aureus</i>	5 (6)	1 (3)	4 (10)		
<i>H. influenzae</i>	2 (2.6)	0	2 (5)		
<i>S. pneumoniae</i>	1 (1.3)	0	1 (2.5)		
<i>P. aeruginosa</i>	1 (1.3)	1 (2.6)	0		
<i>E. faecalis</i>	1 (1.3)	1 (2.6)	0		
<i>S. mutans</i>	1 (1.3)	1 (2.6)	0		
<i>Sphingomonas sp</i>	1 (1.3)	1 (2.6)	0		

Identification of *A. otitidis* by current diagnostic tools

System	No. (%) correct identifications n=39	Confidence level
BBL Crystal	36 (90)	94.3%
API Strep	38 (97)	95.2%
Vitek2 GP card	39 (100)	98.3%
Maldi-tof	39 (100)	99%+

Susceptibility of *A. otitidis* to macrolide antibiotics

Antibiotic E-Test®	<i>S. pneumoniae</i>			staphylococcus		
	R	I	S	R	I	S
erythromycin	19 (49)	11 (28)	9 (23)	12 (31)	7 (18)	20 (51)
clarithromycin	17 (44)	6 (15)	16 (41)	12 (31)	1 (2)	26 (67)
azithromycin	14 (36)	5 (13)	20 (51)	13 (33)	0 (0)	26 (67)

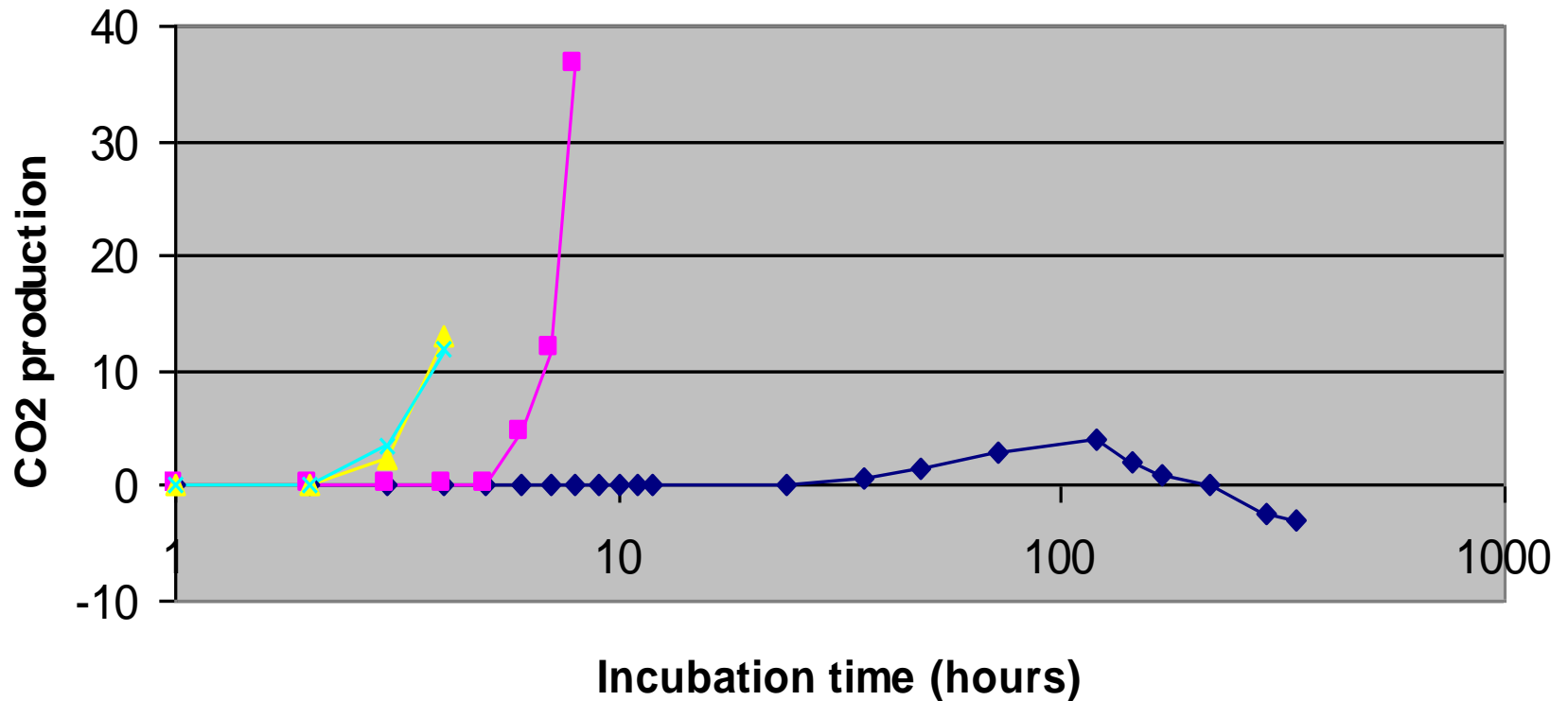
Co-infections and susceptibility to penicillins

- Alloiococcus 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 $\mu\text{g ml}^{-1}$

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 O₂, lack of nutrients, adverse temperatures

Activity in broth medium; otopathogens compared to *A.otitidis*



◆ *A.otitidis* ■ *M.catarrhalis* ▲ *H.influenzae* × *S.pneumoniae*

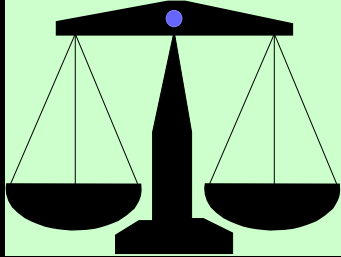
A. otitidis – a persistent organism?


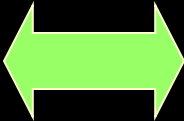

- Slow growth on agar or in liquid medium
- Long term storage - 2 years at 4°C in BHI, 34/36 (94%) grew on HBA
- Anaerobic – at 14 days, 8/36 (22%) grew

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]

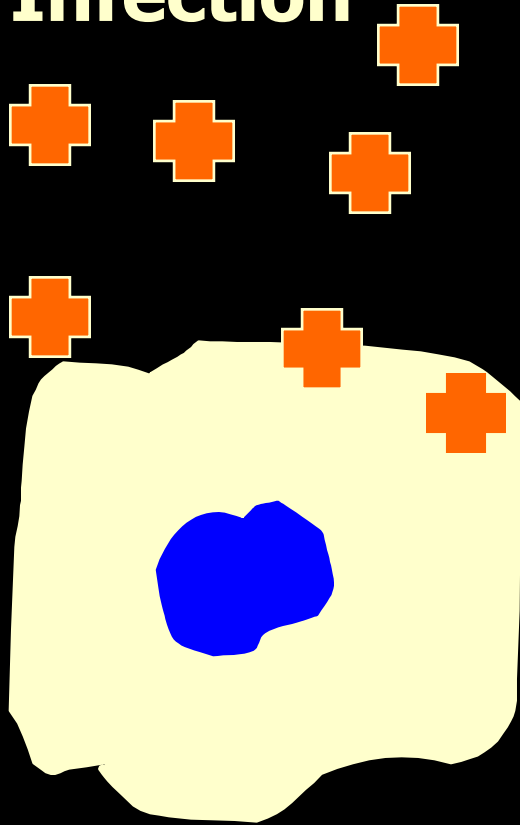
Inflammatory responses: first line of defence



	LOW	BALANCED	HIGH
INFECTION	+	+	+
INFLAMMATION			
DISEASE	invasive	control but no harm	tissue damage

Inflammatory responses to infection

Infection



LEUKOCYTES

TNF α

IFN γ

IL- 1 β

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)

pg m⁻¹ mean (SD)

	IL-1 β	IL-6	IL-8	TNF- α
<i>S. pneumoniae</i>				
ATCC 49619	63 (16)	846 (95)	5981(1373)	29 (19)
“wild” SP2	320 (15)	5288 (422)	>26,000	536 (54)
<i>A. otitidis</i>				
White type	627 (46)	5643 (371)	>26,000	1605 (137)
Green type	283 (11)	4161 (110)	>26,000	17 (30)

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-1 β ($P < 0.05$).

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**