

## The 3rd International Online Conference on Vaccines

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### Strengthening Indigenous Children: Development of a Novel Multi-Component Vaccine for Ear Infection Prevention

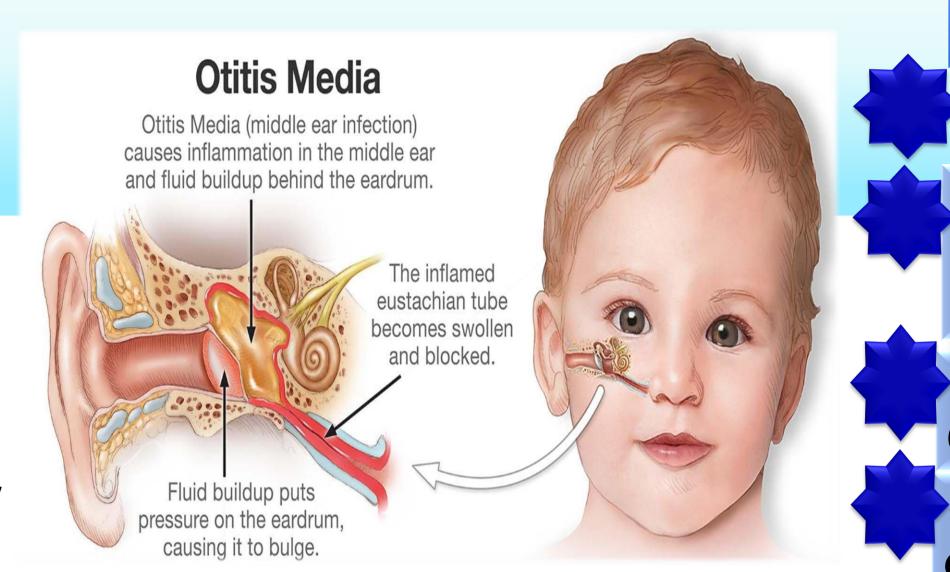


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**Institute for** Biomedicine and Glycomics

#### Introduction

- Otitis media (OM) is an inflammation of the middle ear, often caused by bacterial or viral pathogens, leading to ear pain, fluid buildup, and possible hearing loss.
- Leading cause of antibiotic prescriptions and surgery among children.
- As per WHO, 50% of permanent hearing loss cases are caused by OM.
- Australian Indigenous children suffer chronic middle ear infections at amongst the highest rates in the developed world.



#### Research Problem

**High Disease Morbidity** 

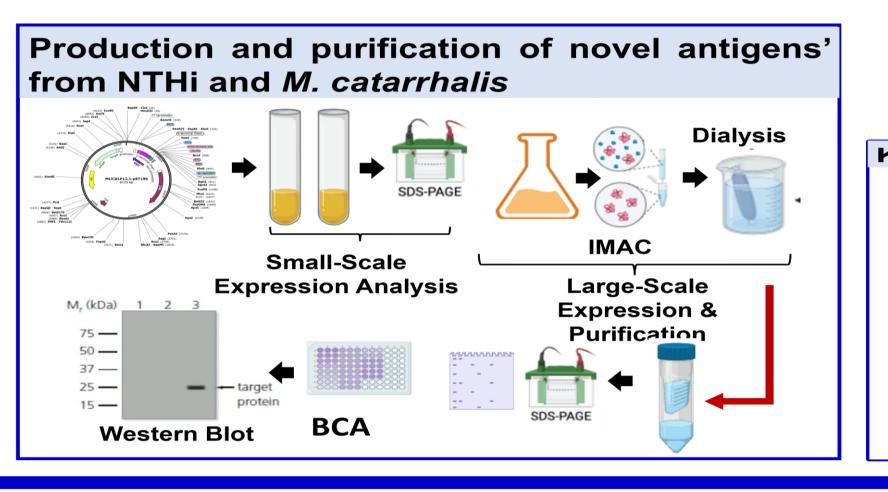
Frequent use of antibiotics for OM is leading to alarming rise in AMR among causative bacteria

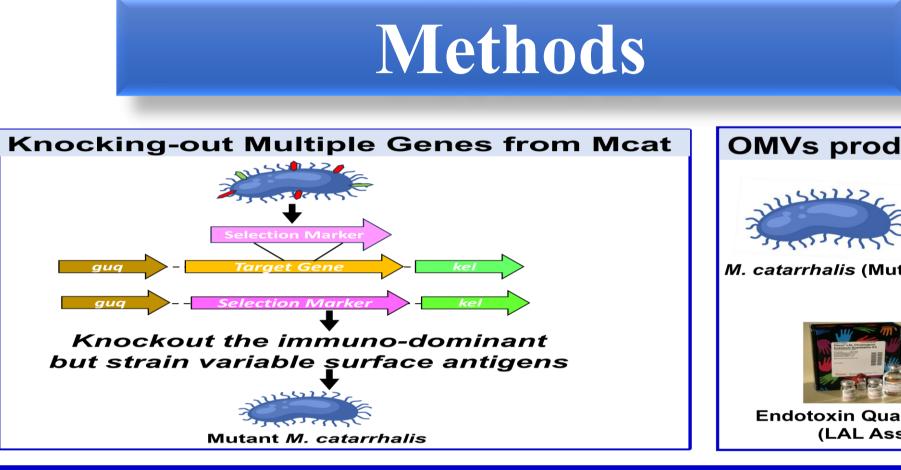
No Specific Licensed Vaccines for

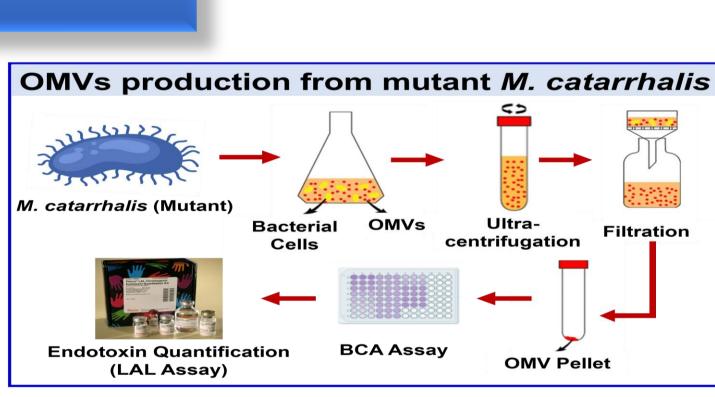
Lack of good target antigens for *M*. catarrhalis

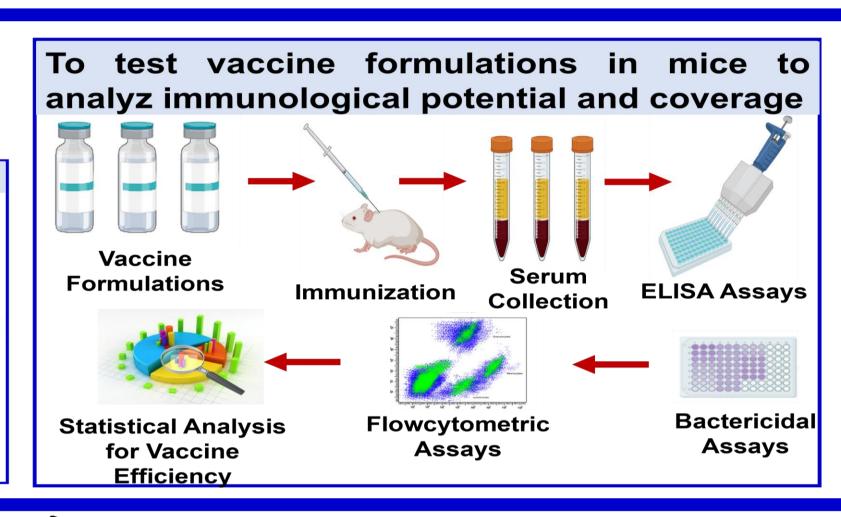
## Project Objective

The overall aim of this project is to develop an effective multi-component vaccine comprising novel protein antigens and genetically modified outer-membrane vesicles to provide better disease coverage and vaccine efficacy, thereby drastically reducing middle ear infections in Australian Indigenous children and all children in general.









# Results Colonies 1-7 Figure 1: (A, B) pET19b and pIDT containing target genes were

digested with Ndel and Xhol followed by agarose gel electrophoresis, band excision and purification (C) The image shows positive colony PCR using T7 primer pair for one cloned gene. All positive clones were verified by sanger sequencing. **Constructs Development** 

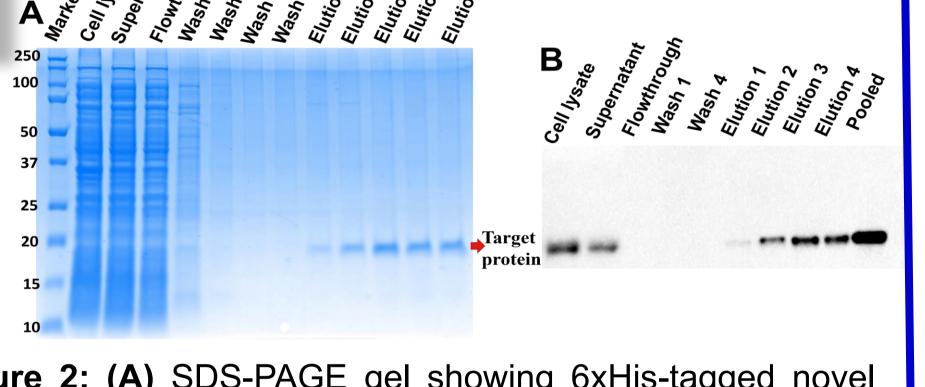


Figure 2: (A) SDS-PAGE gel showing 6xHis-tagged novel protein antigen from Mcat purified under native conditions. **(B)** Detection of His-tagged antigen with anti-His antibody.

Protein Expression and purification of Mcat antigen A

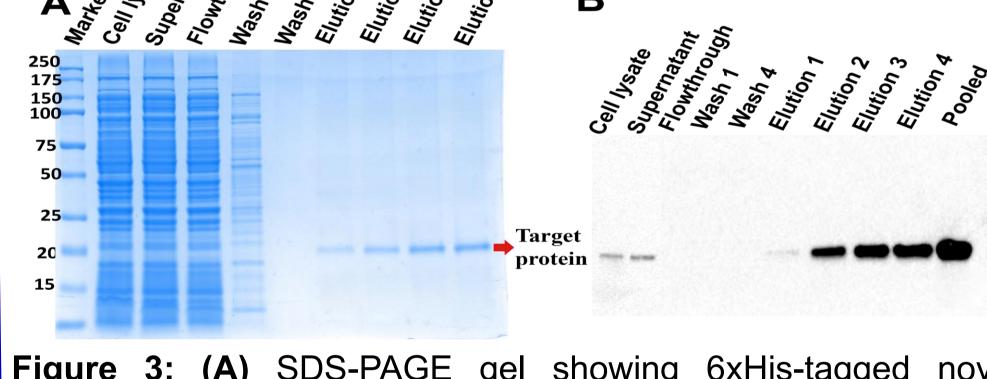


Figure 3: (A) SDS-PAGE gel showing 6xHis-tagged novel antigen from *Mcat* purified under native conditions. (B) Detection of His-tagged antigen with anti-His antibody.

Protein Expression and purification of Mcat antigen B

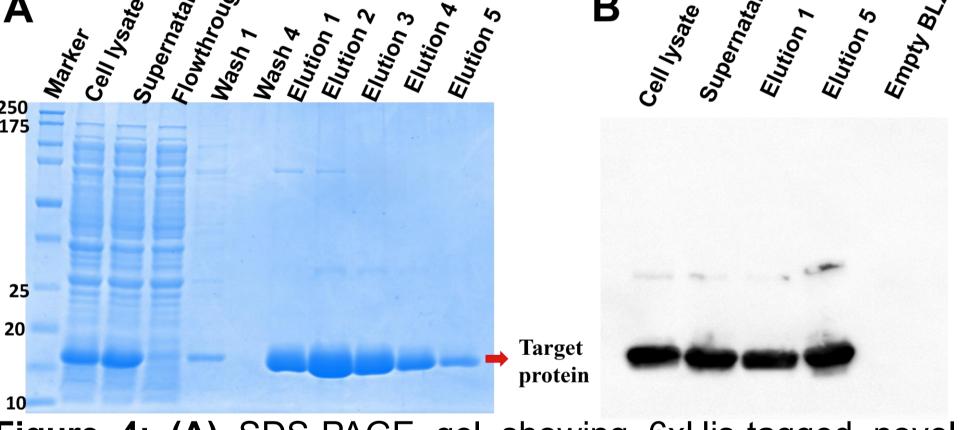


Figure 4: (A) SDS-PAGE gel showing 6xHis-tagged novel antigen from *Mcat* purified under native conditions. (B) Detection of His-tagged antigen with anti-His antibody. Protein Expression and purification of Mcat antigen B

**Deletion cassette design** Figure 5: (A) Design of the deletion construct for immuno-dominant genes of Mcat. (B, C) Digestion of deletion cassette and vector

**Target Band** 

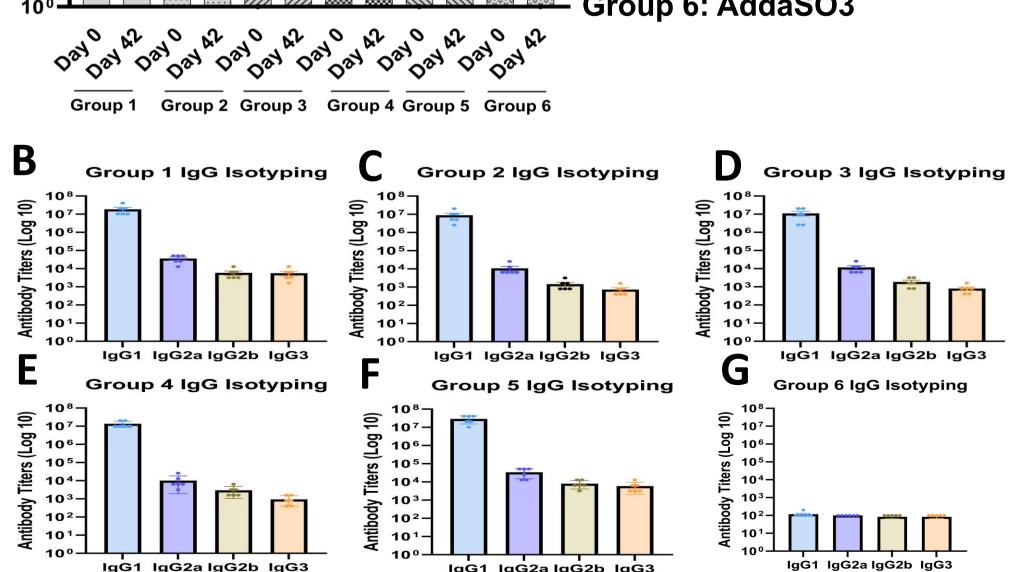
containing selection marker. (D) The image shows growth of positive clones in the presence of selection marker. (E) The image shows

Development of Mcat

**Mutants and** OMV **Purification** 

**ELISA with Mcat 25239** Study 1: Protein Antigens **Group 1: Antigen A** 

**Group 2: Antigen B Group 3: Antigen C Group 4: Antigens B+ C Group 5: Antigens A+B+C Group 6: AddaSO3** 



(n = 6/group; female, 4–6 weeks old) were immunised three times subcutaneously responses were measured by ELISA and are represented as mean + SEM. in 6 different groups. Serum was collected via cardiac puncture at day 42. Antigenspecific IgG antibody responses were measured by ELISA and are represented as mean ± SEM. Statistical analysis was performed using a parametric, one tailed paired t test to compare test groups to their respective day pre-bleeds (serum collected before first immunisation, dark circles) (ns; \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001; \*\*\*\*p < 0.0001). **(B)** Antibody isotyping for G1-G6. Serum IgG1, IgG2a, IgG2b and IgG3 antibody responses were measured by ELISA and are represented as mean + SEM.

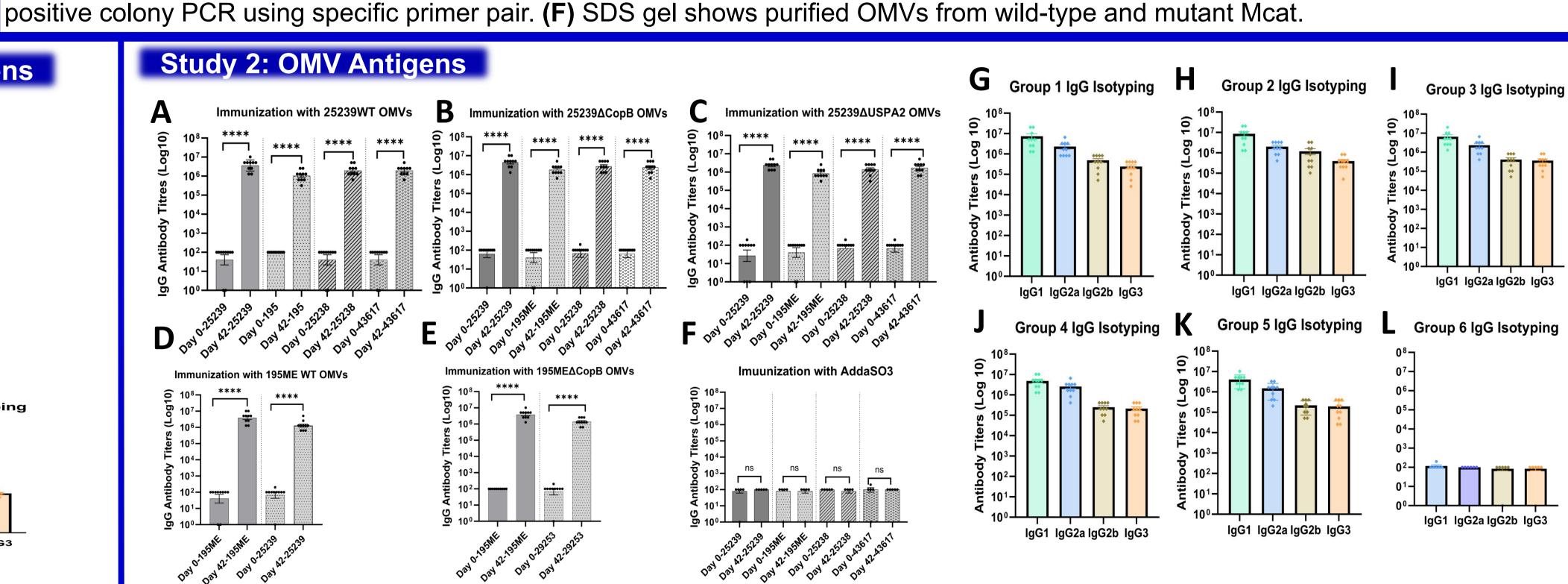


Figure 7: (A-F) Immunogenicity following subcutaneous immunisation with OMV-based antigens. BALB/c mice (n = 10/group; female, 4–6 weeks old) were immunised three times subcutaneously with (A) 25239WT OMVs, (B) 25239∆CopB OMVs, (C) 25239∆USPA2 OMVs, (D) 195MEWT OMVs, (E) 195ME∆CopB OMVs or (F) Adjuvant only. Serum was collected via cardiac puncture at day 42. Antigen-specific IgG antibody responses were measured by ELISA and are represented as mean ± SEM. Statistical analysis was performed using a parametric, one tailed paired t test to compare test groups (light circles) to their respective day -1 pre-bleeds (ns; \*p < Figure 6: (A) Immunogenicity following subcutaneous immunisation. BALB/c mice 0.05; \*\*p < 0.001; \*\*\*p < 0.0001). (G-K) Antibody isotyping for G1-G6. Serum IgG1, IgG2a, IgG2b and IgG3 antibody

Conclusions

Our multicomponent formulation integrates novel recombinant antigens with engineered OMVs to overcome the pathogen diversity that limits current vaccines. Preliminary data indicate strong antibody responses against OM pathogens.

Acknowledgements

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