ECM 2025 Conference
The 3rd International Electronic Conference on Microbiology
01–03 April 2025 | Online
MDPI

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

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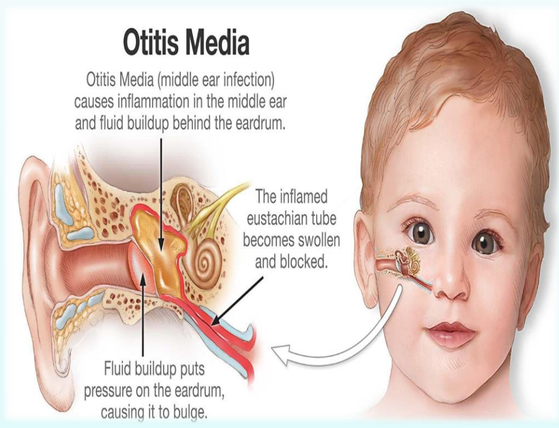
INTRODUCTION

- 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

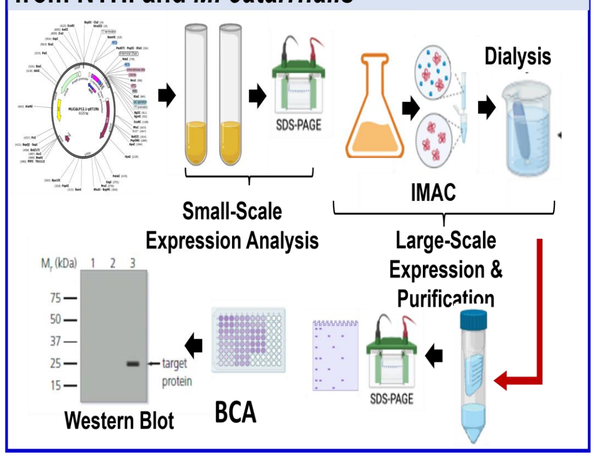
- High disease morbidity
- Frequent use of antibiotics for OM is leading to alarming rise in AMR among causative bacteria
- No specific licensed vaccines for OM
- Lack of good target antigens for NTHi & *M. catarrhalis*

AIMS & OBJECTIVES

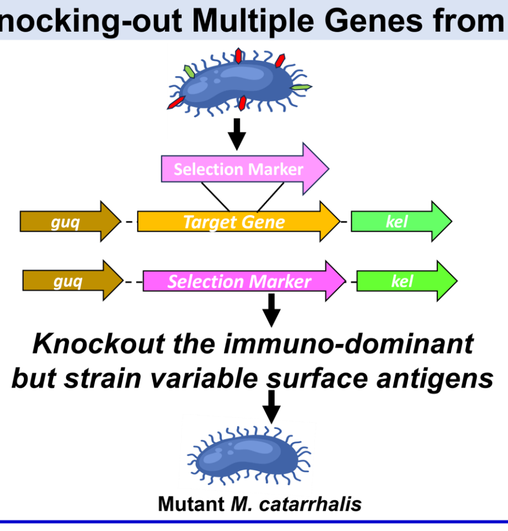
The overall aim of this project is to develop an effective multi-component vaccine comprising novel protein antigens and genetically modified outer-membrane vesicles to provide better disease coverage and enhance vaccine efficacy, thereby drastically reducing middle ear infections in Australian Indigenous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

METHODS

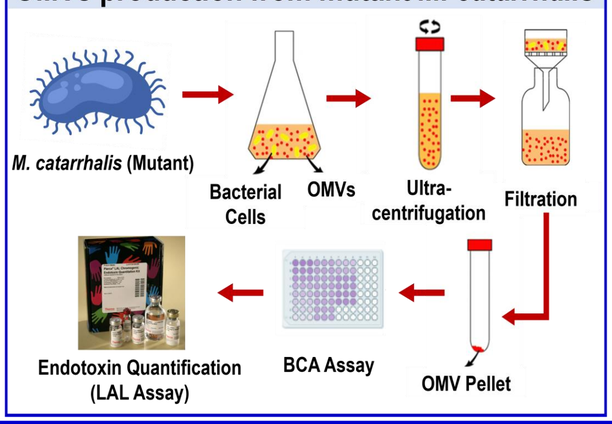
Production and purification of novel antigens' from NTHi and *M. catarrhalis*



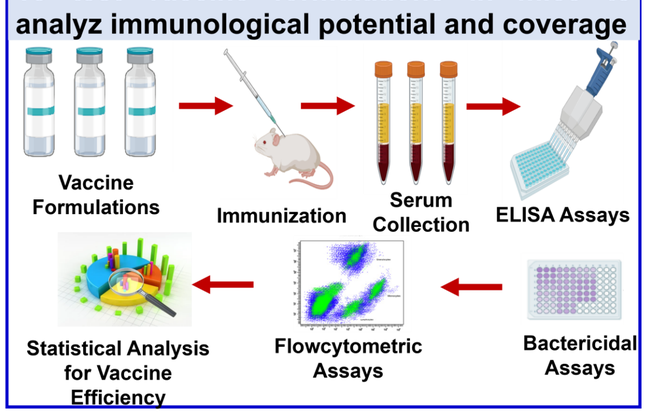
Knocking-out Multiple Genes from Mcat



OMVs production from mutant *M. catarrhalis*



To test vaccine formulations in mice to analyz immunological potential and coverage



RESULTS & DISCUSSION

Constructs Development

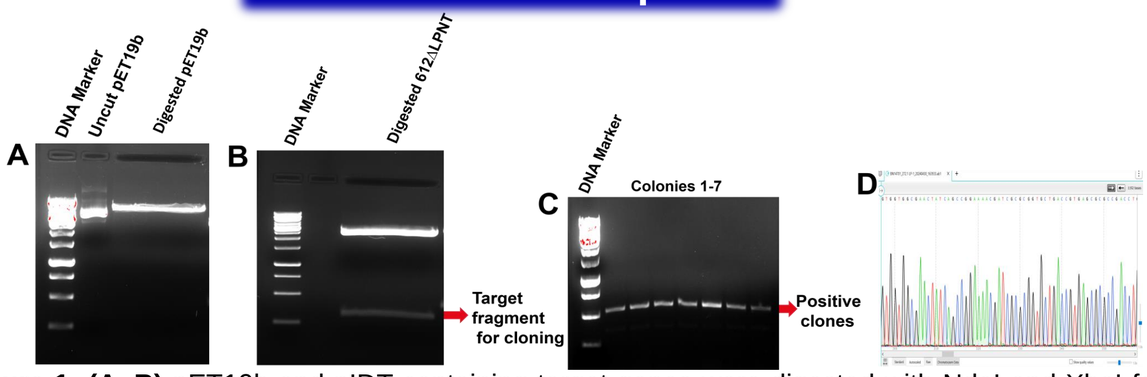


Figure 1: (A, B) pET19b and pIDT containing target genes were digested with NdeI and XhoI followed by agarose gel electrophoresis, band excision and purification using Qiagen gel extraction kit. **(C)** The image shows positive colony PCR using T7 primer pair for one cloned gene. **(D)** All positive clones were verified by sanger sequencing.

Expression and Purification OF NTHi antigens

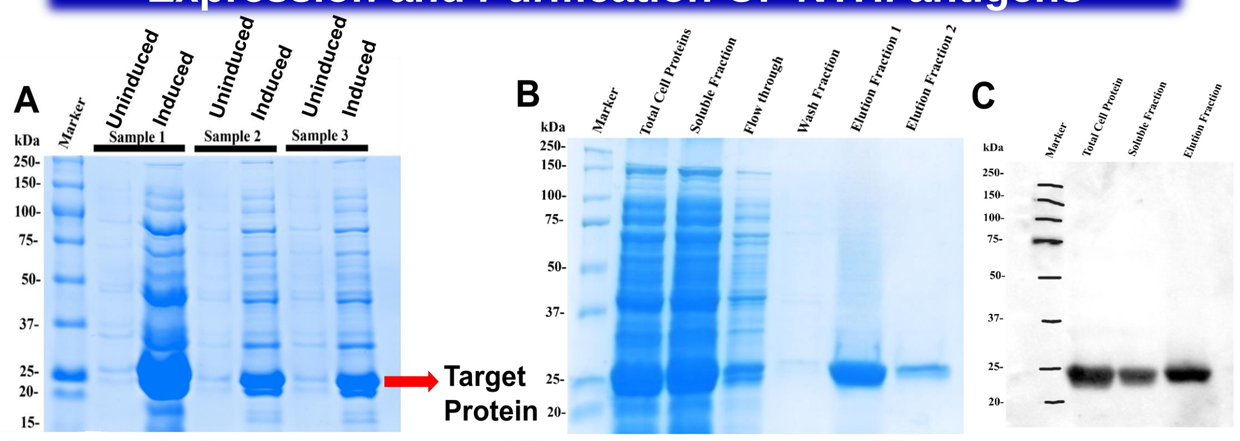


Figure 2: (A) Expression analysis of NTHi protein antigen upon IPTG induction in induced vs uninduced samples using 12% Coomassie-stained SDS-PAGE. **(B)** SDS-PAGE gel showing 6xHis-tagged NTHi antigen purified under native conditions. **(C)** Detection of His-tagged antigen with anti-His antibody.

Expression and Purification of Mcat antigen A

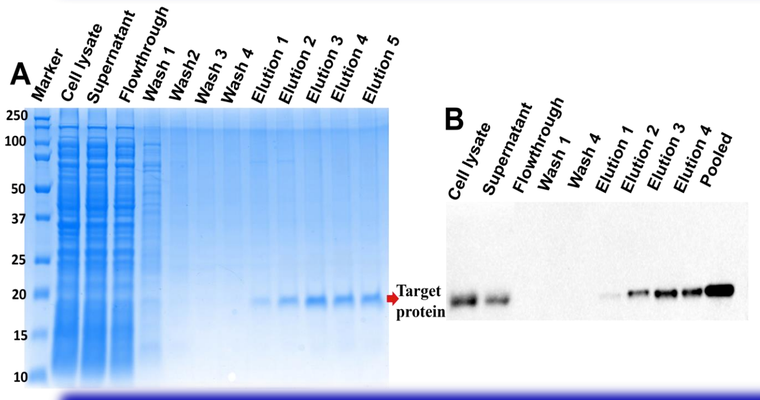


Figure 3: (A) SDS-PAGE gel showing 6xHis-tagged novel protein antigen from *M. catarrhalis* purified under native conditions. **(B)** Detection of His-tagged antigen with anti-His antibody.

Expression and Purification of Mcat antigen B

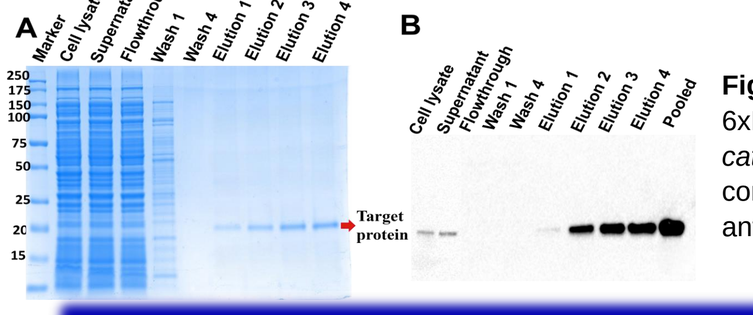


Figure 4: (A) SDS-PAGE gel showing 6xHis-tagged novel antigen from *M. catarrhalis* purified under native conditions. **(B)** Detection of His-tagged antigen with anti-His antibody.

Expression and Purification of Mcat antigen C

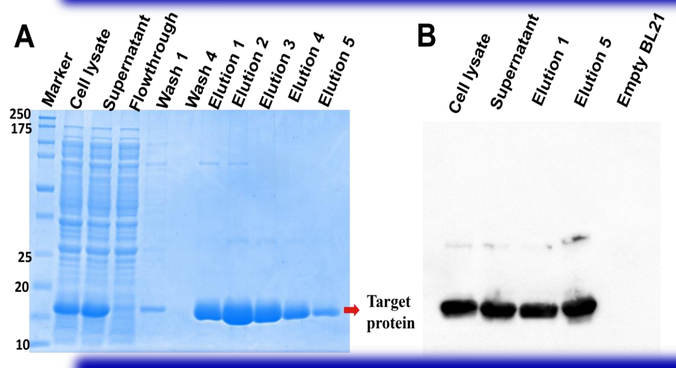


Figure 5: (A) SDS-PAGE gel showing 6xHis-tagged novel antigen from *M. catarrhalis* purified under native conditions. **(B)** Detection of His-tagged antigen with anti-His antibody.

Development of Mcat Mutants for OMV Production

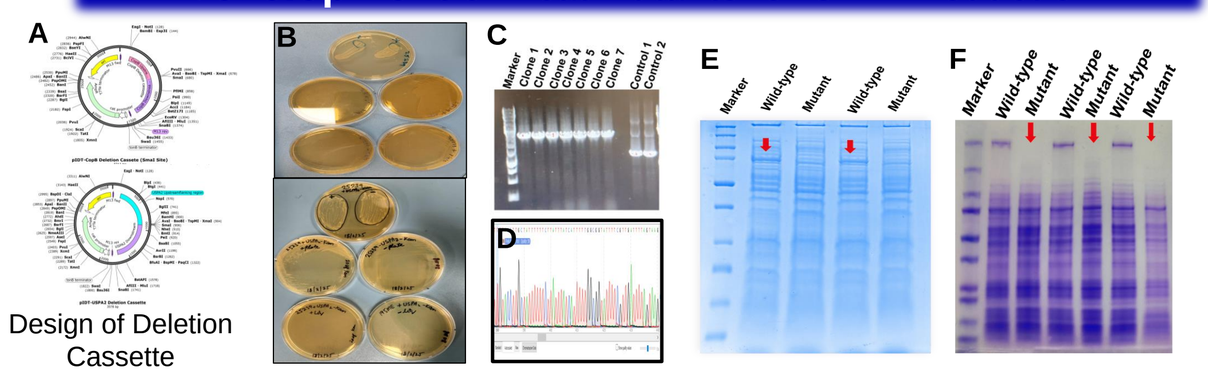


Figure 6: (A) Design of the deletion construct for immuno-dominant genes of Mcat. **(B)** The image shows the growth of positive clones in the presence of a selection marker. **(C)** The image shows positive colony PCR using a specific primer pair. Clones 1-7 show successful deletion of target genes. **(D)** Sanger sequencing data for positive clones **(E, F)** SDS gels showing protein differences between wildtype and mutant Mcat.

CONCLUSION & FUTURE WORK

Given the expansive heterogeneity of pathogens involved in otitis media, a multi-component vaccine is essential for adequate protection. Our vaccine formulation features novel protein antigens derived from *M. catarrhalis,* non-typeable *H. influenzae* and genetically modified outer-membrane vesicles (OMVs) from *M. catarrhalis*. We have successfully expressed and purified recombinant protein antigens from NTHi and *M. catarrhalis* using the *E. coli* expression system and immobilized metal affinity chromatography. We have also generated knockout mutants of *M. catarrhalis* lacking targeted immunodominant strain-variable proteins. These mutants have been used to purify OMVs. These novel protein antigens and modified OMVs will be assessed in preclinical investigations in mice to evaluate vaccine immunogenicity and cross-reactivity against heterologous strains.

ACKNOWLEDGMENTS

We gratefully acknowledge Griffith University for the GUPIRS scholarship and the Earbus Foundation for supporting this research through the Harvey Coates PhD Fellowship.

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