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# Supporting Information

## Supplementary methods and results



Appendix to: Lynch KD, Morotti W, Brian G, et al. Clinical signs of trachoma and laboratory evidence of ocular *Chlamydia trachomatis* infection in a remote Queensland community: a serial cross-sectional study. *Med J Aust* 2023; doi: 10.5694/mja2.51735.

**Supplementary methods**

Community engagement and approval

The community selected is a strong community of approximately 1405 people built on family, leadership, and culture and residents are strongly bonded to their ancestral heritage. Before conducting research activities, we undertook consultation with and sought consent from community leaders, local clinicians, Indigenous health workers, and the local Aboriginal Community Controlled Health Service to ensure their support and seek input into the design of the project. The Aboriginal Shire Council provided community approval for the research to progress. As required by local lore and cultural protocols, advance notice of in-community activities was provided to the Aboriginal Shire Council.

Initial contact with the community about the research project was made via a stakeholder yarning meeting. This was followed up with individual stakeholder consultation and a letter of invitation to families in the community. A population list was compiled using the medical clinic list and local knowledge of Indigenous health workers and school staff.

Feedback between the researchers and the community occurred throughout all stages of the project and was a two-way process. Prior to publication of this manuscript, in-person community meetings occurred to share results and, after results had been analysed, an event was held to recognise the contribution of the community and celebrate the success of the project. Community members were actively involved in the screening and research process, and without their engagement with, and support of, the work, it would not ae been possible. Contributions included working in partnership with the team to refine the population list, assisting with the consent and screening process, and contributing to community engagement activities.

Specimen collection and handling

For conjunctival swab collection, the upper lid was held in the everted position by the ophthalmologist's thumb, positioned away from the lid margin and pressing the lashes against skin in the region of the orbital rim. To collect specimens for polymerase chain reaction (PCR) testing, a polyester-coated cotton swab [Medical Wire & Equipment, MW104], held by the plastic stopper top, was rotated while rubbing it over the everted upper tarsal conjunctiva as many times as required to cover as much of the tarsal plate as possible, avoiding the lid margin. Swabs were immediately placed into a COBAS PCR media tube without further contact. Using a sterile rayon tipped swab (Transystem M40 408C; Copan], the same approach was used to collect specimens for bacterial culture. These swabs were immediately placed into sterile tubes of swab medium (Amies transport gel) without further contact. All swabs were kept cool and dry before transfer within 24 hours of collection to the local Pathology Queensland laboratory for processing.

Swabs and dried blood spots were carried with the teams for up to 8 hours and stored at room temperature at the end of each day. Dried blood spot cards were air-dried overnight, then packed into

individual Whatman foil barrier reusable bags containing two 1g silica desiccant sachets. Dried blood spots were stored for up to one week at room temperature. Specimens were transported at ambient temperature to St Vincent's Centre for Applied Medical Research, Sydney, and stored at −80°C until analysis. Samples were brought to room temperature prior to processing.

Polymerase chain reaction testing

Tubes were vortexed for 3 seconds, then nucleic acid was extracted and amplified using real-time PCR (Cobas 6800, Roche Diagnostics). Results were classified according to the manufacturer's instructions.

Laboratory staff were blinded to all clinical results and samples were de-identified.

Bacterial culture

Organisms for identification and antimicrobial susceptibility testing included *Staphylococcus aureus*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Moraxella catarrhalis*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Corynebacterium macginleyi*, as well as pure growth of Gram-negative enteric organisms. Isolates were identified using the VitekMS instrument and antimicrobial susceptibility testing, using the Vitek2 system for non-fastidious isolates, or by EUCAST disc diffusion methods for fastidious organisms.[1] Beta-lactamase testing was performed on *Haemophilus influenzae*, *Moraxella* sp., and *Neisseria* isolates using the nitrocefin method.[2]

Serological testing for anti-Pgp3 antibodies

A finite mixture model was used to classify the samples as seropositive or seronegative based on normalized absorbance values. Dried blood spot samples collected from the 2019 survey were batch tested, with samples received from the 2021 survey tested in a second batch. The cutoff for seropositivity was determined to be 0.175 and 0.209 for batch 1 and batch 2, respectively, by taking the mean of the Gaussian distribution of the seronegative population plus four standard deviations above the seronegative population. Laboratory staff were blinded to all clinical results and samples were de-identified.

**References**


1. European Committee on Antimicrobial Susceptibility Testing. Breakpoints tables for interpretation of MICs and zone diameters. Version 9.0; 2019. https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_9.0_Breakpoint_Tables.pdf (viewed May 2022).
2. Isenberg HD. *Clinical microbiology procedures handbook*. Washington, DC: American Society for Microbiology, 1992.

## Supplementary results

### Table. Non chlamydial bacterial organisms identified in survey participants from a northwest Queensland community during trachoma screening, 2019–2021[a]

| | Survey year and age group (years) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2019 | | | | 2020 | | | | 2021 | | | |
| **Participant characteristic** | **1–4** | **5–9** | **10–14** | **≥ 15** | **1–4** | **5–9** | **10–14** | **≥ 15** | **1–4** | **5–9** | **10–14** | **≥ 15** |
| **Bacterial swab collected** | 2 | 14 | 6 | 5 | 0 | 11 | 0 | 0 | 5 | 29 | 7 | 0 |
| **Total number of participants with any non-chlamydial bacterial organism detected** | 1 | 6 | 2 | 1 | | 3 | | | 2 | 9 | 4 | |
| **Organism identified** | | | | | | | | | | | | |
| *Staphylococcus aureus* | | 1[c] | 1 | 1 | | 1 | | | 2[f] | 1 | 1 | |
| *Staphylococcus aureus* (MRSA)[b] | | | | | | | | | | 3[g,h,i] | | |
| Non-multiresistant MRSA | | | | | | | | | | 2[g,i] | | |
| *Streptococcus pneumoniae* | | 2 | 1[e] | | | 1 | | | | 2[j] | 2 | |
| *Streptococcus pyogenes* (Group A) | | | | | | | | | 1[f] | 2[h,i] | | |
| *Haemophilus influenzae* | 1 | 4[c,d] | | | | 1 | | | | 2[j] | | |
| *Pantoea* sp. | | | | | | | | | | 1 | | |
| *Acinetobacter baumannii* complex | | | | | | | | | | 1 | 1 | |
| *Acinetobacter haemolyticus* | | | | | | | | | 1[f] | | | |
| *Pseudomonas stutzeri* | | | | | | | | | | 1[h] | | |
| *Corynebacterium diphtheriae* | | | | | | | | | | 1[h] | | |
| **Total number of participants with no organism detected** | 1 | 8 | 4 | 4 | | 8 | | | 3 | 20 | 3 | |

[a]The same individual may be counted more than once if multiple organisms were present

[b]Methicillin-resistant *Staphylococcus aureus*

[c]This individual had both *Staphylococcus aureus* and *Haemophilus influenzae* detected in the left eye

[d]Includes two individuals who had *Haemophilus influenzae* detected bilaterally

[e] This individual had *Streptococcus pneumoniae* detected bilaterally

[f]This individual had *Staphylococcus aureus* detected bilaterally with *Acinetobacter haemolyticus* in the left eye and *Streptococcus pyogenes* (Group A) in the right eye

[g]This individual had both *Staphylococcus aureus* (MRSA) and non-multiresistant MRSA identified in the right eye

[h]This individual had *Pseudomonas stutzeri* detected bilaterally with *Corynebacterium diphtheriae* in the left eye and *Staphylococcus aureus* (MRSA) and *Streptococcus pyogenes* (Group A) in the right eye

[i]This individual had *Staphylococcus aureus* (MRSA) detected bilaterally with non-multiresistant MRSA in the left eye and *Streptococcus pyogenes* (Group A) in the right eye.

[j]This individual had *Haemophilus influenzae* detected in the left eye and *Streptococcus pneumoniae* in the right eye