High Resolution genotyping methods rule out urogenital *Chlamydia trachomatis* infection of an eight-year-old child recently resident in Afghanistan

Chloe Manning[1], Helen Morris[2], Joe Abbott[2], Mitul Patel[2], Ian Clarke[1], Peter Marsh[1,3]

University of Southampton, Faculty of Medicine[1], Birmingham Children's Hospital NHS Foundation Trust[2], Public Health England Laboratory, Southampton[3]

Chlamydia trachomatis (CT) is the leading cause of sexually transmitted infections worldwide, and *C. trachomatis* serovars A, B, Ba and C primarily infect ocular epithelial cells causing **trachoma**. Serovars A, B and Ba have been shown to cause urogenital infection in sporadic cases, whilst serovar C has never before been associated with urogenital infection. Trachoma is the dominant infectious cause of blindness worldwide. Whilst rare in developed countries; trachoma is common in poverty-stricken regions including India, North Africa, and South East Asia. Symptoms of early stages of infection include conjunctivitis which can result in corneal opacity and blindness if untreated.

Aim: To determine the *C. trachomatis ompA* genotype of a *C. trachomatis* nucleic acid amplification test (NAAT) confirmed positive conjunctival swab from a child with established corneal inflammation who recently emigrated to the UK from Afghanistan**.** Neonatal conjunctivitis caused by vertical transmission of *C. trachomatis* from mother to neonate is rare in a child of this age, therefore sexual acquisition of *C. trachomatis* must be ruled out.

Objectives: (a) *Extract and purify C. trachomatis DNA from the conjunctival swab;* ***(b)*** *ompA PCR the extracted DNA;* ***(c)*** *purify PCR products;* ***(d)*** *ompA sequence analysis to determine C. trachomatis genotype.*

Materials and Methods

Sample: A conjunctival swab was collected from an eight-year old child with established corneal inflammation who had recently migrated to the UK from Afghanistan. The swab tested positive for *C. trachomatis* using the RealTime CT/NG assay (Abbott).

Extract *C. trachomatis* DNA from conjunctival swab	• **NucleoSpin DNA Trace Tissue Kit** (Macherey-Nagel) • Visualised on 1% agarose gel
ompA PCR extracted DNA	• **Phusion High-Fidelity PCR** using PCTM3 and NRI primers • Visualised on 1% agarose gel
Purify PCR products	• **Wizard SV gel and PCR clean-up system (Promega)** • Visualised on 1% agarose gel
ompA sequence	• Primers and purified DNA sent to **Source BioScience**
ompA sequence analysis	• **Nucleotide BLAST search** • **ExPASy translation** of nucleotide sequence • **Protein BLAST** of open reading frame (ORF)

Results

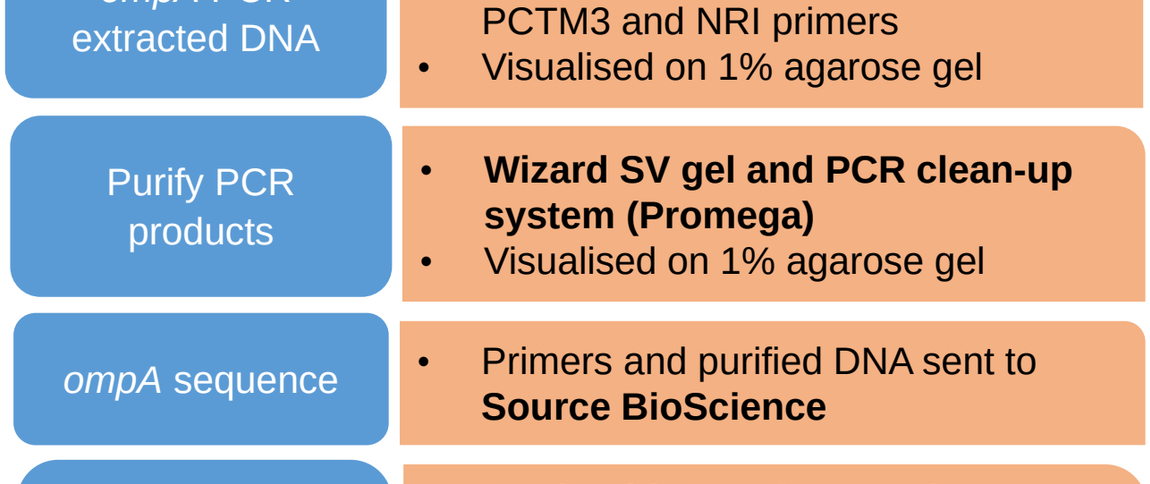

Figure 1. Agarose gel analysis of conjunctival swab DNA, and three reaction mixtures following *ompA* PCR amplification.
[**Lane 1**=Hyperladder1 band size standard; **Lane 2**= conjunctival swab DNA; **Lane 3=** 1:8 dilution of sample; **Lane 4=** positive control (transformed *C. trachomatis* L2 P- pSW2GFP lab strain[1]); **Lane 5=** negative control; **Lane 6=** Hyperladder 1]

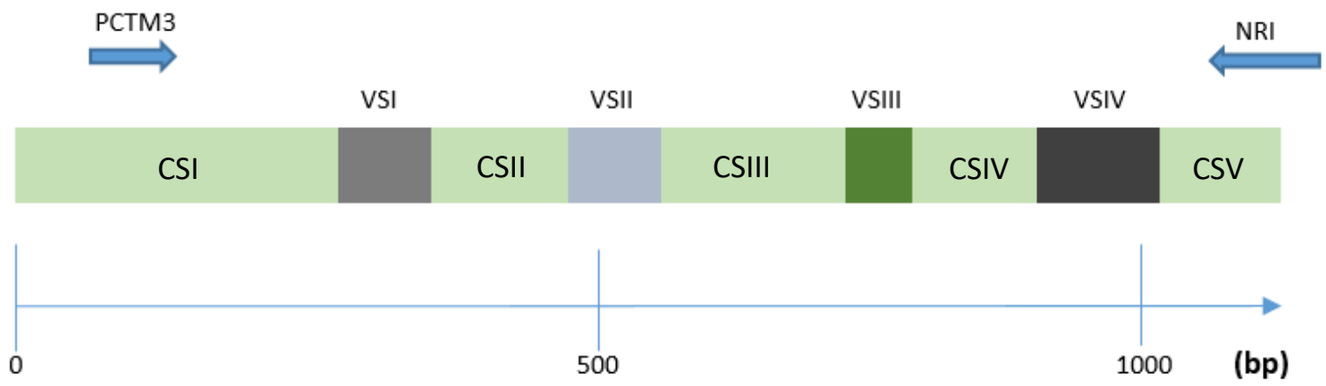

Figure 2. Schematic of *ompA* gene indicating the positions of variable sequences I-IV, and the target sites of the PCTM3 forward primer[2] and the NRI reverse primer[2] for *ompA* gene PCR amplification.
The *ompA* gene is comprised of four variable segments (VSI-IV) interspersed by five membrane-spanning conserved segments (CS). VS regions encode four variable protein domains that contain serovar-specific epitopes[3]. Scale bar indicates number of nucleotide base pairs.

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Sample  235  YRLNMFTPYIGVKWSRVSFDADTIRIAQPKLAEAILDVTTLNPTIAGRGNVVSSGTDNEL  56
Con.         YRLNMFTPYIGVKWSRVSFDADTIRIAQPKLAEAILDVTTLNPTIAG+G+VVSSGTDNEL
Ref      275 YRLNMFTPYIGVKWSRVSFDADTIRIAQPKLAEAILDVTTLNPTIAGKGSVVSSGTDNEL  334

Sample   55  ADTMQIVSLQLNKMKSR  5
Con.         ADTMQIVSLQLNKMKSR
Ref      335 ADTMQIVSLQLNKMKSR  351

                                NPTIAGRGNVVSSGT
                                NPTIAG+G+VVSSGT
                                NPTIAGKGSVVSSGT
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Figure 3. *ompA*-encoded amino acid sequence alignment of variable sequence IV (VSIV) from the patient sample to a reference conjunctival isolate belonging to *ompA* genotype C, using the Basic Local Alignment Search Tool (BLAST).
[**Row 1**=patient sample; **Row 2**=consensus sequence showing location of amino acid changes between translated patient sample sequence and reference isolate; **Row 3**=reference conjunctival isolate] . The sample showed the closest sequence homology (99%) with this reference *ompA* genotype C conjunctival isolate.

Conclusions

- The *C. trachomatis* DNA present in the sample shared the closest identity to ***ompA* genotype C**, a known trachoma-causing *ompA* genotype that has never before been associated with urogenital infection
- **Ruled out sexual acquisition** of *C. trachomatis* in the child
- The strain was identical to known *ompA* genotype C sequences except in 2 locations within variable sequence IV (VSIV) of the *ompA* gene
- Limited epidemiological information recorded for trachoma status in Afghanistan – this strain represents the **first reported** trachoma-causing *C. trachomatis* strain to be *ompA*-sequenced from the region

References:

[1] Wang et al. 2011. *PLoS Pathogens*. 7(9):e1002258
[2] Wang et al. 2011. *PLoS One*. 6(2):e16971
[3] Stephens et al. 1987. *J Bacteriol*. 169(9):3879-85

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