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

Chloe Manning¹, Helen Morris², Joe Abbott², Mitul Patel², Ian Clarke¹, Peter Marsh^{1,3}
University of Southampton, Faculty of Medicine¹, Birmingham Children's Hospital NHS Foundation Trust²,
Public Health England Laboratory, Southampton³

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 <i>C. trachomatis</i> DNA from conjunctival swab	<ul style="list-style-type: none">NucleoSpin DNA Trace Tissue Kit (Macherey-Nagel)Visualised on 1% agarose gel
<i>ompA</i> PCR extracted DNA	<ul style="list-style-type: none">Phusion High-Fidelity PCR using PCTM3 and NRI primersVisualised on 1% agarose gel
Purify PCR products	<ul style="list-style-type: none">Wizard SV gel and PCR clean-up system (Promega)Visualised on 1% agarose gel
<i>ompA</i> sequence	<ul style="list-style-type: none">Primers and purified DNA sent to Source BioScience
<i>ompA</i> sequence analysis	<ul style="list-style-type: none">Nucleotide BLAST searchExPASy translation of nucleotide sequenceProtein BLAST of open reading frame (ORF)

Figure 2. Schematic of *ompA* gene indicating the positions of variable sequences I-IV, and the target sites of the PCTM3 forward primer² and the NRI reverse primer² for *ompA* gene PCR amplification.
The *ompA* gene is comprised of four variable segments (VS I-IV) interspersed by five membrane-spanning conserved segments (CS). VS regions encode four variable protein domains that contain serovar-specific epitopes³. Scale bar indicates number of nucleotide base pairs.

Sample	235	YRLNMFPTYIGVKWSRVSFADTIRIAQPKLAEAILDVTTLNPTIAGRNVVSSGTDNEL	56
Con.		YRLNMFPTYIGVKWSRVSFADTIRIAQPKLAEAILDVTTLNPTIAG+G+VVSSGTDNEL	
Ref	275	YRLNMFPTYIGVKWSRVSFADTIRIAQPKLAEAILDVTTLNPTIAGKGSVVSSGTDNEL	334
Sample	55	ADTMQIVSLQLNKMKS	5
Con.		ADTMQIVSLQLNKMKS	
Ref	335	ADTMQIVSLQLNKMKS	351

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.

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 strain¹); Lane 5= negative control; Lane 6= Hyperladder 1]

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:

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