Elucidating the chlamydial growth characteristics, infection factors, and host responses to persistent chlamydial infection in women

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Certificate of original authorship

I, Mark Thomas, declare that this thesis is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Life Sciences at the University of Technology Sydney. This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis. This document has not been submitted for qualifications at any other academic institution. This research is supported by the Australian Government Research Training Program.

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List of abbreviations

% v v^{-1}	Percentage volume of total volume
% w v^{-1}	Percentage weight of total volume
16S rRNA	16S ribosomal ribonucleic acid
AB	Aberrant body
ACTS	Australian Chlamydia Treatment Study
bp	Base pair
Bpdl	Bipyridal
BSA	Bovine serum albumin
BSC	Biosafety cabinet
cDNA	Complementary deoxyribonucleic acid
CST	Community state type
Ct	Chlamydia trachomatis
CtD	Chlamydia trachomatis D serovar
CXCL9	Chemokine (CXC motif) ligand 9
DAPI	4‟,6-diamidino-2-phenylindole
DC	Dendritic cell
DMEM	Dulbecco‟s Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
DPBS	Dulbecco‟s phosphate-buffered saline

dsDNA	Double-stranded deoxyribonucleic acid
EB	Elementary body
euo	Early upstream open reading frame
FCS	Fetal calf serum
FITC	Fluorescein isothiocyanate
FRT	Female reproductive tract
FTH1	Ferritin heavy chain 1
g	Gravity of Earth
GAG	Glycosaminoglycans
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
gDNA	Genomic deoxyribonucleic acid
GFP	Green fluorescent protein
GLUT1	Glucose transporter-1
h	Hours
h PI	Hours post infection
H+L	Heavy and light chains
HctA	Histone Hc1-like *Chlamydia trachomatis* protein
HctB	Histone Hc2-like *Chlamydia trachomatis* protein
HIV	Human immunodeficiency virus
HREC	Human Research Ethical Committees
Hsp	Heat shock protein
Hsp60	60-kilodalton heat shock protein
Hsp70	70-kilodalton heat shock protein
HSV	Herpes simplex virus

htrA	High temperature requirement A
HIF-1α	Hypoxia inducible factor-1α
IB	Intermediate body
IDO1	Indoleamine 2,3-dioxygenase
IF	Immunofluorescence (microscopy)
IFNG	Interferon gamma
IFU	Inclusion forming unit
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL1A	Interleukin-1-α
IL6	Interleukin 6
IL8	Interleukin 8
IL10	Interleukin 10
inc	Inclusion (protein)
IRF1	Interferon regulatory factor 1
kDa	Kilodalton
kU	Kilo unit
LPS	Lipopolysaccharide
maU ml^{-1}	Milli-Anson Units per milliliter
MCC	Minimum chlamydicidal concentration
MEC	2-*C*-methylerythritol 2,4-cyclodiphosphate
MEP	Melavonate methylerythritol 4-phosphate
MIC	Minimum inhibitory concentration

MOI	Multiplicity of infection
MOMP	Major outer membrane protein
NAAT	Nucleic acid amplification testing
NK	Natural killer (cell)
NTC	No-template control
OM	Outer membrane
omcB	Outer membrane protein B
omp	Outer membrane protein
ompA	Outer membrane protein A
PAMP	Pathogen-associated molecular pattern
PBMC	Peripheral blood mononuclear cell
PBP	Penicillin-binding protein
PCR	Polymerase chain reaction
PID	Pelvic inflammatory disease
Pmp	Polymorphic membrane protein
Pmp21	Polymorphic membrane protein 21
qPCR	Quantitative polymerase chain reaction
QUT	Queensland University of Technology
RB	Reticulate body
RNA	Ribonucleic acid
RNase	Ribonuclease
RT-qPCR	Reverse transcription quantitative polymerase chain reaction
s	Seconds

SNP	Single nucleotide polymorphism
SPG	Sucrose phosphate glutamate
SRA	Sequence read archive
STI	Sexually transmitted infection
SYBR	Synergy Brands
T3SS	Type three secretion system
TARP	Translocated actin-recruiting protein
TBE	2-amino-2-hydroxymethyl-propane-1,3-diol, boric acid ethylenediaminetetraacetic acid
TFI	Tubal factor infertility
TLR	Toll-like receptor
TNC	Tenascin-C
TNFA	Tumour necrosis factor alpha
Tris	2-amino-2-hydroxymethyl-propane-1,3-diol, boric acid ethylenediaminetetraacetic acid
TRITC	Tetramethylrhodamine
trpBA	Tryptophan synthase B/A
U	Unit
USC	University of the Sunshine Coast
UTS	University of Technology Sydney

Abstract

Chlamydia trachomatis is an obligate intracellular parasite and the leading cause of sexually transmitted bacterial infections in the human urogenital tract. Clinical manifestations of chlamydial infection include urethritis, cervicitis, pelvic inflammatory disease and tubal factor infertility. These pathological conditions are caused by the immune response to both acute and chronic chlamydial infections. Evidence suggests a high proportion of infections remain subclinical until spontaneous resolution or the commencement of symptoms leads to a diagnosis. Therefore, a substantial proportion of the morbidity and burden associated with chlamydia can likely be attributed to unresolved and untreated infections. While treatment with azithromycin is highly effective, treatment failure does occur. The mechanisms of treatment failure and its effects on fertility are poorly understood. Unlike other bacterial pathogens, *C. trachomatis* lacks stable genotypic resistance to macrolides. Another key difference between *Chlamydia* and many other bacteria is the constant interaction with its host cell. Thus, it was hypothesised that the unique intracellular niche and developmental cycle of *C. trachomatis* are important microbial factors which could affect treatment efficacy. To test this, several host and chlamydial factors were investigated. 16S rRNA gene amplicon sequencing of vaginal and cervical swabs and endometrial biopsies from participants of a case-control fertility study revealed that similarities in the microbial populations of the vagina and cervix were not predictive of those in the endometrium. While there was no association

between microbial community compositions and fertility status identified, *Ureaplasma* spp. were overrepresented amongst infertile women. The endometrial expression of several genes involved in immunity and reproductive function showed no association with microbial community composition, however, the gene which encodes tenascin-C was over-expressed in women who had a self-reported history of miscarriage. Comparisons of clinical isolates from women treated for chlamydial infections showed no significant differences in developmental or stress phenotypes but suggested that the subtle differences observed using *in vitro* models may not truly reflect the complexity of *in vivo* infectious processes. Finally, analysis of host and chlamydial gene expression before and after antibiotic treatment showed no association with outcome but yielded valuable information about the host and pathogen during the period following treatment. In particular, chlamydial gene expression was upregulated after they had survived treatment with azithromycin. This project has contributed towards current knowledge and increases the field"s understanding of the host and chlamydial factors involved in treatment failure and infertility. Additionally, it provides insight for future investigations of these important and complex interactions between humans and the bacteria which have evolved alongside us.

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Thesis statement

I am submitting my work titled “Elucidating the chlamydial growth characteristics, infection factors, and host responses to persistent chlamydial infection in women” as a conventional thesis, in accordance with the University of Technology Sydney (UTS) Graduate Research School (GRS) Research Candidature Management, Thesis Preparation and Submission Procedures.

Author contribution statements

Chapter 3

Wee, B. A.*, **Thomas, M**.*, Sweeney, E. L., Frentiu, F. D., Samios, M., Ravel, J., . . . Huston, W. M. (2018). A retrospective pilot study to determine whether the reproductive tract microbiota differs between women with a history of infertility and fertile women. *Aust N Z J Obstet Gynaecol, 58*(3), 341-348. doi:10.1111/ajo.12754

*these authors contributed equally to the study.

BAW conducted all bioinformatics analysis and contributed to the drafting of the manuscript. MT conducted laboratory components, RT-qPCR and related statistical analysis and contributed to the drafting of the manuscript. ELS contributed to the design and interpretation of RT-qPCR and contributed to the drafting of the manuscript. FDF contributed to the design and interpretation of RT-qPCR and contributed to the drafting of the manuscript. MS contributed to participant recruitment, questionnaire design and data collection, and contributed to the drafting of the manuscript. JR contributed to bioinformatics design and analysis of the microbiota data and contributed to the drafting of the manuscript. PG contributed R scripts, bioinformatics design and analysis of the microbiota data and contributed to the drafting of the manuscript. GM contributed to bioinformatics design and analysis of the microbiota data and contributed to the drafting of the manuscript. PT contributed to design and interpretation of RT-qPCR and contributed to the drafting of the manuscript. JAA conceived the concept of the study, conducted clinical

recruitment, epidemiological and questionnaire analysis and contributed to the drafting of the manuscript. WMH developed the case-control study design, contributed to statistical analysis and interpretation and contributed to the drafting of the manuscript.

Chapter 4

Amba Lawrence (QUT) conducted the initial isolation of clinical isolates from ACTS swabs and the growth and infectivity assays. Sam Kroon (UTS) performed the iron deprivation chlamydial persistence assays and analysed the results. Mark Thomas (UTS) was responsible for assisting with aspects of experimental design, expansion of the clinical isolates for use in persistence models, penicillin persistence growth assays, the penicillin-azithromycin recovery assays, all confocal imaging shown, interpretation and analysis of data, construction and standardization of figures, and drafting of the paper with the intent of future publication. Wilhelmina Huston (UTS) developed major experimental design components, drafting of the paper, analysis and interpretation of findings. Peter Timms assisted with drafting and aspects of the experimental design. Jane Hocking conceived ACTS and provided access to the clinical isolates of *C. trachomatis* used in this study.

Chapter 5

Mark Thomas (MT) was involved in experimental design, analysis and interpretation of the results, drafting of the manuscript for future publication, and all of the technical components and procedures described. Wilhelmina Huston was responsible for the study conception, analysis and interpretation of the results, and drafting. Peter

Timms assisted with drafting of the manuscript and some components of the experimental design. Jane Hocking conceived ACTS and provided feedback on drafted versions of the paper.

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