UTS UNIVERSITY OF TECHNOLOGY SYDNEY

Pelvic inflammatory disease in women; improving diagnosis and better understanding the disease process.

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the degree of

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under the supervision of Associate Professor Wilhelmina Huston
and Dr. Catherine Burke

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CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Rami Mazraani declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy in the school of life science 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.

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Secondly to my partner Brad, you should have not let me continue my PhD, what were you thinking, I lost 80% of my hair, 80% of wrinkles grew, and I think I discovered a white hair when writing this. However, your support in listening about my research and issues has been non-stop. Thank you! Of course, I would like to extend my sincerest thanks to my family for helping me throughout the years of education. The friends I have made along the way have also been of great assistance, and I wish to personally thank Mark Thomas for teaching me cell culture, and my dearest friend William King for helping me consistently through correct analysis. I would like to thank my friends who have dealt with my complaints and rants, Jessica Pederson, Natalie Strange and Nicole Lima.

“Imagination is more important than knowledge. For knowledge is limited, whereas imagination embraces the entire world” – Albert Einstein

Disclaimer: Although this study and its works focus on PID in cis women, I would like to show my respect to my fellow LGBTQA+ community and understand that trans men can have pelvic inflammatory disease.

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Abstract

Pelvic inflammatory disease (PID) is a condition that involves inflammation of the upper genital tract in women that can be debilitating. This condition can result in reproductive health sequelae such as tubal factor infertility, ectopic pregnancy, and chronic pelvic pain. Diagnosis of PID is a complex and subjective clinical process. This includes a bimanual examination to test for cervical, uterine, and adnexal motion tenderness which is considered a critical diagnostic criterion to indicate PID. The current treatment protocol for PID raises concerns as it is immediately treated with several antibiotics prior to and regardless of infections diagnosed. The increase of antibiotic resistant bacteria in the world and the rise of multiple drug-resistant sexually transmitted infections may be at further risk from this treatment protocol. The antibiotics used for PID include ceftriaxone, cefotaxime, metronidazole, doxycycline, azithromycin and moxifloxacin, with a minimum of three used in conjunction. PID can be associated with sexually transmitted infection, respiratory pathogens, instrumentation (e.g. IUD), and/or an idiopathic aetiology.

This project conducted a prospective pilot study of women with PID, by comparing reproductive tract specimens from women with PID to healthy controls (Case-Control study design) and also further compared the findings in against a separate test group of women with asymptomatic or mild sexually transmitted infections. This involved three main objectives, the first being examining the bacterial compositions of

the female genital tract and their potential relationship with PID. Secondly, investigating expression of a selection of human immune genes for associations with PID. Lastly, the third objective involved characterising the pathogenicity of chlamydial variants using *in vivo* and *in vitro* methods, with a goal to determine if a mouse model could show large pathogenic differences that will establish a system of mouse infections ready to be adapted to looking for traits in chlamydial isolates from PID Cases or Controls.

Human research ethic committee approvals were granted and the recruitment resulted in 15 Cases, 31 Controls, and 13 Test group members (total of 59). Self-completed questionnaire data on behaviour and relevant medical history was received from a total of 59 participants. Biospecimens were successfully collected and received from 59 participants (resulting in 249 bio-specimens, consisting of 177 cervical and 59 vaginal swabs). The demographic, self-reported questionnaire and medical record review datasets were compiled and analysed for each of the groups to evaluate any differences in known risk factors for PID, or other substantial differences between the groups and no confounding epidemiological data was found. DNA was successfully extracted from cervical and vaginal biospecimens. Based on 16S rRNA gene amplicon sequencing, the cervicovaginal microbial composition was found to be variable across women regardless of disease status However, quantitative PCR confirmed that a higher bacterial load of *Atopobium vaginae* and *Prevotella species'* was associated with PID.

RNA was successfully extracted from the cervical swabs of participants. Attempts to extract RNA from the cervical swabs from the test group were unsuccessful, likely due to contamination and extended storage. The gene for lysozyme was found to be expressed

at higher levels in PID cases than controls, as the sole significantly different gene. Albeit, pathway analysis was conducted to identify pathways that had different gene expression levels between cases and controls, and significantly different pathways between cases and controls included those with cell communication and detection of bacteria and viruses.

Chlamydia trachomatis variants were examined to establish a framework approach to evaluate pathology of isolates. Fatty acids were successfully extracted from the bacterial strains and analysis found that there were different concentrations of four unsaturated acids relative to wild-type. Variants were found to have distinct susceptibilities to the fatty acid synthesis inhibitor type II (triclosan) compared to wildtype. Analysis of the *in vivo* properties (survival and reproductive tract pathology) of the variants found no significant differences compared to the wildtype in C57BL/6 and C3H/HeJ mouse vaginal infection models. Establishment of a suitable animal model for *C. trachomatis* virulence to profile if certain strains or isolates have distinct pathogenic traits requires further development.

Overall, this pilot study has established that the recruitment, specimen processing and analysis protocols are effective. The results indicate that there are potential microbiota that may influence the disease. Secondly, human immune gene expression profiles were found to correlate with PID, and these biomarkers could be used for future molecular diagnostic of PID with further supporting data. Finally, this study results and conclusions support that there is a need for a in vivo model of PID, especially in terms of investigating different organisms and potential pathogenic factors