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THE EPIDEMIOLOGY OF CERVICITIS IN SEXUALLY TRANSMITTED INFECTION CLINIC POPULATIONS IN SYDNEY, AUSTRALIA

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A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

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Faculty of Medicine

School of Public Health

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Includes emendations submitted 27/06/2015
DEDICATION

This PhD is dedicated to the writers in my life, past and present who continue to inspire me.

To Dr Jane Adcock, dear friend and mentor (neurologist, Oxford), brothers Jon Lusk (music journalist, English teacher, London) and Dr Christopher Lusk (forest ecologist, editor, academic, NZ), Reihana Robinson (poet, writer, environmentalist, NZ), Geoffrey Robinson (newspaperman, environmentalist, NZ) and to the memories of my parents, my Mother (storyteller, actress) and my Father who taught me the value of quiet achievement, tenacity and authenticity. Arohanui

It is also dedicated to the women who generously gave their time and enrolled in this study, sharing my passion for answers and to the gracious and talented staff of the Short Street Centre, St George Hospital without whom I could never have completed this study and PhD.
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THE CERVICITIS STUDY- INTRODUCTION & ACKNOWLEDGEMENTS

The Cervicitis Study upon which this thesis is based, is an original clinical study designed and implemented by myself (Chief Investigator) and co-investigator and PhD co-supervisor, Dr Pam Konecny. It is the only study of cervicitis in Australian women.

The idea for the study arose in early 2006 at the Short Street Centre on-site laboratory as Dr Konecny and I were discussing the microscope slide of cervicitis in front of us. What was the significance of this finding? What should I do about it in relation to my patient? These were questions that had troubled me since my early microscopy work in sexual health medicine. A quick review of the literature at the time revealed that these were questions commonly asked with little consensus to the answers.

Plans for the Cervicitis Study slowly took shape with a comprehensive literature review and discussion with a potential collaborator, Professor William Rawlinson at the Virology Research Division, SEALS Laboratory, Prince of Wales Hospital. Together we proposed a study utilizing the findings of routine clinical practice and additional use of multiplex PCR tests for genitor-urinary infections, in particular the herpes viruses and the mollicutes, including *Mycoplasma genitalium* (MG), that SEALS laboratory was interested in developing as in-house tests. As a novice investigator and unpublished clinician, I was not successful at attracting NHMRC funding or St George Hospital Research funding for this project, but was fortunate enough to be awarded a one-off Novartis-sponsored Sexual Health College Scholarship in 2006.
My thanks to key collaborators for providing invaluable input to the study design, implementation and analysis, in particular Dr Pam Konecny (co-supervisor and colleague) who has been a tireless enthusiast, mentor and co-writer with me throughout the study, Professor Bob Cumming (supervisor and epidemiologist, University of Sydney) for his essential input to the study planning and guidance on the papers and thesis, Frances Garden (statistician), the generosity and expertise of Professor William Rawlinson’s Virology Research Division (including scientists Christopher McIver, Nicholas Rismanto and Zin Naing) at SEALS Laboratory, Prince of Wales Hospital and to William Pauuwe for his expert computing and personal support throughout this project.

Central to the project’s success was the support of the Sexual Health Clinics where I was employed as a Registrar and then Staff Specialist during the four-year recruitment period, in particular the clinical staff of Short Street Centre at St George Hospital, where most recruitment occurred. By funding necessity the Cervicitis Study utilized a sample of women already attending the Sexual Health clinics, with minimal deviation in the most part from routine clinical practice. This facilitated recruitment and staff involvement. During the recruitment period I was posted for 2 years to RPA and Liverpool Sexual Health Clinics, initially as a Registrar and then Staff Specialist where recruitment started after Ethics Approval, and recruitment continued at the Short Street Service under the supervision of Dr Konecny and myself. Recruitment at these other clinics was hampered by the time constraints of ethical approval and lesser support for the project.

Acknowledgement and thanks to the Sydney HIV/AIDS and Related Programme Units (HARP Units) and New South Wales Health who are the financial sponsors of this wonderful
network of publically-funded Sexual Health clinical services where this opportunistic research occurred.

Special thanks must also go to the staff and patients of the Short Street clinic, in particular Nurse Unit Manager Suzy Wilds, previous Director Dr Chris Carmody, Drs Ruby Uddin and Judith Gardiner, nurses Jeanette Ball, Sara Hristov, Wendy Jarrett, Robyn Dever and receptionist Helen Rayner who welcomed participants and provided invaluable clerical support. Christa McPherson (Scientist, SEALS laboratory, St George Hospital) provided quality control on Gram stain slides from all study sites.

Kevin McGeechan from the University Of Sydney School Of Public Health initially provided statistical support for the study, but from 2009 onwards I hired Frances Garden, PhD candidate at the University of Sydney, who has provided expert, patient and always timely assistance and quality control for my statistical workings with SAS over the project.

Recruitment for the Cervicitis Study started in July 2006 and completed in February 2010. The first paper related to the study was published in 2008, Cervicitis: A Review. This paper was commissioned for a special STI and AIDS Edition in Current Opinion in Infectious diseases. It was my first publication.
THESIS STRUCTURE

This thesis is based on the original clinical study called the Cervicitis Study, of which I was the Chief Investigator.

The Cervicitis Study was a multi-site cross-sectional study of 558 women attending Sexually Transmitted Infection (STI) services in Sydney between 2006 and 2010. The thesis is of hybrid structure containing 8 chapters plus an Appendix. Three chapters (1,3 &7) contain original papers as published in peer-reviewed journals and chapter 4 a pre-publication paper (with published version in Appendix). Chapter 6 includes a paper submitted for publication currently at ‘under review’ status and chapter 7 contains a draft manuscript in preparation for submission for publication.

The first chapter is in two sections. It contains a literature review written as the Cervicitis Study was being planned which was published in early 2008 and a more up to date review of literature concerning cervicitis and related matters from 2008-2014.

Chapter 2 outlines the methods of the Cervicitis Study. Details of the scientific methods are contained in the Appendix in the methods paper of McIver et al. I was 6th author on the methods paper but made a substantial contribution to it.

Chapter 3 contains the first paper from interim findings in the Cervicitis Study, which discusses the epidemiology of Trichomonas vaginalis and diagnostic considerations.

Chapter 4 contains the original mollicute paper, again with late interim findings from the Cervicitis Study which was abridged to a Short Report format for publication in Sexually Transmitted Infections (the published Short Report is included in the Appendix). The initial
full paper is included in the thesis for its valuable examination of the mollicute organisms and their association with the conditions of cervicitis and bacterial vaginitis.

Chapters 5 and 6 contain the final papers of the cervicitis study, using data from the fully recruited study of 558 women. These papers address the main study aims. The paper from Chapter 5 has been submitted for publication to the peer-reviewed journal *Obstetrics and Gynecology* and the paper from chapter 6 is a draft manuscript in preparation for submission for publication.

Chapter 7 presents a paper related to the Cervicitis Study, published in 2013 which outlines the changing epidemiology of heterosexual *Neisseria gonorrhoea* (NG) noted at our clinical service. It is of relevance to help explain the very low rates of NG found during the Cervicitis Study and includes a discussion of the overlooked pharyngeal reservoir of this infection in women. During study recruitment, rates of cervical NG were very low (prevalence of 1.1%). Although in keeping with low rates of heterosexual NG in Sydney during this period, we noted a trend of increasing NG rates and reports of increasing acquisition through oral-genital contact. As these trends occurred around the time of the cervicitis study using the same clinic population, the inclusion of the NG study is relevant to offer a wider epidemiological understanding on NG in women in this population at this time.

Chapter 8 is a concluding discussion and includes a guideline for the management of cervicitis based on the findings of this study in the context of my literature reviews.

The appendix has three parts and includes conference and meeting abstracts from the Cervicitis Study, participant approach, information and consent forms and finally, copies of the five published papers as they appear in journals.
THESIS ABSTRACT

Background

There is much to clarify about cervicitis including its etiology, significance and optimal management. Current management guidelines are inconsistent in their recommendations for affected women and their partners, reflecting lack of clarity concerning the etiology and best-case definition for cervicitis. The commonly used microscopic Gram stain diagnosis for cervicitis of > 30 polymorphonuclear leucocytes/ high-powered field (>30 pmnl/hpf) has little practical application in many clinical settings and cervicitis diagnoses based on clinical assessments such as ‘mucopurulent discharge’ or ‘yellow discharge’ may have wider utility.

Study Aims

With a cross-sectional study, explore infectious and non-infectious associations of cervicitis using different cervicitis case definitions to determine key etiological exposures and the cervicitis definition with best clinical utility.

With a prospective observational sub-study, assess the effect of presumptive treatment with azithromycin 1 G PO of women with cervicitis and non-specific cervicitis (NSC) on the outcomes of cervicitis persistence or genital symptoms and to assess benefit on cervicitis persistence of additional presumptive male partner treatment with azithromycin 1 G PO.
Methods

558 consecutive consenting women attending three publically-funded STD and HIV services in Sydney were enrolled in a cross-sectional study between 2006 and 2010. Infectious and non-infectious associations of cervicitis were examined using the cervicitis definition >30 pmnl/ hpf on cervical Gram stain and these associations were assessed using other commonly used cervicitis case definitions (yellow discharge, mucopurulent discharge (MCP). Women underwent testing for multiple infections including *Chlamydia trachomatis* (CT), *Neisseria gonorrhoea* (NG), *Mycoplasma genitalium* (MG), *Mycoplasma hominis* (MH), *Ureaplasma urealyticum* (UU), *Ureaplasma parvum* (UP), HSV1, HSV2, EBV, VZV, CMV, HPV and *Trichomonas vaginalis* (TV). Data was collected from clinical record sheets modified for the study, on presence of any genital symptoms and potential non-infectious etiological exposures including age group, current smoking, commercial sex work (CSW) and douching status, extent of condom use (always/sometimes/never) and number of sexual partners (1, >1) in the last three months, timing of last intercourse (</1 week ago), current use of combined oral contraceptive (COC), injectable depo-medroxyprogesterone acetate (DMPA)/Progestagen only pill (POP) or Implanon, phase of menstrual cycle (follicular/luteal) and stated history of past chlamydia infection and abnormal Pap smear in the preceding two years.

In the treatment sub-study, women diagnosed with cervicitis (definition>30 pmnl/hpf) were offered presumptive treatment with azithromycin 1 G PO. For this analysis cervicitis was defined as >30 pmnl/hpf on cervical Gram stain with or without known pathogens. NSC was defined as the subset of women with >30 pmnl/hpf in the absence of CT, NG, MG or TV. Treatment outcomes at follow-up included persistence of cervicitis (>30 pmnl/hpf) or
presence of any genital symptoms. Women who tested positive at follow-up for CT, MG, NG or TV (incident or persistent infections) were excluded from this analysis.

Male partners of women with cervicitis were also offered presumptive treatment with 1 G azithromycin and study testing.

Results

Cervicitis defined as >30 pmnl/hpf was present in 268/558 (48.0%), yellow discharge in 129/540 (23.9%), mucopurulent discharge (MCP) in 102/540 (18.9%) and ectropion (visibility of the cervical squamo-columnar junction) in 162/530 (30.6%).

Prevalence of pathogens at initial enrolment was: CT 5.8% (95%CI 3.8-7.7%), NG 1.1% (95% CI 0.2-1.9%), MG 3.8% (95% CI 2.2-5.4%) and TV 3.9% (95% CI 2.2-5.5%). Any genital symptoms were present in 59.9%. Cervicitis was strongly associated with the any genital symptoms (RR 1.58 (95% CI 1.40-1.78) p<0.0001).

Interim study papers found PCR testing for TV to be four to five times more effective in detecting TV than traditional methods of detection and TV was more common in women of culturally and linguistically diverse (CALD) background (p=0.003). MG was found to be significantly associated with women being HIV positive (P=0.033) but not with age, vaginal discharge, commercial sex work, being of culturally and linguistically diverse background or concurrent chlamydia infection.

CT, MG and TV were significantly associated with increased cervicitis risk on multivariate analysis, the strongest associations with the cervicitis definition MCP: CT adjusted Relative
Risk (ARR) =2.61 (95% CI 1.57-4.35) p=0.0002, MG ARR =2.25 (95% CI 1.12-4.54) p=0.003, TV ARR=2.86 (95% CI 1.61-5.09) p=0.0003. NG (RR=3.66 (95% CI 2.02-6.62) p<0.0001), CMV, HPV and HIV increased cervicitis risk on univariate analysis only. Condom use was associated with reduced cervicitis risk in univariate analysis and in multivariate analysis by the yellow discharge definition (ARR=0.68 (95% CI 0.50-0.92) p=0.013). Positive predictive values (PPV) and specificities for significant pathogens were consistently higher for cervicitis ‘tests’ yellow discharge and MCP. Exposures not associated with cervicitis included bacterial vaginosis, HSV1, HSV2, EBV, candida, *Ureaplasma urealyticum*, age, smoking, past chlamydia infection and hormonal contraceptive & cycle phase.

Population Attributable Risk% (PAR%) of significant pathogens in the etiology of cervicitis, assuming causative effects were as follows: CT 8.5%, NG 3.6%, MG 4.5%, TV 6.7%, yielding a total PAR% of the four significant pathogens of only 23.4% in the etiology of cervicitis.

Presumptive treatment of women with azithromycin 1 G orally showed a non-significant reduction in cervicitis persistence in women with cervicitis, RR=0.74 (95% CI 0.46-1.21) p=0.235 and in women with NSC, RR=0.60 (95% CI 0.35-1.02) P=0.058. Presumptive treatment was associated with a non-significant reduction of symptoms in women with cervicitis, RR=0.67 (95% CI 0.44-1.01) p=0.054 and in women with NSC, RR=0.91 (95% CI 0.46-1.79) p=0.780. Addition of presumptive partner treatment did not reduce cervicitis persistence in women with cervicitis, RR=1.18 (95% CI 0.70-2.00) p=0.528 or those with NSC, RR= 1.02 (95% CI 0.54-1.90) p=0.961.
Conclusions

There is significantly increased cervicitis risk with CT, MG, NG and TV, however much cervicitis remains unexplained. Condom use reduces cervicitis risk. The cervicitis case definitions of yellow discharge or mucopurulent discharge have the highest clinical utility with consistently higher associations, PPVs and specificities for significant pathogens and are more practical than the microscopy definition >30 pmnl/hpf.

Although we found presumptive azithromycin treatment was associated with a 30% to 40% reduction in cervicitis persistence in women with NSC and reduction of symptoms for women with cervicitis, these effects did not reach statistical significance. A larger RCT study may show more conclusive results. If azithromycin treatment of women with cervicitis and NSC is associated with a reduction of the outcomes assessed, our results suggest it is not a large effect.
PUBLICATIONS from the Cervicitis Study

I was first author on the following publications and papers


(Journal Impact Factor 5.0)

(51 citations, including 2015 CDC STD Management Guidelines)


(Journal Impact Factor 2.6) (12 citations, Editor’s Choice Award)


(Journal Impact Factor 2.6)

(24 citations including 2015 CDC STD Management Guidelines )

4. Lusk MJ, Uddin RNN, Lahra MM, Garden FL, Kundu RL, Konecny P. Pharyngeal gonorrhea in women: an important reservoir for increasing Neisseria gonorrhoea infection in urban

(Journal Impact Factor 4.8)

Draft manuscript in preparation for submission

Methods paper (6th author)

(Journal Impact Factor 4.2) (60 citations)
CANDIDATE CONTRIBUTIONS of M Josephine Lusk

The following is an outline of my contribution to the body of research.

- Principal and corresponding author on all five clinical papers relating to the Cervicitis Study, three already published in peer-reviewed journals (Chapters 1, 3 & Appendix) one paper submitted and currently under review (chapter 5) and one in preparation for submission (Chapter 6). I was 6th author on the scientific methods paper (in Appendix) but had significant input to this paper. I was the principal and corresponding author on the related paper on gonorrhoea epidemiology (Chapter 7).

- Chief Investigator of the Cervicitis Study. Study inception jointly with Dr Pam Konecny.

- Cervicitis Study design was shared by Dr Konecny and myself during a detailed joint initial literature review (published). Dr Robert Cumming had input on Study design and sample size.

- Completion and submission of all Ethics approval documents and all ongoing correspondence with the South East Sydney Illawarra Ethics committee and the Ethics Review Committee at the Royal Prince Alfred and Liverpool Hospitals.

- Completion and submission of funding applications to National Health and Medical Research Council (NHMRC) 2006 and 2007, St George Medical Research Committee (2007&2008) and Australian Sexual Health College Research Scholarships (2006-successful) 2007 and 2008.
• Design of all the study participant approach and information forms, consent forms and clinical data entry sheet.

• Upkeep of participant eligibility, participation and decline log, on-site laboratory log of participant details and findings, Gram stain slide quality assurance database and clinical database. Nikolas Rismanto and Zin Naing maintained the SEALS Laboratory multiplex PCR database. I performed the manual entry of the combined clinical and laboratory database for analysis in SAS. Frances Garden assisted in cleaning the database.

• Collection of all the clinical data from patient files and design and completion of the patient database.

• Regular meetings with Hospital Scientist Christa McPherson at SEALS microbiology Lab, St George for quality assurance of Gram stain slides.

• Regular meetings with clinic staff to maintain protocol practice and trouble-shoot.

• Regular laboratory meetings with Dr Pam Konecny and Prof Bill Rawlinson’s team at SEALS during the PCR formulation, validation and operation phases.

• SAS software supplied by the University of Sydney was used for all statistical analysis of results. Statistician Frances Garden, at my direction of what was required to be analyzed, largely wrote the programme in SAS. I ran the programme for statistical analyses and Frances Garden checked all my results and interpretations for accuracy and authenticity and provided trouble-shooting for problems as they arose. I paid Frances privately for her statistical input.
Communication of dedicated study multiplex results to patients. I directed and assisted the nursing staff in this process of contacting patients for clinical review and treatments as required.
ABBREVIATIONS

ARR  adjusted relative risk
BV   bacterial vaginosis
CDC  Centers for Disease Control
CMV  cytomegalovirus
CALD culturally and linguistically diverse
COC  combined oral contraceptive pill
CI   confidence interval
CSW  commercial sex worker
CT   Chlamydia trachomatis
Cx   cervix
DMPA depomedroxyprogesterone acetate
EBV  Epstein Barr virus
G    gram
HSV  herpes simplex virus
HPV  human papilloma virus
HIV  human immunodeficiency virus
IUCD intrauterine contraceptive device
MCP  mucopurulent
MG   Mycoplasma genitalium
MH   Mycoplasma hominis
NG   Neisseria gonorrhoea
NAAT nucleic acid amplification technique
NPV  negative predictive value
NSC  non-specific cervicitis
NSU  non-specific urethritis
OR   odds ratio
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
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<tbody>
<tr>
<td>PAR</td>
<td>population attributable risk</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PID</td>
<td>pelvic inflammatory disease</td>
</tr>
<tr>
<td>pmnl/hpf</td>
<td>polymorphonuclear leucocytes/high powered field</td>
</tr>
<tr>
<td>PO</td>
<td>Per oral (orally)</td>
</tr>
<tr>
<td>POC</td>
<td>point of care</td>
</tr>
<tr>
<td>POP</td>
<td>progestagen only pill</td>
</tr>
<tr>
<td>PPV</td>
<td>positive predictive value</td>
</tr>
<tr>
<td>RCT</td>
<td>randomized controlled trial</td>
</tr>
<tr>
<td>RR</td>
<td>relative risk</td>
</tr>
<tr>
<td>RPA</td>
<td>Royal Prince Alfred</td>
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<tr>
<td>SEALS</td>
<td>South East Area Laboratory Services</td>
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<tr>
<td>STD</td>
<td>sexually transmitted disease</td>
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<tr>
<td>STI</td>
<td>sexually transmitted infection</td>
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<tr>
<td>TV</td>
<td>Trichomonas vaginalis</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
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<tr>
<td>US</td>
<td>United States</td>
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<tr>
<td>UP</td>
<td>Ureaplasma parvum</td>
</tr>
<tr>
<td>UU</td>
<td>Ureaplasma urealyticum</td>
</tr>
<tr>
<td>VZV</td>
<td>varicella zoster virus</td>
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CERVICITIS STUDY-AIMS

Review of the literature when the study started in 2006 revealed that there was still much to clarify about the etiology, definition, significance of and best clinical management of women with cervicitis. This study was designed to clarify the epidemiology of cervicitis in STI clinic populations in Sydney, and provide a clearer picture of the factors associated with cervicitis, best case definition and a practical guideline for clinicians on how to manage the condition.

STUDY AIMS

1. With a large multi-site cross-sectional study of women presenting to Sexual Health Services, explore infectious and non-infectious exposures putatively associated with cervicitis, using the commonly accepted working cervicitis case definition of >30 polymorphonuclear leucocytes /high powered field (pmnl/hpf) on cervical Gram stain and compare these associations using other commonly used cervicitis case definitions (yellow discharge, mucopurulent discharge and ectropion), to determine the cervicitis definition with best clinical utility.

2. With a prospective observational sub-study assess the effect of presumptive treatment with azithromycin 1 G PO of women with cervicitis (30 pmnl/hpf) and non-specific cervicitis (NSC) (women with CT, NG, MG and TV excluded), on the outcomes of cervicitis persistence or genital symptoms and to assess benefit on cervicitis persistence of additional presumptive male partner treatment with azithromycin 1 G PO.
CHAPTER 1

Literature reviews

1. Cervicitis: A review

As published in *Current Opinion in Infectious Diseases* 2008; 21:49-55.

2. Literature review update: 2008-2014-update
1. Cervicitis – A Review

M. Josephine Lusk and Pam Konecny

*Current Opinion in Infectious Diseases* 2008; 21:49-55.

ABSTRACT

**Purpose of Review**

Sexually transmitted infections (STIs) impact significantly on global health. Whilst *Chlamydia, N.gonorrhoea* and syphilis have been extensively examined, there remains a paucity of knowledge of non-chlamydial and non-gonococcal cervicitis, prevalent but poorly characterized condition, with uncertain clinical implications. With increasing application of molecular diagnostic methods for the detection of STIs and a growing body of literature on cervicitis, a cervicitis review is timely.

**Recent findings**

The number of putative aetiological agents implicated in cervicitis is growing and includes *Mycoplasma genitalium*, Herpes Simplex Virus, Cytomegalovirus, bacterial vaginosis and Trichomonas. The potential role of cervicitis in HIV transmission has been highlighted. Increasing broad-spectrum antibiotic usage with associated emergence of antimicrobial resistance reinforces the need for targeted antibiotic therapies, including cervicitis management.

**Summary**

As our understanding of the etiology and significance of cervicitis, particularly non-specific cervicitis (NSC) improves, management will be refined. Advances in molecular diagnostic testing will facilitate this process but urinary nucleic acid amplification testing (NAAT) should
not replace clinical examination while cervicitis prevalence and significance is not yet established. A standardized approach to cervicitis research, particularly with consensus of case definition, may facilitate outcomes that can be more generally applied in clinical practice.
INTRODUCTION

Cervicitis was first recognized as an important clinical entity in 1984 by Brunham [1]. Since then much controversy has existed. Interpretation and comparison of published studies is hampered by the lack of consensus in the case definition, variability in populations sampled and methods used for pathogen detection [2**]. Advances in molecular diagnostics have created an opportunity to further clarify cervicitis. However, while there is an understandable desire for rapid, sensitive, potentially clinician-independent testing for Chlamydia and gonorrhoea, supplanting speculum-guided cervical specimens with urinary nucleic acid amplification technique (NAAT) testing in asymptomatic women attending STI clinics [3] may result in lost opportunity for detecting cervicitis before its implications are fully realized. It is thus timely to review cervicitis. An extensive literature search was conducted using MEDLINE, Embase and Cochrane Library on the topics of cervicitis, reproductive health and STIs.

BACKGROUND

Cervicitis is a frequently asymptomatic, inflammatory condition of the cervix [4]. It is common with rates as high as 30%-45% in some STI clinic populations [1,2,5] and is generally considered to be associated with sexually transmissible pathogens. [1,2**4,6,7*]. However, Chlamydia and N.gonorrhoea account for less than half of cervicitis cases, with a largely undefined aetiology in the remainder [1,2**8,9], referred to as ‘non-chlamydial, non-gonococcal cervicitis’ or non-specific cervicitis (NSC). The clinical significance of the finding of NSC, especially in asymptomatic ‘low risk’ women, has been debated [8, 9, 10]. Other organisms variably implicated in the pathogenesis of NSC, include Mycoplasma genitalium (MG), Mycoplasma hominis (MH), Ureaplasma urealyticum (UU), bacterial
vaginosis (BV), herpes simplex virus (HSV), Cytomegalovirus (CMV), *Trichomonas vaginalis* and Adenovirus. This raises concerns about the appropriateness of empirical treatments currently used to treat women with cervicitis and their sexual partner(s). Importantly, Bradshaw and colleagues [11**], in a recent Australian study of non-specific urethritis (NSU), the analogous clinical condition in men, highlighted the role of Adenovirus, HSV-1, macrolide-resistant *Mycoplasma* species and oral sex in NSU, factors yet to be fully characterised in NSC. A lack of consensus on definitive treatments for cervicitis in STI Treatment Guidelines [12, 13], reflect these uncertainties with the potential for over-use of antibiotics. Furthermore, the psychological impact of empirically treating cervicitis as an STI has recently being examined [14*].

**Significance of cervicitis**

Complications of cervicitis include endometritis, pelvic inflammatory disease (PID) and adverse outcomes of pregnancy and the newborn. The synergy between genital infection and enhancement of HIV transmission is well recognised. There is also literature implicating cervical inflammation in the pathogenesis of cervical cancer.

**PID**

Cervicitis may serve as an important marker of subclinical PID. Mucopurulent cervicitis and endometritis may be the only signs of PID in some women [4,9,10,15]. Many women with tubal factor infertility, ectopic pregnancy or chronic pelvic pain do not give a history of PID, but subclinical PID is likely to be an important contributing factor [16,17*]. The reported risk of women with lower genital tract infection developing PID ranges from 20-80% depending on method used for detection, delay in diagnosis, treatment, co-infection and other host factors [reviewed in 17*]. Peipert and colleagues’ analysis of women with pelvic pain in the
PEACH study (PID Evaluation and Clinical Health Study) found lower genital tract leukorrhoea (>10 leucocytes per high-powered field on microscopic examination of vaginal fluid) had a high sensitivity (89%) but low specificity (19%) for predicting histologically proven endometritis [18]. Other researchers have confirmed the high PPV of leukorrhoea for PID in the high-risk STI settings [19], particularly in the setting of BV [20]. Thus, vaginal leucorrhoea may be a useful adjunctive tool in the diagnosis of upper genital tract infection. Whilst there is disparate literature concerning chlamydial and gonococcal contribution to PID, even less is known about the relationship of NSC to upper genital tract infection. This is an important consideration when 20-30% cases of NSC are refractory to empiric ‘cervicitis’ treatment [9]. Furthermore, a recent novel study modelling different management algorithms for cervicitis in a hypothetical teen clinic population has emphasised the important psychological ramifications associated with diagnosis and empiric treatment of cervicitis for women and their partners, verses the PID-prevention benefits where there is a low prevalence of *Chlamydia* [14*].

**Pregnancy**

*Chlamydia* infection has been associated with a doubling of ectopic pregnancy rates in a Norwegian study [21] and while an association of chlamydial infection with pre-term delivery is suggested, its role is not yet fully elucidated [17]. Nugent and Hillier’s analysis [10] of a large cohort of high-risk pregnant women found that cervicitis was significantly associated with the delivery of low birth weight babies (adjusted RR 2.11, 95%CI 1.10-4.04). This was despite a low sensitivity (25%) and low PPV (24%) for *Chlamydia* infection, implicating NSC. A recent Chilean study [22] examining the benefit of antibiotic administration to women with preterm labor, found that whilst there was no overall benefit
on composite neonatal morbidity/mortality outcome, a subgroup of women with NSC without amniotic fluid infection and intact membranes derived benefit from antibiotic administration with significantly lower frequency of neonatal morbidity and mortality. This finding suggests that cervicitis may be a useful clinical marker for women at risk who might benefit from antibiotic intervention.

**HIV transmission**

Cervicitis is thought to play an important role in the transmission of HIV infection, by increasing susceptibility to HIV infection and increased HIV viral shedding. The association of genital ulceration, particularly HSV-related, with increased risk of HIV transmission risk is well recognised [23]. A significant correlation between cervical HIV DNA and microscopic evidence of cervical inflammation (adjusted OR 8.7) has been demonstrated [24].

Mechanisms by which cervicitis may increase HIV-1 shedding include increased viral replication in the context of infection or inflammation particularly in the presence of elevated pro-inflammatory cytokines, disruption of normal mucosa and increased numbers of HIV-infected cells in cervical secretions. Effective treatment of chlamydial or gonococcal cervicitis correlated with a greater than six-fold decrease in cervical HIV-1 RNA and with normalisation of cervical polymorphonuclear counts, but no reduction in HIV-1 infected cells as measured by presence or absence of HIV-1 proviral DNA in one study [25]. Conversely and somewhat perplexingly, in the same study, treatment of NSC in these women did not significantly reduce HIV-1 viral shedding but did significantly reduce the number of HIV-1 infected cells. These data raise intriguing questions about the relationship between HIV-1 infected cells HIV-1 gene expression and thus HIV transmission risk in different causes of cervicitis.
Endometritis in HIV-1 positive Kenyan women was associated with a 15-fold increase in HIV viral shedding (95% CI 2-120) which is concerning as asymptomatic endometritis may be more common in HIV positive women. [26*]. Thus implementation of effective STI screening, management and prevention strategies could significantly impact on HIV transmission, particularly in resource poor settings.

**HPV and Cervical cancer**

Chronic inflammation has been linked to many epithelial cancers, and thus the role of chronic cervicitis as a cofactor in cervical cancer seems plausible [27]. HPV, predominantly types 16 and 18, is required for the development of the majority of cervical carcinomas [28]. The role of HPV in cervicitis is less clear. Studies have noted a positive association between measures of cervical inflammation and squamous intraepithelial lesions (SIL) [28, 29]. A case control study of Costa Rican women found that in women infected with oncogenic HPV types, the likelihood of developing high grade SIL increased with degree of cervical inflammation [29]. *Chlamydia*, HSV, Trichomonas, bacterial vaginosis and CMV-associated cervical inflammation have been reported as potential cofactors in development of cervical carcinoma in past predominantly sero-epidemiological based studies. Recent, more convincing evidence for a causal link of any of these pathogens is lacking. *Ureaplasma urealyticum* has been suggested as a possible co-factor in the development of abnormal cervical cytology in the presence of HPV [30*].

**Case Definition of Cervicitis**

The case definition of cervicitis varies widely and is considered by many a clinical diagnosis evidenced by cervical ectopy (extension of the columnar epithelium of the endocervix onto the visible ectocervix), a ‘friable cervix’, presence of ‘mucopurulent’ or yellow discharge or a
combination of these signs. However overt signs of cervicitis may be overlooked given the variance in signs considered indicative of cervicitis. The presence of a yellow discharge, indicating neutrophil production of myeloperoxidase, has been suggested as a good predictor of Chlamydia or N.gonorrhoea infection [1, 4, 15]. However a recent evaluation of syndromic management using signs and symptoms of vaginal discharge in women in an antenatal setting in Botswana, failed to satisfactorily identify women with Chlamydia or gonorrhoea infection [31**].

Another approach adopted by clinicians is microscopic analysis of mucous taken from the endocervical canal. Leukocytes are normally present throughout the reproductive tract including cervical tissue [32] but are considered pathological when present in high numbers (usually >30/high-powered field) [1, 4]. Polymorph assessment of cervical mucous is not affected by the phase of the menstrual cycle except for during menstruation when it becomes less reliable [1]. The microscopic diagnosis of cervicitis is determined by Gram stain of the number of polymorphonuclear leucocytes per high-powered field (PMNL/hpf (x1000, oil immersion)) in cervical mucous using different cutoff thresholds, usually >30 PMNL/hpf [4, 5, 8, 10, 25] but often >10 pmnl/hpf [1, 4, 32]. The validity of each cut off has been reviewed [2**, 9] Consensus in the literature appears to favour the former, which has greater specificity at the expense of reduced sensitivity.

The availability of microscopy to facilitate cervicitis diagnosis will be impacted in resource-poor clinical settings. Microscopy may be open to intra-observer variability and error with vaginal epithelial cell contamination of cervical samples and counting polymorphs outside of cervical mucous. Studies reporting low positive predictive value (PPV) of microscopic cervicitis in detection of Chlamydia and N.gonorrhoea in low STI prevalence settings [8, 10]
are based on ability to predict *Chlamydia* infection detected by culture laboratory methods. The PPV of cervical findings could be considerably improved with the use of NAAT methods of *Chlamydia* detection. Additionally, PPVs are often given in terms of *Chlamydia* and *N. gonorrhoea* detection only and do not consider the increasing array of recently described pathogens, such as *Mycoplasma* spp.

Therefore, it would seem prudent to diagnose cervicitis using a combination of microscopy (>30pmnl/hpf) and at least one of the abovementioned clinical signs. Using these diagnostic criteria for cervicitis may improve the PPV for detecting disease.

**Non-infectious Etiology**

It has been suggested that mucopurulent discharge could be caused by exposure of the cervical columnar epithelium to non-infectious factors in the vagina, such as smoking, douching and combined oral contraception (COC). Earlier large prospective studies found a significant association with COC use: Paavonen’s adjusted OR 2.5 (p=0.02) [33] and Castle and Hillier’s OR 2.9, (95%CI 1.4-5.9) [29]. Interestingly, however, authors of a subsequent large cross sectional study found no such association after adjusting for the presence of cervical ectopy [34].

There is conflicting literature concerning the association between vaginal douching and cervicitis, endometritis and PID. PID was found to be significantly associated with current, frequent douching in a formative Seattle study [35]. A similar association with cervical *Chlamydia* infection and douching was observed in a separate large cross sectional study [36]. However, in contradiction, no association between douching and gonococcal or *Chlamydia* cervicitis or PID was found recently in a large prospective observational study of predominantly African-American women who commonly douche [37].
Although smoking had been previously linked to an increased risk of PID, no link between smoking and cervicitis, dysplasia or *Chlamydia* infection was found, after adjustment for confounders, in a UK cross sectional study [38].

**Infectious Etiology**

Of the known infectious agents of cervicitis, *Chlamydia* and gonorrhoea have been the most widely studied.

**Chlamydia trachomatis and Neisseria gonorrhoea**

*Chlamydia* is the most frequently identified cause of cervicitis with rates of *Chlamydia* in women with cervicitis varying widely in the literature from 11-50% [1,6,17,33] depending on population sampled, cervicitis definition and detection methods. However only 10-20% of *Chlamydia* infections may be associated with obvious clinical signs of cervicitis [6]. This may be explained in part by infections with a lower quantity of infectious organisms and strain variability, including ‘non-fusing variants’ of *Chlamydia* (about 1.5% of isolates) [39].

Increasing application of NAAT testing for *Chlamydia* has resulted in enhanced ability to detect this pathogen, but this does not completely account for the concerning continued increase in *Chlamydia* infection [17*].

Whilst *N.gonorrhoea* (NG) is known to cause cervicitis, the proportion of cervicitis attributable to NG is highly variable, in keeping with the markedly different prevalence of NG in different populations.

**Mycoplasma genitalium**

There is strong support for the role of *Mycoplasma genitalium* (MG) in the aetiology of cervicitis [5,40,41,42*,43], endometritis [43,44], PID [42*,45**], genital tract disease in men [11**,42*] and more recently in tubal factor infertility [46]. However a recent serological
study found a trend but not a significant association of MG with PID and ectopic pregnancy [47*]. The organism fulfils Koch’s postulates for pathogenicity and the balance of current evidence supports the use of antibiotics if MG is detected [11, 12, 42*, 48**]. A recent Danish prevalence study found the likelihood of MG infection in women was associated with increasing numbers of recent sexual partners and partners with symptoms [48**]. However, the low MG prevalence of 2.3% could not justify routine screening. Similar low prevalence rates of MG in asymptomatic women are reported, 6% in a Swedish study [40] and 7% in US STD clinic population study [5], where in the latter, MG infection conferred a three-fold greater risk of cervicitis. A recent large British study of antenatal women found a prevalence of only 0.7% and suggested that MG is not a risk factor for adverse pregnancy outcome in healthy women [49]. A higher prevalence of 6.2% in pregnant women in Guinea-Bissau was significantly associated with HIV-1 infection but not with adverse pregnancy outcomes [50]. Importantly, prevalence rates of MG in symptomatic and high-risk populations appear considerably higher, 13-25% [41, 44, 51, 52].

**Mycoplasma hominis**

*Mycoplasma hominis* (MH) is commonly found in the genital tract of sexually experienced females and a role in PID and post-abortal fever [45**] has been suggested. Reports of the prevalence of MH in women with cervicitis vary widely between 2.3% in Turkish study and 26% in a small Wisconsin College population [41]. Nugent and Hillier found MH to be significantly associated with cervicitis (RR 2.96 95% CI 1.76-4.99) in a study of high-risk pregnant women [10]. It is suggested that MH may exist symbiotically with the mixed bacteria of bacterial vaginosis (BV) [45**, 53]. MH serum antibody titres and vaginal leucorrhoea have been found to be higher in women with BV, than women without BV [53].
It is difficult to determine the pathogenic role of MH given its frequent association with BV [45**].

**Bacterial vaginosis**

Bacterial vaginosis (BV) is found in up to 50% of women with cervicitis [33], and may play a role in the etiology [7*, 33, 54]. The association of BV with endometritis, PID and adverse pregnancy outcome is increasingly accepted [16, 45**, 55]. Even where a significant association between PID and BV was not found, in a large observational cohort of African-American women, BV was suggested as a marker for women at high risk of PID [56]. The authors found that in women with BV, the presence of *Chlamydia* or *N. gonorrhoea* was associated with a three-fold risk of PID. The strongest risk for PID in the presence of BV was carriage of pigmented, anaerobic Gram-negative rods (*Porphyromonas, Prevotella, Bacteroides*). Schwebke’s study found an association between BV and cervicitis and between use of metronidazole gel and resolution of cervicitis [54]. A reduction in pro-inflammatory vaginal cytokines with treatment of BV has been noted in one recent RCT involving pregnant women suggesting that BV is associated with inflammatory changes at the cervix [57]. Marrazzo’s group investigating risk factors for cervicitis in women with BV identified older age, new male or female partner, recent oral sex and absence of H$_2$O$_2$-producing lactobacilli [7]. The loss of H$_2$O$_2$-producing lactobacilli in conjunction with increase in sialidases and glycosidases produced in BV may break down the protective cervical mucus barrier [58]. In keeping with this literature, the CDC guidelines recommend that BV be treated if found in the presence of cervicitis [12].
**Ureaplasma urealyticum**

*Ureaplasma urealyticum* (UU) is commonly found in the genital tracts of symptomatic and asymptomatic men and women, associated with lifetime number of sexual partners. UU is suggested as a pathogen associated with cervicitis with OR 2.7, P<0.0133 in study [33], adverse pregnancy outcome [45**, 59] and postpartum sepsis [45**]. However there is little evidence of its role in PID [45**].

**HSV, CMV and Adenovirus**

HSV-1 and HSV–2 have been associated with cervicitis [4, 6,34]. Cervical HSV shedding is thought to be generally asymptomatic. Several studies suggest an association between cytomegalovirus (CMV) and cervicitis [6, 34, 60, 61]. CMV accounted for 7.6% of cases of cervicitis in one large cross sectional study [34]. Another study of cervical biopsies of HPV-associated cervical neoplasia identified CMV DNA in 8.7% of specimens [61]. CMV shedding has also been found to be significantly greater in HIV-positive than HIV-negative women [62]. The development of molecular diagnostic techniques, particularly multiplex polymerase chain reaction (mPCRs) will aid in the detection of these viruses, which are also associated with significant congenital infection [63]. Adenovirus has been implicated in NGU in males [11**], and it is thought to have a role in cervicitis [64] but it is not well defined and an area of potential interest.

**Trichomonas & Candidiasis**

*Trichomonas* is associated with cervical inflammation [2,33,65*,66*] and increased risk of HIV transmission [65]. Its reported contribution to the aetiology of cervicitis is highly variable reflecting local prevalence, and it is considered to be frequently under-diagnosed due to the relatively low sensitivity of wet-mount microscopy. New methods for
*Trichomonas* detection, specifically NAAT testing and the new rapid ‘Point-of-Care’ (POC) bedside immunochromographic tests could help clarify local prevalences [66*]. Very little is published on cervicitis and yeast, but a negative association with cervicitis has been suggested [33].

**Management of cervicitis**

In Australasia, standard empiric treatment for cervicitis is azithromycin for affected women and their sexual partners [13]. As the local heterosexual prevalence of gonorrhoea is very low [3], concurrent treatment for gonorrhoea is not routinely given empirically. Azithromycin failure in 28% of men with MG-related urethritis has been reported and occurred more frequently when the MG originated from South East Asia, where there is emerging macrolide resistance [11**,67**]. This has important treatment implications when MG is associated with cervicitis. There are reports of improved clearance rates of MG with extended courses of azithromycin and moxifloxacin [42, 67**].

Persistent cervicitis, despite ‘standard’ empirical treatment is not infrequently encountered (personal observation) and reported by others [6, 8, 12]. The natural history of cervicitis is not defined, nor is the benefit of further treatment for unresponsive cases and their partners. Most STI guidelines suggest gynaecological review to exclude underlying pathology such as malignancy or the consideration of chemical irritant or idiopathic causes. Ablative therapy of the cervix has been used to treat chronic cervicitis [68], but there is a paucity of literature concerning the rationale and effectiveness of this intervention, which presumably relates to the association between ectopy and cervicitis. Returning to the concept that cervicitis may be an indicator of silent PID, STI guidelines could perhaps give consideration to recommending PID treatments for persistent cases of cervicitis. The management of PID
has recently been reviewed and emphasis placed on achieving high rates of clinical as well as microbiological cures [69].

CONCLUSION

In conclusion, cervicitis remains a condition yet to be fully characterised. It is common, often asymptomatic and may be associated with significant adverse outcomes for women. Research in NSC is a particular area of need. Wide variations of case definition, study populations and methods for pathogen isolation, hinder the ability to draw conclusions on the aetiology, natural history and best management of cervicitis on a population basis. Certainly the evidence suggests it is a multifactorial condition. We suggest future research should combine a microscopic definition of >30 pmnl/hpf, the more frequently cited criterion, with at least one of the accepted clinical signs such as yellow mucopus.

Urinary-based NAAT methods of STI testing have revolutionized the process of STI testing, but ironically without internal genital examination, the diagnosis of cervicitis cannot be made. With the streamlining of clinical services and in some practices, replacement of genital examination with urinary NAAT testing particularly for asymptomatic screening, we risk overlooking significant pathology in women with ‘negative STI screening tests’. In the meantime further research is needed to elucidate the contribution of new putative aetiological agents, such as MG and BV and their antibiotic susceptibility patterns and non-infectious factors implicated in the aetiology of cervicitis, in order to improve diagnosis and management of this condition and thus ultimately improve health outcomes for women and their partners.
REFERENCES


** Excellent, up to date, expert discussion of the dilemmas currently faced in defining aetiology, clinical significance and management of cervicitis


* This study proposes streamlining of STI screening by potentially replacing internal cervical sampling with urinary NAAT testing, challenging current standards of practice.


Emerging evidence linking BV and cervicitis. They characterized the risk factors for cervicitis amongst women with BV, which included new male or female partner, lack of H2O2 producing lactobacilli, older age and recent oral sex.


Nyirjesy P, Non-gonococcal and non-chlamydial cervicitis. Current Infectious Disease Reports 2001; 3:540-545


This landmark paper examines the aetiology of the analogous condition of NGU in men. It provides important data supporting the roles of MG, adenovirus and HSV-1 in the aetiology of NGU and provides impetus for similar focus and investigations of the role of these organisms in cervicitis in women.


The latest version of widely accepted guidelines used by clinicians and which undergo continual review and evaluation.


A novel approach modelling 3 algorithms for management of cervicitis addressing the less explored psychological morbidity of an STI diagnosis and the impact this has on health in young women. Although this analysis was based on the assumption that non-chlamydial cervicitis did not confer a risk of subsequent morbidity, the need for further clarification of the significance of a diagnosis of ‘cervicitis’ is highlighted.


Very clear, thorough review of the literature on Chlamydia, the epidemiology, pathophysiology, management, serious reproductive sequelae and strategies for screening for this pathogen.


21 Bakken IJ, Skjeldestad FE, Nordbo SA. Chlamydia trachomatis infections increase the risk for ectopic pregnancy: A population-based, nested case-control study. Sex Transm Dis 2007; 34:166-9


* Interesting study suggesting that non-specific endocervical inflammation (defined as >10 PMNL/hpf) in pregnant women in preterm labour may be a useful clinical marker for women who might benefit from antibiotic intervention.


This small cross-sectional study demonstrates an association between upper genital tract infection (plasma cell endometritis) and increased HIV shedding, which has important implications for heterosexual transmission.


Reviews previous reported associations between cervical organisms and HPV associated cervical carcinoma. The association of UU with increasing level of cervical cellular abnormality in the context of HPV infection suggested a co-factor role for UU in oncogenesis.

Romoren M, Sundby J, Velauthapillai M. Chlamydia and gonorrhoea in pregnant Botswana women: time to discard the syndromic approach? BMC Infectious Diseases 2007; 7:27
Large comprehensive evaluation of vaginal signs & symptoms and socio-demographic factors for predicting infection with *Chlamydia* and *N. gonorrhoea* in the syndromic management of a population of antenatal women where cervical infection prevalence is around 10% and pathogen testing is not available. The present syndromic algorithms were not found to be satisfactorily predictive, and the authors called for urgent availability of rapid, cheap pathogen testing.


Expert review covering the evidence for MG being considered an STI. The authors felt testing for MG is warranted in symptomatic patients, with the need for standardised testing kits and specific treatment.


The most comprehensive update on the role of Mycoplasma genitalium, M hominis and Ureaplasma species and BV and their role in female genital tract disease and adverse pregnancy outcome.

* Given the importance of dwindling fertility rates in developed countries worldwide, this is a very topical study linking MG with tubal factor infertility.


* Another very topical investigation into the potential relationship of MG with PID and ectopic pregnancy. Limited by small study size and using stored serum


** Excellent large prevalence study in women and men using samples from a previous population based study, with thoughtful discussion of topical issues.


60 McGalie CE, McBride HA, McCluggage WG. Cytomegalovirus infection of the cervix: morphological observations in 5 cases of a possibly under-recognised condition. J Clin Pathol 2004; 57:691-4


62 Clarke LM, Duerr A, Feldmen J et al. Factors associated with Cytomegalovirus infection among Human immunodeficiency virus type 1 seronegative and seropositive women from an urban minority community. JID 1996; 173:77-82


64 Swenson PD, Lowens MS, Celum CL, Hierholzer JC. Adenovirus types 2,8 and 37 associated with genital infections in patients attending a sexually transmitted disease clinic. J Clin Microbiol 1995; 33:2728-2731

65 McClelland RC, Sangere L, Hassan WM. Infection with Trichomonas vaginalis increases the risk of HIV-1 acquisition. JID2007; 195:698-702

In response to lack of a demonstrable link between trichomoniasis and HIV-1 acquisition in previous studies, the authors analysed data from a large 11 year prospective study of female sex workers in Nairobi. They demonstrated a 1.5(adjusted) fold increased risk of HIV acquisition with trichomoniasis, which was
also associated with cervicitis, a significant result given the relatively high *Trichomonas* prevalence in resource limited settings.


* Highly topical discussion of the advances made in new generation ‘Point-of-Care’ (POC) bedside test kits for STI screening, particularly for use in resource and laboratory poor settings.


** Increased MICs to azithromycin were demonstrated in this study. The researchers have pursued the aetiology of persistent NSU and widened the horizons of STI research.


** A comprehensive review of more recent randomized controlled trials examining efficacies of treatment regimens for PID. The authors (and Editorial commentary) emphasized the need to focus upon gynaecological and reproductive outcomes, rather than short-term clinical and microbiological cure.
2 Literature review update: Cervicitis 2008-2014

The following literature review is an update of papers examining the epidemiology and treatment of cervicitis and related topics, since the publication of the article ‘Cervicitis: A review’ Lusk and Konecny. Current Opinion in Infectious Diseases 2008; 21:49-55. This review paper has been successfully received with 46 citations to date including citation in the 2010 CDC STD Guidelines for the management of cervicitis.

In the field of cervicitis little progress has been made in the last 6 years in clarifying the etiology, optimum management and clinical relevance of the finding of cervicitis. Undoubtedly, with self-collected vaginal and urine samples, there is less need to examine women so fewer clinicians actually make the diagnosis of cervicitis. However, given its association with upper genital tract infection \(^1,^2\), preterm birth \(^3\) HIV transmission \(^4\) and symptoms in many women, it remains an important area for further research.

The two most important recent contributions to the field have been a large US cross-sectional study by Gaydos in 2009 \(^5\) and a 2013 randomized control trial (RCT) of treatment outcomes for cervicitis of unknown etiology \(^6\). Gaydos’s study in a Baltimore STD service of 324 women examined the prevalence and associations of \textit{Chlamydia trachomatis} (CT), \textit{Neisseria gonorrhoea} (NG), \textit{Trichomonas vaginalis} (TV) and \textit{Mycoplasma genitalium} (MG) with cervicitis (definition cervical discharge or mucopurulent discharge or cervical friability). They found only MG to be associated with cervicitis in multivariate analysis (AOR= 2.5, p=0.002) and a high cervicitis prevalence of 41%. STDs were highly prevalent in this population: CT 11.1%, NG 4.6%, TV 15.3% and MG 19.2 %. Interestingly, factors not found to be associated with cervicitis included CT (AOR 1.81 (95% CI 0.79-4.14) p=0.16), NG (AOR
1.01 (95% CI 0.29-3.53) p=0.99, TV (AOR =1.74 (95% CI 0.92-3.31) p=0.09, bacterial vaginosis (BV) (AOR =1.51 (95% CI 0.95-2.41) p=0.08, and age. Some of these factors, in particular CT and TV may have been significant if sample size had been larger. This paper was accompanied by an editorial 7 by Manhart on MG. Our Cervicitis Study was similarly designed to the Gaydos study but larger with more comprehensive examination of a number of other variables and examination of associations using several commonly accepted cervicitis definitions, including microscopic Gram stain diagnosis 30 polymorphonuclear leucocytes /high powered field (30pmnl/hpf), yellow discharge and mucopurulent discharge.

Taylor’s cervicitis treatment paper 6 was published in 2013 in STD with an Editorial Commentary from Wiesenfeld 8. This study was a multicenter RCT attempting to examine the treatment outcome of cervicitis of unknown etiology (women with CT, NG, MG, TV, symptomatic BV, active HSV excluded). Unfortunately the study proved too ambitious with recruitment of only 53 women with cervicitis of unknown etiology and closed early with 33 women completing the study. Women with ‘cervicitis of unknown etiology’ (cervicitis definition used was mucopus and/or easily induced bleeding and >30 pmnl/hpf) were randomized to placebo or treatment with Azithromycin 1G plus cefixime 400mg, with follow-up at 2 and 8 weeks. Clinical cure and failure rates of treatment were not significantly different in the 2 arms at 8 weeks.

Taylor also published a 2014 review on cervicitis 9 highlighting the lack of consensus case definition for cervicitis, un-explained etiology for a large proportion of cases and lack of current evidence to support presumptive treatment of cervicitis. A UK survey of Sexual Health physicians questioned about cervicitis 10 also demonstrated a lack of consensus in
diagnosis and management, and concluded that diagnosis by microscopy should be abandoned.

**Cervicitis, Subclinical PID and PID**

Despite the lack of studies focusing on the etiology and significance of cervicitis itself there have been some excellent papers on PID, subclinical PID and associated adverse reproductive outcomes. This is of relevance to the understanding of cervicitis given that lower genital tract inflammation (cervicitis or leucorrhoea) is a sensitive indicator of endometritis or subclinical pelvic infection $^{1,11}$. As for cervicitis, studies of PID are limited by the inconsistent diagnostic criteria used for this diagnosis.

In a prospective observational treatment trial of 418 women with or at high risk of CT or NG or with BV, but without clinical PID, Wiesenfeld and Hillier’s group $^{12}$ identified a 40% reduction in pregnancy rate at median follow-up time of 2.8 years (HR=0.6 (95 % CI 0.4 - 0.8)) in women with subclinical PID (indicated on endometrial biopsy) despite presumptive treatment for CT, NG and BV. Women with proven CT or NG, in the absence of subclinical PID, had no reduction in fertility. BV in the absence of subclinical PID also did not affect pregnancy rates. They concluded that subclinical PID decreases fertility despite presumptive treatment for STDs and that current treatments for seemingly uncomplicated cervical CT and NG might be inadequate for preventing the long-term outcome of infertility. This study adds support to the argument for presumptive treatment of cervicitis including non-specific cervicitis.(NOT SURE!!)

Another recent study in 298 women with confirmed endometritis utilizing data from the PEACH study $^{13}$ investigated the effect of delayed treatment (>14 days symptoms) to cover
CT, MG and NG endometritis, on reproductive outcomes. In the entire cohort they found non-significant increases in infertility, recurrent PID and chronic pelvic pain in those women delaying treatment. Results relating to single pathogens were not significant due to small numbers. Treatment was more commonly delayed in women with CT and MG, probably due to fewer symptoms.

There have been three interesting recent papers on Chlamydia and PID. A widely cited 2010 BMJ RCT (The POPI, Prevention Of Pelvic Infection Study) by Oakeshott and colleagues of 2500 young sexually active female university students in the UK, offered baseline self-collected vaginal swab for chlamydia with immediate testing and treatment if required in the intervention group and for the control group, sample storage and analysis after one year (with the caveat that controls could seek testing and treatment in the interim, which 22% of controls wisely did). The ethics of this study seem marginal, and the authors pointed out that it was at the limits of acceptability. The follow-up outcome was incident PID over 1 year largely based on patient account and some medical records. There was a non-significant reduction in PID amongst women immediately screened and treated, (RR 0.65 (95% CI 0.34-1.22) over those with deferred analysis and treatment of baseline samples, with similar baseline chlamydia rates in the two groups. Most women with PID were negative for chlamydia at baseline (79%) and they concluded that a single screen for chlamydia was not effective in high-risk women, suggesting more frequent screening for this group to pick up incident infection. A weakness of this study was failure to take into account other PID-related infections such as NG and MG.

Another UK study examining PID used data from the UK General Practice Research Database (covers 5.5 % of the population) examining numbers of diagnostic coding for
definite, probable and possible PID diagnoses in General Practice between 2000 and 2008. They found the rate of definite/probable PID decreased by 10.4% per year, most pronounced in women 16-19 years, with rates of possible PID increasing. Some of the findings could be explained by symptomatic people seeking care preferentially in Genitourinary (GUM) Clinics (given the easier access with the 48-hours-wait target introduced to GUM services in 2004 and more PID diagnoses reported in this setting) and trends of less specific diagnoses being made by GPs with the obvious limitations of relying on such coding for reliable PID diagnosis. The most likely explanation for reducing PID rates was cited as the substantial increase in chlamydia testing and diagnosis over this decade, particularly in younger women.

More generally, Haggerty and colleagues\(^\text{16}\) produced a 2010 review of 24 papers on the risk of sequelae after chlamydia infection. They found no prospective studies directly assessing risk of long-term reproductive sequelae after untreated chlamydia infection (reflecting the difficulty of conducting such studies). They concluded that in high-risk settings 2-5% of untreated women with chlamydia infection developed PID within the first 2 weeks between testing positive and being treated but rate of progression to PID in asymptomatic women was lower. Despite our knowledge of the adverse outcomes associated with delayed or untreated PID, a 2011 study of adherence to US CDC guidelines for PID treatment in the US Emergency departments revealed a dismal 30% adherence to guidelines\(^\text{17}\).

Haggerty also reviewed PID management in 2007\(^\text{18}\). The dual regimen doxycycline/metronidazole has been found to have poor clinical and microbiological cure rates, one UK observational study finding a cure rate of 55%, this increasing to 72% with the additional of ceftriaxone\(^\text{19}\). Doxycycline has poor cover for streptococci, E coli, anaerobes and NG and
since PID is thought to be a poly-microbial infection, broad cover is required. Another excellent discussion on PID appeared in 2010, by Soper\textsuperscript{20}, which reminded us that symptoms of PID are not always the traditional pain and fever and can be more subtle such as abnormal discharge, cycle change, heavy menstruation and urinary symptoms, but the diagnosis is clinched by signs of lower genital tract infection in conjunction with pelvic organ tenderness. Best outpatient treatment was suggested as broad-spectrum cephalosporin plus doxycycline or azithromycin. A UK study\textsuperscript{21} comparing the efficacy of azithromycin monotherapy against azithromycin/metronidazole combination for treatment of PID (mostly diagnosed by laparoscopy) found a 97% clinical cure rate and clearance of pathogens with azithromycin alone (but didn’t include testing for MG or TV) and 96% cure with azithromycin/metronidazole regimen. However it was not clear from this study which azithromycin regimen was most optimal (one trial used 500mg IV then 250 mg orally for 6 days a second trial used IV Azithromycin for 2 days then oral for 5 days) and involvement from Pfizer was not accompanied by a conflict of interest declaration. Additionally details on what constituted a ‘cure’ were unclear in this study. Azithromycin provides good cover for CT, NG and anaerobes and is a single dose, well tolerated treatment with a 68-hour half-life and accordingly seems to be an essential part of any PID treatment regimen.

Current Guidelines in Australia for outpatient management of PID mostly recommend azithromycin stat plus 14 days of doxycycline and metronidazole BD, plus ceftriaxone 500 mg IMI if severe or NG isolated/suspected.

**TV and HIV.** Naturally, much research of the last decade has focused on the role of STIs in enhancement of HIV transmission. A 2012 large STI prevalence study in asymptomatic HIV positive men and women in South Africa examined prevalence and associated risk factors
for cervicitis and urethritis-associated pathogens, finding pathogens more prevalent amongst women and TV to be the most prevalent pathogen overall (7.6%). The authors recommended STI screening in HIV positive men and women, given the large burden of asymptomatic STIs in this population. Although not common in urban Australia, TV is important because of its prevalence worldwide, often with an asymptomatic presence, relatively overlooked pathogenic role and likely association with HIV transmission. McClelland summarized nicely, the role of TV in a 2008 Editorial alongside Van Der Pol’s large definitive case control study demonstrating the significant association between the presence of TV infection and increased risk of HIV acquisition in a general population of African women (AOR 2.74 95% CI 1.25-6.00). This and the high prevalence of TV in HIV-endemic areas implicate TV as a major contributor to heterosexual HIV transmission and McClelland suggested that the control of TV presents a relatively easy opportunity to reduce HIV transmission.

Our findings concerning the under-diagnosis of TV with wet mount preparation compared to PCR testing, were corroborated in a US study of high risk women, also finding a four to five fold increase in TV case detection using PCR detection methods rather than traditional detection methods and finding many women with TV to be asymptomatic. They recommended more widespread TV PCR screening inclusion in high-risk populations. However there has been little recent investigation of the association of TV with cervicitis. A 2013 cross-sectional study in Peruvian CSWs did not accurately assess the role of TV, focusing on BV and there were no statistically significant findings.
**BV and Cervicitis, PID and HIV transmission**

The relationship between cervicitis and BV remains controversial, with few recent papers examining this association and most studies examining the role of BV in genital tract inflammation and PID. A detailed Chinese paper using PCR analysis of the microflora in BV in women with and without cervicitis, found that lactobacilli were significantly reduced in both conditions but did not find evidence of direct involvement of BV with cervicitis. The 2009 Gaydos cross-sectional study found an association between BV and cervicitis (ARR=1.51 (95% CI 0.95-2.41) p=0.08) but it was not statistically significant.

The relationship between BV and PID is much more solid, demonstrated in a multicenter US study of over 1100 women comparing incident PID rates over 3 years in women with and without BV, where they found carriage of BV-associated microorganisms significantly increased PID risk (ARR 2.03(95% CI 1.16-3.53)). This risk was particularly increased for women with a new sexual partner. In a longitudinal study over 1 year of 3620 women, Schwebke’s group demonstrated BV was associated with a significantly increased risk (adjusted hazard ratio (AHR) 1.73 (95% CI 1.42-2.11) for acquisition of TV, NG and/or CT infection, with similar estimates for isolated TV, NG and CT infections.

There has also been mounting evidence of the role of BV as an STI with a Melbourne based study finding BV absent in truly sexually inexperienced women and US study of 335 women who have sex with women finding BV to be associated with sexual practices that efficiently transmit vaginal fluid (sharing toys and lubricant use). A 2008 review and meta-analysis of 43 studies looking at associations between sexual risk factors and BV found a summary estimate of BV and new/ multiple male partners was 1.6 (95% CI 1.5-1.8), BV and
female partners 2.0 (95% CI 1.7-2.3) and condom use 0.8 (0.8-0.9), suggesting that BV has an 
edemiological profile similar to that of an STI.

The association between BV and enhanced HIV acquisition seems well established, as 
summarized in a 2008 meta-analysis of 23 HIV incidence studies, which found BV to be 
associated with increased HIV acquisition (RR 1.6, 95% CI 1.2-2.1) and 21 HIV prevalence 
studies showing a positive association \(^{36}\). There was also a large 2008 African study of 4531 
women which found that women with BV (Hazard ratio (HR) 2.5 (95% CI 1.68-3.72) or yeast 
(HR 2.97 (95% CI 1.67-5.28) were more likely to acquire HIV \(^{37}\).

**MG and Cervicitis, PID and STD co-infection**

MG is increasingly recognized as a genital tract pathogen, with a consistent picture 
emerging of similar or greater prevalence \(^{7,38-42}\) and pathogenicity to CT, and associations 
with upper and lower genital tract inflammation in women, risk of preterm birth \(^{43}\), NSU in 
men and enhanced risk of HIV transmission. Haggerty reviewed MG and PID \(^{44}\), finding 
strong evidence for its role and suggested MG as an important contributor to treatment 
failure for PID, suggesting the need for MG antibiotic cover to be included in PID treatment 
regimens. This is important given that MG testing is not available in many settings and co-
infection with other STIs, in particular chlamydia is being increasingly reported, as is 
resistance of MG to azithromycin. In contrast, a side study from the POPI study analyzed PID 
incidence after baseline MG infection in a large group of UK students and concluded that 
MG was not likely to be a significant risk factor for PID (RR 2.35 (95% CI 0.74-7.46) p=0.14), 
but this study had a number of limitations as mentioned above, and the prevalence of MG 
was much lower (3.3%) than that reported in many developing world studies \(^{45}\). Manhart’s
review \(^{46}\) of MG and cervicitis (14 studies) and MG and PID (9 studies) in 2011 concluded MG’s role in both conditions to be conflicting. Odds ratios for association between MG and cervicitis ranged from 1.2 to 5.7, but were often un-adjusted, and almost half the studies reported no association. Of the six studies using MG PCR to investigate association with PID, ORs ranged from 4.6-6.3 in five studies, supporting the association.

There has only been one study \(^{47}\) with weak support of MG as a cause of ectopic pregnancy, finding antibodies to MG (and CT) associated with risk of ectopic pregnancy, OR 1.6 (95% CI 0.6-4.0).

MG is variably reported to be associated with CT infection, symptoms and with cervicitis. A US study of 381 mainly Afro-American women in an STD service \(^{48}\) with very high STD rates, found MG co-infections in 30% of women with NG, 25% of women with CT and 20 % of women with TV. MG as a sole infection was associated with age <22 years (AOR 2.53(1.25-5.12) and cervicitis (AOR 2.11(1.04-4.26). Vaginal swab specimens had superior MG detection rates compared to endocervical swab specimens. In a US study of 331 high-risk adolescents from a hospital- based teen health center (again with very high STD prevalence rates), Huppert et al \(^{39}\) found MG to be significantly associated with CT co-infection (OR 2.3 95% CI 1.4-4.4) but not with TV, NG, cervicitis (defined as mucopurulent discharge or cervical friability) or symptoms. Another US study involving quarterly visits for two years of 383 adolescent women in a primary care setting and 117 of their male partners \(^{49}\) also found MG significantly associated with CT co-infection (AOR 1.89 (95% CI 1.07-3.34) P=0.029) but not with NG, TV or symptoms. Male partners of MG-positive women were more likely to have MG than male partners of women not infected (P<0.02), supporting sexual transmission. They found 25% of male partners of MG positive women to be infected.
compared to our figure of 40% (un-reported data, see Abstract 2010, Appendix, Part 1). In Hitti’s large case-control study of MG in Peruvian sex workers, MG was associated with CT co-infection (<0.001) and importantly preterm birth (AOR 2.5 (95% CI 1.2-5.0) P=0.014).

Closer to home, a NZ study of women under 25 years having terminations of pregnancy found MG was not significantly associated with CT co-infection. Additionally our 2010 interim findings paper found a significant relationship between MG and cervicitis (ARR 1.24 (95% CI 1.04-1.48 p=0.02) and between MG and HIV (p=0.03) but not with CT (p=0.44) or vaginal discharge (p=0.32).

**MG and HIV**

There have been several papers arising from a hormonal contraceptive and HIV acquisition study of women in Uganda and Zimbabwe. This study also looked at various STIs and their contributions to HIV transmission, including a nested case-control study which found a two-fold increase in HIV acquisition risk in women who had MG immediately prior to their HIV diagnosis (AOR 2.41(95% CI 1.01-5.80) p=0.05). They also found HSV2 and NG to be strongly associated with HIV acquisition. The same authors also published a meta-analysis of 19 studies in AIDS 2009 on the association of MG with HIV and found an overall OR of 2.01 (95% CI 1.44-2.79). Manhart has written prolifically on the topic of MG and HIV. Most interesting was an original study in 2008 of 303 HIV positive Kenyan women, finding a high MG prevalence (17%) and a three-fold increase in risk of cervical HIV shedding (AOR 2.9 (95% CI 1.1-7.6) p=0.03) in women with high cervical MG organism load. Low cervical MG organism load and cervicitis were not associated with cervical HIV shedding. MG was much more prevalent than CT (7%) or NG (10%).
Manhart and McClelland \textsuperscript{52} wrote a perceptive editorial accompanying a paper by Vandepitte et al on the natural history of MG infection in Ugandan CSW \textsuperscript{42}. The editorial recommended priority review of antibiotic regimens currently used in syndromic management in Africa, given the high prevalence of both MG and HIV in these areas and increasing reports of MG resistance to azithromycin. The natural history study \textsuperscript{42} involved MG testing of samples from a previous cohort study of 1027 CSW. Prevalence of MG was 14%. At three months 55% had spontaneously cleared infection, 83% within 6 months and 93 \% within 1 year, however 39 \% became re-infected. HIV positive women cleared MG more slowly than HIV negative women and clearance was slower with CD4 counts <350. Women had antibiotics during the study to treat other STIs, but were not specifically treated at the time for MG with azithromycin. This paper includes a detailed discussion of the antibiotic resistance mechanisms of MG.

\textbf{MG treatment}

The body of literature dealing with MG treatment is growing. MG resistance has been reported and investigated since 2005 by Bradshaw \textsuperscript{53}, Jensen \textsuperscript{54}, Bjornelius \textsuperscript{55} and Jernberg \textsuperscript{56}. The Melbourne study \textsuperscript{53} recruited males with NSU and females with cervicitis/PID and contacts of MG and found MG prevalence in these groups 11\% and 10\% respectively. Azithromycin 1 G was effective in eradicating MG in 84\%. Jensen \textsuperscript{54} isolated the mutation responsible for MG azithromycin resistance and in analysis of nine specimens of men with NGU obtained before and after azithromycin treatment, found only two initially had the mutation, suggesting single dose azithromycin treatment induced this resistance mutation. A Japanese group also suggested a possible increased resistance with a 1 G azithromycin
dose \(^{57}\). This obviously has implications for the current widespread use of azithromycin 1 G therapy for cervicitis and NSU.

A longer five day treatment regimen of azithromycin 500mg on day one followed by four days of 250 mg proved to be 100% effective for MG eradication in women and 96% effective (95% CI 85-99%) in men, in a 2008 Scandinavian study of 155 men and 60 women with MG, recruited from six STI services in 2002-2004 \(^{55}\). A single 1 G dose of azithromycin was effective in 85% (95% CI 69-94%) in men and 88% (95% CI 64-99%) in women. A consideration here though is the relatively small numbers of women especially, and the fact that people given extended azithromycin therapy had already been given and failed doxycycline treatment. As a result of this study, the extended 5 day course of azithromycin was adopted in Scandinavia for treatment of MG. It would be good to see a larger study of the efficacy of this regimen, as it is preferable to widespread moxifloxacin use. A somewhat different result was found in a 2008 retrospective survey of treatment efficacy in 10,109 STI patients in Oslo 2005-2006 \(^{56}\) which examined the microbiological cure rates of several different regimens for MG treatment, and found the following eradication rates: azithromycin 1 G (79%), azithromycin 1 G repeated after five to seven days (74%), azithromycin 500mg then 250 mg for further four days (78%), ofloxacin 200mg BD for 10 days (56%) and moxifloxacin 400mg daily for seven days (100%). Only the first 2 regimens were used first line over the observed period. If the five day regimen was used 2\(^{nd}\) or 3\(^{rd}\) line, the cure rate dropped to 34%, with the suggestion that these patients were likely already resistant to azithromycin. MG prevalence was 4.5%. Although retrospective, this was a huge sample and this country’s health policies facilitated high follow-up rates. The
findings did concur that a five day azithromycin regimen is an effective first line treatment for MG, although in this study, no more so that a single 1G dose of azithromycin.

Manhart reviewed MG treatment in 2011, concluding that single dose or five day azithromycin treatments were probably of similar efficacy and was cautious about the small number and observational nature of the data supporting moxifloxacin use. Accordingly she recommended prudent use of moxifloxacin only for 2nd line treatment of resistant cervicitis/PID or NGU with proven MG infection persisting after test of cure at four weeks.

There are also reports of moxifloxacin resistance appearing.

An exciting new development was described in a large French study evaluating the clinical performance of the Bio-Rad Dx CT/NG/MG multiplex assay in urogenital samples in comparison with the Roche COBAS® TaqMan® CT assay for CT and an in-house TaqMan PCR assay for MG. They found very high sensitivity and specificity of the multiplex, recommending this assay as suitable in urogenital specimens of symptomatic and asymptomatic men and women.

A discussion of the potential role of cytokines in cervicitis appears in the main findings paper (chapter 5). Finally a search of the literature does not yield any studies examining the potential effects of trauma of sex, toxic effect of semen per se or timing of last intercourse as factors associated with cervicitis.
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CHAPTER 2

METHODS
METHODS

The Cervicitis Study was designed from mid-2006 to 2008 and commenced recruitment at the Short Street Centre in July 2008. This study had to be of an opportunistic nature, utilizing my role as a Senior Registrar and then Staff Specialist at Sexual Health Services in Sydney and relied upon the supervision and mentorship of my colleague and PhD co-supervisor Dr Pam Konecny. Part of my job description was to be a researcher, so this fitted in as part of my employment. Whilst posted to other clinics I maintained a supervisory and support presence at the Short Street Centre. This clinic is a small but very dynamic service dedicated to the support of research with just over six full time staff members, attached to the St George Hospital. It forms part of a network of publically funded-Sexual Health Services in New South Wales.

All the STI clinics sites used for this study provide STI and HIV services by triage to patients from ‘priority populations’ defined in the 2006-2009 NSW Sexually Transmissible Infections Strategy, symptomatic patients, STI contacts and those referred from primary care and hospital specialists. They operate in areas with a high proportion (40%) of patients from culturally and linguistically diverse cultural backgrounds.

As a novice researcher, the only funding I secured was a one-off research scholarship through the Sexual Health College in 2006 (sponsored by Novartis) which helped towards
the cost of multiplex laboratory testing during the study and to cover my statistical expenses. Collaboration with Prof. William Rawlinson’s Virology Research Laboratory was critical to give us access to the multiplex PCR tests and limited number of HPV testing kits used for comprehensive pathogen detection.

By necessity the protocol for the study had to follow standard clinical practice in the clinics, in order to fit in with everyday workflow and not financially impact upon the clinic budget. Thus the study was observational in its design. This facilitated staff involvement and clinic support and importantly meant decline rates for the study were low because there was minimal intervention or disruption for participants.

The study aims were as follows:

1. With a multi-site cross-sectional study of women presenting to Sexual Health Services, explore infectious and non-infectious exposures putatively associated with cervicitis on literature review[^1], using the commonly accepted working cervicitis case definition of >30 polymorphonuclear leucocytes /high-powered field (pmnl/hpf) on cervical Gram stain and compare these associations using other commonly used cervicitis case definitions (yellow discharge, mucopurulent discharge and ectropion), to determine the cervicitis definition with best clinical utility.

2. With an observational prospective sub-study assess the effect of presumptive treatment with azithromycin 1 G PO of women with cervicitis (30 pmnl/hpf) and non-specific cervicitis (NSC), on the outcomes of cervicitis persistence or genital symptoms and assess benefit on cervicitis persistence of additional presumptive male partner treatment with azithromycin 1 G PO.
We initially intended to include recruitment from a gynaecology service as well as STI clinics, but logistics proved too difficult and recruitment was confined to the services where I was working during the recruitment period, primarily Short Street Centre, and secondary sites Royal Prince Alfred (RPA) Hospital Sexual Health (April-November 2008) and Liverpool Sexual Health clinics (November-December 2008). Ethics approval delays and local clinical service restrictions limited recruitment in these secondary sites to 89 women, with 83% of participants being recruited at the primary site Short Street Centre.

The study protocol is also described in the papers from Chapters 3-6 and the Laboratory methods for the dedicated study PCR tests are described in McIver’s paper, included in the Thesis appendix 3.

**Selection of Study Subjects**

The study enrolled 558 women between July 2006 and February 2010. All recruiting clinicians underwent standardized on-site training in diagnostic microbiology, study protocol and sample collection. Inclusion criteria included consecutive consenting women over 18 years requiring vaginal examination based on presenting complaint or requesting examination. During the study it was clinic policy for clinicians to collect speculum samples on most women for screening or investigation of symptoms, with patient self-collected samples less commonly offered. (Re-enrolment was permitted if more than six months had elapsed since initial enrolment and a new sexual partner reported.) Exclusion criteria included PID, menstruation, pregnancy, no vaginal sex in prior three months, antibiotic use in prior month, sexual assault, Intrauterine contraceptive device (IUCD) or cervical surgery in prior 3 months. The exclusion criteria were chosen to exclude women with conditions where
the cervical environment may have been altered (recent antibiotic use, surgery, pregnancy, menstruation, IUCD pelvic infection). Women who had not had vaginal sex for 3 months were excluded, as they were less likely to have infection present.

There were 1327 consecutive women approached with 630 (47.5%) eligible and 697 (52.5%) ineligible. Of 630 eligible women 558 (88.6%) were enrolled (including 21 enrolled twice) and 72 (11.4%) declined. A log was kept of reasons for not enrolling and declines. Most common reasons for ineligibility were: not requiring examination (36.7%), recent antibiotic use (13.5%) and menstruation/ pregnancy (12.8%).

**Data collection and laboratory methods**

The cervix was visualized using a sterile speculum and excess exudate removed using a non-sterile cotton swab before taking four endocervical sterile cotton swabs (Copan California). The first endocervical swab was taken for Gram stain to define women as having cervicitis or not by the working definition >30 pmnl in 3 non-adjacent high-powered fields and for NG culture on selective media (lysed horse blood agar containing vancomycin, colistin, nystatin and trimethoprim (VCNT)). A second endocervical swab was collected for *Chlamydia trachomatis* (CT) / *Neisseria gonorrhoea* (NG) PCR testing (Amplicor CT/NG Test (Roche)). NG positivity was defined by positive culture +/- PCR test result. NG Culture was routinely performed in the clinic at the time, with PCR results carrying less reliability in the low prevalence setting. The third endocervical swab was taken for multiplex PCRs for MG, *M hominis* (MH), *Ureaplasma urealyticum* (UU), *Ureaplasma parvum* (UP), HSV1, HSV2, EBV, VZV, CMV and TV. A 4th endocervical swab was also taken to store for any future testing as required. Swabs three and four were transported in viral 2SP viral transport medium (0.5 ml
sucrose phosphate + vancomycin maintenance buffer) and batch tested non real-time. All samples were processed at the research laboratory within 48 hours of being taken.

A high vaginal swab was taken for Gram stain and candida culture and wet preparation for TV if clinically suspicious (see chapter 3). Bacterial vaginosis (BV) was defined on Gram stain using Nugent’s criteria. Laboratory scientists were blinded to clinical information.

From June 2008 onwards, women at the Short Street site were also asked to submit an additional first-void urine sample to be tested in tandem. Results for a pathogen were taken to be positive if they were positive on endocervical testing, urine testing or both from this time onwards. Comparison of the pathogen detection from endocervical and urine sample testing will be the topic of a future publication.

Gram stain slides from consultations were verified for diagnosis of cervicitis and BV at a later time by a laboratory scientist blinded to clinician findings (Christa McPherson at St George Hospital SEALs Microbiology). All discrepant slides were re-examined and consensus Gram stain cervicitis diagnosis reached. The cervicitis diagnosis >30 pmnl/hpf was robust, 87.2% of clinician Gram stain slides being examined by the laboratory scientist and high agreement between clinician and laboratory diagnosis of cervicitis or not (kappa 0.79 (95% CI 0.74-0.84)

Testing for high risk Human Papilloma Virus (HPV) subtypes 16, 18, 31, 33, 35, 39, 45, 51, 56, 58, 59, 68 (Human Genetic Signatures High Risk HPV Detection Kit, Sydney, Australia) was performed in a subset (n=191) of women as we had access to a limited number of kits from the manufacturers. This test was in the process of being TGA- approved at the time of the study. HPV was included based on biological plausibility of a potential role in inflammation of the cervix and literature support and is discussed further in Chapter 5.
Clinical and history information was collected from clinical history sheets, modified for the purposes of the study. Yellow discharge was recorded if this was seen on examination of the cervix (yellow staining of a white cotton swab) or a woman complained of yellow discharge. Mucopurulent discharge (MCP) (subjective clinician observation of purulent looking cervical secretions) and ectropion (visibility of the squamocolumnar junction) were recorded if this was the noted on the cervix by the examining clinician.

Data was collected on presence of any genital symptoms and potential non-infectious etiological exposures including age group, current smoking, commercial sex work (CSW) and douching status, extent of condom use (always/sometimes/never) and number of sexual partners (1, >1) in the last three months, timing of last intercourse within the last week or >1 week ago, current use of combined oral contraceptive (COC), injectable depot-medroxyprogesterone acetate (DMPA) /Progestagen only pill (POP) or Implanon, phase of menstrual cycle (follicular/luteal) and stated history of past chlamydia infection and abnormal Pap smear in the preceding two years.

Male partners of women diagnosed with cervicitis were also offered testing with standard clinic tests of chlamydia and gonorrhoea plus by consent, dedicated study multiplex PCR tests, all from a first void urine sample. Samples were collected from 52 male partners.

**The Treatment Sub-study**

All women diagnosed with cervicitis were offered presumptive treatment with azithromycin 1 G PO stat on the day of assessment and follow-up at 6 to 8 weeks for re-testing for CT/NG, study multiplex PCRs and assessment for persisting cervicitis and persistence of any genital symptoms. If NG or TV were diagnosed at initial assessment by on-site microscopy, women
were treated accordingly with ceftriaxone 500mg IM or tinidazole 2G PO respectively and not offered presumptive azithromycin.

In keeping with clinic practice at the time women were not actively recalled unless they required a test of cure or treatment for an identified pathogen. Time to follow-up of some women with positive study multiplex results was delayed due to batch testing but all CT/NG results were acted upon real time.

Cervicitis was defined as >30 pmnl/hpf with or without known pathogens. NSC was defined as >30 pmnl/hpf in the absence of CT, MG, NG or TV (pathogens found to be significantly associated with cervicitis from the Cervicitis Study and literature review). Women with positive results for CT, MG, NG or TV (ie persistent or interim incident infection) at follow-up were excluded from the follow-up analysis.

Follow-up outcomes were defined as persistence of cervicitis by the same cervicitis definition, >30pmnl/hpf on cervical Gram stain or report of any genital symptoms. All participants diagnosed with STIs during the study were contacted and offered treatment and follow-up as required according to clinic guidelines.

Male partners of women with cervicitis were also offered presumptive treatment with 1 G azithromycin and study testing if they attended, with discussion concerning the lack of a firm guideline in this area. Women diagnosed with cervicitis were advised of the uncertainty of benefit of presumptive treatment for themselves and presumptive partner treatment. Women who returned for follow-up were asked if they had taken the azithromycin treatment supplied as directed and if their sexual partner(s) had taken this same treatment (‘partner treatment’).
Statistical methods and databases

I collected all clinical data from patient notes onto temporary clinical datasheets, and then entered this into a clinical Excel database with some clerical assistance from Helen Rayner at the Short Street Centre. I then manually transferred data from the laboratory database into a final combined database for analysis in SAS.

Associations of infectious and non-infectious exposures with cervicitis (the ‘disease’) were estimated using relative risks (RR) and $\chi^2$ testing. As this was a cross-sectional study and the outcome of cervicitis very common, RRs were calculated rather than odds ratios as the most valid measure of association. Relative risks were estimated by log-binomial regression. Multivariate analysis involved forward selection of covariates using exposures from the univariate analysis with $p < 0.05$. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the ‘test’ (different cervicitis definitions >30 pmnl/hpf, yellow discharge, mucopurulent discharge) for the significant pathogens, were calculated using 2x2 tables.

Sample size calculations indicated an ideal and logistically possible sample size of 600 women and this was powered (80%, $p<0.05$) to detect RR of 3.0 (assuming cervicitis prevalence of 20% and pathogen prevalence 5%) or RR 2.4, assuming pathogen prevalence of 10%. A review of findings at sample size of 558 indicated that relative risks were static and that sample size was sufficient for the primary study aim. Thus, at this point recruitment was halted.

For the treatment sub-study analysis, differences between baseline characteristics of women with cervicitis who were followed up or not, and of women with cervicitis followed
up who had azithromycin treatment or not, were assessed using χ² tests. The effect of presumptive treatment of women with cervicitis and the additional effect of partner treatment were estimated using relative risks (RR) and 95% confidence intervals (CI). All calculations were performed with SAS version 9.3 (SAS Institute, Cary, NC USA).

ETHICAL APPROVALS

Ethics approval for the three clinical sites was granted by the South Eastern Sydney and Illawarra Area Health Service Human Research Ethics Committee (Short Street Clinic) and the Sydney South West Area Heath Service Ethics Review Committee (RPA and Liverpool clinics). Copies of study participant approach, information and consent forms are included at the beginning of the thesis appendix.

REFERENCES


CHAPTER 3

*Trichomonas vaginalis*: under-diagnosis in urban Australia could facilitate re-emergence

As published in *Sexually Transmitted Infections* 2010;86(3):227-30
FOREWORD

This chapter comprises the second publication from the Cervicitis Study and was published in *Sexually Transmitted Infections* in early 2010, but appeared initially online November 2009 and received the ‘Editor’s Choice’ award.

This paper presents interim findings from the Cervicitis Study from the data of 356 women and highlighted the discrepancy in *Trichomonas vaginalis* case detection between the traditional diagnostic method of discretionary wet mount preparation verses PCR detection method, which was found to be four to five fold more effective in detecting *Trichomonas vaginalis*. We also found a significant association between cervicitis and TV and found TV to be significantly more common in women with culturally and linguistically diverse cultural backgrounds.

TV has tended to be the forgotten STI, particularly in urban environments in developed countries. This paper aimed to raise the profile of TV in the urban Australian setting given the important potential impacts of this infection upon adverse health outcomes in women including preterm birth and enhanced risk of HIV transmission, not forgetting troublesome symptoms for many.

We suggest that TV is likely to be under-diagnosed presently, which could pose a threat to the re-emergence of this infection and accordingly recommended development of commercially available PCR tests for TV and wider uptake of these tests.
AUTHOR CONTRIBUTIONS:

MJ Lusk was the principal and corresponding author.

MJ Lusk and PK designed and implemented the clinical research project, collected clinical samples and clinical data and supervised designated clinicians in sample and data collection, undertook data entry and statistical analysis. PK assisted in writing the manuscript. ZN, NR, BR, CJM and WDR processed and stored samples and undertook the PCR testing of the clinical samples and contributed to writing the manuscript (laboratory methods section).

RGC assisted with the design of the study, choice and of statistical methods, and contributed to reviewing the manuscript. KM assisted with database management and statistical analysis and reviewed manuscript.
Trichomonas vaginalis: under-diagnosis in urban Australia could facilitate re-emergence

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Sexually Transmitted Infections 2010;86(3):227-30

ABSTRACT:

Objectives: Trichomonas vaginalis (TV) has a low profile in urban sexually transmitted infection (STI) clinics in many developed countries. The objective of this study was to determine true prevalence of TV in an Australian urban sexual health setting using sensitive molecular diagnostic techniques.

Methods: A cross-sectional study investigating the etiology of cervicitis in women attending two urban sexual health clinics in Sydney, Australia, enrolled 356 consecutive eligible women from 2006-2008. The diagnostic yield from the standard clinical practice of discretionary high vaginal wet preparation microscopy in women with suspicious vaginal discharge was compared with universal use of nested polymerase chain reaction (PCR) for TV of cervical samples.

Results: TV was detected by PCR in 17/356 women (4.8%, 95% Confidence Interval (CI) 2.8-7.5%), whereas only 4 cases (1.1%, 95% CI 0.3-2.8%) were detected by discretionary wet preparation microscopy. Eleven of the 17 women (p=0.003) were of culturally and...
linguistically diverse (CALD) background. Additionally, cervicitis was found to be significantly associated with TV, RR 1.66(1.14-2.42), p=0.034

Conclusions: Traditional TV detection methods underestimate TV prevalence in urban Australia. The TV prevalence of 4.8% by PCR testing in this study exceeds previously reported urban Australian TV rates of <1%. An increase in trichomoniasis-associated adverse reproductive outcomes and enhanced HIV transmission poses a salient public health threat. Accordingly TV warrants a higher profile in urban STI clinic settings in developed countries and we suggest priority be given to development of standardized molecular TV detection techniques and that these become part of routine STI testing.

Key Messages

- We report a higher than previously reported prevalence of *Trichomonas vaginalis* (TV) of 4.8% by PCR methods in an urban Australian STI clinic setting.

- TV is likely under-diagnosed in urban STI clinic settings using only traditional methods of detection. This may presage re-emergence with important Public Health consequences.

- We find TV is significantly associated with women of culturally and linguistically diverse (CALD) background, and cervicitis is significantly associated with TV (RR 1.66, p=0.034).

- TV warrants a higher profile. Priority should be given to the development of standardized molecular TV detection techniques for inclusion in routine STI testing.
**INTRODUCTION**

*Trichomonas vaginalis* (TV) is a sexually transmitted infection (STI) causing significant morbidity worldwide. Trichomonads are highly site-specific protozoan parasites. In women, TV infects the lower urogenital tract, causing superficial vaginal and cervical ulceration. Typical symptoms include frothy yellow discharge, itch, odour, dyspareunia and occasionally vaginal bleeding. Infection of the urethra and para-urethral glands causes dysuria and frequency \(^1\). However, at least one third of infected women may be asymptomatic \(^2\). Trichomoniasis has been associated with premature rupture of membranes \(^3\), pelvic inflammatory disease (PID) \(^4\) cervicitis \(^5,6\) and enhanced risk of HIV transmission \(^7\). Men may present with balanitis, urethral discharge or dysuria, but again, high rates of asymptomatic carriage have been reported. A recent US STI clinic study detected TV in 72% of male partners of infected females and of these men, 77% were asymptomatic \(^8\). The duration of TV infection in women may be prolonged for up to 3-5 years but only about four months in men \(^9\). The natural history of TV infection is not well defined.

Trichomoniasis is the most common curable sexually transmitted infection. In 1999, WHO estimated 174 million new cases per year, more than double the number of *Chlamydia trachomatis* cases and treble the cases of gonorrhoea. The high TV prevalence worldwide is concentrated in developing countries and socioeconomically disadvantaged groups, with a dramatic decline in TV rates in some developed countries in the past few decades \(^10\). TV prevalence in urban Australia is reportedly low based upon routine wet preparation diagnostic method \(^9,10\) Australian rates of TV peaked in the 1950s at 20-30% and rapidly declined through the 1960s and 1970s to below 1% in 1990 \(^10\). This has been attributed to
the combination of widespread use of the Nitroimidazoles, and increased surveillance through Papanicolaou smears\(^9,10\). This decline and the fact that TV is not a notifiable disease in Australia have led to the present situation where testing for TV has assumed a very low priority in urban Australian settings. An audit of commercial sex workers (CSW) undergoing regular STI screening at a sexual health clinic in Melbourne in 2003 reported a very low incidence of TV at 0.11 per 100 person months\(^11\). By contrast, TV prevalence in indigenous Australian women, in remote Northern Territory, was high at 25% in a self-sampling PCR method based study\(^12\). Prevalence of TV by PCR amongst US women of reproductive age was recently found to be 3.1%, with an even lower prevalence of 1.3% in the sub-group of non-Hispanic white women\(^13\).

We are conducting a prospective study investigating the prevalence and etiology of cervicitis using molecular diagnostic techniques in women attending two urban STI clinics in Sydney, Australia. In this paper we report the prevalence of TV by PCR testing against traditional methods of detection, and compare our findings to previous data from other Australian urban STI clinics.

**METHODS**

This study was conducted in two urban Sydney STI clinics from July 2006-December 2008. Ethics approval was granted by the South Eastern Sydney and Illawarra Area Health Service Human Research Ethics Committee and the Sydney South West Area Heath Service Ethics Review Committee (Royal Prince Alfred Hospital Zone).
Subject selection

Women were eligible for this study if they were 18 years or older, had been sexually active in the previous three months and required an internal examination. The parent cervicitis study protocol excluded women if they had been previously enrolled, had clinical pelvic inflammatory disease (PID), had received antibiotics or undergone gynaecological intervention in the previous month, had an intrauterine contraceptive device, were currently menstruating or pregnant, attending about sexual assault or unsuitable for enrolment due to psychosocial ill health or comprehension difficulties. Of a total of 957 consecutive first attendances, 561 women (58.6%) were ineligible 40 (4.2%) declined and 356 (37.2%) were enrolled. Most common reasons for ineligibility included examination not required (30.7%), antibiotics in previous month (14.2%), pregnancy (8.6%) and no sex in previous 3 months (8.3%). Women with clinical PID comprised 3.1% of ineligible women. This report is an interim analysis of 356 consecutive first attendances of women enrolled in the parent cervicitis study.

Sampling procedure

A sterile speculum was used to visualize the vagina and cervix. Gram stain was performed on all cervical and high vaginal swabs (HVS). Due to the nature of the parent study, HVS for wet preparation microscopy was discretionary based upon clinical suspicion of TV infection, in keeping with usual clinical practice, and so not performed for all women. The endocervix was sampled in all women, with a sterile cotton swab (Copan, CA, USA) for Gram stain and bacterial agents. An additional endocervical sterile cotton swab was taken and placed in viral transport medium 199 (GIBCO Invitrogen, NY, USA) and stored at -70°C for subsequent
PCR testing for TV by the method described below. Swabs from all women were tested for TV by PCR.

Cervicitis was defined as >30 polymorphonuclear cells per high-powered field (pmnl/hpf) in at least 3 non-adjacent fields of cervical mucus on Gram stain of the first endocervical swab. Bacterial vaginosis (BV) was defined by Nugent score from Gram stain of the HVS.

Nucleic acid extraction and PCR amplification

Swabs were suspended in 500µl of viral transport medium (above) before extraction of the total nucleic acid using a robotic extraction machine (MagNaPure LC, Roche, Germany) applying the Total NA protocol according to the manufacturer’s instructions (Roche, Germany). Extracts were stored at 4°C before testing within 48 hours of collection.

Detection of TV was performed using a nested PCR. Briefly, the first round reaction comprised 10µl of template in a 50µl of PCR reaction mixture containing 9µl of Nuclease-free water, 25µl of 2x iScript reaction mix (BioRad, Australia), 0.5µM each primer: TricV-OF (5’ CTATTGTGAACTGTTACCCCTAC 3’) and TricV-OR (5’ TCTGCTGCACTTACCCCTAC 3’) and 1µl of iScript RT enzyme (BioRad, Australia). (This commercial master mix is used in our laboratory for the amplification of both RNA and DNA templates). Cycling conditions were 50°C for 30 min; denaturation at 95°C for 15 min, then 35 cycles of 94°C for 45 secs, 57°C for 45 secs, and 72°C one min; a final extension of 7 min at 72°C; and a 4°C hold. A second round reaction comprised of 2µl first-round product included in a 50µl reaction mixture containing 18µl of nuclease-free water, 25µl of Ampliataq gold PCR Master Mix (Applied Biosystems), and 0.5µM each primer: TricV-IF (5’ CTCAGGTCAAAGGCACTTACCTTGAA
3’) and TricV-IR (5’ GCTTGGAGGACATGAACTTCGGA 3’)\(^{14}\). Cycling conditions included denaturation and activation at 95°C for 5 min, 33 cycles of: 94°C for 20 secs, 57°C for 20 secs, 72°C for 20 secs; a final extension at 72°C for 10 min and a 4°C hold. PCR products of 206 bp were expected for *Trichomonas vaginalis* positives and were visualised by gel electrophoresis.

Using positive controls from either culture-proven or molecularly proven sources, sensitivity was assessed by measuring the limit of detection (10\(^2\) copies per reaction) of plasmid constructs of the target sites as previously described\(^{14}\) and is estimated to be between 95 to 98%. The specificity was determined as follows: confirmation by probe hybridization following the PCR amplification, and DNA sequencing was performed on PCR products of the first 10 TV positive samples from the study. Nucleotide BLAST on NCBI site (for all 10 samples) confirmed that all DNA sequences produced from sequencing were *Trichomonas vaginalis*. The results from above methods confirmed the assay has 100% specificity.

**Analysis**

We report the prevalence with 95% confidence intervals of TV by traditional methods and PCR methods. Population characteristics of women with and without TV were compared using Chi-square testing. P values < 0.05 were considered statistically significant. Data were analyzed with SAS software, SAS Institute Inc. USA.
RESULTS

Prevalence of TV by PCR testing was 17/356 (4.8%, 95% CI 2.8-7.5%). Clinical suspicion prompting discretionary wet mount preparation microscopy identified TV in only 4/356 women (1.1%, 95% CI 0.3-2.8%). PCR identified a higher percentage of women with TV (p=0.0003).

Of the 17 women positive for TV by PCR testing, only 11 had wet preparation microscopy performed. Use of discretionary wet preparation was not significantly different in women with and without TV by PCR (p=0.498). Detection by Papanicolaou smear occurred in only 2 of the 11 pap smears done in women with TV. The mean age of the women with TV, 33.2 years, was not significantly different from the mean age of women without TV, 30.7 years (p=0.221) (Table 1).

Significantly, 11/17 women with TV (p=0.003) were of culturally and linguistically diverse (CALD) background and identified consorts from populations of higher TV prevalence overseas (Africa, China, Sri Lanka, South America, Lebanon, Black American). Three women identified rural or ‘bush’ Australian contacts. Five women were commercial sex workers (CSW). No cases of TV were indigenous women. Six cases had bacterial vaginosis (BV) and three had concurrent STIs (two with Chlamydia and one with HIV and active genital herpes). Dysuria was significantly associated with women with TV (p=0.014). Prevalence of cervicitis in women without TV was 39%, compared to 65% (11/17) in women with TV, giving a RR of cervicitis in the presence of TV of 1.66 (95% CI 1.14, 2.42) P=0.034.
TABLE 1. Characteristics of women with and without *Trichomonas vaginalis* by PCR

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Women with TV n=17</th>
<th>Women without TV n=339</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (yrs)</td>
<td>33.4</td>
<td>30.7</td>
<td>0.221</td>
</tr>
<tr>
<td>CALD ¹</td>
<td>11 (65%)</td>
<td>104 (31%)</td>
<td>0.003</td>
</tr>
<tr>
<td>Condoms always</td>
<td>4 (24%)</td>
<td>75 (22%)</td>
<td>0.892</td>
</tr>
<tr>
<td>&gt;1 partner last 3 mth</td>
<td>6 (35%)</td>
<td>95 (28%)</td>
<td>0.516</td>
</tr>
<tr>
<td>CSW</td>
<td>5 (29%)</td>
<td>58 (17%)</td>
<td>0.195</td>
</tr>
<tr>
<td>Concomitant STI ²</td>
<td>2 (12%)</td>
<td>24 (7%)</td>
<td>0.397</td>
</tr>
<tr>
<td>Signs &amp; symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dysuria</td>
<td>7 (41%)</td>
<td>59 (17%)</td>
<td>0.014</td>
</tr>
<tr>
<td>Vaginal discharge</td>
<td>11 (65%)</td>
<td>143 (42%)</td>
<td>0.067</td>
</tr>
<tr>
<td>Bacterial vaginosis ³</td>
<td>6 (35%)</td>
<td>79 (23%)</td>
<td>0.258</td>
</tr>
</tbody>
</table>

*p values <0.05 statistically significant at the 95% confidence level.

1. CALD (culturally and linguistically diverse) defined as women identifying at clinic registration as preferring a language other than English, speaking a language other than English at home, or identifying non English Ethnic background.

2. Concomitant Chlamydia or Gonorrhoea

3. Defined by Nugent’s score on Gram stain of high vaginal swab.
DISCUSSION

Our data show that traditional methods of detection greatly underestimate the prevalence of *Trichomonas vaginalis*. We report a TV prevalence of 4.8% by PCR testing whereas clinical suspicion prompting discretionary wet preparation microscopy identified TV in only 4/356 women (1.1%) in the same study population. The lower case detection rate by discretionary use of wet preparation microscopy (and Pap smear) observed here reflect the inadequacy of relying solely upon these methods for detecting TV. Wet mount microscopy has sensitivity as low as 52% depending on specimen handling, skill of the microscopist and TV organism load in specimen. Additionally, wet preparation microscopy is not routinely performed even in many STI clinic settings and laboratories.

These TV prevalence rates by PCR are higher than previously reported rates of <1% in other urban Australian STI clinic studies, sampling CSWs using traditional diagnostic methods. This difference could signal a true rise in TV prevalence or be attributed to the more sensitive PCR diagnostic techniques employed here as well as sampling a less clinically homogeneous, highly screened population than those represented in previous literature. Women recruited to the study were a convenience sample which was biased towards symptomatic women in an STI service; factors that likely contributed to the high prevalence of both *Trichomans vaginalis* and cervicitis.

However due to the relatively low population prevalence of TV, the positive predictive value of these PCR findings may be reduced. On the other hand these PCR data may potentially underestimate the true prevalence due to the strict inclusion criteria of the parent study protocol and retrospective PCR testing of stored, frozen and thawed
specimens. Cervical specimens were used for this study, but performance of cervical and vaginal specimens for TV PCR testing has been shown to be comparable.

An important finding in our study was the high proportion (11/17, P= 0.003) of women with TV who were of CALD backgrounds. Urban society in Australia, as in many other industrialized countries, is very culturally diverse with a highly mobile population. Accordingly sexual networks, particularly of migrant and first generation Australians often include consorts who come from or travel to populations with higher TV prevalence. We found the symptom of dysuria to be significantly associated with women with TV, highlighting the need for clinicians to consider the possibility of TV especially in the absence of other causes of dysuria. We did not find an association between TV and bacterial vaginosis although others have. BV may mask the presence of TV. Standard BV treatment with nitroimidazoles will effectively eradicate co-existent TV infection however partners of women with concomitant BV and TV will remain untreated, potentially facilitating the persistence and spread of TV.

Cervicitis was associated with TV (RR 1.66, p=0.034) supporting the role of TV in the etiology of cervicitis. Non-gonococcal, non-chlamydia cervical, or non-specific cervicitis (NSC) comprises a major proportion of reported cervicitis and TV has been recently recognized as an etiological agent in this condition. Molecular testing for TV in STI populations is likely to shed further light on the etiology of NSC in women, a current area of research need.

Co-infection with another STI, including TV, remains a significant risk factor for HIV acquisition. Women increasingly carry the burden of the HIV epidemic in many developing
and westernized countries. As many screening programs are targeting younger women, older groups of sexually active women where TV predominates may be overlooked, potentially contributing to the TV reservoir in the community. The recent rise in gonorrhoea in women in some industrialized nations and noted in our own region may indicate relaxation of safer sex practices, possibly presaging a rise in STIs with re-establishment in these sexually active populations and increased opportunity for HIV transmission.

If not specifically sought, TV will not be detected. Presently sensitive, standardized detection methods for TV are seldom commercially available, relying on in-house laboratory molecular methods. This coupled with the low profile of TV has led to infrequent uptake of any testing for TV in many clinical settings. Our findings suggest that when sensitive diagnostic tests are routinely or selectively applied TV may be found to be more prevalent than previously thought. These concerns echo those of others that priority be given to developing standardized, sensitive, cost-effective techniques for TV detection.

In summary, we report a higher than previously reported prevalence of TV, by PCR testing, in an urban Australian STI clinic population. We suggest the current low profile of TV and the variable application of insensitive tests for case detection, have led to under-diagnosis of TV. In turn this could facilitate a re-emergence of TV particularly in culturally diverse urban populations. Trichomoniasis has the potential to impact significantly on reproductive health of women and increase HIV transmission. Accordingly TV warrants a higher profile in urban settings in developed countries. With this demonstration of higher than expected TV prevalence, a call is justified for the development and application of standardized PCR TV detection techniques to facilitate case-finding, surveillance and continued control of this STI.
REFERENCES


5. Marrazzo JM, Martin D. Management of women with cervicitis. CID 2007; 44 (suppl 3):102-110


CHAPTER 4

*Mycoplasma genitalium* is strongly associated with cervicitis and *Mycoplasma hominis* with bacterial vaginosis in an urban Australian STI clinic population.
FOREWORD

This chapter presents a paper based on Cervicitis Study interim findings from 527 women, being the original paper submitted to the journal *Sexually Transmitted Infections*, which was shortened to a Short Report format for publication, focusing on the findings concerning MG. The Short Report appears in the Appendix of the thesis as published in *Sexually Transmitted Infections* in 2011.

One of the initial curiosities of the Cervicitis Study was to explore the group of bacteria called the mollicutes, which include the organisms *Mycoplasma genitalium* (MG), *Mycoplasma hominis* (MH), *Ureaplasma urealyticum* (UU) and *Ureaplasma parvum* (UP). MG has become the organism of most interest, emerging as important genital tract pathogen but UU has also been implicated as a genital tract pathogen with more evidence of its role in men. Both MH and UU have been implicated in cervicitis and bacterial vaginosis. The significance of UP is not defined.

The collaborating Scientists developed a multiplex PCR test for the four mollicutes, which was used in this study (see McIver’s method paper Part 3, Appendix).

This paper was designed to examine the role of these commonly found genital tract agents, and to clarify their associations with the common conditions of cervicitis and bacterial vaginosis. It presents material on the association of the mollicutes with BV that is currently unpublished elsewhere.
AUTHOR CONTRIBUTIONS

MJ Lusk was the principal and corresponding author.

MJ Lusk, PK, RGC and WDR designed the study. MJ Lusk and PK co-ordinated the study and supervised recruitment. ZWN and WDR developed and carried out the PCR testing. MJ Lusk performed the statistical analysis and interpretation with input from FLG and RGC. All authors contributed to and reviewed the final manuscript.
Mycoplasma genitalium is strongly associated with cervicitis and Mycoplasma hominis with bacterial vaginosis in an urban Australian STI clinic population

ABSTRACT

Objectives: To elucidate the prevalence and associations of the genital mollicutes, Mycoplasma genitalium (MG), Mycoplasma hominis (MH), Ureaplasma urealyticum (UU) and Ureaplasma parvum (UP) with cervicitis and bacterial vaginosis (BV) and to characterize the clinical associations of MG in a Sydney STI clinic population.

Methods: 527 women were enrolled in a cross-sectional study at two Sydney Australian Sexually Transmitted Infection (STI) services. Genital mollicutes were detected by multiplex PCR testing of cervical swabs and associations with cervicitis and bacterial vaginosis (BV) analysed.

Results: MG was found in 4.0% of women, MH in 17.1%, UU in 14.1% and UP in 51.8%. MG was the only mollicute associated with cervicitis (RR 1.85, 95% CI 1.52-2.26, P<0.0001). MG was significantly associated with women being HIV positive (P=0.033) but not with age, vaginal discharge, commercial sex work, being of culturally and linguistically diverse background or concurrent chlamydia infection. Two of the 21 women with MG had ectopic pregnancies. MH was significantly associated with BV. Women with MH were almost four
times more likely to have BV than women without MH (RR 3.61 (95% CI 2.80-4.65) P<0.0001). None of the other genital mollicutes were significantly associated with the presence of BV.

**Conclusions:** We recommend wider application of PCR testing for *Mycoplasma genitalium* in STI services in high-risk women, particularly those with cervicitis or HIV infection. Further longitudinal studies of the impact of MG infection upon female reproductive health are needed.
INTRODUCTION

Substantial evidence implicating *Mycoplasma genitalium* (MG) as a genital tract pathogen has emerged in the last decade. However the potentially pathogenic roles of the other genital mollicutes, *Mycoplasma hominis* (MH), *Ureaplasm a urealyticum* (UU) and *Ureaplasm a parvum* (UP) are less well characterized. The consequences of any mollicute infection for female reproductive health require clarification.

The mycoplasmas and ureaplasmas belong to the class of mollicutes. There are 16 species detected in humans, of which seven are primarily found in the urogenital tract\(^1\), the four of most interest being MG, MH, UU, UP. Occasionally these may be found in the oropharynx due to orogenital contact and transient colonization of the neonate may occur during passage through the birth canal. However, establishment of the mollicutes in the urogenital tract occurs after sexual debut.

Of the genital mollicutes, MG has been most strongly implicated as a sexually transmitted infection (STI) with associations with cervicitis\(^2-6\), endometritis\(^7\), pelvic inflammatory disease (PID)\(^8\), non-gonococcal urethritis\(^9\), tubal factor Infertility\(^10\) and preterm birth\(^11\). Cervicitis is common particularly in STI clinic populations and has been shown to be associated with endometritis, pelvic inflammatory disease (PID)\(^12\), adverse pregnancy outcomes\(^13\) and enhanced transmission of HIV infection\(^14\). However less than half of cervicitis cases are associated with chlamydia or gonorrhoea; the remainder are termed non-specific cervicitis (NSC). Aetiological organisms implicated in NSC include *Trichomonas vaginalis*, Herpes viruses, MG, MH and UU\(^15\).
There appear to be relationships between the genital mollicutes and bacterial vaginosis (BV). Mycoplasma hominis has been associated with BV \(^{1,16,17}\) but the roles of the other mollicutes in BV are unclear \(^{1,18}\). The pathogenic potential of BV and enhanced HIV transmission associated with BV, have been recently recognized \(^{19}\). However the pathogenic mechanisms of the genital mollicutes are to date, variably characterized \(^1\) and the clinician is faced with the uncertainty of the significance of detecting a mollicute in the female genital tract. Thus elucidation of their disparate roles as commensals or STI pathogens is of importance.

Using a cross sectional study of 527 attendees of three Australian STI services, we determined the prevalence of the genital mollicutes MG, MH, UU and UP and their associations with the conditions of cervicitis and BV and examined the clinical correlates of MG infection.

**METHODS:**

A cross-sectional study was undertaken in three urban Sydney STI clinics from 30 June 2006 to 31\(^{st}\) January 2010. Ethics approval was granted by the South Eastern Sydney and Illawarra Area Health Service Human Research Ethics Committee and the Sydney South West Area Heath Service Ethics Review Committee (Royal Prince Alfred Hospital Zone).

**Participant selection**

527 consecutive eligible consenting women were enrolled with 83% recruited from a primary study site. Inclusion criteria were women 18 years or older, sexually active in the previous three months and requiring or requesting an internal examination. Exclusion criteria were clinical PID, antibiotics or gynaecological intervention in the previous month,
an intrauterine contraceptive device, current menstruation or pregnancy, attendance concerning sexual assault or unsuitability due to psychosocial ill health.

Of 570 eligible women 527 consented (92.5%). Of women who were ineligible (889), the most common reasons were internal examination not required (33.2%), antibiotics in the previous month (12.6%), pregnancy (9.2%), not sexually active (7.9%) and sexual assault (6.0%). Thirty one women (3.5%) were excluded due to clinical PID.

**Sampling procedure**

The study required two cervical swabs in addition to routine clinic tests. Seven clinicians trained in study protocol recruited women to the study. A sterile speculum was used to visualize the vagina and cervix. A high vaginal swab (HVS) was taken for Gram stain and wet preparation. The endocervix was sampled in all women, with a sterile cotton swab (Copan, CA, USA) for routine gonorrhoea and chlamydia testing. An additional endocervical sterile cotton swab was taken and placed in viral transport medium 199 (GIBCO Invitrogen, NY, USA) and stored at -70°C for subsequent study PCR testing. Gram stain was performed on all cervical and HVS and these were examined real-time in the clinic laboratory for the presence of cervicitis, bacterial vaginosis, yeast and *T vaginalis*. Cervicitis was defined as >30 polymorphonuclear leucocytes per high-powered field (pmnl/hpf) in at least three non-adjacent fields of cervical mucus on Gram stain. Bacterial vaginosis was defined by Nugent score from Gram stain of the HVS. A single laboratory scientist blinded to clinical diagnosis later assessed clinicians’ cervical and vaginal Gram stains for diagnostic accuracy.

**Nucleic acid extraction and PCR amplification**

Nucleic acid extraction from swab specimens (suspended in 500 µl of viral transport medium) was carried out using a robotic extraction machine (MagNaPure LC, Roche,
Germany) applying the Total NA protocol according to the manufacturer’s instructions (Roche, Germany). Specimens were extracted within 48 hours of receipt, then stored and subsequently tested in batches.

Detection of mollicute species MG, MH, UU and UP was performed using a single-round multiplex PCR (mPCR) \(^{20}\) followed by PCR enzyme-linked immunosorbent assay (ELISA). VDL06 mPCR reaction comprised 10μl of template in a 50μl of PCR reaction mixture containing 14.8μl of Nuclease-free water, 14μl of 2x iScript reaction mix (BioRad, Australia), 0.4μM of each primer (Table 1), 0.2μl digoxigenin-11-dUTP (Roche, Germany), and 1μl of iScript RT enzyme (BioRad, Australia). (This is a commercial master mix used in our laboratory for the amplification of both RNA and DNA templates). Cycling conditions were 50°C for 30 min; denaturation at 95°C for 15 min, then 50 cycles of 94°C for 30 secs, 57°C for 45 secs, and 72°C one min; a final extension of 7 min at 72°C; and a 4°C hold. PCR products were visualized by gel electrophoresis, and the amplicons were identified by probe hybridization (Table 1) using the PCR ELISA assay (digoxigenin detection, Roche, Germany). Positive controls from either culture-proven or PCR proven sources were used as PCR controls.

Sensitivity of VDL06 mPCR was assessed by measuring the limit of detection \((10^3\) copies per reaction) of plasmid constructs of the target sites as previously described \(^{20}\) and is estimated to be between 95 to 98%. The specificity was determined by DNA sequencing on PCR products of the first 10 positive samples of each MG, MH, UU and UP from the study. Nucleotide BLAST on NCBI site (for all 40 samples) confirmed that all DNA sequences produced from sequencing were of correct mollicutes species. The results from DNA sequencing confirmed the assay has 100% specificity.
Table 1. Primers and probes used in identification of mollicute species (VDL06 mPCR)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Primer name</th>
<th>Size</th>
<th>Promer sequence 5' -&gt; 3'</th>
<th>Target gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycoplasma genitalium/hominis</td>
<td>My-ins</td>
<td>520</td>
<td>GTA ATA CAT AGG TCG CAA GCG TTA TC</td>
<td>16S tRNA gene</td>
</tr>
<tr>
<td></td>
<td>MGSO-2-B</td>
<td>520</td>
<td>CAC CAT CTG TCA CTC TGT TAA CCT C</td>
<td></td>
</tr>
<tr>
<td>Ureaplasma parvum</td>
<td>UMS-170</td>
<td>396</td>
<td>GTA TTT GCA ATC TTT ATA TGT TTT CG</td>
<td>Multiple Banded Antigen Gene</td>
</tr>
<tr>
<td></td>
<td>UMA222</td>
<td></td>
<td>GTTA GCA GCA TTA AAT TCA ATG</td>
<td></td>
</tr>
<tr>
<td>Ureaplasma urealyticum</td>
<td>UMS-170</td>
<td>476</td>
<td>GTA TTT GCA ATC TTT ATA TGT TTT CG</td>
<td></td>
</tr>
<tr>
<td></td>
<td>UMA263</td>
<td></td>
<td>TTT GTT GTT GCG TTT TCT G</td>
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</table>

<table>
<thead>
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<th>Organism</th>
<th>Probe name</th>
<th>Probe sequence 5' -&gt; 3'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycoplasma genitalium/hominis</td>
<td>Mgen-P3-Am</td>
<td>bTCGGAGCGATCCCTTCGTT</td>
</tr>
<tr>
<td>Mycoplasma hominis</td>
<td>Mhom-P10-Am</td>
<td>bGACACTAGCAAACATAGGTT</td>
</tr>
<tr>
<td>Ureaplasma parvum</td>
<td>UPPROBE1</td>
<td>bCTGAGCTATGACATGGAGTTACC</td>
</tr>
<tr>
<td>Ureaplasma urealyticum</td>
<td>UUPROBE1</td>
<td>bCTGAATTCAATGTTGCAATACATCAGCTGA</td>
</tr>
</tbody>
</table>

Statistical methods

The prevalence and exact 95% confidence intervals (CI) of mollicutes and bacterial vaginosis in the study sample were calculated. The associations of mollicutes with BV and cervicitis were estimated using relative risks (RR), 95% confidence intervals and the associated P value. As this was a cross-sectional study and the outcomes of cervicitis and bacterial vaginosis were both common, we calculated RR rather than odds ratios as the most valid measure of association. Comparisons between woman with and without MG were made using chi-squared tests and t-tests. All analyses were performed with SAS version 9.2 (SAS
Institute, Cary, NC USA). The Genmod procedure was used to enable analysis of RR using SAS.

RESULTS

Of women enrolled, 40.6% presented as asymptomatic and 59.4% had symptoms. MG was found in 4.0% of women, MH in 17.1%, UU in 14.1% and UP in 51.8% of women (Table 2). MG was the only mollicute found to be associated with cervicitis (RR 1.85 95% CI 1.52-2.26, p<0.0001). The prevalence of BV was 25.5% and BV was not associated with cervicitis. Examining the relationship between the genital mollicutes and BV (Table 2), women with MH were almost four times more likely to have BV than women without MH (RR 3.61 95% CI 2.80-4.65, p<0.0001). None of the other genital mollicutes were significantly associated with BV.

Correlates of MG infection were examined (Table 3). MG was significantly associated with cervicitis (p<0.0001) and with women being HIV positive (p=0.033). There was no association between MG and age, vaginal discharge, commercial sex work (CSW), being culturally and linguistically diverse (CALD) or concurrent chlamydia infection (prevalence 5.7% in our study sample). It was noted that two of the 21 women with MG had ectopic pregnancies near the time of recruitment.

Mollicutes were found in 346/527 (65.7%) women and of these women 95/346 (27.5%) had more than one mollicute detected (Table 4). Of the mollicutes, UP occurred significantly more often (p<0.0001) as a single mollicute infection (72% of UP infections).

The prevalence of cervicitis was 47.7%. Agreement between the clinician and laboratory scientists’ Gram stain diagnosis of cervicitis was high at 85.4% (95% CI 80.1-90.0).
Table 2. Prevalence and associations of Mollicutes with cervicitis and BV

<table>
<thead>
<tr>
<th>Bacterial Exposure</th>
<th>Prevalence (%) (95%CI)</th>
<th>RR of cervicitis</th>
<th>p value</th>
<th>RR of BV</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>M genitalium</td>
<td>21/527 4.0% (2.5-6.0)</td>
<td>1.85 (1.52-2.26)</td>
<td>&lt;0.0001</td>
<td>1.11 (0.56-2.22)</td>
<td>0.764</td>
</tr>
<tr>
<td>M Hominis</td>
<td>90/527 17.1% (14.0-20.6)</td>
<td>1.00 (0.79-1.27)</td>
<td>0.993</td>
<td>3.61 (2.80-4.65)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>U. Urealyticum</td>
<td>74/527 14.1% (11.2-17.3)</td>
<td>0.96 (0.73-1.25)</td>
<td>0.747</td>
<td>1.38 (0.96-1.97)</td>
<td>0.080</td>
</tr>
<tr>
<td>U parvum</td>
<td>273/527 51.8% (47.4-56.1)</td>
<td>1.09 (0.91-1.31)</td>
<td>0.342</td>
<td>0.85 (0.64-1.14)</td>
<td>0.278</td>
</tr>
<tr>
<td>Bacterial vaginosis</td>
<td>134/527 25.5% (21.9-29.4)</td>
<td>1.01 (0.83-1.24)</td>
<td>0.889</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Characteristics of women with and without MG

<table>
<thead>
<tr>
<th></th>
<th>Women with MG (n=21)</th>
<th>Women without MG(n=506)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (yrs)</td>
<td>28.1</td>
<td>30.4</td>
<td>0.279</td>
</tr>
<tr>
<td>Vaginal discharge§</td>
<td>11</td>
<td>210</td>
<td>0.322</td>
</tr>
<tr>
<td>Cervicitis ¥</td>
<td>18</td>
<td>234</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>concurrent Chlamydia</td>
<td>2</td>
<td>28</td>
<td>0.443</td>
</tr>
<tr>
<td>Commercial sex work</td>
<td>3</td>
<td>92</td>
<td>0.666</td>
</tr>
<tr>
<td>CALD t</td>
<td>7</td>
<td>164</td>
<td>0.945</td>
</tr>
<tr>
<td>HIV positivity</td>
<td>2</td>
<td>11</td>
<td>0.033</td>
</tr>
<tr>
<td>Ectopic pregnancy</td>
<td>3</td>
<td>Unknown</td>
<td></td>
</tr>
</tbody>
</table>

*P value from a t-test for age and chi-squared tests for all other variables.

§ Symptom of vaginal discharge
¥ Cervicitis defined >30 polymorphonuclear leucocytes per high-powered field (pmnl/hpf) in at least 3 non-adjacent fields of cervical mucus on Gram stain

‡ CALD (culturally and linguistically diverse) defined as women indicating they speak a language other than English at home, or prefer to speak a language other than English, or identify a specific ethnic background that is non-English.
Table 4. Mollicute co-infections

<table>
<thead>
<tr>
<th>Mollicute(s) detected</th>
<th>Number of women (n=527)</th>
<th>% of total women</th>
<th>Proportion of Mollicutes as single infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>MG only</td>
<td>6</td>
<td>1.1</td>
<td>6/21 (28.6%)</td>
</tr>
<tr>
<td>MH only</td>
<td>19</td>
<td>3.6</td>
<td>19/90 (21.1%)</td>
</tr>
<tr>
<td>UP only</td>
<td>197</td>
<td>37.4</td>
<td>197/273 (72.2%)</td>
</tr>
<tr>
<td>UU only</td>
<td>29</td>
<td>5.5</td>
<td>29/74 (39.2%)</td>
</tr>
<tr>
<td>MG+MH</td>
<td>2</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>MG+UP</td>
<td>6</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>MG+UU</td>
<td>2</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>MG+UP+UU</td>
<td>2</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>MG+MH+UU</td>
<td>1</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>MH+UP</td>
<td>42</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>MH+UU</td>
<td>14</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>MH+UP+UU</td>
<td>10</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>UP+UU</td>
<td>14</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>MG+MH+UP+UU</td>
<td>2</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>181</td>
<td>34.3</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>527</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

In this study a prevalence of MG of 4.0% was detected. This is similar to other populations: 6.0% in a Swedish STI population $^{4,5}$ 7.0% in a Seattle STI population $^3$ and 4.5% in a Norwegian STI clinic setting $^{21}$. More locally, a study of under-25 year old women undergoing abortions in New Zealand found a MG prevalence of 8.7% $^{22}$. The prevalence of MG in this study is approaching that of Chlamydia, which has been noted in other STI clinic populations $^{4,5,23}$. Additionally 4.0 % MG prevalence is likely to be an underestimate in this population due to the strict inclusion criteria of the study.

While the prevalence of cervicitis by the case definition used was very high (47.7%), this is similar to other high-risk STI clinic populations $^2,21$. The strong agreement (85.4%) between the clinician and laboratory scientists’ Gram stain diagnosis of cervicitis suggests that we used a robust and reproducible case-definition.

MG was found to be to be significantly associated with cervicitis. None of the other genital mollicutes or bacterial vaginosis were associated with cervicitis (Table 2). As noted by Manhart $^{24}$ the evidence supporting the role of MG in cervicitis is conflicting. Studies supporting this association $^2-5$ have used variable definitions of cervicitis and methods of MG detection and some use archived specimens $^3$. There is little information on the potential associations between the Ureaplasmas and cervicitis.

When the relationships between the genital mollicutes and BV were analysed, women with MH were almost four times more likely to have BV than women without MH. (Table 2) This supports the purported role of MH as a marker for BV $^1,17$ but the extent to which MH is a pathogen in itself remains ill-defined $^{16}$. Although we found a strong MH-BV association, 27
of the 90 women (30%) with MH did not have concurrent BV, and our measurements for MH were qualitative rather than quantitative. Rosenstein’s group found that in higher grade BV, numbers of MH organisms increased dramatically \(^{17}\). In our study, there was no association of UU or MG with BV, which has been observed previously \(^3,^{18}\). However, others have reported associations of MG with BV \(^{25}\) and of UU with BV \(^{17}\). This study found UP to be very common (52%). Although not found to be associated with cervicitis or BV, UP was significantly more likely to occur as a single mollicute infection (72.2%) in contrast to the other mollicutes which were more likely to coexist with other mollicutes (p<0.0001). (Table 4) This raises the question of whether UP may have a protective probiotic effect with respect to infections with other mollicutes, although there is no literature supporting this. (The presence of UP did not significantly affect the associations of MG, *Chlamydia trachomatis*, *Trichomonas vaginalis* or *Neisseria gonorrhoea* with cervicitis (working not shown)).

We examined correlates of MG infection (Table 3) and found MG to be significantly associated with women being HIV positive. The main study clinic serves as a referral centre for HIV positive women; hence the HIV prevalence found in this study (2.5%) is higher than the background HIV prevalence in this population (0.1-0.2 %) \(^{26}\). This association of MG with HIV positivity has also been noted in a study of Nairobi STI clinic attendees with salpingitis \(^{27}\) in a study of West African sex workers \(^6\) and more recently in a prospective study of MSM in Brighton, UK \(^{23}\). As HIV shedding is associated with high MG organism burden \(^{28}\), it is possible MG has the potential to be a significant contributor to heterosexual and homosexual HIV transmission. We did not find MG to be associated with concurrent
chlamydia infection, which is consistent with other studies \(^{22,25}\) but recently Chlamydia co-infection was found to be associated in a large study of preterm birth in Peru \(^{11}\).

Interestingly, we found two of the women with MG had ectopic pregnancies (both early undiagnosed pregnancies at the time of recruitment) and another woman gave a history of an ectopic pregnancy in the year prior to diagnosis of MG. There is some evidence to support the association of ectopic pregnancy and MG \(^{1}\). Jurstrand’s group in a serological study of archived samples \(^{29}\) found no significant association with ectopic pregnancy, but a tendency to an association in younger women. A large UK community based study of low risk pregnant women \(^{25}\) was unable to assess an association between MG and adverse pregnancy outcome, perhaps due to very low MG prevalence. Similarly, a large study of pregnant women in Guinea-Bissau \(^{30}\) did not show any association of MG with adverse pregnancy outcome. Neither the British or African study examined ectopic pregnancy outcome. This observation in our data is quite striking; however we did not collect information on ectopic pregnancy in all study participants and so are unable to evaluate the significance of this finding at this point. Given the disastrous reproductive outcome of a tubal pregnancy, high MG prevalence found in some populations of pregnant women \(^{22}\) and lack of research in this area, the potential association between MG and ectopic pregnancy warrants further investigation.

In summary, we present important prevalence data and clarification of the associations of the genital mollicutes with the common female genital tract conditions of cervicitis and BV. We found MG to be the only mollucute significantly associated with cervicitis and only MH to be associated with BV. MG was also found more frequently in HIV positive women and our data suggest a possible association between MG and ectopic pregnancy. In conclusion, we
recommend wider application of PCR testing for *Mycoplasma genitalium* in STI services and the development of a commercially available test, for use in high-risk women, particularly those with cervicitis or HIV infection. The longer-term impact of MG infection on reproductive health warrants further investigation.
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CHAPTER 5

Cervicitis: Can we move to a consensus case definition?
Infectious and non-infectious associations and clinical utility
of commonly used case definitions for cervicitis.

As submitted to Obstetrics and Gynecology 6 August 2014 - under review
FOREWORD

This paper is the final summary paper for the Cervicitis Study and encompasses the first study aim using data from the full recruitment of 558 women. All infectious and non-infectious exposures putatively associated with cervicitis from review of the literature are included in this comprehensive analysis including multivariate analysis. These associations are also compared using other commonly used case definitions for cervicitis including yellow discharge, mucopurulent discharge and ectropion, in an attempt to provide guidance for a consensus case definition for cervicitis.

We found a consistent pattern of higher positive predictive values (PPV) and specificities for the significant pathogens, using the case definitions yellow discharge and mucopurulent discharge. This demonstration of better utility of these case definitions concurs with recent literature suggesting the traditional definition by microscopy of >30 pmnl/hpf lacks prediction of pathogens and is impractical. Importantly it is not available in most clinical practice and overcalls disease.

AUTHOR CONTRIBUTIONS

MJ Lusk was the principal and corresponding author.

MJ Lusk, PK, RGC and WDR designed the study. MJ Lusk and PK co-ordinated the study and supervised recruitment. ZWN and WDR developed and carried out the PCR testing. MJ Lusk performed the statistical analysis and interpretation with input from FLG and RGC. All authors contributed to and reviewed the final manuscript.
Cervicitis: Can we move to a consensus case definition? Infectious and non-infectious associations and clinical utility of commonly used case definitions for cervicitis.

M Josephine Lusk, Frances L Garden, William D Rawlinson, Zin W Naing, Robert G Cumming, Pam Konecny

ABSTRACT

Objective

To investigate associations and case definition with best utility for cervicitis.

Methods

558 women enrolled in a cross-sectional study examining infectious and non-infectious associations of cervicitis using the cervicitis definition >30 pmnl/hpf on cervical Gram stain. All women underwent testing for multiple pathogens. These associations were assessed using other cervicitis definitions (yellow discharge, mucopurulent discharge (MCP)) to identify the definition with best clinical utility.

Results

Chlamydia trachomatis (CT), Mycoplasma genitalium (MG) and Trichomonas vaginalis (TV) were significantly associated with increased cervicitis risk by multivariate analysis, the strongest associations with the definition MCP: CT Adjusted Relative Risk (ARR) = 2.61 (95% CI 1.57-4.35) p=0.0002, MG ARR =2.25 (95% CI 1.12-4.54) p=0.003, TV ARR = 2.86 (95% CI 1.61-5.09) p=0.0003. NG (RR= 3.66 (95% CI 2.02-6.62) p<0.0001), CMV, HPV and HIV were
associated with increased cervicitis risk on univariate analysis only. Condom use reduced cervicitis risk on multivariate analysis (ARR=0.68 (95% CI 0.50-0.92) p=0.013). Positive predictive values (PPV) and specificities for significant pathogens were higher for cervicitis ‘tests’ yellow discharge and MCP. Exposures not associated with cervicitis included bacterial vaginosis, HSV1, HSV2, EBV, candida, *Ureaplasma urealyticum*, age, smoking, past chlamydia infection, hormonal contraceptive and cycle phase.

**Conclusion**

There is significantly increased cervicitis risk with CT, NG, MG and TV, however much cervicitis remains unexplained. Condom use reduces cervicitis risk. The cervicitis case definitions of yellow discharge or mucopurulent discharge have the highest clinical utility with consistently higher associations, PPV and specificities for significant pathogens and are more practical than the microscopy definition >30 pmnl/hpf.
INTRODUCTION

Thirty years on from its debut there is still much to clarify about cervicitis including its etiology, significance and optimal management. Cervicitis is clinically important being associated with pelvic inflammatory disease (PID) \(^1,^2\) preterm birth \(^3\) and enhanced risk of HIV transmission \(^4\). *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoea* (NG) are consistently associated with cervicitis \(^5,^6\) but *Mycoplasma genitalium* (MG) \(^7,^8\) and *Trichomonas vaginalis* (TV) \(^5,^7,^9\) less so. Most diagnoses of cervicitis occur in the absence of these pathogens and are referred to as non-chlamydial non-gonococcal cervicitis \(^5\), non-specific cervicitis \(^4\) or cervicitis of unknown etiology \(^10\). Lack of consensus regarding cervicitis case definition currently limits interpretation of studies and accordingly estimates of the cervicitis prevalence and its associations vary widely depending on definition used and population studied \(^5,^6\).

With the increasing trend of self-collected vaginal or urine samples and molecular diagnostic testing, vaginal examination is less frequently performed with fewer clinicians making the diagnosis of cervicitis. The commonly used microscopic Gram stain diagnosis of > 30 polymorphonuclear leucocytes /high-powered field (>30 pmnl/hpf) has little practical application in many clinical settings, particularly primary care, but remains popular in Sexually Transmitted Disease (STD) clinics. Cervicitis diagnoses based on non-microscopic clinical assessments such as ‘mucopurulent discharge’ or ‘yellow discharge’ may have wider utility \(^6,^11^-^13\). This research responds to the call for further clarification of the etiology and best-case definition for the diagnosis of cervicitis \(^5,^11,^12\).
This study aimed to explore infectious and non-infectious associations of cervicitis using the working cervicitis case definition >30 pmnl/hpf on cervical Gram stain, for exposures putatively associated with cervicitis from current literature review\(^6\). These associations were examined using other commonly accepted cervicitis case definitions (yellow discharge, mucopurulent discharge and ectropion) in order to determine the cervicitis definition with best clinical utility.

**MATERIALS AND METHODS**

The study protocol is described previously\(^9,14\). In brief a cross sectional study in three publicly-funded STD clinics in Sydney, enrolled 558 women between July 2006 and February 2010. These clinics provide STD and HIV services by triage to patients from ‘priority populations’ defined in the 2006-2009 NSW Sexually Transmissible Infections Strategy\(^15\), symptomatic patients, STD contacts and those referred from primary care and hospital specialists. They operate in catchments with a high proportion (40%) of patients from culturally and linguistically diverse cultural backgrounds.

All recruiting clinicians underwent standardized on-site training in diagnostic microbiology. Inclusion criteria included consecutive consenting women over 18 years requiring vaginal examination based on presenting complaint or requesting examination. During the study it was clinic policy for clinicians to collect speculum samples, with patient self-collected samples less commonly offered. (Re-enrolment was permitted if more than six months had elapsed since initial enrolment and a new sexual partner reported). Exclusion criteria included PID, menstruation, pregnancy, no vaginal sex in prior three months, antibiotic use in prior month, sexual assault, IUCD or cervical surgery in prior 3 months.
**Sampling and laboratory methods**

The cervix was visualized using a sterile speculum and excess exudate removed using a non-sterile cotton swab before taking three endocervical sterile cotton swabs (Copan California). The first endocervical swab was taken for Gram stain to define women as having cervicitis or not by the working definition >30 pmnl in 3 non-adjacent high-powered fields and for NG culture on selective media (lysed horse blood agar containing vancomycin, colistin, nystatin and trimethoprim (VCNT)). A second endocervical swab was collected for CT/NG PCR testing (Amplicor CT/NG Test (Roche)) according to usual clinic practice. NG positivity was defined by positive culture +/- PCR test result. NG Culture was routinely performed in the clinic at the time, with PCR results carrying less reliability in the low prevalence setting. The third endocervical swab was taken for multiplex PCRs \(^\text{14}\) for MG, *M hominis* (MH), *Ureaplasma urealyticum* (UU), *Ureaplasma parvum* (UP), HSV1, HSV2, EBV, VZV, CMV and TV. A high vaginal swab was taken for Gram stain and candida culture and wet preparation for TV if clinically suspicious \(^\text{9}\). Bacterial vaginosis (BV) was defined on Gram stain using Nugent’s criteria.

A laboratory scientist blinded to clinician findings verified gram stain slides for diagnosis of cervicitis and BV at a later time. All discrepant slides were reexamined and consensus Gram stain cervicitis diagnosis reached. Testing for high risk Human Papilloma Virus (HPV) subtypes 16,18,31,33,35,39,45,51,56,58,59,68 (Human Genetic Signatures High Risk HPV Detection Kit, Sydney, Australia) was performed in a subset (n=191) of women \(^\text{16}\). All women diagnosed with STDs during the study were treated according to clinic guidelines.
Yellow discharge was recorded if this was seen on examination of the cervix (cervical discharge yellow colour on a white cotton swab) or the woman complained of yellow discharge. Mucopurulent discharge (MCP) (subjective clinician observation of purulent looking cervical secretions) and ectropion (visibility of the squamocolumnar junction) were recorded if this was noted on the cervix by the examining clinician.

Data was collected from clinical history sheets modified for the study, on presence of any genital symptoms and potential non-infectious etiological exposures. These included age group, current smoking, commercial sex work (CSW) and douching status, extent of condom use (never or sometimes/always) and number of sexual partners (1, >1) in the last three months, current use of combined oral contraceptive (COC), injectable depomedroxyprogesterone acetate (DMPA) /Progestagen only pill (POP) or Implanon, phase of menstrual cycle (follicular/luteal) and stated history of past chlamydia infection and abnormal Pap smear in the preceding two years.

**Statistical methods**

Associations of infectious and non-infectious exposures with cervicitis (the ‘disease’) were estimated using relative risks (RR) and $\chi^2$ testing. As this was a cross-sectional study and the outcome of cervicitis very common, RR$^2$s were calculated rather than odds ratios as the most valid measure of association. Relative risks were estimated by log-binomial regression. Multivariate analysis involved forward selection of covariates using exposures from the univariate analysis with $p < 0.05$. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the ‘test’ (different cervicitis definitions >30 pmnl/hpf, yellow discharge, mucopurulent discharge) for the significant pathogens, were calculated.
using 2x2 tables. The Laboratory Excel database was maintained by ZWN and MJL entered all clinical data and combined the two databases manually. The study was powered (80%, p<0.05) to detect RR of 3.0 (assuming cervicitis prevalence of 20% and pathogen prevalence 5%) or RR 2.4, assuming pathogen prevalence of 10%. All calculations were performed with SAS version 9.3 (SAS Institute, Cary, NC USA).

Ethics approval for the three clinical sites was granted by the South Eastern Sydney and Illawarra Area Health Service Human Research Ethics Committee and the Sydney South West Area Health Service Ethics Review Committee.

RESULTS

There were 1327 consecutive women approached with 630 (47.5%) eligible and 697 (52.5%) ineligible. Of 630 eligible women 558 (88.6%) were enrolled (including 21 enrolled twice) and 72 (11.4%) declined. Most common reasons for ineligibility were: not requiring examination (36.7%), recent antibiotic use (13.5%), menstruation/ pregnancy (12.8%).

Cervicitis defined as >30 pmnl/hpf was present in 268/558 (48.0%), yellow discharge in 129/540 (23.9%), mucopurulent discharge (MCP) in 102/540 (18.9%) and ectropion in 162/530 (30.6%). The cervicitis diagnosis >30 pmnl/hpf was robust, 87.2% of clinician Gram stain slides being examined by a laboratory scientist and high agreement between clinician and laboratory diagnosis of cervicitis or not (kappa 0.79 (95% CI 0.74-0.84)).

Prevalence of pathogens at enrolment was: CT 5.8% (95% Confidence Interval (CI) 3.8-7.7%), NG 1.1% (95% CI 0.2-1.9%), MG 3.8% (95% CI 2.2-5.4%) and TV 3.9% (95% CI 2.2-5.5%). Any genital symptoms were present in 59.9%. Cervicitis was strongly associated with the any genital symptoms (RR 1.58 (95% CI 1.40-1.78) p<0.0001).
Prevalence of infectious and non-infectious exposures and their associations with cervicitis, by different cervicitis case definitions are shown in Table 1 and 2 respectively. In univariate analysis using the cervicitis definition >30 pmnl/hpf, cervicitis risk was significantly increased for CT (RR=1.47 (95% CI 1.14-1.89) \( p=0.003 \)), NG (RR=2.06 (95% CI 1.89-2.25) \( p=0.012 \)), MG (RR=1.84 (95% CI 1.51-2.23) \( p<0.0001 \)), TV (RR=1.41 (95% CI 1.03-1.93) \( p=0.033 \)), CMV (RR=1.36 (95% CI 1.02-1.82) \( p=0.038 \)) and High Risk HPV genotype (RR=1.88 (95% CI 1.34-2.62) \( P=0.0002 \)).

Notably the RR of cervicitis increased for most of these exposures using the cervicitis case definitions yellow discharge and MCP, being highest for MCP: CT (RR= 2.39 (95% CI 1.52-3.78) \( p= 0.0002 \)), NG (RR= 3.66 (95% CI 2.02-6.62) \( p<0.0001 \)), MG (RR=2.37 (95% CI 1.40-4.02) \( p=0.001 \)), TV (RR=2.39 (95% CI 1.41-4.06) \( p=0.001 \)), CMV (RR 2.00 (95% CI 1.17-3.40) \( p=0.011 \)).

Presence of HIV infection was associated with increased cervicitis risk only by the MCP definition (RR 2.10 (95% CI 1.03-4.27) \( p=0.041 \)) and interestingly also with ectropion (RR=2.05 (95% CI 1.31-3.22) \( p=0.002 \)). Use of condoms ‘sometimes or always’ was associated with a significant reduction in cervicitis risk by case definition >30 pmnl/hpf (RR=0.82 (95% CI 0.68-0.98) \( p=0.027 \)) and yellow discharge definition (RR=0.71 (95% CI 0.52-0.96) \( p=0.027 \)) and also a reduction in symptoms (RR=0.82 (95% CI 0.71-0.95) \( p=0.006 \)). Current CSW was associated with reduced cervicitis risk by yellow discharge definition only (RR=0.58 (95% CI 0.35-0.96) \( p=0.035 \)) and douching an increase (RR=1.57 (95% CI 1.05-2.34) \( p=0.028 \)). Douching was uncommon (11.2%).
Reporting sexual intercourse in the last week was associated with decreased cervicitis risk only by the MCP definition (RR 0.63 (95% CI 0.44-0.90) p=0.012 and reporting >1 partner in the previous 3 months was also associated with a reduction in cervicitis, only by the yellow discharge definition (RR=0.57 (95% CI 0.40-0.83) p=0.003).

None of the exposures BV (and MH), UU, UP, HSV1, HSV2, EBV, VZV, candida, age less than 25 years, smoking, COC, DMPA /POP/Implanon, menstrual phase, stated history of ever having chlamydia or abnormal Pap smear in the last two years, were associated with cervicitis by any case definitions in univariate analysis. BV was strongly associated with MH (RR=3.7 (95% CI 2.90-4.73) p<0.0001) but not with any of the other mollicutes MG, UP or UU.

Multivariate analysis (Table 3) of significant exposures with different cervicitis case definitions, found only CT, MG and TV to be significantly associated with increased cervicitis risk. (NG could not be modelled due to small data numbers, but achieved the highest unadjusted RR). The highest RR for cervicitis were again seen for the case definition MCP: CT Adjusted relative risk (ARR) = 2.61 (95% CI 1.57-4.35) p=0.0002, MG ARR =2.25 (95% CI 1.12-4.54) p=0.003 and TV ARR = 2.86 (95% CI 1.61-5.09) p=0.0003. Using the working cervicitis definition of 30pmnl/hpf, only CT (ARR =1.30 (95% CI 1.01-1.68) p=0.045) and MG (ARR=1.55 (95% CI 1.24-1.95) p=0.0002) were associated with increased cervicitis risk. Use of condoms sometimes/ always was associated with reduced cervicitis risk by the >30 pmnl/hpf (ARR=0.86 (95% CI 0.74-0.99) p=0.047) and yellow discharge cervicitis definitions only (ARR = 0.68 (95% CI 0.50-0.92) p=0.013). CMV was not significantly associated with cervicitis by any case definition.
Table 4 outlines the sensitivities, specificities, PPV and NPV of different cervicitis case definitions (‘tests’) for the significant pathogens MG, CT, NG and TV. This shows the strongest PPV and specificities for all significant pathogens were consistently higher for the cervicitis ‘tests’ yellow discharge and MCP.

Population Attributable Risk% (PAR%) of significant pathogens in the etiology of cervicitis, assuming causative effects, were calculated using ARR for the case definition MCP from table 3 and unadjusted RR for NG, and are as follows: CT 8.5%, NG 3.6%, MG 4.5%, TV 6.7%, yielding a total PAR% of the four significant pathogens of 23.4% in the etiology of cervicitis.
Table 1

Univariate Analysis. Exposure prevalence and unadjusted relative risk (RR) of cervicitis by different case definitions (30 pmnl/hpf, yellow discharge and mucopurulent discharge (MCP), ectropion and presence of symptoms) for infectious exposures

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Prev %</th>
<th>RR Cx &gt;30 pmnl</th>
<th>95% CI</th>
<th>p</th>
<th>RR yellow d/c</th>
<th>p</th>
<th>RR MCP</th>
<th>p</th>
<th>RR ectrop</th>
<th>p</th>
<th>RR Sympt</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MG</td>
<td>552</td>
<td>3.8</td>
<td>1.84</td>
<td>1.51-2.23</td>
<td>&lt;.0001</td>
<td>2.10</td>
<td>0.002</td>
<td>2.37</td>
<td>.0013</td>
<td>1.24</td>
<td>0.445</td>
<td>0.96</td>
<td>0.820</td>
</tr>
<tr>
<td>MH</td>
<td>552</td>
<td>16.9</td>
<td>0.99</td>
<td>0.78-1.25</td>
<td>0.94</td>
<td>1.18</td>
<td>0.387</td>
<td>1.40</td>
<td>0.103</td>
<td>0.82</td>
<td>0.284</td>
<td>1.28</td>
<td>.0011</td>
</tr>
<tr>
<td>UU</td>
<td>550</td>
<td>14.0</td>
<td>0.95</td>
<td>0.73-1.23</td>
<td>0.685</td>
<td>0.99</td>
<td>0.875</td>
<td>1.04</td>
<td>0.886</td>
<td>1.04</td>
<td>0.844</td>
<td>1.05</td>
<td>0.591</td>
</tr>
<tr>
<td>UP</td>
<td>552</td>
<td>52.4</td>
<td>1.14</td>
<td>0.95-1.36</td>
<td>0.158</td>
<td>1.00</td>
<td>0.999</td>
<td>1.04</td>
<td>0.826</td>
<td>0.94</td>
<td>0.617</td>
<td>1.03</td>
<td>0.657</td>
</tr>
<tr>
<td>CT</td>
<td>555</td>
<td>5.8</td>
<td>1.47</td>
<td>1.14-1.89</td>
<td>.003</td>
<td>1.84</td>
<td>0.073</td>
<td>2.39</td>
<td>0.0002</td>
<td>1.76</td>
<td>0.003</td>
<td>0.83</td>
<td>0.295</td>
</tr>
<tr>
<td>NG</td>
<td>554</td>
<td>1.1</td>
<td>2.06</td>
<td>1.89-2.25</td>
<td>.012</td>
<td>3.60</td>
<td>&lt;.0001</td>
<td>3.66</td>
<td>&lt;.0001</td>
<td>0.54</td>
<td>0.501</td>
<td>1.4</td>
<td>0.072</td>
</tr>
<tr>
<td>TV</td>
<td>545</td>
<td>3.9</td>
<td>1.41</td>
<td>1.03-1.93</td>
<td>.033</td>
<td>2.06</td>
<td>0.0029</td>
<td>2.39</td>
<td>0.0012</td>
<td>0.98</td>
<td>0.956</td>
<td>1.21</td>
<td>0.187</td>
</tr>
<tr>
<td>HPV</td>
<td>191</td>
<td>8.4</td>
<td>1.88</td>
<td>1.34-2.62</td>
<td>.0002</td>
<td>1.91</td>
<td>0.108</td>
<td>2.33</td>
<td>0.078</td>
<td>1.45</td>
<td>0.230</td>
<td>1.22</td>
<td>0.271</td>
</tr>
<tr>
<td>CMV</td>
<td>542</td>
<td>5.2</td>
<td>1.36</td>
<td>1.02-1.82</td>
<td>.038</td>
<td>1.71</td>
<td>0.030</td>
<td>2.00</td>
<td>0.011</td>
<td>1.57</td>
<td>0.036</td>
<td>1.22</td>
<td>0.118</td>
</tr>
<tr>
<td>BV</td>
<td>555</td>
<td>25.4</td>
<td>1.02</td>
<td>0.83-1.24</td>
<td>0.855</td>
<td>1.00</td>
<td>0.995</td>
<td>1.21</td>
<td>0.323</td>
<td>1.11</td>
<td>0.465</td>
<td>1.32</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>HSV1</td>
<td>542</td>
<td>1.3</td>
<td>0.90</td>
<td>0.38-2.13</td>
<td>0.809</td>
<td>1.82</td>
<td>0.177</td>
<td>1.53</td>
<td>0.485</td>
<td>0.93</td>
<td>0.910</td>
<td>1.21</td>
<td>0.439</td>
</tr>
<tr>
<td>HSV2</td>
<td>543</td>
<td>2.0</td>
<td>0.95</td>
<td>0.49-1.82</td>
<td>0.864</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.59</td>
<td>0.416</td>
<td>0.61</td>
</tr>
<tr>
<td>EBV</td>
<td>542</td>
<td>4.8</td>
<td>0.97</td>
<td>0.63-1.48</td>
<td>0.881</td>
<td>0.97</td>
<td>0.929</td>
<td>1.24</td>
<td>0.056</td>
<td>1.61</td>
<td>0.028</td>
<td>0.97</td>
<td>0.858</td>
</tr>
<tr>
<td>VZV</td>
<td>542</td>
<td>2.2</td>
<td>0.70</td>
<td>0.31-1.56</td>
<td>0.377</td>
<td>0.70</td>
<td>0.576</td>
<td>-</td>
<td>-</td>
<td>0.27</td>
<td>0.170</td>
<td>0.70</td>
<td>0.293</td>
</tr>
<tr>
<td>Candida</td>
<td>555</td>
<td>18.9</td>
<td>1.10</td>
<td>0.89-1.36</td>
<td>0.366</td>
<td>1.02</td>
<td>0.929</td>
<td>1.03</td>
<td>0.887</td>
<td>0.69</td>
<td>0.061</td>
<td>1.45</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>HIV</td>
<td>554</td>
<td>2.5</td>
<td>1.11</td>
<td>0.60-2.04</td>
<td>0.740</td>
<td>1.64</td>
<td>0.170</td>
<td>2.10</td>
<td>0.041</td>
<td>2.05</td>
<td>0.002</td>
<td>0.47</td>
<td>0.076</td>
</tr>
</tbody>
</table>

Blank entries where RR could not be calculated

n varies according to data numbers for that variable

*Neisseria gonorrhoea* (NG), *Chlamydia trachomatis* (CT), *Trichomonas vaginalis* (TV) *Mycoplasma genitalium* (MG), *Mycoplasma hominis* (MH), *Ureaplasma urealyticum* (UU), *Ureaplasma parvum* (UP), High Risk Human Papilloma Virus (HPV) herpes simplex virus type 1 (HSV1), herpes simplex virus type 2 (HSV2), Epstein Barr virus (EBV), varicella zoster virus (VZV), cytomegalovirus (CMV), human immunodeficiency virus (HIV), bacterial vaginosis (BV)
**Table 2**

Univariate analysis. Exposure prevalence and unadjusted relative risk (RR) of cervicitis by different case definitions (30 pmnl/hpf, yellow discharge and mucopurulent discharge (MCP), ectropion and presence of any genital symptoms) for non-infectious exposures

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Prev %</th>
<th>RR Ex &gt;30 pmnl</th>
<th>95% CI</th>
<th>p</th>
<th>RR yellow d/c</th>
<th>p</th>
<th>RR MCP</th>
<th>p</th>
<th>RR ectrop</th>
<th>p</th>
<th>RR Sym</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age group (&lt;25yrs)</strong></td>
<td>558</td>
<td>30.8</td>
<td>1.15</td>
<td>0.97-1.38</td>
<td>0.115</td>
<td>0.92</td>
<td>0.606</td>
<td>1.18</td>
<td>0.383</td>
<td><strong>1.50</strong></td>
<td>0.002</td>
<td>1.03</td>
<td>0.700</td>
</tr>
<tr>
<td><strong>Smoke</strong></td>
<td>549</td>
<td><strong>41.7</strong></td>
<td>0.97</td>
<td>0.81-1.17</td>
<td>0.775</td>
<td>1.01</td>
<td>0.679</td>
<td>0.93</td>
<td>0.698</td>
<td><strong>0.76</strong></td>
<td>0.049</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>CSW</strong></td>
<td>553</td>
<td>17.5</td>
<td>0.83</td>
<td>0.65-1.08</td>
<td>0.158</td>
<td><strong>0.58</strong></td>
<td><strong>0.035</strong></td>
<td>0.89</td>
<td>0.629</td>
<td>0.71</td>
<td>0.092</td>
<td>0.81</td>
<td>0.053</td>
</tr>
<tr>
<td><strong>douche</strong></td>
<td>492</td>
<td><strong>11.2</strong></td>
<td>1.02</td>
<td>0.77-1.36</td>
<td>0.884</td>
<td><strong>1.57</strong></td>
<td><strong>0.028</strong></td>
<td>1.51</td>
<td>0.885</td>
<td>0.88</td>
<td>0.622</td>
<td>1.07</td>
<td>0.511</td>
</tr>
<tr>
<td><em>Condom sometime/always</em></td>
<td>528</td>
<td><strong>65.9</strong></td>
<td>0.82</td>
<td>0.68-0.98</td>
<td><strong>0.027</strong></td>
<td><strong>0.71</strong></td>
<td><strong>0.027</strong></td>
<td>0.94</td>
<td>0.747</td>
<td>1.21</td>
<td>0.200</td>
<td><strong>0.82</strong></td>
<td><strong>0.006</strong></td>
</tr>
<tr>
<td><strong>Partner&gt;1</strong></td>
<td>545</td>
<td>35.2</td>
<td>0.87</td>
<td>0.72-1.06</td>
<td>0.162</td>
<td><strong>0.57</strong></td>
<td><strong>0.003</strong></td>
<td>0.87</td>
<td>0.470</td>
<td><strong>0.71</strong></td>
<td><strong>0.021</strong></td>
<td>0.96</td>
<td>0.608</td>
</tr>
<tr>
<td><strong>Last sex&lt;1 week ago</strong></td>
<td>542</td>
<td>52.6</td>
<td>0.90</td>
<td>0.75-1.07</td>
<td>0.215</td>
<td>0.76</td>
<td>0.083</td>
<td><strong>0.63</strong></td>
<td><strong>0.012</strong></td>
<td>0.90</td>
<td>0.433</td>
<td>0.91</td>
<td>0.188</td>
</tr>
<tr>
<td><strong>COC</strong></td>
<td>550</td>
<td>28.2</td>
<td>1.11</td>
<td>0.92-1.33</td>
<td>0.292</td>
<td>0.88</td>
<td>0.491</td>
<td>0.74</td>
<td>0.171</td>
<td><strong>1.76</strong></td>
<td>&lt;.0001</td>
<td>0.89</td>
<td>0.154</td>
</tr>
<tr>
<td><strong>Cycle phase 2</strong></td>
<td>426</td>
<td>60.1</td>
<td>0.85</td>
<td>0.69-1.04</td>
<td>0.109</td>
<td>0.80</td>
<td>0.216</td>
<td>0.88</td>
<td>0.502</td>
<td>0.99</td>
<td>0.965</td>
<td>1.03</td>
<td>0.678</td>
</tr>
<tr>
<td><strong>POP/DMPA/implanon</strong></td>
<td>545</td>
<td>5.9</td>
<td>0.97</td>
<td>0.66-1.42</td>
<td>0.890</td>
<td>1.23</td>
<td>0.478</td>
<td>0.84</td>
<td>0.678</td>
<td>0.83</td>
<td>0.600</td>
<td>0.99</td>
<td>0.972</td>
</tr>
<tr>
<td><strong>Hx CT</strong></td>
<td>384</td>
<td>13.8</td>
<td>0.91</td>
<td>0.67-1.23</td>
<td>0.532</td>
<td>1.40</td>
<td>0.124</td>
<td>1.15</td>
<td>0.603</td>
<td>1.26</td>
<td>0.263</td>
<td>0.96</td>
<td>0.725</td>
</tr>
<tr>
<td><strong>Abnormal pap within last 2 yrs</strong></td>
<td>487</td>
<td>12.9</td>
<td>0.95</td>
<td>0.72-1.27</td>
<td>0.735</td>
<td>0.83</td>
<td>0.494</td>
<td>0.79</td>
<td>0.462</td>
<td><strong>1.58</strong></td>
<td><strong>0.008</strong></td>
<td>0.97</td>
<td>0.794</td>
</tr>
</tbody>
</table>

n varies according to data numbers for that variable

*a* condoms used “sometimes/always” in the last 3 months

*b* partner >1 means more than one partner in the last 3 months

commercial sex worker (CSW), combined oral contraceptive (COC), depo-medroxyprogesterone acetate (DMPA), progestogen only pill (POP)/Implanon™, Hx chlamydia is reported history of chlamydia ever
Table 3
Multivariate analysis. Adjusted relative risk (ARR) of cervicitis by different case definitions (30pmnl/hpf, yellow discharge and mucopurulent discharge (MCP)) for significant infectious and non-infectious exposures

<table>
<thead>
<tr>
<th>Variable</th>
<th>30 pmnl/hpf</th>
<th>95% CI</th>
<th>Yellow d/c</th>
<th>95% CI</th>
<th>MCP</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ARR</td>
<td>p</td>
<td>ARR</td>
<td>p</td>
<td>ARR</td>
<td>p</td>
</tr>
<tr>
<td>MG</td>
<td>1.55</td>
<td>1.24-1.95</td>
<td>0.002</td>
<td>2.13</td>
<td>1.27-3.55</td>
<td>0.004</td>
</tr>
<tr>
<td>CT</td>
<td>1.30</td>
<td>1.01-1.68</td>
<td>0.045</td>
<td>1.81</td>
<td>1.12-2.92</td>
<td>0.015</td>
</tr>
<tr>
<td>TV</td>
<td>1.34</td>
<td>0.96-1.87</td>
<td>0.086</td>
<td>2.30</td>
<td>1.40-3.78</td>
<td>0.001</td>
</tr>
<tr>
<td>CMV</td>
<td>1.16</td>
<td>0.89-1.50</td>
<td>0.275</td>
<td>1.50</td>
<td>0.89-2.51</td>
<td>0.128</td>
</tr>
<tr>
<td>Condoms sometimes/always a</td>
<td>0.86</td>
<td>0.74-0.99</td>
<td>0.047</td>
<td>0.68</td>
<td>0.50-0.92</td>
<td>0.013</td>
</tr>
<tr>
<td>(HIV) b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.68</td>
<td>0.74-3.80</td>
</tr>
</tbody>
</table>

Multivariate model includes exposures with p < 0.05 from univariate analysis using the cervicitis case definition 30 pmnl/hpf. NG excluded from model as data too sparse. HPV excluded as n=191

*Neisseria gonorrhea* (NG), *Chlamydia trachomatis* (CT), *Trichomonas vaginalis* (TV) *Mycoplasma genitalium* (MG)

acondoms used “sometimes/always” in the last 3 months

bHIV included in model for MCP as unadjusted RR of cervicitis with HIV significant by this case definition.
Table 4

Sensitivities, specificities, negative predictive values (NPV) and positive predictive value (PPV) of different cervicitis case definitions (*tests*) 30 pmnl/hpf, yellow discharge, mucopurulent discharge (MCP) for the significant infectious exposures MG, CT, NG and TV

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cx case Def (test)</th>
<th>Sens (%)</th>
<th>Spec (%)</th>
<th>ppv (%)</th>
<th>npv (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MG</td>
<td>30 pmnl</td>
<td>86</td>
<td>54</td>
<td>7</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>Yellow discharge</td>
<td>48</td>
<td>77</td>
<td>8</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>MCP</td>
<td>43</td>
<td>82</td>
<td>9</td>
<td>97</td>
</tr>
<tr>
<td>CT</td>
<td>30pmnl</td>
<td>69</td>
<td>54</td>
<td>8</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>Yellow discharge</td>
<td>42</td>
<td>77</td>
<td>10</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>MCP</td>
<td>43</td>
<td>82</td>
<td>13</td>
<td>96</td>
</tr>
<tr>
<td>NG</td>
<td>30 pmnl</td>
<td>100</td>
<td>53</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Yellow discharge</td>
<td>83</td>
<td>77</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>MCP</td>
<td>67</td>
<td>82</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>TV</td>
<td>30 pmnl</td>
<td>67</td>
<td>53</td>
<td>5</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>Yellow discharge</td>
<td>48</td>
<td>77</td>
<td>8</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>MCP</td>
<td>43</td>
<td>82</td>
<td>9</td>
<td>97</td>
</tr>
</tbody>
</table>

*Neisseria gonorrhea (NG), Chlamydia trachomatis (CT), Trichomonas vaginalis (TV) Mycoplasma genitalium (MG)*
DISCUSSION

Cervicitis is very common and frequently symptomatic, necessitating better understanding of its etiology. Infectious exposures significantly associated with increased cervicitis risk in multivariate analysis were CT, NG, MG and TV, with all these associations being strongest for the cervicitis case definition MCP. Use of condoms sometimes/always reduced cervicitis risk. Infectious exposures not associated with cervicitis included HSV1, HSV2, EBV, BV, MH, UU, UP and candida. Non-infectious exposures not associated with cervicitis included age less than 25, smoking, COC, DMPA/POP/Implanon use, reported history of CT or abnormal Pap smear in the preceding two years and menstrual phase.

Our results concerning HSV and BV are contrary to others but Gaydos et al also recently found BV was not associated with cervicitis. Our finding of MH being the only mollicute associated with BV has also been noted previously. Both BV and MH have been implicated in PID so their association with cervicitis might be expected.

After adjusting for other significant exposures CMV was not significantly associated with cervicitis by any case definition, supporting its role as a bystander rather than cause of cervicitis, despite an important role in neonatal infection. The cervical CMV prevalence of 5.2% is remarkably similar to the CMV IgM positivity rate of 5.5% found in a local study of pregnant women.

Infection with HIV was associated with cervicitis using the MCP cervicitis definition but was not significant when adjusted for STDs. This is an important potential association given that STDs enhance HIV shedding from the cervix contributing to horizontal and vertical HIV
transmission. In earlier studies we demonstrated a significant association between MG and HIV, as well as MG and cervicitis. Manhart et al described increased HIV shedding in women with high cervical MG burden; it may be that MG-associated cervicitis in particular is an important HIV transmission driver.

Cervical infection with High-Risk HPV was associated with increased cervicitis risk by the >30 pmnl/hpf definition only in univariate analysis. This is a biologically plausible association. Cervical inflammation has previously been suggested as a possible co-factor in the development of high grade cervical lesions in women with oncogenic HPV, with uncertain temporality to this association. We were limited to 191 High-Risk HPV testing kits, but with more data this might have been a stronger finding and worth exploring further.

A curious finding was >1 partner being associated with a reduction in cervicitis by the yellow discharge definition (RR=0.57 (95% CI 0.40-0.83) p=0.003). A possible explanation besides chance, was that women with more partners might use condoms more, but there was no significant interaction between number of partners and condoms use (p=0.426). Also, unexplained was the finding that sex in the previous week reduced cervicitis risk, but this was only for the MCP definition, and has not been implicated as a factor previously.

A case definition for cervicitis with the best clinical utility is one easily made in primary care with good PPV for pathogens that does not over diagnose ‘disease’. Using the definition of >30 pmnl/hpf, 48% of women had cervicitis, a very high rate of ‘disease’ with incumbent ramifications for individuals and couples with the diagnosis of an ‘STD’ and potential for antibiotic overuse. Because most women diagnosed with cervicitis will routinely have testing for at least CT and NG with the opportunity for recall for treatment, the specificity of
a bedside ‘test’ for cervicitis is probably of greater importance than the sensitivity. Our data show that the MCP ‘test’ for cervicitis (and yellow discharge ‘test’) show consistently higher ARR, PPV and specificities than the traditional cervicitis ‘test’ of 30 pmnl/hpf, for the significant pathogens CT, NG, MG and TV, but lower sensitivities. Importantly cervicitis or ‘disease’ was diagnosed in only 18.9% of women using the MCP case definition. Marrazzo et al. also found cervical signs such as mucopus to have better PPV for pathogens chlamydia or gonorrhea than >30 pmnl/hpf, particularly in women under 25. Our findings suggest the case definition >30 pmnl/hpf over-calls disease and lacks prediction of pathogens. It follows that MCP or yellow discharge are the cervicitis case definitions with better all-round clinical utility. NPV for significant pathogens were high for all cervicitis ‘tests’, reflecting low pathogen prevalence in this population.

As rapid point of care (POC) tests become more available, such a test to predict disease would be a welcome development in the first-line management of women with vaginal symptoms, given these complaints are often poorly managed initially. Vaginal leucocytes predict endometritis and so act as a useful triage system, particularly in the presence of BV and other STDs (CT and NG). A blind vaginal swab with a rapid POC test for both leucocytes and pH could provide an effective, immediate and less invasive way of predicting serious infection and guiding immediate management than a speculum examination. MCP cervicitis or raised vaginal leucocytes both provide us with ‘tests’ at the bedside with reassuringly high NPV and specificities for pathogens in the absence of ultimate solution of cheap reliable POC multiplex tests for CT, NG, MG and TV.

In our STD population, using the most predictive cervicitis definition of MCP, the PAR% of the significant pathogens CT NG, MG and TV (assuming causative roles), might still only
account for 23.4% of cervicitis. However as PAR% is prevalence driven, this figure would increase with higher pathogen prevalence and highly triaged situations.

Given this result it is reasonable to postulate that factors other than infective agents play a role in cervicitis. A very extensive range of potential pathogens were considered in this study, but the low cumulative PAR % of the significant pathogens leave about 75 % of cervicitis unexplained in this setting and not necessarily synonymous with pathogen infection. We have however demonstrated some important negative findings, particularly concerning the potential roles of BV, MH, UU and HSV. With expanding knowledge of the microbiome, in particular the vaginal microbiome, other pathogens may be uncovered, much as MG has emerged as a pathogen in the last 15 years.

Inflammatory cytokines have been implicated in cervical inflammation. Pro-inflammatory cytokines and cervical monocyte activation have been associated with ectopy and menstrual cycle phase in healthy women which may afford protection to the female genital tract under these conditions, but ironically enhance HIV acquisition risk. Higher RANTES and lower secretory leukocyte protease inhibitor (SLPI) were associated with HIV transmission in a large study of African women (seen in DMPA users, possibly contributing to increased HIV transmission risk in this group), but the study did not examine cervicitis associations. Elevated Interleukin 10 has been described in women with non-ulcerative STDs and BV and also proposed as a possible mechanism for increased susceptibility to HIV. A small Japanese study of pregnant women found significantly elevated levels of IL-8, IL6, lactoferrin and μ-DF in cervicitis. A recent small Indian study found elevated levels of interlekin-1β, tumor necrosis factor-α and interleukin 6 to be significantly associated with cervicitis, but they did not control for infections with CT, NG, HSV or MG, thereby limiting
the significance of their findings. Whether these inflammatory markers contribute to or are simply present in cervicitis is not clear.

There is also the possibility that ‘cervical inflammation’ or cervicitis may be part of a normal continuum in some women. Little is known about the natural history of cervicitis. The three different definitions of cervicitis used in this study yielded widely differing cervicitis prevalence, from 48% (30 PMNL) down to 18.9% from mucopurulent discharge. Such divergent results suggest inaccuracies and uncertainties with the criteria used to define a condition, labelled as pathological by many, but as a poorly explained enigma by others. This study has tried to address the merits of the different definitions widely used and propose a best working definition for cervicitis. Only from this point, can the management of this condition be clarified.

**Study strengths and limitations**

This study was cross sectional with limited inference on causality, but sample size was large and appropriately powered (80% p<0.05) to detect pathogen RR of 2.5 to 3.0. We included in the study all relevant infectious and non-infectious exposures. Clinical practice directed examination of a convenience sample of all symptomatic women and some asymptomatic women, so enrolment was biased towards symptomatic women (60%) likely contributing to high cervicitis rates, by all definitions. Recruitment occurred at three related STD clinics but 83% at the primary clinic (Short Street Centre) using a small group of clinicians with advantages for consistency but also potential biases. HPV data on all women would have been optimal but funding precluded this.
In summary we have demonstrated significantly increased cervicitis risk with CT, NG, MG and TV however much cervicitis remains unexplained. We recommend the cervicitis case definitions with best clinical utility are yellow discharge or mucopurulent discharge, which have consistently higher associations, PPV and specificities for significant pathogens than the traditional case definition >30 pmn/l/hpf. We hope this study further supports a consensus case definition for cervicitis.
REFERENCES


CHAPTER 6

Cervicitis: A prospective observational study of empiric treatment with azithromycin in women with cervicitis and non-specific cervicitis and their male partners, in Sydney STD clinic populations.

Draft manuscript in preparation for submission
FOREWORD

This paper presents the findings of an observational treatment study which was a sub-study of the Cervicitis Study. It aimed to investigate the benefit of presumptive treatment of women with cervicitis and non-specific cervicitis (NSC) with azithromycin 1 G orally and determine if there is any benefit of addition of presumptive azithromycin treatment of male partners. At the time the Cervicitis Study was planned it was common practice to presumptively treat women diagnosed with cervicitis with azithromycin. Support for this practice waned as it has become more apparent that only a small proportion of cervicitis occurs in the presence of chlamydia infection and although azithromycin covers chlamydia and a large proportion of Mycoplasma genitalium (MG) infection, there is growing evidence of azithromycin resistance to MG.

Cervicitis was defined as >30 pmnl/hpf with or without known pathogens. NSC was defined as cervicitis (>30 pmnl/hpf) occurring in the absence of the pathogens CT, MG, NG or TV. This assumed findings from the main Cervicitis Study paper (chapter 5) that these pathogens are significantly associated with cervicitis.

The outcomes examined in this study were the persistence of cervicitis (30 pmnl/hpf) or the presence of any genital symptoms. Analysis of follow-up outcomes in women excluded any women who were diagnosed as having one of the pathogens CT, MG, NG or TV at follow-up visit. Although we found presumptive azithromycin treatment was associated with a 30% to 40% reduction in cervicitis persistence in women with NSC and a similar reduction of symptoms for women with cervicitis, these effects did not reach statistical significance. A larger RCT study may show more conclusive results. If azithromycin treatment of women
with cervicitis and NSC is associated with a reduction of the outcomes assessed, our results suggest it is not a large effect.

**Study limitations**

The findings of this study have significant limitations primarily because it was not a randomized controlled trial (RCT), follow-up numbers were very small (particularly the non-treatment group) and there was a long and variable time to follow-up for many women. In a more formalized and resourced study, routine follow-up of women at four to six weeks would be desirable to more reliably link treatment with effect on follow-up outcomes. This study was an observation of the results of clinical practice (a ‘real life’ observational study) and suffers the limitations of this; however it represents a solid effort to answer questions about presumptive treatment.

In the context of the literature reviews it seems likely that cervicitis predicts upper genital tract infection and the long-term sequelae associated with this and so it is possible that azithromycin treatment in women with cervicitis and NSC could have benefits in the reduction of sequelae of subclinical PID. These are more difficult treatment outcomes to assess but may be more important than the outcomes assessed in this study.

**AUTHOR CONTRIBUTIONS**

MJ Lusk wrote the manuscript and PK, RC and FG contributed to the final draft. The study was designed by MJ Lusk, PK & RGC. MJ Lusk and FLG performed and checked all statistical workings and interpretations.
Cervicitis: A prospective observational study of empiric treatment with azithromycin in women with cervicitis and non-specific cervicitis and their male partners, in Sydney STD clinic populations.

M Josephine Lusk, Frances L Garden, Robert G Cumming, Pam Konecny

ABSTRACT

Background and Aim

Cervicitis management guidelines reflect its uncertain etiology with unclear benefit of presumptive azithromycin treatment of women and their partners. The aim of this prospective observational study in women attending three STD services in Sydney 2006-2010 was to assess the effect of presumptive azithromycin 1G orally for women with cervicitis and non-specific cervicitis (NSC) and effect of additional partner presumptive treatment.

Methods

Cervicitis was defined as >30 pmnl/hpf with or without known pathogens. NSC was defined as >30 pmnl/hpf in the absence of Chlamydia trachomatis (CT), Mycoplasma genitalium (MG), Neisseria gonorrhoea (NG) or Trichomonas vaginalis (TV). Testing for CT, MG and TV was by PCR and NG by PCR and culture. The follow-up outcomes were persistence of cervicitis or persistent of any genital symptoms. Women with positive tests for CT, MG, NG or TV at follow-up (incident or persistent infection) were excluded from the follow-up analysis. Effect on the outcomes was also assessed after additional reported presumptive partner treatment with azithromycin 1G.
**Results:**

558 women were enrolled. Prevalence of pathogens at enrolment of all women was: CT 5.8%, NG 1.1%, MG 3.8%, TV 3.9%. Cervicitis prevalence at enrolment was 268/558 (48.0%). Of these women, 131 (48.9%) returned for follow-up and after exclusion of women with positive pathogens at follow-up or missing data, 125 women with cervicitis and 89 women with NSC could be included in the follow-up analysis.

Presumptive treatment of women with azithromycin 1 G orally showed a non-significant reduction in cervicitis persistence in women with cervicitis, RR=0.74 (95% CI 0.46-1.21) p=0.235 and in women with NSC, RR=0.60 (95% CI 0.35-1.02) P=0.058. Presumptive treatment was associated with a non-significant reduction of symptoms in women with cervicitis, RR=0.67 (95% CI 0.44-1.01) p=0.054 and in women with NSC, RR=0.91 (95% CI 0.46-1.79) p= 0.780. Addition of presumptive partner treatment did not reduce cervicitis persistence in women with cervicitis, RR=1.18 (95% CI 0.70-2.00) p=0.528 or those with NSC, RR= 1.02 (95% CI 0.54-1.90) p=0.961.

**Conclusions:** Although not reaching statistical significance, our findings suggest presumptive azithromycin treatment of women with NSC may be associated with reduced cervicitis persistence and a reduction of symptoms in women with cervicitis. We did not find a benefit of partner presumptive treatment. A larger study, ideally a RCT may show significant benefit of presumptive treatment of women particularly in higher STD prevalence populations.
INTRODUCTION

Cervicitis is associated with clinical disease such as pelvic inflammatory disease (PID), endometritis and preterm birth \(^1\)\(^-\)\(^4\) and increased risk of HIV transmission \(^5\). It is common and often asymptomatic but many women have troubling symptoms which require a clinician’s action. Accordingly it remains relevant to pursue better understanding of how to manage cervicitis at the time of initial consultation.

Lower genital tract complaints in women are extremely common and often inaccurately diagnosed and presumptively treated in the first instance \(^6\). This has the disadvantages of antibiotic overuse, cost and increased potential for reinfection if clear diagnoses are never made and partners not concurrently treated. Management guidelines for cervicitis reflect the uncertainty of the etiology of cervicitis and are inconsistent in their recommendations for management. The 2010 CDC guidelines \(^7\) recommend presumptive treatment of cervicitis with azithromycin 1 G stat for women less than 25 years of age, those reporting new or frequent partner change and unprotected sex, HIV positive women and women unlikely to return for follow-up. The addition of presumptive treatment for *Neisseria gonorrhea* (NG) is suggested in populations of >5% NG prevalence and partner management is informed by pathogen isolation. Interestingly the management of the analogous condition in males, non-specific urethritis (NGU), is much more clearly defined with the recommendation of presumptive treatment of affected men and their female partners, assuming high *Chlamydia trachomatis* (CT) infection rates. Many guidelines for cervicitis are not so prescriptive and questions remain as to whether there is benefit in presumptive treatment of women with cervicitis and presumptive treatment of their male sexual partners \(^8\)\(^-\)\(^11\).
The aim of this prospective observational study was to assess the effect of presumptive treatment with azithromycin 1 G PO of women with cervicitis and non-specific cervicitis (NSC), on the outcome of cervicitis persistence or genital symptoms and to assess if there was a benefit on cervicitis persistence of additional presumptive male partner treatment with azithromycin 1 G PO.

METHODS

Recruitment and Laboratory methods

This was an observational treatment-outcome sub-study within a larger study examining infectious and non-infectious exposures associated with cervicitis and utility of commonly used definitions of cervicitis (submitted elsewhere for publication). The methods have been previously described 12,13: in brief a cross-sectional study in three STD services in Sydney enrolled 558 consecutive consenting women from July 2006 to February 2010. These services are publically-funded STD and HIV management clinics serving suburban populations of average socio-economic status including a large proportion (40%) of culturally and linguistically diverse people.

Women over 18 years consecutively attending the clinics, requiring vaginal examination based on presenting complaint or requesting examination, were approached for the study. The exclusion criteria were PID, menstruation, pregnancy, no vaginal sex in the prior 3 months, antibiotic use in the prior month, sexual assault, IUCD or cervical surgery in the prior 3 months. Women were diagnosed as having cervicitis or not by the assessment of a cervical swab Gram stain using the definition >30 polymorphonucleocytes/ high-powered
field (pmnl/hpf) in 3 non-adjacent fields, with this diagnosis verified at a later time by a laboratory scientist blinded to clinician findings.

For the purposes of analysis cervicitis was defined as the finding of >30 pmnl/hpf on cervical Gram stain with or without known pathogens. NSC was defined as the subset of women with >30 pmnl/hpf in the absence of Chlamydia trachomatis (CT), Mycoplasma genitalium (MG), Neisseria gonorrhoea (NG) or Trichomonas vaginalis (TV). Women with positive results for CT, MG, NG or TV (ie persistent or interim incident infection) at follow-up, were excluded from the follow-up analysis.

Women were tested real time for CT and NG by PCR (Amplicor CT/NG PCR test (Roche)), NG by culture (on selective media of lysed horse blood agar containing vancomycin, colistin, nystatin and trimethoprim (VCNT)), bacterial vaginosis (Amsel’s Criteria) and candida (culture). They had non-real time testing for a number of other infectious agents putatively associated with cervicitis, using dedicated study multiplex PCRs 13 for Mycoplasma genitalium (MG), M hominis (MH), Ureaplasma urealyticum (UU), Ureaplasma parvum (UP), HSV1, HSV2, EBV, VZV, CMV and Trichomonas vaginalis (TV). NG status was considered positive if a positive PCR result was confirmed by culture.

Clinical data was collected during patient clinical consultations by a group of trained clinic doctors and nurses using clinical history sheets modified for the purposes of the study. Variables included age, presence of any genital symptoms, current commercial sex work (CSW), current smoking, current douching status, condom use (always/sometimes/never) and number of sexual partners (1, >1) in the last 3 months.
Treatment and follow-up

All women diagnosed with cervicitis were offered presumptive treatment with azithromycin 1 G PO stat on the day of enrolment but if NG or TV were diagnosed by on-site microscopy, women were treated accordingly with ceftriaxone 500mg IM or tinidazole 2G PO respectively and not offered presumptive azithromycin. Women with cervicitis were encouraged to return for follow-up in 6 to 8 weeks’ time but were not actively recalled unless they required a test of cure or treatment for a pathogen identified from non-real time study test results. Time to follow-up of some women with positive study multiplex results was delayed due to batch testing but all routine tests for CT and NG were acted upon in real time.

Follow-up outcomes were defined as persistence of cervicitis by the same cervicitis definition >30pmnl/hpf on cervical Gram stain or report of any genital symptoms.

Women taking presumptive azithromycin treatment were given a contact information slip for their male partners offering male partners presumptive treatment and study testing. Women diagnosed with cervicitis were advised of the uncertainty of benefit of presumptive treatment for themselves and presumptive partner treatment. Women who returned for follow-up were asked if they had taken the azithromycin treatment supplied as directed and if their sexual partner(s) had taken this same treatment (‘partner treatment’).

Statistical analysis

Differences between baseline characteristics of women with cervicitis who were followed up or not, and of women with cervicitis followed up who had azithromycin treatment or not, were assessed using χ² tests. The effect of presumptive treatment of women with cervicitis
and the additional effect of partner treatment were estimated using relative risks (RR) and 95% confidence intervals (CI). Significant associations were those for which the p-value was <0.05. All calculations were performed with SAS version 9.3 (SAS Institute, Cary, NC USA).

Ethics approval was granted by the South Eastern Sydney and Illawarra Area Health Service Human Research Ethics Committee and the Sydney South West Area Health Service Ethics Review Committee.
1327 consecutive women approached

630 women eligible

558 women enrolled (88.6%)

268 women diagnosed with cervicitis (48%)

125 women with all cervicitis
follow-up

114 women treated with azithromycin
11 women not treated

89 women with non-specific cervicitis
follow-up (CT, NG, MG, TV excluded)

82 women treated with azithromycin
7 women not treated

697 women ineligible

72 women declined (11.4%)
RESULTS

Of 1327 consecutive women approached, 630 were eligible and 558 enrolled (88.6%). 72/630 (11.4%) of eligible women declined participation. Of women enrolled 268/558 (48.0%) were diagnosed with cervicitis.

Of the 268 women with cervicitis, 131 (48.9%) returned for follow-up, and after exclusion of women with positive pathogens at follow-up and missing data, 125 women with cervicitis and 89 women with NSC could be included in the follow-up analysis (Figure 1). (Three women in the cervicitis group had infections at follow-up (two with CT and one with MG) and none in the NSC group). Presumptive treatment with azithromycin 1 G orally was taken by 114/125 women with cervicitis and by 82/89 women with NSC. Reasons for not taking presumptive treatment included other diagnosis made at time of consultation (eg. NG, TV, HSV) prompting alternative treatment, declined treatment or misclassified as not having cervicitis at initial consultation.

Baseline prevalence of pathogens in all women at initial enrolment was as follows: of CT 5.8%, NG 1.1%, MG 3.8 % and TV 3.9%. Gram stain diagnosis of cervicitis was verified in 87.2% of all women with high agreement between clinician and laboratory diagnosis (kappa = 0.79 (95% CI 0.74-0.84).

There were no significant differences in the following baseline characteristics of women with cervicitis who followed-up/didn’t follow-up: age <25 years, presence of any symptoms, CSW, smoking, douching, condoms use sometimes/always and partner number >1 in the previous 3 months (Table 1). For all women with cervicitis who followed up who had presumptive treatment /didn’t have presumptive treatment, there were also no significant
differences for these same characteristics. However women reporting current CSW or more than 1 partner in the prior 3 months were more likely to have cervicitis treated with azithromycin than not.

Time between initial and follow-up visit was variable with a mean of 24.9 weeks and median 10.1 weeks.

Presumptive treatment of women with azithromycin 1 G orally showed a non-significant reduction in cervicitis persistence in women with cervicitis, RR=0.74 (95% CI 0.46-1.21) p=0.235 and in women with NSC, RR=0.60 (95% CI 0.35-1.02) P=0.058 (Table 2). A parallel analysis looking at the effect of presumptive treatment on the outcome of presence of symptoms at follow-up found a non-significant reduction in women with cervicitis RR=0.67 (95% CI 0.44-1.01) p=0.054 and in women with NSC, RR=0.91 (95% CI 0.46-1.79) p=0.780 (Table 3).

Of 114 women with cervicitis in the follow-up analysis, 78 were able to report partner treatment. There was no effect of stated partner presumptive treatment on the follow-up outcome of persistent cervicitis, RR 1.18 (95% CI 0.70-2.00) p=0.528. For the 89 women with NSC, 56 could report partner treatment status. There was no additional effect of partner treatment on persistence of cervicitis in this NSC group, RR=1.02 (95% CI 0.54-1.90) p= 0.961 (Table 4).

The mean age of all women enrolled was 30.3 years and median 28.0 years with two thirds (69.2%) being 25 years or older and 30.8% less than 25 years of age. Neither the diagnosis of cervicitis nor presence of one of the main pathogens MG, NG, CT or TV were more likely in women in the age group less than 25 years (p=0.115 and p=0.439 respectively). However, CT
alone was significantly more likely in women under 25 years (prevalence 9.3%) than in women older than 25 years (prevalence 4.2%), $p=0.017$. 
Table 1

**Baseline characteristics of all women with cervicitis (Cx)** (30 pmnl/hpf) (n=268) who were followed up (n=131) or not (n=137), and of all women with cervicitis followed up who had azithromycin (azith) treatment (n=117) or not (n=13).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Women who followed up</th>
<th>Women not followed up</th>
<th>P value</th>
<th>Women with Cx treated azith</th>
<th>Women with Cx not treated azith</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group &lt;25 yrs</td>
<td>47/131 (35.9%)</td>
<td>44/137 (32.1%)</td>
<td>0.516</td>
<td>42/117 (35.9%)</td>
<td>5/13 (38.5%)</td>
<td>0.999</td>
</tr>
<tr>
<td>Any symptoms</td>
<td>92/131 (70.2%)</td>
<td>97/136 (71.3%)</td>
<td>0.844</td>
<td>81/117 (69.2%)</td>
<td>10/13 (76.9%)</td>
<td>0.754</td>
</tr>
<tr>
<td>Current CSW</td>
<td>23/129 (17.8%)</td>
<td>17/137 (12.4%)</td>
<td>0.216</td>
<td>22/115 (19.1%)</td>
<td>1/13 (7.7%)</td>
<td>0.461</td>
</tr>
<tr>
<td>Current smoker</td>
<td>55/128 (43.0%)</td>
<td>52/135 (38.5%)</td>
<td>0.463</td>
<td>48/115 (41.7%)</td>
<td>7/12 (58.3%)</td>
<td>0.361</td>
</tr>
<tr>
<td>Current douche</td>
<td>15/119 (12.6%)</td>
<td>12/118 (10.2%)</td>
<td>0.555</td>
<td>13/117 (12.2%)</td>
<td>2/11 (18.2%)</td>
<td>0.630</td>
</tr>
<tr>
<td>Condom use sometimes/always last 3 months</td>
<td>80/124 (64.5%)</td>
<td>75/129 (58.1%)</td>
<td>0.298</td>
<td>71/111 (64.0%)</td>
<td>8/12 (66.7%)</td>
<td>0.999</td>
</tr>
<tr>
<td>&gt;1 partner last 3 months</td>
<td>44/129 (34.1%)</td>
<td>40/132 (30.3%)</td>
<td>0.511</td>
<td>40/115 (34.8%)</td>
<td>3/13 (23.1%)</td>
<td>0.541</td>
</tr>
</tbody>
</table>

*Numbers vary according to data availability for baseline characteristic

Table 2

**Effect of presumptive azithromycin (azith) treatment** in women with cervicitis (Cx)* and non-specific cervicitis (NSC) (CT, MG, NG, TV excluded) on follow-up outcome of persistent cervicitis.

<table>
<thead>
<tr>
<th>Followup number</th>
<th>Treatment with azith number</th>
<th>Cx persistence with Rx azith</th>
<th>Cx persistence not Rx azith</th>
<th>RR of cervicitis persistence with Rx azith</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All women with Cx n=268</td>
<td>125</td>
<td>114</td>
<td>54/114</td>
<td>7/11</td>
<td>0.74(0.46-1.21)</td>
</tr>
<tr>
<td>Women with NSC n=208</td>
<td>89</td>
<td>82</td>
<td>35/82</td>
<td>5/7</td>
<td>0.60(0.35-1.02)</td>
</tr>
</tbody>
</table>

*Diagnosis of cervicitis > 30 pmnl/hpf on cervical gram stain

# Women with CT, MG, NG or TV at follow up (ie persistent or incident infections) were excluded
Table 3

**Effect of presumptive azithromycin (azith) treatment** in women with cervicitis (Cx)* and non-specific cervicitis (NSC) (MG, CT, NG, TV excluded) on follow-up outcome of any genital symptoms

<table>
<thead>
<tr>
<th></th>
<th>Follow-up number#</th>
<th>Treatment with azith number</th>
<th>Symptoms at follow-up with Rx azith</th>
<th>Symptoms at follow-up not Rx azith</th>
<th>RR Symptoms at follow-up</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All women with Cx</td>
<td>122</td>
<td>114</td>
<td>54/111</td>
<td>8/11</td>
<td><strong>0.67(0.45-1.03)</strong></td>
<td>0.054</td>
</tr>
<tr>
<td>Women with NSC</td>
<td>86</td>
<td>82</td>
<td>41/79</td>
<td>4/7</td>
<td><strong>0.91(0.46-1.79)</strong></td>
<td>0.780</td>
</tr>
</tbody>
</table>

*Diagnosis of cervicitis > 30 pmn/hpf on cervical gram stain

# Women with CT, MG, NG or TV at follow up (ie persistent or incident infections) were excluded. Missing data on symptoms at follow-up in 3 women
Table 4

Effect of additional stated partner presumptive azithromycin (azith) treatment, in women presumptively treated with azithromycin for cervicitis (Cx)* and non-specific cervicitis, on follow-up outcome of persistent cervicitis

<table>
<thead>
<tr>
<th></th>
<th>Number of women with Cx followed up and Rx azith</th>
<th>Number of women able to report partner Rx status</th>
<th>Cx persistence with women stated partner Rx azith</th>
<th>Cx persistence in women stated partner not Rx azith</th>
<th>RR of Cx persistence in women whose partners Rx azith</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cervicitis</td>
<td>114</td>
<td>78</td>
<td>21/45</td>
<td>13/33</td>
<td>1.18 (0.70-2.00)</td>
<td>0.528</td>
</tr>
<tr>
<td>NSC</td>
<td>82</td>
<td>56</td>
<td>12/29</td>
<td>11/27</td>
<td>1.02 (0.54-1.90)</td>
<td>0.961</td>
</tr>
</tbody>
</table>

*Diagnosis of cervicitis > 30 pmnl/hpf on cervical gram stain
DISCUSSION

Although we found presumptive azithromycin treatment was associated with a 30% to 40% reduction in cervicitis persistence in women with NSC and a similar reduction in symptoms for women with cervicitis, these effects did not reach statistical significance. The strongest effect of presumptive treatment was seen in reduction of cervicitis persistence in women with NSC. If azithromycin treatment of women with cervicitis and NSC is associated with a reduction of the outcomes assessed, our results suggest it is not a large effect. A larger RCT study may have more conclusive results.

The benefits of presumptive treatment may be greater in younger women with their reproductive years ahead, particularly in populations with high CT and MG prevalence. There may also be benefits for symptomatic women wanting resolution of their symptoms.

We did not find any benefit on cervicitis persistence of presumptive partner treatment with azithromycin for women with cervicitis or NSC. Given the lack of clear benefit of treating affected women, lack of added benefit of presumptive partner treatment is not surprising. Numbers were small and so confidence intervals wide and there was reliance on stated history of partner treatment.

Cervicitis was a very common diagnosis (48.8%) but prevalence of pathogens commonly associated with cervicitis (CT, MG, NG or TV) was low compared to some other STD populations. 8, 9 The age of women in this study was relatively high with two thirds of women being older than 25 years. We did not find cervicitis or isolation of one of the main pathogens (CT, MG, NG or TV) to be more likely in women younger than 25 years, however CT alone was more likely in women younger than 25 years. Marrazzo 10, 14 and the CDC
guidelines \(^7\) recommended presumptive treatment of cervicitis in women less than 25 years. These recommendations reflect generally higher chlamydia rates in younger women, but as cervicitis is also associated with pathogens other than chlamydia, this age cutoff for presumptive azithromycin treatment may be less applicable to populations where chlamydia rates are low.

An anti-inflammatory role of macrolides including azithromycin has been demonstrated in the respiratory tract \(^{15, 16}\) and a benefit of azithromycin treatment in cervicitis is plausible, arising from anti-inflammatory effects particularly in cases of NSC, not just from antimicrobial action. The post-treatment outcomes examined in this study were persistence of cervicitis and persistence of any symptoms. If lower genital tract inflammation (cervicitis or leucorrhoea) is an indicator of endometritis or subclinical pelvic infection, even in the absence of known pathogens \(^2, 17\), azithromycin treatment in women with cervicitis and NSC could have potential benefits in the reduction of sequelae of subclinical PID. This is a more difficult treatment outcome to assess but may be more important than the outcomes assessed in this study.

**Study strengths and limitations**

The main limitations of this work are that it was not a randomized controlled trial (RCT) and due to loss to follow-up, sample size of women in treatment and particularly in the non-treatment groups, was very small. Additionally mean follow-up time was long and highly variable. This study was part of a larger study investigating the etiology of cervicitis and so was not specifically powered for this treatment outcome question. In a more formalized and resourced study, strict follow-up of women at four to six weeks would be desirable to
increase sample size and more reliably link treatment effect with follow-up outcomes. Additionally factors including new partner exposure, condom use and interim antibiotic use, could not be controlled for prior to follow-up. This study was an observation of the results of clinical practice (a ‘real life’ observational study) and suffered the limitations of this.

Reliance on women’s stated history of partner treatment lessened the reliability of this analysis but our result provides further confidence in adopting an expectant approach with screening the male partner rather than presumptive antibiotic treatment.

Strengths include the inclusion of STD testing of women at follow-up visit so that women with incident or persistent infections at follow-up were excluded from the analysis. Baseline characteristics in women who followed up/not and women who had presumptive treatment/not, were not significantly different. Enrolment rates for the main study were high; prospective design with minimal deviation from standard clinical practice facilitated subject participation and staff involvement. Recruitment occurred in three STD clinics within metropolitan Sydney, but 83% of enrolments were at a primary clinic (Short Street Centre) and study clinicians were a limited trained group, which has advantages from a consistency point but also potential biases.

**CONCLUSIONS**

Although not reaching statistical significance, our findings suggest presumptive azithromycin treatment of women with NSC may be associated with reduced cervicitis persistence and a reduction of symptoms in women with cervicitis. We did not find a benefit of partner presumptive treatment. A larger study, ideally a RCT, may show significant benefit of presumptive treatment of women, particularly in higher STD prevalence populations.
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7. Sexually Transmitted Diseases Treatment Guidelines, 2010. Department of Health and Human Services Centres for Disease Control and Prevention, Dec 17, 2010


CHAPTER 7

Pharyngeal gonorrhoea in women: an important reservoir for increasing Neisseria Gonorrhoea prevalence in urban Australian heterosexuals?

As published in Journal of Sexually Transmitted Diseases 2013,

doi.org/10.1155/2013/967471
FOREWORD

This paper is included in the thesis as it is related to the main body of work and was commenced at the Short Street Centre towards the end of recruitment for the Cervicitis study. Prevalence of *N. gonorrhoea* (NG) in women recruited to the Cervicitis Study was very low (1.1%) and we noticed subsequently that NG prevalence in women was increasing, particularly isolation of NG from the pharynx in women and cases of NG in heterosexual males with increasing reports of acquisition from oral sex. From detailed sexual histories it became apparent that we might be missing a proportion of NG in women because we were not routinely testing for infection in the pharynx (apart from routine pharyngeal screening in female commercial sex workers). Pharyngeal screening in men who have sex with men (MSM) has been routine screening practice for a number of years due to the well documented reservoir of pharyngeal infection in this group with reports of increasing Minimum Inhibitory Concentrations (MIC) to ceftriaxone in extra genital sites. We postulated a similar reservoir in females, especially those regularly engaging in oral sex and changing partners. This study was a collaboration with Monica Lahra at the Neisseria Reference Laboratory, SEALS Laboratory, Prince of Wales Hospital. The study aimed to demonstrate this change in NG epidemiology and also to determine if NG resistance was occurring in this population of heterosexuals alongside the rising reports of ceftriaxone resistance in MSM.
AUTHOR CONTRIBUTIONS

MJ Lusk wrote the manuscript and RU, PK, FG, ML and RK contributed to the manuscript and revisions. MJ Lusk identified trends in the heterosexual client population. RU and MJ Lusk collated the clinical data. MJ Lusk and FG performed data analysis. ML and RK provided antibiotic susceptibility data.
Pharyngeal gonorrhoea in women: an important reservoir for increasing *Neisseria gonorrhoea* prevalence in urban Australian heterosexuals?

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**ABSTRACT**

We aim to characterize sexual behavioral aspects of heterosexual *Neisseria gonorrhoea* (NG) acquisition in two Sexually Transmitted Diseases clinics in Sydney, Australia, 2008-2012. Of 167 NG cases 102 were heterosexually acquired with a trend of increasing NG prevalence in heterosexuals from 1.1% (95%CI 0.6-2.1) in 2008 to 3.0% (95% CI 2.0-4.0) in 2012 (p=0.027). Of heterosexual male cases unprotected fellatio was the likely sexual activity for NG acquisition in 21/69 (30.4%) and commercial sex work (CSW) contact the likely source in 28/69 (40.6%) NG prevalence overall in CSW (2.2%) was not significantly higher than in non-CSW (1.2%) (p=0.15) but in 2012 there was a significant increase in NG prevalence in CSW (8.6%) compared to non-CSW (1.6%) (p<0.001). Pharyngeal NG was found in 9/33 (27.3%) female cases.

Decreased susceptibility to ceftriaxone (MIC $\geq 0.03$ mg/L) occurred in 2.5% NG isolates, none heterosexually acquired. All were azithromycin susceptible.
A significant trend of increasing prevalence of heterosexual gonorrhoea in an urban Australian STD clinic setting is reported. We advocate maintenance of NG screening in women, including pharyngeal screening in all women with partner change who report fellatio, as pharyngeal NG may be an important reservoir for heterosexual transmission. Outreach to CSW should be enhanced.
INTRODUCTION

Latest surveillance indicates rising rates of Neisseria gonorrhoea (NG) in New South Wales, Australia. The risk of HIV transmission is significantly enhanced by co-infection with NG and so the control of NG particularly in light of increasing minimum inhibitory concentration (MIC) values to ceftriaxone, is a major public health concern. The predominance of gonorrhoea amongst Australian urban men who have sex with men (MSM) is well documented but heterosexual gonorrhoea in urban settings is less well characterised. NG is a notifiable disease in Australia but data is only collected by age, sex and region of diagnosis and so heterosexual trends are poorly defined. Trends of increasing prevalence of heterosexually acquired NG and acquisition from fellatio and commercial sex worker (CSW) contact were noted in our suburban STD services in 2009, prompting this investigation specifically aimed at examining sexual behavioral aspects of heterosexual NG acquisition.

METHODS

A case series was conducted from patient records at two STD services in South Eastern Sydney over a 5 year period, 1 January 2008 to 31 December 2012. Data was collected prospectively from late 2009 when the study started but retrospectively prior to this. These clinics operate in a culturally diverse suburban environment and offer free services by triage to high risk patients defined by ‘priority populations’ specified in the 2010-2013 NSW STI (Sexually Transmitted Infection) strategy (ie MSM, youth, CSW, Multi-partnered heterosexuals, intravenous drug users, HIV positive, indigenous) and other symptomatic patients, contacts of STDs or those referred by General Practitioners (GPs).
NG cases were identified from the clinic database. All NG cases were included whether detected by routine screening or testing in symptomatic patients. Heterosexual acquisition was defined as sexual activity not involving any same sex contact in the preceding 12 months. Heterosexual patient numbers were derived from total client numbers, minus MSM and women who have sex with women (WSW). Likely acquisition source and activity was identified from detailed sexual histories, which routinely seek information on the nature and timing of recent sexual contacts including number and sex of consorts, type of sexual contact (oral, vaginal, anal, insertive/receptive) and condom use for each activity. In deciding the likely transmission mode and source of NG, we took into account onset of symptoms and NG disease incubation time.

Receipt of oral sex (fellatio) was considered the likely route of NG infection when this activity occurred in isolation without a condom or if this occurred concurrently with vaginal or anal sex where a condom was used for the latter activities but not for oral sex. A female commercial sex worker was defined as a woman who stated she was currently engaged in sex work. Local contact was defined as sexual contact with a person in Australia.

Clinic policy states that all symptomatic patients are tested for NG in the relevant anatomical site. MSM are screened for NG in the rectum, urine and throat, CSW in the throat and cervix or urine, heterosexual men in the urine and heterosexual women in the cervix or urine. Cases diagnosed by PCR are also cultured where possible, in order to ascertain antimicrobial susceptibility data. NG was treated during the study with ceftriaxone 250mg IMI and this was increased to 500 mg IMI from early 2010 in keeping with local recommendations. Cefixime is not available in Australia.
NG was cultured on selective media of lysed horse blood agar containing vancomycin, colistin, nystatin and trimethoprim (VCNT) inhibitors. Antimicrobial susceptibility testing was performed prospectively at the Neisseria Reference Laboratory, Randwick, Sydney a WHO Collaborating Centre for STD, using published methodology [8]. Decreased susceptibility of NG to ceftriaxone in extra genital sites was reported when the MIC value was ≥0.03 mg/L and ≥0.06 mg/L in genital sites. All samples positive for NG using Nucleic Acid Amplification Techniques (NAAT) (Roche Amplicor PCR from study commencement to June 2011 and then from July 2011 by Roche Cobas 4800) were confirmed by supplementary assays targeting porA and opa genes as required by the National Testing Guidelines.

Proportions were compared using Chi-Square tests and trends identified using a Mantel-Haenszel Chi-Square test with SAS (version 9.2; SAS Institute Inc., Cary, NC, USA).

Ethics approval was granted by the South Eastern Illawarra Area Health Service Ethics Committee.

RESULTS

During the study 6164 patients were seen of which 5118 (83.0%) were classified as heterosexual and 1046 (17.0%) MSM or WSW, with approximately equal numbers of male (53.1%) and female (46.9%) heterosexual patients (table 1). There were 167 cases of NG, 102 (61.1%) heterosexually acquired (overall prevalence 2.0%) and 65 (38.9%) MSM acquired (overall prevalence 6.9%). Over the 5 year period there was a significant trend of increasing NG prevalence in heterosexuals rising from 1.1% (95%CI 0.6-2.1) in 2008 to 3.0% (95% CI 2.0-4.0) in 2012 (p=0.027).
Of heterosexual cases, 69 were male and 33 were female (Male: Female ratio 2.1:1). 67/69 (97.1%) of heterosexual males and 25/33 (75.8%) of females had genital symptoms.

Receipt of unprotected fellatio was the likely source of acquisition for 21/69 (30.4%) of heterosexual males (11/21 CSW related and 10/21 non-CSW related). Commercial sex work (CSW) contact was the probable NG source for 28/69 (40.6%) of heterosexual males (18 CSW contacts local, and 10 whilst overseas). Only 7/33 (21.2%) of female cases reported current CSW. Importantly, NG prevalence during the study overall in CSW (2.2%) was not significantly higher than in non-CSW (1.2%) (p=0.15) but in 2012 there was a significant increase in NG prevalence in CSW seen (8.6%) compared to non CSW (1.6%), p<0.001.

Of female cases, 31/33 (93.9%) reported unprotected vaginal sex. Pharyngeal NG was found in 9/33 (27.3%) women, 5 of these CSW. NG was acquired locally in 24/33 (72.7%) of females and 47/69 (68.1%) of heterosexual males.

137/167 (82%) of NG cases were diagnosed by positive culture and 30/167 (18%) by positive PCR alone. Antimicrobial susceptibility data was available in 122/137 (89%) of NG cases diagnosed by culture. Decreased susceptibility to ceftriaxone was reported in 3/122 (2.5%), (two pharyngeal and one rectal isolate) all MSM related isolates, none heterosexually acquired. Of the 122 isolates with antibiotic susceptibility data, 55/122 (45.1%) were MSM related and 67/122 (54.9%) were heterosexually related. All NG isolates were azithromycin susceptible.
Table 1. Summary of heterosexual patients and NG cases 2008-2012

<table>
<thead>
<tr>
<th>Year</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Hetero patients (male &amp; female)</td>
<td>875</td>
<td>1009</td>
<td>1102</td>
<td>1041</td>
<td>1091</td>
<td>5118</td>
</tr>
<tr>
<td>No. hetero male patients</td>
<td>473</td>
<td>529</td>
<td>577</td>
<td>539</td>
<td>599</td>
<td>2717</td>
</tr>
<tr>
<td>No. hetero female patients</td>
<td>402</td>
<td>480</td>
<td>525</td>
<td>502</td>
<td>492</td>
<td>2401</td>
</tr>
<tr>
<td>Total NG cases</td>
<td>18</td>
<td>35</td>
<td>27</td>
<td>33</td>
<td>54</td>
<td>167</td>
</tr>
<tr>
<td>Total hetero NG</td>
<td>10</td>
<td>26</td>
<td>13</td>
<td>20</td>
<td>33</td>
<td>102</td>
</tr>
<tr>
<td>Hetero male NG</td>
<td>5</td>
<td>19</td>
<td>11</td>
<td>13</td>
<td>21</td>
<td>69</td>
</tr>
<tr>
<td>Hetero female NG</td>
<td>5</td>
<td>7</td>
<td>2</td>
<td>7</td>
<td>12</td>
<td>33</td>
</tr>
<tr>
<td>Prevalence of NG in heterosexuals (%)*</td>
<td>1.14</td>
<td>2.58</td>
<td>1.18</td>
<td>1.92</td>
<td>3.02</td>
<td>2.0</td>
</tr>
<tr>
<td>No. female CSW</td>
<td>50</td>
<td>63</td>
<td>76</td>
<td>75</td>
<td>58</td>
<td>322</td>
</tr>
<tr>
<td>Mean age (yrs) heterosexuals with NG**</td>
<td>32.4</td>
<td>33.1</td>
<td>38.2</td>
<td>44.3</td>
<td>37.4</td>
<td>37.3</td>
</tr>
<tr>
<td>Mean age (yrs) heterosexual female with NG***</td>
<td>32.4</td>
<td>39.0</td>
<td>44.0</td>
<td>25.7</td>
<td>32.8</td>
<td>33.2</td>
</tr>
</tbody>
</table>

*A significant trend of increasing NG prevalence in heterosexuals was noted over the 5 year period (p=0.027).

**No significant age trend in heterosexual male NG cases (p=0.122)

***No significant age trend in heterosexual female cases (p=0.387)
DISCUSSION

This study found an increasing prevalence of heterosexual gonorrhoea in an urban Australian setting from 2008-2012, a trend which may be contributing significantly to rising NG notifications in Australia. At our services heterosexual acquisition accounted for 61.0% (102/167) of NG cases. The male: female ratio in heterosexual cases of 2.1:1 is comparable to the overall national Australian surveillance ratio of 2:1 \(^9\). The overall study male: female ratio, including the MSM cases was 4.0:1, which is in marked contrast to our South East Sydney local health district reported ratio of 8:1 where NG detection predominates in the large MSM population \(^1,10\). This reflects the lower proportion of MSM attendances at our clinics (25.8%) compared to inner city services. The persistence of the 2:1 male: female case ratio in heterosexuals is interesting. Factors contributing to this male predominance in heterosexual NG case detection might include increased likelihood of symptomatic disease and therefore detection in males (and more asymptomatic disease in females), lack of pharyngeal screening in females, suboptimal screening of CSW and high risk females and possibly some misclassification of ‘heterosexual’ acquisition. Enhanced surveillance of NG might help to clarify this enigma.

Receipt of unprotected fellatio was the likely sexual activity resulting in NG acquisition for 30% of heterosexual men. Additionally the number of heterosexual male NG cases due to fellatio may be underestimated in this study if receipt of unprotected oral sex occurred in conjunction with unprotected vaginal or anal sex. The pharyngeal NG reservoir in MSM is well recognized \(^11,12\) but this reservoir could also be important in all women with partner change who practise fellatio, not just CSW. This theory is supported by the high transmission rate in heterosexual men receiving fellatio and noting that equal numbers of fellatio-related
transmissions occurred in men reporting contact with CSW and non-CSW females. Oral sex is frequent amongst heterosexuals and is typically unprotected. This combined with lack of awareness of the associated STD transmission risk, common perception that oral sex is not sex and infrequent pharyngeal screening in women, may facilitate heterosexual NG transmission via this route. We isolated pharyngeal NG from 27% of female cases (by culture) but this is likely to be an underestimate due to clinic policy at the time of only undertaking pharyngeal screening in CSW and MSM. Increased uptake of more sensitive NAAT testing in the pharynx is also likely to improve female NG detection. Our findings in both men and women suggest a NG pharyngeal reservoir in women may be a common source of NG infection for heterosexual males. A recent UK study also suggested the pharynx may be an important NG reservoir in heterosexual women with a similar finding of 30% of female NG cases being pharyngeal. We found NG Infections in heterosexual men were almost always associated with genital symptoms (97.1%) but women less commonly so (75.8%). Hence asymptomatic screening in women may be particularly important. Accordingly, our clinic guideline has changed to recommend the maintenance of NG screening in heterosexual women with additional pharyngeal NG screening in those women reporting partner change and fellatio. As female cases generally reported unprotected vaginal sex (93.9%), condom use is also reiterated.

CSW in Australia have low rates of STDs reflecting good condom use. However a recent study of CSWs providing fellatio in Sydney found Cantonese speaking women were significantly less likely to use condoms for this service than Thai-speaking and English-speaking CSW. Additionally, women who don’t identify as being CSW (eg. working in massage) may be less likely to engage in safe sex, including safe oral sex. Our study found
40% of heterosexual males reported CSW contact but only 21% of female cases were CSW, an inconsistency which could reflect suboptimal testing rates and outreach to CSW in our population. Importantly NG prevalence in female CSW overall was no different from that in female non-CSW, except for the significant rise noted in 2012. Of concern however was the finding that 2/3 of the NG infections related to CSW contact occurred locally in Sydney, the rest acquired from overseas contacts. This would suggest a need to enhance local educational and testing services available to women engaged in CSW.

Three quarters of all infections were acquired from local contact, reflecting the increasing local heterosexual NG prevalence noted in this study and from local surveillance. Numbers of NG cases rose in 2012 which is also in keeping with the rise in local NG prevalence.

Antimicrobial susceptibility data was available for 89% of cultured isolates. Decreased susceptibility to ceftriaxone occurred in 3/122 (2.5%) isolates, all MSM related cases from extra genital sites. No decreased susceptibility was noted in heterosexually acquired isolates but numbers are too small to speculate on the significance of any difference in antibiotic susceptibilities between these populations at this time, but this should be the subject of ongoing monitoring and surveillance to inform treatment recommendations in these different populations. The finding of three isolates with decreased susceptibility to ceftriaxone is consistent with the right shift in MIC values to ceftriaxone reported locally and globally and cause for concern for disease control in the absence of viable treatment alternatives if resistance to ceftriaxone develops. All isolates were sensitive to azithromycin.

We recommend cases positive by PCR should also be cultured where possible for purposes of monitoring NG isolate susceptibility. The widespread supplanting of culture methods with PCR has the advantages of greater sensitivity and no fuss specimen transport, but at the
cost of antimicrobial surveillance. STD services are best placed to maintain this NG antimicrobial resistance surveillance role.

This study is limited by small numbers, reliance on patient sexual histories and its partially retrospective nature in two triaged STD clinic populations within the same local health district. Inevitably some definitions, particularly relating to transactional sex can become blurred and sexual histories are reliant on patient recall and propensity to disclose the exact nature of the contact. Some cases were unwilling or unable to identify the likely source of the infection. Clinical judgment was applied to determine the likely source of infection based on disease incubation, onset of symptoms and detailed recent sexual history. Triage processes were unchanged over the study period and priority population groups remained relatively stable. Importantly antibiotic resistance testing was prospective and performed in a reference laboratory, a WHO Collaborating Centre for STD.

**CONCLUSION**

A significant trend of increasing prevalence of heterosexual gonorrhoea in an urban Australian STD clinic setting is reported. This study suggests the pharynx may be an important reservoir for heterosexual NG transmission and we advocate maintenance of NG screening in women, particularly inclusion of pharyngeal screening in women with partner change who practise fellatio. Case detection, enhanced surveillance and health promotion are pivotal to NG control. Health promotion efforts should include messages concerning STD transmission risks associated with oral sex in heterosexuals and we recommend enhanced CSW engagement with education and STD testing opportunities.
REFERENCES


CHAPTER 8

SUMMARY and CONCLUSIONS

CERVICITIS MANAGEMENT GUIDELINE
SUMMARY and CONCLUSIONS

The aims of the Cervicitis Study have been addressed in these chapters and papers as well as exploration of side topics including the associations and epidemiology of the mollicutes, diagnostic recommendations concerning *Trichomonas vaginalis* and changing epidemiology of *Neisseria gonorrhoea* in STI clinic populations in Sydney.

This body of work has contributed to the literature, both internationally and nationally, informing Australian clinicians on the cervicitis prevalence and associations we may expect to find in our urban populations. To date this is the only study of cervicitis in Australian women.

Chapters 5 and 6 present the summary papers of the full Cervicitis Study findings and make some substantial contributions to our knowledge of the etiology, consensus definition and management of cervicitis. Using a large multi-site cross-sectional study we comprehensively examined all infectious and non-infectious exposures putatively associated with cervicitis in the literature and were able to demonstrate by multivariate analysis significantly increased risks of cervicitis for CT, MG, NG and TV and a reduction in cervicitis risk with condom use. Important negative findings were the lack of associations of cervicitis with BV, CMV, HIV, HSV and young age.

The Study also demonstrated the superior utility of the cervicitis case definitions mucopurulent discharge and yellow discharge. These definitions diagnose fewer women with a ‘disease’ and have consistently higher PPVs and specificities for the significantly
associated pathogens than the traditional microscopy case definition of >30 pmnl/hpf. These findings provide evidence for a consensus case definition for cervicitis and concur with the growing opinion that cervicitis case definition by microscopy lacks practicality and utility in most clinical settings.

Despite this comprehensive study of potential causative infections associated with cervicitis, the population attributable risks for cervicitis of the significant pathogens, namely CT, NG, MG and TV (assuming causative roles), might still only account for 23.4% of cervicitis in our STI population, using the most predictive cervicitis definition of MCP. This leaves the majority of cases unexplained which is a slightly disappointing result. Pathogen prevalence was low in this study (reflecting the relatively high socioeconomic status of our population with good access to health care) and the population attributable risk% (PAR%) figure would rise in populations with higher STI rates. It does raise the question though, of other factors at play (such as cytokines and mechanical factors) and the possibility that cervicitis could be part of a normal continuum in some women. There is also the likelihood of other organisms involved in the etiology of cervicitis that are yet to be found. MG is the ‘new chlamydia’ and there may be more pathogen discoveries as ability to detect and study our microbiome expands.

The results of the observational treatment study of women and their male partners with azithromycin 1 G orally, are interesting and add to our current sparse knowledge of how best to manage this very common and often symptomatic condition.

Although we found presumptive azithromycin treatment was associated with a 30% to 40% reduction in cervicitis persistence in women with NSC and a similar reduction in symptoms
in women with cervicitis, these effects did not reach statistical significance. A larger RCT study may show more conclusive results. If azithromycin treatment of women with cervicitis and NSC is associated with a reduction of the outcomes assessed, our results suggest it is not a large effect. Longer term reproductive and genital tract health outcomes in women may be more relevant outcomes to measure in assessing the benefit or not of presumptively treating women with cervicitis and NSC.

From the literature review it seems likely there are benefits of azithromycin treatment stemming from its anti-inflammatory properties as well as the treatment of undiagnosed subclinical PID and endometritis, outcomes that have not been assessed in this study. Evidence suggesting that cervicitis may predict upper genital tract infection is quite compelling and logical and this adds to argument for treating women with cervicitis. These potential benefits of treating women must be weighed against with the disadvantages of labelling them as having an ‘STI’ and potentially overusing antibiotics. The benefits of presumptive treatment may be greater in younger women with their reproductive years ahead, particularly in populations with high CT and MG prevalence. There may also be benefits for symptomatic women wanting resolution of their symptoms.

The finding of no benefit of additional male partner treatment was also useful and not unexpected given the non-significant benefit in women themselves. Our result provides further confidence in adopting an expectant approach with screening male partners rather than giving them presumptive antibiotic treatment.
Study strengths and weaknesses

This research was not commissioned and was minimally supported by specific funding, borne out of a genuine curiosity and desire to provide clarity and practical solutions for a commonly encountered problem in clinical practice. Accordingly it had to be designed to take place opportunistically as part of routine practice, in publically-funded STI services in Sydney and by necessity involved considerable collaboration with SEALS Laboratory.

Recruitment of women to the study involved a convenience sample of 558 of consecutively presenting women >18 years of age who required vaginal examination based on presenting complaint or who requested examination. Hence the sample was biased towards the recruitment of symptomatic women and in fact 60 % of women recruited had genital symptoms. This biased could contribute to the high rates of Trichomans vaginalis and also to the high rates of cervicitis prevalence, particularly by the microscopy definition of (> 30 pmnl/hpf). This high prevalence of cervicitis is fully discussed in Chapter 5 of the thesis, where findings are compared using different cervicitis definitions; in particular cervicitis by the definition MCP was found to be much less prevalent and more predictive of significant pathogens.

The main study was cross-sectional and so had limited inference on causality, but sample size was large and appropriately powered. Recruitment occurred at three related STI clinics but 83% at the primary clinic (Short Street Centre) using a small group of trained recruiting clinicians with advantages for consistency but also potential biases.

The findings of the treatment study do have significant limitations and need to be interpreted in the context of the main Cervicitis Study paper (chapter 5). Main limitations
were that it was not a RCT, follow-up numbers were small and there was a long and variable time to follow-up for many women (reflecting clinical medicine as it really happens). However, women with incident or persistent infections at follow up were excluded from the analysis. In a more formalized and resourced study, strict follow-up of women at 4 to 6 weeks would be desirable to more reliably link treatment with effect on follow-up outcomes. For the analysis of effect of additional partner treatment we were reliant on patient report. This study was an observation of the results of clinical practice and suffered the limitations of this.

The completion of the study was a result of the dedication and hard work of all the investigators and collaborators and represents quite a unique effort. There were no conflicts of interest.

**Areas of future research**

A vast amount of clinical and laboratory data was collected for this study and accordingly there are several areas of potential future study from the existing data and other research questions generated.

There is a subset of women who had multiplex PCR testing in cervical as well as urine samples, and a comparison of the yields from these specimens would be a useful study of test performance and help to guide testing practice. It would be good to see SEALS Laboratory develop a multiplex PCR for CT/NG/MG and TV as a flow-on from this study. It would be a big seller!
There were a large number of women in the Cervicitis Study who had persistent cervicitis and persistent symptoms who were followed up over several visits. Most clinicians will be familiar with these cases who present a diagnostic and management dilemma and often end up being referred to gynecologists for investigations and surgical interventions. A hypothesis I have concerning these women is that a number of them have gynecological pathology, in particular conditions such as chronic PID, endometriosis, adenomyosis and polycystic ovarian syndrome which may be associated with chronic discharge. A case-control study of these potential associations could be considered but such a plan might be hampered somewhat by the disconnect between gynaecology and STI research and would require collaboration with a gynaecology service.

In the study of women with MG (Chapter 4) two women with MG were found to have ectopic pregnancies, one at the time of diagnosis and one just after diagnosis of MG. There is only one previous study examining the relationship between MG and ectopic pregnancy and this was inconclusive. Given that MG seems to behave very similarly to CT, it is possible that untreated MG may be associated with ectopic pregnancy, a disastrous health and reproductive outcome. A case-control study of this association would be relatively straightforward and something I am considering in the future dependent upon funding opportunities.
CERVICITIS MANAGEMENT GUIDELINE

The following is a guideline for the diagnosis, testing and management of women with cervicitis, based on the findings of this Cervicitis Study in the context of my extensive literature review chapters.

Background

Cervicitis is a common, often symptomatic condition that can be associated with the infections CT, MG, NG and TV. However many cases are present in the absence of these pathogens (non-specific cervicitis) and so cervicitis should not be considered synonymous with STI infection. In STI populations prevalence can range from 20 to 40% of women. Cervicitis can be an indicator of upper genital tract infection in some women.

Testing & Diagnosis

Diagnostic criteria differ but the presence of a mucopurulent discharge or yellow discharge (observed by clinician or noted by the patient) are case definitions for cervicitis with better prediction and higher specificity for the pathogens CT, MG, NG and TV than the microscopy Gram stain diagnosis of >30 pmnl/hpf.

Microscopy is still an extremely useful tool at the point of consultation for diagnosing many causes of vaginal discharge and should be encouraged where available to confirm the presence of cervicitis, bacterial vaginosis and candida by Gram Stain and TV by wet preparation. PH measurement is also very useful and will be elevated in BV, TV and frequently secondary to douching and presence of semen or leucorrhoea.
All women presenting for screening or symptomatic assessment should be tested for CT and NG. If cervicitis is present, laboratory testing should also be performed for MG and TV by PCR, if available. Selective testing for MG and TV is particularly suggested for women with sexual contact or reporting consort contacts in higher prevalence areas such as Asia, Africa, USA and South America. TV may be more likely after rural Australian or Pacific sexual contacts.

PCR testing from a vaginal swab is more sensitive than testing a first void urine sample.

**Treatment/management**

The benefit of presumptive azithromycin treatment of women with cervicitis is unclear, but there may be benefit of treating women for resolution of cervicitis and/or vaginal symptoms. Presumptive treatment should only be offered to women who are unlikely to return for follow-up, women of reproductive age reporting recent partner change and unprotected vaginal sex or women with HIV or at high risk of HIV acquisition.

If a woman is low-risk sexually and/or has asymptomatic cervicitis, presumptive treatment should be deferred pending laboratory testing results and treatment given if informed by test results.

Condom use reduces the risk of cervicitis.

Follow-up of women with asymptomatic cervicitis, in the absence of isolation of a pathogen, is not necessary. If a woman has persistent symptoms, follow-up is recommended in 4 to 6 weeks.

**The recommended treatment for cervicitis is azithromycin 1 G orally.**
If a woman has additional genital symptoms such as dyspareunia, pelvic pain, changed menstrual pattern (heavy/painful/spotting) or urinary symptoms in the presence of cervicitis, the clinician should have a low threshold for treating for subclinical PID particularly in women of reproductive age.

**Treatment if PID is suspected:**

**Azithromycin 1 G orally**

Repeat dose in 1 week (or add doxycycline 100mg BD for 10 days)

PLUS metronidazole for 10 days orally

PLUS ceftriaxone 500mg IMI

The presumptive treatment of male sexual partners is not recommended, however if a pathogen is isolated on testing or a woman has persistent cervicitis, partners should be tested and their treatment informed by pathogen isolation.

**Persistent Cervicitis**

If cervicitis is persistent after treatment with azithromycin 1 G orally, testing for MG and TV should be done and tests for CT and NG repeated if reinfection is possible or a new partner reported. Make sure she has had a recent pap smear and pelvic examination.

If a woman has persistent asymptomatic cervicitis and tests for CT, NG, MG and TV are negative it is reasonable to give no further treatment and review as required.
If a woman has persistent cervicitis accompanied by other symptoms such as pelvic pain, menstrual irregularity, urinary symptoms or dyspareunia, broader spectrum antibiotic treatment should be used to cover the possibility of MG-associated PID (in the absence of access to MG testing).

*Treatment for MG-associated PID*

*Azithromycin 500mg on day 1 then 250 mg for a further 5 days*

PLUS *Metronidazole 400mg BD for 10 days*

PLUS *ceftriaxone 500mg IMI*

If MG is isolated a test of cure should be performed in 4 to 6 weeks from a vaginal/cervical swab and check that partner(s) have also been treated for MG. If MG is not responsive to azithromycin treat with moxifloxacin 400mg once daily for 7 days and again perform a test of cure at 4 to 6 weeks post treatment.

If treatment is unsuccessful in resolving symptoms, high resolution pelvic ultrasound is recommended and consideration of gynaecological investigation.
REFERENCES


5. Sexually Transmitted Diseases Treatment Guidelines, 2010. Department of Health and Human Services Centres for Disaes Control and Prevention, Dec 17, 2010


7. Lusk MJ, Garden FL, Cumming RG, Konecny P. A non randomised prospective controlled study of empiric treatment with azithromycin in women with crervicitis and non specific cervicitis amnd their male partners in sydney STD clinic populations.

APPENDIX PART 1

Conference and meeting abstracts
Conference and meeting abstracts

FOREWORD

In 2009 I was invited to give two oral presentations at the Australasian Sexual Health Conference in Brisbane. These were interim findings from the Cervicitis Study and were also oral platform presentations that same year at the St George Hospital Research Symposium.

In 2010 the abstract ‘Mycoplasma genitalium: association with cervicitis, response to azithromycin treatment and partner carriage’ was accepted for poster presentation at the Australasian Sexual Health Conference.

On 19 June 2015 I presented the findings of the NG Epidemiology study (Chapter 7) to the National Neisseria Network meeting in Sydney, Australia.
Mycoplasma genitalium is associated with cervicitis in a metropolitan Sydney Sexual Health Clinic Population.

2009 Oral presentations Australasian Sexual Health Conference and St George Hospital Research Symposium

Mycoplasma genitalium (MG) is a recognised causative agent of sexually transmissible infection (STI). Its role in male non-specific urethritis has been recently established, but the epidemiology of MG in women and its role in cervicitis is less well characterised. The development of molecular diagnostic testing has facilitated identification of a wider range of organisms potentially implicated in urogenital disease.

A prospective cross-sectional study investigating the prevalence and aetiology of cervicitis in two Sydney STI clinics enrolled 423 women from 2006-2009. A multiplex polymerase chain reaction (mPCR) assay designated VDL06 was configured and validated for the detection of MG, Ureaplasma parvum, Ureaplasma urealyticum and Mycoplasma hominis. Cervical samples were analysed using VDLO6. Cervicitis was defined as >30 polymorphonuclear leucocytes per high-powered field (>30 pmnl/hpf) on three non-adjacent fields in a Gram stain of cervical mucous.

Cervicitis prevalence in this cohort was 39%. MG was detected in 14/423 women, a prevalence of 3.31% (95 % CI 1.82%, 5.49%). The unadjusted prevalence ratio of cervicitis in women with MG was 2.09 (95 % CI 1.55, 2.82 P=0.002). The frequency of MG in the women
with cervicitis was 11/165 (6.7%). The clinical and demographic data for the women with MG will be presented. Data will also be presented on the prevalence and cervicitis association of the other mollicutes studied in this cohort.

This is the first Australian study to demonstrate a significant association of MG with cervicitis and to determine prevalence of MG in urban Australian women. The application of a mPCR increased our ability to investigate a range of organisms potentially associated with cervicitis. The capacity to detect MG in women with cervicitis will facilitate better targeted therapy for this common condition. Molecular testing for MG should be available routinely for clinical services to improve STI management, particularly for cervicitis and urethritis.
Trichomonas vaginalis: under-diagnosis in urban Australia could facilitate re-emergence

2009 Oral presentations Sexual Health conference and St George Hospital Research Symposium.

Trichomonas vaginalis (TV) has a low profile in urban sexually transmitted disease (STD) clinics in Australia and in many developed countries. TV prevalence in urban Australia is reportedly low based largely upon routine wet preparation direct microscopy in highly screened CSW populations.

A prospective study in a metropolitan Sydney STD clinic, TV was detected by a polymerase chain reaction (PCR) assay in 16/356 women (4.5%, 95% Confidence Interval (CI) 2.6% - 7.2%), whereas discretionary wet preparation microscopy in women with clinically suspicious vaginal discharge found only four cases (1.1%, 95% CI 0.3%-2.8%). This PCR result exceeds recently reported urban Australian rates of <1%. The clinical and demographic features of the TV positive women will be presented.

In this cohort a high proportion of women with TV were not from a recognized geographic risk group but their consorts were. Local acquisition of TV was common. Urban society in Australia, as in other developed countries, is very culturally diverse. Accordingly, sexual networks particularly of migrant and first generation Australians may include contacts from populations with higher TV prevalence.
This data suggests that the current low profile of TV in urban Australian STD clinics and the variable application of insensitive tests for case detection may be leading to under-diagnosis of TV. This could facilitate a re-emergence of TV in diverse urban populations. An increase in trichomoniasis-associated adverse reproductive outcomes and enhanced HIV transmission could pose a salient public health threat. Accordingly we propose that standardized molecular detection tests for trichomoniasis be part of routine testing in urban Australian STD settings.
Mycoplasma genitalium: association with cervicitis, response to azithromycin treatment and partner carriage’

2010 poster presentation Australasian Sexual Health Conference

AIM

To assess Mycoplasma genitalium (MG) prevalence, association with cervicitis, response to Azithromycin treatment and partner carriage in women enrolled in the Cervicitis Study in an STI clinic setting in Sydney.

METHODS

Twenty one women from a cross-sectional study of 541 women at two STI clinics in Sydney tested positive on cervical swabs for MG. Prevalence of MG, association with cervicitis, response to Azithromycin treatment, and partner carriage rates were analyzed.

RESULTS

The prevalence of MG was 21/541 (3.9%). MG was significantly associated with cervicitis (18/21) (RR 1.85 (1.53-2.28) p<0.0001). Of the 21 women with MG, 16 were treated with Azithromycin (1G stat) for cervicitis at the time of consultation. Follow-up MG testing with clinical assessment was possible in 13 women (of whom 11 had received Azithromycin treatment and two had received doxycycline and metronidazole as cover for PID). All 13 women were negative for MG although nine had persistent cervicitis. Of the five male partners of women with MG, asymptomatic MG carriage was found in 2/5 (40%).
CONCLUSION

Azithromycin appears to be an effective treatment for *Mycoplasma genitalium* infection. MG is common in an STI clinic population and is associated with cervicitis. Carriage in 40% of male partners supports sexual transmission. The significance of persistent cervicitis after microbiological cure in these women warrants further investigation and will be discussed.
APPENDIX PART 2

Cervicitis Study Ethics-approved documents
(Short Street Centre and RPA sites)
Participant Approach
Participant Information and Consent
Partner Information
Infectious organisms associated with Cervicitis in Sexual Health populations in Sydney.

To women attending the service, we are now running an approved research study to try to improve the health of Women.

We need your help.

Please read on ....
Infectious organisms associated with Cervicitis in Sexual Health populations in Sydney.

Cervicitis Study Eligibility

Eligible for study (tick any that apply to you)

- □ > 18 yrs
- □ Sexually active in last 3 months
- □ Require/requesting vaginal examination today

Not Eligible for study (tick any that apply to you)

- □ < 18 yrs
- □ Not sexually active in last 3 months
- □ Not requiring/requesting vaginal examination today
- □ Have period currently
- □ Pregnant
- □ Have IUD contraceptive device
- □ Cervical surgery in last month
- □ Termination of Pregnancy (TOP) in last month
- □ Clinical pelvic infection (PID)
- □ Antibiotics in previous month
- □ Hysterectomy
- □ Previously enrolled

Patient Enrolment

- □ Consent ing to take part in study
- □ Not consenting to take part in study
- □ Defer
Infectious organisms associated with Cervicitis in a metropolitan Sydney Sexual Health Clinic population.

Participant Information statement

As a female patient attending the Short Street Centre, you are invited to participate in a Cervicitis Study we are conducting at this clinic.

We hope to learn more about the condition of CERVICITIS (inflammation of the cervix). This is a poorly understood, relatively common condition affecting the cervix (neck of the womb), which can be caused by the sexually transmitted infections chlamydia and gonorrhoea. However in over half of the cases of cervicitis other infective agents may be involved, which are often not identified in routine testing and their role is not well defined in this condition, termed ‘non-specific cervicitis’. Some of these less well known infections can also lead to pelvic infection and have an effect on subsequent pregnancies if not treated. Due to recent advances in modern laboratory technology we now have the capacity to more easily identify these organisms.

Presently when cervicitis is diagnosed, we offer routine antibiotic treatment (azithromycin tablets). By finding out more about the possible causes of cervicitis, we hope to contribute information to improve understanding, definition and current treatment and so reduce potential complications of this condition.

Cervicitis is diagnosed on an internal vaginal examination with a speculum (like having a Pap test). Depending on your reasons for attending today, an internal examination may be appropriate and only if so, will we invite you to participate in this study (provided you fulfil eligibility criteria).

Your participation in the study is completely voluntary and optional and will in no way affect the quality of the care you receive at this Clinic. We cannot guarantee that you will gain any direct benefit from participation. There is no financial incentive provided for participation.

If you decide to participate, you will be offered our routine clinic tests and in addition two extra swabs will be taken from the cervix for the ‘additional tests’. These ‘additional tests’ are looking for other infectious agents believed to be associated
Infectious organisms associated with Cervicitis in a metropolitan Sydney Sexual Health Clinic population.

with cervicitis. We plan to look at how common cervicitis is in women with and without these infections. By participating you will only have two additional cervical swabs. There are no other interventions involved or changes from routine clinical practice, hence we believe there to be minimal additional risk of harm to you by participation. Sometimes after swabs are taken from the cervix, there may be some slight spotting or discomfort.

If cervicitis is diagnosed you will be offered the standard treatment for cervicitis (azithromycin tablets), regardless of participation in the study. Additional treatment will be provided to you if the additional test results identify any infectious agent where there is treatment of known benefit.

We advise that current sexual partners be offered treatment also. We will provide you with a contact/information slip for your partner, who can receive testing and treatment at this clinic or elsewhere. We are hoping to test partners as part of this Cervicitis study and so would encourage partners return to this clinic.

We will request that women diagnosed as having cervicitis return for follow-up testing and review at 4 to 6 weeks which is routine clinical practice.

During your consultation today we will ask you if you wish to participate in this study and if so, after full discussion, will ask you to sign a participant consent form (attached).

Dr Josephine Lusk

Senior Sexual Health Registrar
Cervicitis Study Chief Investigator
BSc FRNZCGP DRCOG
Infectious organisms associated with Cervicitis in a metropolitan Sydney Sexual Health Clinic population.

Participant Consent form

1. I,…………………………………………………...of…………………………………………………. …………aged ……….years, agree to participate as a subject in the study described in the subject information statement ,attached to this form.

2. I acknowledge that I have read the Subject Information Statement, which explains why I have been selected, the aims of the experiment and the nature and possible risk of the investigation, and I understand the statement to my satisfaction.

3. Before signing this consent form, I have been given the opportunity to ask questions relating to any harm I might suffer as a result of my participation and I have received satisfactory answers.

4. I understand that I can withdraw from the study at any time without prejudice to my relationship to the Short Street Clinic and St George hospital.

5. I agree that research data gathered from the results of the study may be published, provided that I cannot be identified.

6. I understand that if I have any questions relating to my participation in this research, I may contact Dr. Josephine Lusk or Dr. Pam Konecny on ph 9350 2742, who will assist with my queries.

7. I acknowledge receipt of a copy of this consent form and the Subject information sheet.

Complaints may be directed to the South Eastern and Illawarra Area Health Service Human Ethics Committee, Southern Section, St George Hospital, Gray Street, Kogarah, 2217. Ph 9350 2481 fax 9350 3968
Infectious organisms associated with Cervicitis in a metropolitan Sydney Sexual Health Clinic population.

---------------------------------------
Signature of subject

---------------------------------------
Signature of witness

---------------------------------------
Print name

---------------------------------------
Print name

---------------------------------------
Date

Nature of witness

Revocation of consent

I hereby wish to WITHDRAW my consent to participate in the research project (titled above) and described in the patient information statement, and understand that Short Street Clinic and St George Hospital have previously agreed that such withdrawal WILL NOT jeopardize any treatment or my relationship with Short Street clinic or St George Hospital, or my medical attendants.

---------------------------------------
Signature

---------------------------------------
Date

---------------------------------------
Print name

The section for Revocation of consent should be forwarded to Dr Josephine Lusk (Chief Investigator), Short Street Clinic, Ground Floor Prichard Wing, St George Hospital, Kogarah, 2217
Infectious organisms associated with Cervicitis in a metropolitan Sydney Sexual Health Clinic population.

Partner Information Statement

As a sexual partner of a female patient diagnosed as having cervicitis, who attended the Short Street Centre, you are invited to participate in a Cervicitis Study we are conducting at this clinic.

We hope to learn more about the condition of CERVICITIS. This is a poorly understood, relatively common condition affecting the cervix (neck of the womb), which can be caused by the sexually transmitted infections Chlamydia or gonorrhoea (about 50 % of the time). However in over half of the cases of cervicitis other infective agents may be involved, which are often not identified in routine testing and their role is not well defined in this condition, termed ‘non-specific cervicitis’.

Presently when cervicitis is diagnosed, we offer routine antibiotic treatment to the affected women and to her sexual partner. By finding out more about the possible causes of cervicitis, we hope to contribute information to improve understanding, definition and treatment and so reduce potential complications of cervicitis.

If you decide to participate, you will be offered the standard tests for Chlamydia and gonorrhoea, performed on a urine sample. No other examination is necessary unless you have symptoms yourself. We will also test the urine sample for other infectious agents believed to be associated with cervicitis.

You will receive the standard treatment for contacts of cervicitis (azithromycin tablets only), regardless of participation in the study. Additional treatment will be provided to you if the additional test results identify an infective agent where there is treatment of known benefit.

Your participation in the study is completely voluntary and optional and will in no way affect the quality of the care you receive at this Clinic. We cannot guarantee that you will gain any direct benefit from participation.
PARTICIPANT INFORMATION SHEET
1 of 3

Infectious organisms associated with Cervicitis in metropolitan Sexual Health patients in Sydney.

You are invited to participate in a Study.

We hope to learn more about the condition of CERVICITIS (inflammation of the cervix or neck of the womb). This is a poorly understood, common condition which can be caused by the sexually transmitted infections Chlamydia and Gonorrhoea, however in the majority of cases other unidentified infectious organisms, which can be passed between sexual partners, are thought to be involved, hence the term 'non specific cervicitis'. Presently we cannot test for many of these organisms, but recent advances in lab technology have made this easier.

We plan to look at how common cervicitis is in women with and without these infections. Cervicitis is thought be associated with pelvic infection and so affects the reproductive health of women. By finding out more about the possible causes of cervicitis we hope to improve upon present management.

The study is being conducted within this institution by Dr. Josephine Lusk, Staff Specialist in Sexual Health. It is part of a larger study involving one other Sexual Health Services and SEALS Virology Research Laboratory, Prince of Wales Hospital, Randwick.

Cervicitis is diagnosed on an internal vaginal examination with a speculum (like having a Pap test). Depending on your reasons for attending today, an internal examination may be appropriate and only if so, will we invite you to participate in this study (provided you fulfil eligibility criteria).

If you agree to participate in this study, you will be asked to sign a Participant Consent Form. Two extra sterile swabs will then be taken from the cervix during your vaginal examination. These extra study swabs will be used to look for other infectious agents believed to be associated with cervicitis. There are no other interventions involved or changes from routine clinical practice. If cervicitis is diagnosed you will receive the standard treatment of a single dose of an antibiotic, regardless of participation in the study. Results of any routine tests done today will be available to you as per usual. The additional ‘study swabs’ will be tested in batches at a later time, and results only available to you if these additional tests identify organisms where there is further treatment of known benefit.
PARTICIPANT INFORMATION SHEET
2 of 3

Infectious organisms associated with Cervicitis in metropolitan Sexual Health and Gynaecology clinic patients in Sydney.

If you are diagnosed with cervicitis, you will be asked to return for follow-up testing and review 4 to 6 weeks later, which is routine clinical practice. We would advise that your current sexual partner(s) be offered treatment also. We will provide you with a contact/information slip for your partner(s), who can receive testing and treatment at this clinic or elsewhere. We are also hoping to test partners as part of this Cervicitis study and so would encourage partners to return to this clinic, however their participation in this study is completely optional and will not affect their quality of care.
In addition the researchers would like to have access to your medical record to obtain information relevant to this study only.

RISKS

The risks of participating in this study are:
- Sometimes after swabs are taken from the cervix, there may be some slight spotting or discomfort, which usually settles quickly.
- You may receive special study results in addition to routine results at a time later than the initial consultation. These will only be made available to you if they are validated and there is a recognised intervention available of known benefit

BENEFITS

While we intend that this research study furthers medical knowledge and may improve management of cervicitis in the future, it may not be of direct benefit to you.

COSTS

Participation in this study will not cost you anything, nor will you be paid.

VOLUNTARY PARTICIPATION

Participation in this study is entirely voluntary. You do not have to take part in it. If you do take part, you can withdraw at any time without having to give a reason. Whatever your decision, please be assured that it will not affect your medical treatment or your relationship with the staff who are caring for you.
Participant Information Sheet
3 of 3

Infectious organisms associated with Cervicitis in metropolitan Sexual Health and Gynaecology clinic patients in Sydney.

Confidentiality

All the information collected from you for the study will be treated confidentially, and only the researchers named above and the clinician who you see will have access to it. The study results may be presented at a conference or in a scientific publication, but individual participants will not be identifiable in such a presentation.

Further Information

When you have read this information, Dr Josephine Lusk will discuss it with you further and answer any questions you may have. If you would like to know more at any stage, please feel free to contact her 95153131. This information sheet is for you to keep.

Ethics Approval

This study has been approved of the Ethics Review Committee (RPAH Zone) of the Sydney South West Area Health Service. If you wish to discuss your rights as a study participant or if you have concerns or complaints about the conduct of this study, you should contact the Secretary on 02 9515 6766 and quote the protocol number X07-0277/07/RPAH/170.

Dr. Josephine Lusk

Cervicitis Study Chief Investigator
Staff specialist in Sexual Health
BSc MBChB FRNZCGP DRCOG MPH FACSHM
Infectious organisms associated with Cervicitis in a metropolitan Sydney Sexual Health Clinic population.

**Partner Information Statement**

As a sexual partner of a female patient diagnosed as having cervicitis, who attended the Short Street Centre, you are invited to participate in a Cervicitis Study we are conducting at this clinic.

We hope to learn more about the condition of CERVICITIS. This is a poorly understood, relatively common condition affecting the cervix (neck of the womb), which can be caused by the sexually transmitted infections Chlamydia or gonorrhoea (about 50 % of the time). However in over half of the cases of cervicitis other infective agents may be involved, which are often not identified in routine testing and their role is not well defined in this condition, termed ‘non-specific cervicitis’.

Presently when cervicitis is diagnosed, we offer routine antibiotic treatment to the affected women and to her sexual partner. By finding out more about the possible causes of cervicitis, we hope to contribute information to improve understanding, definition and treatment and so reduce potential complications of cervicitis.

If you decide to participate, you will be offered the standard tests for Chlamydia and gonorrhoea, performed on a urine sample. No other examination is necessary unless you have symptoms yourself. We will also test the urine sample for other infectious agents believed to be associated with cervicitis.

You will receive the standard treatment for contacts of cervicitis (azithromycin tablets only), regardless of participation in the study. Additional treatment will be provided to you if the additional test results identify an infective agent where there is treatment of known benefit.

Your participation in the study is completely voluntary and optional and will in no way affect the quality of the care you receive at this Clinic. We cannot guarantee that you will gain any direct benefit from participation.
PARTICIPANT CONSENT FORM
Page 1 of 2

Infectious organisms associated with Cervicitis in metropolitan Sexual Health and Gynaecology clinic patients in Sydney.

1. I .......................................................... of .......................................................... agree to be a participant in the study described in the participant information statement for the research project set out above.

2. I acknowledge that I have read the participant information statement, which explains why I have been selected, the aims of the study and the nature and the possible risks of the investigation, and the statement has been explained to me to my satisfaction.

3. Before signing this consent form, I have been given the opportunity of asking any questions relating to any possible physical and mental harm I might suffer as a result of my participation and I have received satisfactory answers.

4. I understand that I can withdraw from the study at any time without prejudice to my relationship to RPA Sexual Health or any other facility required for my care.

5. I agree that research data gathered from the results of the study may be published or presented provided that I cannot be identified.

6. I understand that if I have any questions relating to my participation in this research, I may contact Dr. Josephine Lusk, who will be pleased to answer them.

7. I acknowledge receipt of a copy of this Consent Form and the Participant Information Statement.

_________________________________________  ____________________________________________
Signature of participant  Signature of witness

_________________________________________  ____________________________________________
Please PRINT name  Please PRINT name

_________________________________________  ____________________________________________
Date  Nature of Witness
PARTICIPANT REVOCATION OF CONSENT FORM

Page 2 of 2

Infectious organisms associated with Cervicitis in metropolitan Sexual Health and Gynaecology clinic patients in Sydney

I hereby wish to WITHDRAW my consent to participate in the research project described above and understand that such withdrawal WILL NOT jeopardize any treatment or my relationship with RPA Sexual Health or my medical or nursing attendants.

______________________________  __________________________
Signature                        Date

Please PRINT Name

The section for Revocation of Consent should be forwarded to Dr. Josephine Lusk, Chief Investigator of Cervicitis Study, RPA Sexual Health, Page Building, RPA Hospital, Camperdown, NSW 2050
APPENDIX PART 3

Papers as published in Peer-reviewed Journals

1. Methods paper
2. Cervicitis review
3. TV paper
4. MG paper
5. NG paper
Multiplex PCR Testing Detection of Higher-than-Expected Rates of Cervical Mycoplasma, Ureaplasma, and Trichomonas and Viral Infections in Sexually Active Australian Women

Christopher J. McIver, Nikola Rismanto, Catherine Smith, Zin Wai Naing, Ben Rayner, M. Josephine Lusk, Pamela Konecny, Peter A. White, and William D. Rawlinson

Virology Division, Microbiology Department (SEALS), Prince of Wales Hospital, School of Medical Sciences, School of Biotechnology and Biomolecular Sciences, University of New South Wales, Department of Immunology and Infectious Diseases, St George Hospital, Sydney, Australia

Received 28 September 2008/Returned for modification 12 November 2008/Accepted 13 February 2009

Knowing the prevalence of etiologic agents of nongonococcal and nonchlamydial cervicitis is important for improving the efficacy of empirical treatments for this commonly encountered condition. We describe four multiplex PCRs (mPCRs), designated VDL05, VDL06, VDL07, and VDL09, which facilitate the detection of a wide range of agents either known to be or putatively associated with cervicitis, including cytomegalovirus (CMV), enterovirus (EV), Epstein-Barr virus (EBV), varicella-zoster virus (VZV), herpes simplex virus type 1 (HSV-1), and herpes simplex virus type 2 (HSV-2) (VDL05); Ureaplasma parvum, Ureaplasma urealyticum, Mycoplasma genitalium, and Mycoplasma hominis (VDL06); Chlamydia trachomatis, Trichomonas vaginalis, Treponema pallidum, and group B streptococci (VDL07); and adenovirus species A to E (VDL09). The mPCRs were used to test 233 cervical swabs from 175 women attending a sexual-health clinic in Sydney, Australia, during 2006 and 2007. The agents detected alone or in combination in all cervical swabs (percentage of total swabs) included CMV (6.0), EV (2.1), EBV (2.6), VZV (4.7), HSV-1 (2.6), HSV-2 (0.8), HSV-2 and VZV (0.4), U. parvum (57.0), U. urealyticum (6.1), M. genitalium (1.3), M. hominis (13.7), C. trachomatis (0.4), T. vaginalis (3.4), and group B streptococci (0.4). Adenovirus species A to E and T. pallidum were not detected. These assays are adaptable for routine diagnostic laboratories and provide an opportunity to measure the true prevalence of microorganisms potentially associated with cervicitis and other genital infections.

Cervicitis, an acute or chronic inflammation of the uterine cervix, is generally viewed as a consequence of infection with sexually transmissible agents. Neisseria gonorrhoeae and Chlamydia trachomatis are the most commonly reported pathogens, possibly because they are most frequently screened for. However, the etiology of most cases is undetermined and could be multifactorial in nature (11, 34, 35, 40). Studies undertaken in other epidemiologic settings indicate significant differences in the prevalences of other cervical infectious agents (1, 41, 44, 45, 58). An underappreciation of the prevalences of and roles possibly because they are most frequently screened for. How- ever, the etiology of most cases is undetermined and could be multifactorial in nature (11, 34, 35, 40). Studies undertaken in other epidemiologic settings indicate significant differences in the prevalences of other cervical infectious agents (1, 41, 44, 45, 58). An underappreciation of the prevalences of and roles potentially jeopardizes the effectiveness of empirical treatments for cervicitis. Unresolved cervicitis can result in ascending infection, endometritis, pelvic inflammatory disease, and salpingitis (11, 23, 46). Furthermore, cervicitis may enhance human immunodeficiency virus susceptibility by the disruption of mucosa, allowing increased viral replication within recruited inflammatory cells (30). The development of molecular methods, such as PCR and DNA hybridization, has allowed the detection of a range of agents whose etiologic roles in genital infections need to be further investigated, including the viruses cytomegalovirus (CMV), herpes simplex virus type 1 (HSV-1) and HSV-2 (4, 43), adenovirus (6, 10, 50), and the Mollicutes Ureaplasma parvum, Ureaplasma urealyticum, Mycoplasma hominis, and Mycoplasma genitalium (1, 28, 59). There have also been reports of genital infections caused by Epstein-Barr virus (EBV) (4, 55), varicella-zoster virus (VZV) (27), and enterovirus (EV) (24). We report here the use of four multiplex PCR (mPCR) assays, designated VDL05, VDL06, VDL07, and VDL09, based on a conventional platform, for the detection of 19 microorganisms in cervical swabs, including Treponema pallidum and C. trachomatis, Trichomonas vaginalis, group B streptococci, and five adenovirus species, in addition to those mentioned above. The assays were developed using cervical swabs from different women taken on one or more occasions during different visits to a sexual-health clinic.

MATERIALS AND METHODS

Patients. Cervical swabs (n = 233) were taken from 175 women consecutively attending a sexual-health clinic in Sydney, Australia (between one and three visits), during 2006 and 2007 who were all eligible for recruitment to an as-yet- unpublished case-control study investigating cervicitis. They included women with and without cervicitis. All of the women were aged ≥18 years, had been sexually active in the preceding 3 months, required an internal examination regardless of symptoms, and had been treated with antibiotics or received gynecologic intervention in the preceding month, did not have an intrauterine contraceptive device in situ, and were not currently menstruating or pregnant. Women with pelvic inflammatory disease were excluded. Written informed consent was obtained from all of the women. The study protocol and data management were approved by the South Eastern Sydney and Illawarra Area Health Service Human Research Ethics Committee.
TABLE 1. Oligonucleotides used in molecular detection methods

<table>
<thead>
<tr>
<th>mPCR</th>
<th>Agent</th>
<th>Oligonucleotidea</th>
<th>Code</th>
<th>Oligonucleotide sequence (5’–3’)</th>
<th>Target (bp)b</th>
<th>LDc Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>For Molluscs</td>
<td>Urea- and</td>
<td>My-ins</td>
<td>CTA CAT TGA TGG TCG CAA CGG TTA TC</td>
<td>16S rRNA gene</td>
<td>(520)</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>M. hominis</td>
<td>MGO-2-Bi</td>
<td>CAC CAT CCG TCA CTC TGT TAA CTT C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M. genitalium</td>
<td>Mhomin-P10-Am</td>
<td>GAC ACT AGG AAA CTA GAG TTA G</td>
<td>103</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>U. parvum</td>
<td>Mgen-P3-Am</td>
<td>TCG GAG CGA TCC TCT CGG T</td>
<td>103</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>U. urealyticum</td>
<td>UMS-57</td>
<td>T(CT)AGA ATC TTA GTG TGG AT A TTA TTT</td>
<td>Multiple banded antigen gene</td>
<td>(326–327)</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>U. urealyticum</td>
<td>UMS-170</td>
<td>T(CT) GGA ATC TTA ATG TGT TTT GG</td>
<td>Multiple banded antigen gene</td>
<td>(476)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>U. urealyticum</td>
<td>UMA223</td>
<td>TTT GGT GCT GTG TT TCT G</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>U. urealyticum</td>
<td>UUM-PROBE1</td>
<td>CTA AAT TCA ATG TCA TTA TTA CAT CAG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CTA A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>For nonviral</td>
<td>C. trachomatis</td>
<td>CT-OF</td>
<td>TGG CAA GCT CTT GCT GTG GGG AAATA</td>
<td>Omp1</td>
<td>(931/378)</td>
<td></td>
</tr>
<tr>
<td>agents</td>
<td></td>
<td>CT-OR</td>
<td>TCA CAT CGG CAG CTC CAG CAA TAG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(VDL07)</td>
<td></td>
<td>CT-IF</td>
<td>ACA TTA GGA GCC ACC AGT GGA TAT C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT-I</td>
<td>ATC CCC ATG CCC ATG CCC ACC ATC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT-PROBE</td>
<td>TGG GTG GAG CTT GGC GCG TCG GGG C</td>
<td>102</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TricV-OF</td>
<td>CTA TGG TCG AAC ATT GGT CCT ACC CTC</td>
<td>G3 (264/266)</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TricV-OR</td>
<td>TCT GGG CCG TCT TCT ATG AGG CCG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TricV-IF</td>
<td>CTC GTC CCG GAA CAG CAG TTT AGG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TricV-IR</td>
<td>GTC TGG AGG GGA CAA CAA CAC CAG G</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TricV-PROBE</td>
<td>CTA CAA CAA ATT CTT CTC CTG C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TP7-OF</td>
<td>CTC ACC ACT GCT GGT AG</td>
<td>Imd gene (616/506)</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TP8-OR</td>
<td>AAC GCC TCC ATC ATC AGA CC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TP3-IF</td>
<td>CAG GTC GAG GAT GCT GAA GT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>TP4-IR</td>
<td>CGT GGC AGT AAC CGC AGT CT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TP5-PROBE</td>
<td>GAC GTG AGG ACT CTC AAA TC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group B</td>
<td></td>
<td>STB-OF</td>
<td>AAC GCA CCA ACC GGG TTG CCA TGG</td>
<td>xpdf (418/260)</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>streptococci</td>
<td></td>
<td>STB-OR</td>
<td>GAC CCA CCT CTT CTG ACT CAG AAA AA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>STB-IF</td>
<td>ACA AGC GAG GGC ACT GCT TT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
<td>STB-IR</td>
<td>GTT TGA GGT GTG TGA CCT GAA CTA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>STB-PROBE</td>
<td>ACA AGC GAG GGC ACT GCT CT</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Adenovirus | Adenovirus sp. A | A  | AdA1          | GCT GAA GAA MCC GAA GAA AAT GA        | Fiber (1,444–1,537)                    | 57          |
| specific   | Adenovirus sp. B | B  | AdA2          | CRT TGG TGG TCT GTG AATG AAC AAT C    | Fiber (670–772)                      | 10         |
| (VDL09)    | Adenovirus sp. C | A  | AdB1          | TST ACC ATG AAAG ATG AGG AAC AA        | Fiber (1,986–2,000)                  | 10          |
|             | Adenovirus sp. C | B  | AdB2          | GGA TAA GCT GTA GTR CTG GCG AT         | Fiber (1,986–2,000)                  | 10          |
|             | Adenovirus sp. C | A  | AdC1          | TAT TAC GCA TCA CCT CCT TCT C         | Fiber (1,986–2,000)                  | 10          |
|             | Adenovirus sp. C | B  | AdC2          | AAG CTA TGT GTG GTG GGC GC            | Fiber (1,986–2,000)                  | 10          |
|             | Adenovirus sp. D | A  | AdD1          | GAT GTC AAA TCT TTC GTC CAC           | Fiber (1,205–1,221)                 | 10          |
|             | Adenovirus sp. E | A  | AdE1          | TAC CCG TGC TGG TGT AAA AAT C         | Fiber (967)                        | 10          |
|             | Adenovirus sp. E | B  | AdE2          | TCT CTA CGA TGC AGA CCA CG            |                                 | 10          |

a A, outer sense primer; B, outer antisense primer; C, inner sense primer; D, inner antisense primer; P, probe.

b First-round product/second-round product.

c LD, limit of detection (number of copies per reaction).

d 32 bp for U. parvum serovars 1 and 3/14 and 327 bp for serovar 6 (28).

e Oligonucleotide designed by Nikolas Rismanto.

f Underlined sequences are modifications of the published primers cited.

Sampling procedure. The cervix was accessed using a sterile metal speculum and was prepared for swabbing by removing exudate with a large nonsterile swab (Multigate Medical Products, China). An initial swab of the endocervix was taken with a sterile cotton swab (Copan Diagnostics), suspended in viral transport medium 199 (Gibco Invitrogen, New York) and stored at 4°C before being placed in viral transport medium for 48 h. The reaction mixtures were prepared in accordance with the manufacturer’s instructions for a 50-μl reaction and consisted of 5.8 μl of RNAse-free water, 10.0 μl of buffer, 2.0 μl of deoxynucleoside triphosphate mix, 2.5 μl of each primer (including primers for internal control at a final concentration of 0.5 μM) (Table 1), 2.0 μl Qiagen OneStep RT-PCR enzyme mix at a final activity of 0.5 U, 0.2 μl digoxigenin-11-dUTP (Roche, Germany), and 10 μl of template. The cycling procedures included an RT step at 50°C for 30 min, denaturation at 95°C for 15 min, and then 50 cycles of 94°C for 30 s, 57°C for 30 s, and 72°C for 1 min, a final extension of 7 min at 72°C, and a 4°C hold. The products were visualized by gel electrophoresis, and the amplicons were identified by probe hybridization, followed by PCR enzyme-linked immunosorbent assay (digoxigenin detection) (Roche, Germany). Known-positive clinical samples were used as reaction controls for the PCR.

Virus detection (VDL05). A nested mPCR of the same designation previously described (38) was used without modification for the detection of CMV, HSV-1, HSV-2, EBV, EV, and VZV. Briefly, a first-round reaction comprising 20 μl of template, 0.5 μl of AmpErase (uracil N-glycosylase) (Applied Biosystems), and 0.10 μl of each primer (38) was included in a 50-μl reaction mixture of the Qiagen OneStep RT-PCR kit (Qiagen, Germany). A second-round reaction was undertaken using 2 μl of first-round product, 0.2 μl digoxigenin-11-dUTP (Roche, Germany), and 0.10 μl of each primer in a 50-μl reaction mixture of AmpliTaq Gold PCR Master Mix (Applied Biosystems). The thermocycling conditions used for each round were as previously described (38). The products were visualized by gel electrophoresis, and the amplicons were identified by probe hybridization (as described above).

Detection of adenovirus species A to E (VDL09). The adenovirus detection method is based on that previously described by Xu et al. (56) and was modified to conform to the above-mentioned protocols. A single-round reaction was performed comprising 5 μl of template and 0.20 μl of each primer (Table 1) in a 50-μl reaction mixture of AmpliTaq Gold PCR Master Mix (Applied Biosys-
TABLE 2. Use of mPCRs for screening nongonococcal agents in cervical swabs

<table>
<thead>
<tr>
<th>Microorganism(s)</th>
<th>mPCR</th>
<th>Total cervical swabs (n = 233)</th>
<th>Total women (n = 175)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mollicutes</td>
<td>VDL06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U. parvum</td>
<td>112 (48.0)</td>
<td>93 (53.1)</td>
<td></td>
</tr>
<tr>
<td>U. urealyticum</td>
<td>6 (2.6)</td>
<td>6 (3.4)</td>
<td></td>
</tr>
<tr>
<td>M. hominis</td>
<td>15 (6.4)</td>
<td>13 (7.4)</td>
<td></td>
</tr>
<tr>
<td>M. genitalium</td>
<td>3 (1.3)</td>
<td>3 (1.7)</td>
<td></td>
</tr>
<tr>
<td>U. parvum + M. hominis</td>
<td>15 (6.4)</td>
<td>13 (7.4)</td>
<td></td>
</tr>
<tr>
<td>U. urealyticum + M. hominis</td>
<td>2 (0.9)</td>
<td>2 (1.1)</td>
<td></td>
</tr>
<tr>
<td>U. parvum + U. urealyticum</td>
<td>6 (2.6)</td>
<td>5 (2.9)</td>
<td></td>
</tr>
<tr>
<td>Viruses</td>
<td>VDL05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV</td>
<td>14 (6.0)</td>
<td>11 (6.3)</td>
<td></td>
</tr>
<tr>
<td>EV</td>
<td>5 (2.1)</td>
<td>5 (2.8)</td>
<td></td>
</tr>
<tr>
<td>EBV</td>
<td>6 (2.6)</td>
<td>6 (3.4)</td>
<td></td>
</tr>
<tr>
<td>HSV-1</td>
<td>6 (2.6)</td>
<td>6 (3.4)</td>
<td></td>
</tr>
<tr>
<td>HSV-2</td>
<td>2 (0.8)</td>
<td>2 (1.1)</td>
<td></td>
</tr>
<tr>
<td>VZV</td>
<td>10 (4.3)</td>
<td>9 (5.1)</td>
<td></td>
</tr>
<tr>
<td>VZV + HSV-2</td>
<td>1 (0.4%)</td>
<td>1 (0.6)</td>
<td></td>
</tr>
<tr>
<td>Adenovirus species</td>
<td>VDL09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A, B, C, D, E</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other agents</td>
<td>VDL07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. trachomatis</td>
<td>1 (0.4)</td>
<td>1 (0.6)</td>
<td></td>
</tr>
<tr>
<td>T. vaginalis</td>
<td>8 (3.4)</td>
<td>7 (4)</td>
<td></td>
</tr>
<tr>
<td>T. pallidum</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Group B streptococi</td>
<td>1 (0.4)</td>
<td>1 (0.6)</td>
<td></td>
</tr>
</tbody>
</table>

RESULTS

The limit of detection (Table 1) for each agent ranged from 10 (for T. vaginalis) to 10^5 (for T. pallidum) copies per reaction. False positives were not detected when between 17 and 96 proven-negative control samples of each agent were tested.

The results of the screening of 233 cervical swabs from 175 women by the four mPCRs are shown in Table 2. The total agents detected among the 175 participating women at the initial and subsequent visits are also shown. The Mollicutes were the most common group of organisms detected, and were recovered from 159 (68.2%) of the 233 cervical swabs tested. Either alone or in combination with another member of the Mollicutes, U. parvum was the species most commonly detected (57.0%), followed by M. hominis (13.7%), U. urealyticum (6.1%), and M. genitalium (1.3%). CMV was the predominant virus detected (6.0%), followed by VZV (4.3%). The remaining viruses (EV, EBV, HSV-1, and HSV-2) were each detected in <3% of the samples, and adenoviruses (A to E) were not detected. T. vaginalis (4.0%) was the commonest agent detected by VDL07 (for other agents). C. trachomatis and group B streptococci were detected in <1% of these samples, while T. pallidum was not detected.

Multiple infections were detected in 42 (24.0%) of the 175 women tested. Two of these patients had multiple infections on two separate occasions. All 44 coinfections included a Mollicutes sp., most commonly U. parvum (88.6%). Of the eight patients diagnosed with trichomoniasis, six (75.0%) had coinfections with U. parvum. Two of these patients were also coinfected with either VZV or CMV.

DISCUSSION

mPCR assays allow simultaneous detection of multiple agents in a single reaction and were applied here to detect a broad range of microorganisms. The mPCRs developed in this study are based on those we previously described for the detection of viruses in a routine diagnostic laboratory (38) utilizing identical reagent and cycling conditions. This simplifies the workflow, allowing performance of these assays in a routine diagnostic laboratory with basic molecular facilities. The choice of a commercial master mix including a reverse transcriptase reaction benefits a busy laboratory environment where both RNA and DNA agents are being detected.

The mPCRs VDL05 and VDL07 are nested PCRs to increase sensitivity, while specificity is enhanced with a post-PCR probe hybridization assay. The adenovirus mPCR (VDL09) was limited to a single-round reaction without post-PCR probe hybridization because of variation in regions targeted by the species-specific primers (57). A single-round PCR was used to detect M. hominis, M. genitalium, U. parvum, and U. urealyticum (VDL06). The method developed by Yoshida et al. (59) was first considered for the detection of these agents. However, our evaluation of this method showed cross-reactions with the hybridization reactions for U. parvum (serotypes 6 and 149) and U. urealyticum and weak reactions for U. parvum with wild strains of M. hominis (data not shown). The mPCR employed in this study utilizes the method of Yoshida et al. for the detection of Mycoplasma spp. and includes specific primers for U. parvum (28) and U. urealyticum (54). These primer sets allow differentiation of the ureaplasmata by characteristic electrophoretic-bend sizes, which are confirmed by probe hybridization.

CMV was the most frequent virus detected using the VDL05 mPCR. CMV is not a common cause of cervicitis in immunocompetent women (37). However, studies in China have shown detection rates of 5.1% in a prospective study of women with cervical human papillomavirus (58) and 14.0% in erosive cervicitis (44), possibly as a result of cervical carriage and reactivation by localized inflammation (36). Infection in pregnancy
may cause spontaneous abortion, and there is a significant risk of fetal infection with congenital abnormalities (3, 8, 37). In this study, CMV was detected in 6.3% of female patients tested, which is a prevalence not previously reported in Australian clinics, suggesting the need to consider routine testing in pregnant high-risk patients.

Previous studies have suggested that most genital HSV infections are caused by HSV-2 (13, 42, 43, 52). Consistent with more recent findings (6, 13, 17, 29, 56) increasingly implicating HSV-1 in genital infection, we detected HSV-1 (3.4%) more commonly than HSV-2 (1.7%) in the female patients tested. An early study in China showed detection rates in erosive cervicitis to be as high as 26.5% (44), with asymptomatic shedding potentially an important means of transmission (13). None of the 175 women in our study had genital erosions or clinical signs of acute HSV infection at the time of testing.

There have been reports of EBV-associated genital ulcers in women (2, 7, 14, 21, 25, 32, 53, 55). This condition is under-recognized and may be incorrectly attributed to HSV infection (7, 32, 53). However, the clinical relevance of our detection of EBV in 3.4% of female patients in this study has yet to be established. A recent study showed strong evidence for sexual transmission of the virus from a partner infected with infectious mononucleosis (55). In a study in Thailand of women with HSV-associated genital herpes, 17/30 (56.7%) cases were infectious mononucleosis (55). In a study in Thailand of women with HSV-associated genital herpes, 17/30 (56.7%) cases were found to have EBV DNA present, although the clinical significance was not determined (25).

The presence of EV in the female genital tract may also be a predisposition to antenatal and perinatal infection (3). An early study in Russia detected antigens of coxsackie A and B virus in the vaginal secretions of 16.3% of young girls with protracted forms of vulvovaginitis (33). More recently, a study in Central Africa detected EV RNA in nearly 10% of women of childbearing age, which may be the basis for possible antenatal or perinatal transmission from mother-to-child (24). Detection of EV in 2.8% of the women in our study indicates the proportion of patients at risk, but again, the clinical relevance has yet to be determined.

The Mollicutes detected in this study are associated with infections of the genitourinary tract, reproductive failure, and neonatal morbidity and mortality. Our detection rates of the four species of the Mollicutes putatively associated with genital infection are consistent with previous studies, with U. parvum being the commonest (26, 45, 49). Detection of M. genitalium is becoming increasingly important because of recent reports of a high prevalence of the organism in women with cervicitis (15, 41, 45). Furthermore, the high prevalence of infected sexual partners supports its role as a sexually transmitted infection (15).

The VDLO7 mPCR screens organisms with larger genomes and was reduced to four detectable agents to minimize template competition. In this assay, T. vaginalis was the most commonly detected agent in women at 4.0% and was included, as there has been a proven advantage of molecular techniques over the insensitive traditional methods of direct visualization and wet-mount microscopy (47, 51) and Pap smear. Inclusion in this assay enabled detection of trichomoniasis, which is sexually transmissible and often asymptomatic. T. vaginalis is associated with pelvic inflammatory diseases and adverse birth outcomes (51) and is also linked to an increased risk of human immunodeficiency virus transmission (48). Vaginal colonization with group B streptococci is not normally symptomatic or associated with sexual transmission. However, cervical colonization is relevant to pathology of the fetus and newborn, and significant morbidity may arise if group B Streptococcus is not detected and eradicated (23).

The detection rate of chlamydial infections (<1%) in this study is lower than expected for this population and could be explained in part by the exclusion of women with pelvic inflammatory disease and recent antibiotic treatment from the study population. As was evident again here, syphilitic cervicitis is uncommon but is important to diagnose because infection may clinically and colposcopically simulate a primary advanced cervical cancer (19, 20). Ideally, the assay for this agent should be more sensitive and should be performed as a monoplex to increase sensitivity for high-risk patients.

Although uncommon, adenovirus has been associated with genital infections (5, 6, 50). We did not detect adenovirus in the women examined here. Recent Australian studies of men with urethritis showed that the infection is uncommon and seasonal (6).

A test for N. gonorrhoeae was not considered in this development because of reports of cross-reactivity in commercial and published methods with closely related strains, such as Neisseria subflava and Neisseria cinerea (16). Furthermore, the diagnosis of this pathogen is a simple and expedient process using conventional microscopy and culture techniques.

As shown here, improved screening has demonstrated higher-than-expected rates of occurrence of organisms, particularly the Mollicutes, in the cervixes of women attending sexual-health clinics. These mPCR assays will facilitate further clarification of the significance of these organisms in genital infections, distinguishing pathogens from commensals. Ultimately, the improvement of the diagnosis of cervicitis and other genital infections will guide the use of appropriate interventions targeted against specific pathogens. Efficacious treatment of cervicitis has important implications for the reduction of gynecologic infections and risk to fetal development, for the control of sexually transmitted diseases, and for improved reproductive health at the public health level.

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Cervicitis: a review
M. Josephine Luska and Pam Konecny

Introduction
Cervicitis was first recognized as an important clinical entity in 1984 by Brunham et al. [1]. Since then much controversy has existed. Interpretation and comparison of published studies is hampered by the lack of consensus in the case definition, and variability in the populations sampled and methods used for pathogen detection [2]. Advances in molecular diagnostics have created an opportunity to further clarify cervicitis. However, given an understandable desire for rapid, sensitive, potentially clinician-independent testing for Chlamydia and Neisseria gonorrhoea, supplanting speculum-guided cervical specimens with urinary nucleic acid amplification technique (NAAT) testing in asymptomatic women attending sexually transmitted infection (STI) clinics [3] may result in a lost opportunity for detecting cervicitis before its implications are fully realized. It is thus timely to review cervicitis. An extensive literature search was conducted using MEDLINE, Embase and the Cochrane Library on the topics of cervicitis, reproductive health and STIs.

Background
Cervicitis is a frequently asymptomatic, inflammatory condition of the cervix [4]. It is common with rates as high as 30–45% in some STI clinic populations [1,2,5] and is generally considered to be associated with sexually transmissible pathogens [1,2,4,6,7]. However, Chlamydia and Neisseria gonorrhoea account for less than half of cervicitis cases, with a largely undefined aetiology in the remainder [1,2,8,9], referred to as nonchlamydial, nongonococcal cervicitis or nonspecific cervicitis (NSC). The clinical significance of the finding of NSC, especially in asymptomatic, ‘low-risk’ women, has been debated [8–10]. Other organisms variably implicated in the pathogenesis of NSC include Mycoplasma genitalium,
**Sexually transmitted diseases and urinary tract infections**

*Mycoplasma hominis, Ureaplasma urealyticum*, bacterial vaginosis, herpes simplex virus (HSV), cytomegalovirus (CMV), *Trichomonas vaginalis* and adenovirus. This raises concerns about the appropriateness of empirical treatments currently used to treat women with cervicitis and their sexual partner(s). Importantly, Bradshaw and colleagues [11**, in a recent Australian study of non-specific urethritis, the analogous clinical condition in men, highlighted the role of adenovirus, HSV-1, macrolide-resistant *Mycoplasma* species and oral sex in nonspecific urethritis, factors yet to be fully characterized in NSC. A lack of consensus on definitive treatments for cervicitis in STI treatment guidelines [12*,13] reflects these uncertainties, with the potential for over-use of antibiotics. Furthermore, the psychological impact of empirically treating cervicitis as an STI has recently been examined [14**].

**Significance of cervicitis**

Complications of cervicitis include endometritis, pelvic inflammatory disease (PID) and adverse outcomes of pregnancy and the newborn. The synergy between genital infection and enhancement of HIV transmission is well recognized. There is also literature implicating cervical inflammation in the pathogenesis of cervical cancer.

**Pelvic inflammatory disease**

Cervicitis may serve as an important marker of subclinical PID. Mucopurulent cervicitis and endometritis may be the only signs of PID in some women [4,9,10,15]. Many women with tubal-factor infertility, ectopic pregnancy or chronic pelvic pain do not give a history of PID, but subclinical PID is likely to be an important contributing factor [16,17]. The reported risk of women with lower-genital-tract infection developing PID ranges from 20 to 80% depending on method used for detection, delay in diagnosis, treatment, co-infection and other host factors (reviewed in [17]). Peipert and colleagues’ [18] analysis of women with pelvic pain in the PID Evaluation and Clinical Health (PEACH) study found lower-genital-tract leucorrhoea (>10 leucocytes per high-powered field (hpf) on microscopic examination of vaginal fluid) had a high sensitivity (89%) but low specificity (19%) for predicting histologically proven endometritis. Other researchers have confirmed the high positive predictive value (PPV) of leucorrhoea for PID in the high-risk STI settings [19], particularly in the setting of bacterial vaginosis [20]. Thus, vaginal leucorrhoea may also be a useful adjunctive tool in the diagnosis of upper-genital-tract infection.

Although there is disparate literature concerning chlamydial and gonococcal contribution to PID, even less is known about the relationship of NSC to upper-genital-tract infection. This is an important consideration when 20–30% cases of NSC are refractory to empirical ‘cervicitis’ treatment [9]. Furthermore, a recent novel study modelling different management algorithms for cervicitis in a hypothetical teen clinic population has emphasized the important psychological ramifications associated with diagnosis and empirical treatment of cervicitis for women and their partners, versus the PID-prevention benefits where there is a low prevalence of *Chlamydia* [14**].

**Pregnancy**

*Chlamydia* infection has been associated with a doubling of ectopic pregnancy rates in a Norwegian study [21] and while an association of chlamydial infection with preterm delivery is suggested, its role is not yet fully elucidated [17]. Nugent and Hillier’s [10] analysis of a large cohort of high-risk pregnant women found that cervicitis was significantly associated with the delivery of low-birthweight babies [adjusted relative risk 2.11, 95% confidence interval (CI) 1.10–4.04]. This was despite a low sensitivity (25%) and low PPV (24%) for *Chlamydia* infection, implicating NSC. A recent Chilean study [22] examining the benefit of antibiotic administration to women with preterm labour found that whereas there was no overall benefit on composite neonatal morbidity/mortality outcome, a subgroup of women with NSC without amniotic fluid infection and intact membranes derived benefit from antibiotic administration with significantly lower frequency of neonatal morbidity and mortality. This finding suggests that cervicitis may be a useful clinical marker for women at risk who might benefit from antibiotic intervention.

**HIV transmission**

Cervicitis is thought to play an important role in the transmission of HIV infection, by increasing susceptibility to HIV infection and increased HIV viral shedding. The association of genital ulceration, particularly HSV-related, with increased risk of HIV transmission risk is well recognized [23]. A significant correlation between cervical HIV DNA and microscopic evidence of cervical inflammation [adjusted odds ratio (OR) 8.7] has been demonstrated [24]. Mechanisms by which cervicitis may increase HIV-1 shedding include increased viral replication in the context of infection or inflammation particularly in the presence of elevated pro-inflammatory cytokines, disruption of normal mucosa and increased numbers of HIV-infected cells in cervical secretions. Effective treatment of chlamydial or gonococcal cervicitis correlated with a greater than 6-fold decrease in cervical HIV-1 RNA and with normalization of cervical polymorphonuclear counts, but no reduction in HIV-1-infected cells as measured by presence or absence of HIV-1 proviral DNA in one study [25]. Intriguingly, treatment of the subgroup of women with NSC in the same study...
Cervicitis

The case definition of cervicitis varies widely and is considered by many a clinical diagnosis to be evidenced by cervical ecotopy (extension of the columnar epithelium of the endocervix onto the visible ectocervix), a ‘friable cervix’, presence of ‘mucopurulent’ or yellow discharge or a combination of these signs. However, overt signs of cervicitis may be overlooked given the variance in signs considered indicative of cervicitis. The presence of a yellow discharge, indicating neutrophil production of myeloperoxidase, has been suggested as a good predictor of Chlamydia or N. gonorrhoea infection [1,4,15]. However, a recent evaluation of syndromic management using signs and symptoms of vaginal discharge in women in an antenatal setting in Botswana failed to satisfactorily identify women with Chlamydia or gonorrhoea infection [31**].

Another approach adopted by clinicians is microscopic analysis of mucus taken from the endocervical canal. Leucocytes are normally present throughout the reproductive tract including cervical tissue [32] but are considered pathological when present in high numbers (usually >30/hpf) [1,4]. Cervical mucus polymorph assessment is not affected by the phase of the menstrual cycle except for during menstruation when it becomes less reliable [1]. The microscopic diagnosis of cervicitis is determined by Gram stain of the number of polymorphonuclear leucocytes per hpf (PMNL/hpf; ×1000, oil immersion) in cervical mucus using different cut-off thresholds, usually >30 PMNL/hpf [4,5,8,10,25] but often >10 PMNL/hpf [1,4,32]. The validity of each cut-off value has been reviewed [2**,9]. Consensus in the literature appears to favour the former, which has greater specificity at the expense of reduced sensitivity.

The availability of microscopy to facilitate cervicitis diagnosis will be impacted in resource-poor clinical settings. Microscopy may be open to intra-observer variability and error with vaginal epithelial cell contamination of cervical samples and counting polymorphs outside of cervical mucus. Studies reporting low PPV of microscopic cervicitis in detection of Chlamydia and N. gonorrhoea in settings of low STI prevalence [8,10] are based on the ability to predict Chlamydia infection detected by culture laboratory methods. The PPV of cervical findings could be considerably improved with the use of NAAT methods of Chlamydia detection. Additionally, PPV’s are often given in terms of Chlamydia and N. gonorrhoea detection only and do not consider the increasing array of recently described pathogens, such as Mycoplasma spp.

Therefore, it would seem prudent to diagnose cervicitis using a combination of microscopy (>30 PMNL/hpf) and at least one of the abovementioned clinical signs. Using these diagnostic criteria for cervicitis may improve the PPV for detecting disease.

Noninfectious aetiology

It has been suggested that mucopurulent discharge could be caused by exposure of the cervical columnar epithelium to noninfectious factors in the vagina, such as smoking, douching and combined oral contraception. Earlier large prospective studies found a significant association with use of combined oral contraception: Paavonen et al. [33] showed an adjusted OR of 2.5 (P = 0.02) and Castle et al. [29] showed an OR of 2.9 (95% CI 1.4–5.9). Interestingly, however, authors of a subsequent large cross-sectional study found no such association after adjusting for the presence of cervical ectopy [34].

There is conflicting literature concerning the association between vaginal douching and cervicitis, endometritis...

Human papillomavirus and cervical cancer

Chronic inflammation has been linked to many epithelial cancers, and thus the role of chronic cervicitis as a cofactor in cervical cancer seems plausible [27]. Human papillomavirus (HPV), predominantly types 16 and 18, is required for the development of the majority of cervical carcinomas [28]. The role of HPV in cervicitis is less clear. Studies have noted a positive association between measures of cervical inflammation and squamous intraepithelial lesions [28,29]. A case–control study of Costa Rican women found that in women infected with oncogenic HPV types, the likelihood of developing high-grade squamous intraepithelial lesions increased with the degree of cervical inflammation [29]. Chlamydia, HSV, Trichomonas, bacterial vaginosis and CMV-associated cervical inflammation have been reported as potential cofactors in development of cervical carcinoma in past predominantly seroepidemiological-based studies. Recent, more convincing evidence for a causal link of any of these pathogens is lacking. U. urealyticum has been suggested as a possible cofactor in the development of abnormal cervical cytology in the presence of HPV [30**].

Endometritis in HIV-1-positive Kenyan women was associated with a 15-fold increase in HIV viral shedding (95% CI 2–120), which is concerning as asymptomatic endometritis may be more common in HIV-positive women [26*]. Thus implementation of effective STI screening, management and prevention strategies could significantly impact on HIV transmission, particularly in resource-poor settings.

Case definition of cervicitis

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and PID. PID was found to be significantly associated with current, frequent douching in a formative Seattle study [35]. A similar association with cervical Chlamydia infection and douching was observed in a separate large cross-sectional study [36]. However, in contradiction, no association between douching and gonococcal or Chlamydia cervicitis or PID was found recently in a large prospective observational study of predominantly African-American women who commonly douche [37].

Although smoking had been previously linked to an increased risk of PID, no link between smoking and cervicitis, dysplasia or Chlamydia infection was found, after adjustment for confounders, in a UK cross-sectional study [38].

Infectious aetiology

Of the known infectious agents of cervicitis, Chlamydia and N. gonorrhoea have been the most widely studied.

Chlamydia trachomatis and Neisseria gonorrhoea

Chlamydia is the most frequently identified cause of cervicitis with rates of Chlamydia in women with cervicitis varying widely in the literature from 11 to 50% [1,6,17,33] depending on population sampled, cervicitis definition and detection methods. However only 10–20% of Chlamydia infections may be associated with obvious clinical signs of cervicitis [6]. This may be explained in part by infections with a lower quantity of infectious organisms and strain variability, including ‘non-fusing variants’ of Chlamydia (about 1.5% of isolates) [39]. Increasing application of NAAT testing for Chlamydia has resulted in enhanced ability to detect this pathogen, but this does not completely account for the concerning continued increase in Chlamydia infection [17*].

Whereas N. gonorrhoea is known to cause cervicitis, the proportion of cervicitis attributable to N. gonorrhoea is highly variable, in keeping with the markedly different prevalence of N. gonorrhoea in different populations.

Mycoplasma genitalium

There is strong support for the role of M. genitalium in the aetiology of cervicitis [5,40,41,42**,43], endometritis [43,44], PID [42**,45**], genital-tract disease in men [11**,42**] and more recently in tubal-factor infertility [46*]. A recent serological study found a trend but not a significant association of M. genitalium with PID and ectopic pregnancy [47*]. The organism fulfils Koch’s postulates for pathogenicity and the balance of current evidence supports the use of antibiotics if M. genitalium is detected [11**,12*,42**,45**]. A recent Danish prevalence study found the likelihood of M. genitalium infection in women was associated with increasing numbers of recent sexual partners and partners with symptoms [48**].

However, the low M. genitalium prevalence of 2.3% could not justify routine screening. Low prevalence rates of M. genitalium in asymptomatic women are reported, 6% in a Swedish study [40] and 7% in US STD clinic population study [5]; in the latter, M. genitalium infection conferred a 3-fold greater risk of cervicitis. A recent large British study of antenatal women found a prevalence of only 0.7% and suggested that M. genitalium is not a risk factor for adverse pregnancy outcome in healthy women [49]. A higher prevalence of 6.2% in pregnant women in Guinea-Bissau was significantly associated with HIV-1 infection but not with adverse pregnancy outcomes [50]. Impor-tantly, prevalence rates of M. genitalium in symptomatic and high-risk populations appear considerably higher, 13–25% [41,44,51,52].

Mycoplasma hominis

M. hominis is commonly found in the genital tract of sexually experienced females and a role in PID and postabortal fever [45**] has been suggested. Reports of the prevalence of M. hominis in women with cervicitis vary widely between 2.3% in a Turkish study and 26% in a small Wisconsin college population [41]. Nugent and Hillier [10] found M. hominis to be significantly associated with cervicitis (relative risk 2.96, 95% CI 1.76–4.99) in a study of high-risk pregnant women. It is suggested that M. hominis may exist symbiotically with the mixed bacteria of bacterial vaginosis [45**,53]. M. hominis serum antibody titres and vaginal leucorrhoea have been found to be higher in women with bacterial vaginosis, than women without bacterial vaginosis [53]. It is difficult to determine the pathogenic role of M. hominis given its frequent association with bacterial vaginosis [45**].

Bacterial vaginosis

Bacterial vaginosis is found in up to 50% of women with cervicitis [33], and may play a role in the aetiology [7*,33,54]. The association of bacterial vaginosis with endometritis, PID and adverse pregnancy outcome is increasingly accepted [16,43**,55]. Even though a significant association between PID and bacterial vaginosis was not found in a large observational cohort of African-American women, bacterial vaginosis was suggested as a marker for women at higher risk of PID [56]. The authors found that in women with bacterial vaginosis, the presence of Chlamydia or N. gonorrhoea was associated with a 3.1-fold risk of PID compared to a 1.91-fold risk in the presence of normal vaginal flora. The strongest risk for PID in the presence of bacterial vaginosis was carriage of pigmented, anaerobic Gram-negative rods (Porphyromonas, Prevotella, Bacteroides). The study of Schwabke and Weiss [54] found an association between bacterial vaginosis and cervicitis and between use of metronidazole gel and resolution of cervicitis. A reduction in pro-inflammatory vaginal cytokines with
treatment of bacterial vaginosis has been noted in one recent randomized controlled trial involving pregnant women, suggesting that bacterial vaginosis is associated with inflammatory changes at the cervix [57]. Marrazzo’s group [77], investigating risk factors for cervicitis in women with bacterial vaginosis, identified older age, new male or female partner, recent oral sex and absence of \( \text{H}_2\text{O}_2 \)-producing lactobacilli. The loss of \( \text{H}_2\text{O}_2 \)-producing lactobacilli in conjunction with increase in sialidases and glycosidases produced in bacterial vaginosis may break down the protective cervical mucus barrier [58]. In keeping with this literature, the Center for Disease Control and Prevention guidelines recommend that bacterial vaginosis be treated if found in the presence of cervicitis [12*].

**Ureaplasma urealyticum**

*U. urealyticum* is commonly found in the genital tracts of symptomatic and asymptomatic men and women, associated with lifetime number of sexual partners. *U. urealyticum* is suggested as a pathogen associated with cervicitis with an OR of 2.7 (\( P < 0.0133 \)) in study [33], adverse pregnancy outcome [45**,59] and postpartum sepsis [45**]. However there is little evidence of its role in PID [45**].

**Herpes simplex virus, cytomegalovirus and adenovirus**

HSV-1 and HSV-2 have been associated with cervicitis [4,6,34]. Cervical HSV shedding is thought to be generally asymptomatic. Several studies suggest an association between CMV and cervicitis [6,34,60,61]. CMV accounted for 7.6% of cases of cervicitis in one large cross-sectional study [34]. Another study of cervical biopsies of HPV-associated cervical neoplasia identified CMV DNA in 8.7% of specimens [61]. CMV shedding has also been found to be significantly greater in HIV-positive than HIV-negative women [62]. The development of molecular diagnostic techniques, particularly multiplex polymerase chain reaction (mPCR), will aid in the detection of these viruses, which are also associated with significant congenital infection [63]. Adenovirus has been implicated in nongonococcal urethritis in males [11**], and it is thought to have a role in cervicitis [64] but it is not well defined and is an area of potential interest.

**Trichomonas**

*Trichomonas* is associated with cervical inflammation [2**,33,65**,66] and increased risk of HIV transmission [65**]. Its reported contribution to the aetiology of cervicitis is highly variable, reflecting local prevalence, and it is considered to be frequently under-diagnosed due to the relatively low sensitivity of wet-mount microscopy. New methods for *Trichomonas* detection, specifically NAAT testing and the new rapid point-of-care (POC) bedside immunochromographic tests, could help clarify local prevalences [66**]. Very little is published on cervicitis and yeast, but a negative association with cervicitis has been suggested [33].

**Management of cervicitis**

In Australasia, standard empiric treatment for cervicitis is azithromycin for affected women and their sexual partners [13]. As the local heterosexual prevalence of gonorrhoea is very low [3*], concurrent treatment for gonorrhoea is not routinely given empirically. Azithromycin failure in 28% of men with *M. genitalium*-related urethritis has been reported and occurred more frequently when the *M. genitalium* originated from southeast Asia, where there is emerging macrolide resistance [11**,67**]. This has important treatment implications when *M. genitalium* is associated with cervicitis. There are reports of improved clearance rates of *M. genitalium* with extended courses of azithromycin and moxifloxacin [42**,67**].

Persistent cervicitis, despite ‘standard’ empirical treatment is not infrequently encountered (M.J. Lusk and P. Konecny, personal observation) and reported by others [6,8,12*]. The natural history of cervicitis is not defined, nor is the benefit of further treatment for unresponsive cases and their partners. Most STI guidelines suggest gynaecological review to exclude underlying pathology such as malignancy or the consideration of chemical irritant or idiopathic causes. Ablative therapy of the cervix has been used to treat chronic cervicitis [68], but there is a paucity of literature concerning the rationale and effectiveness of this intervention, which presumably relates to the association between ectopy and cervicitis. Returning to the concept that cervicitis may be an indicator of silent PID, STI guidelines could perhaps give consideration to recommending PID treatments for persistent cases of cervicitis. The management of PID has recently been reviewed and emphasis placed on achieving high rates of clinical as well as microbiological cures [69**].

**Conclusion**

In conclusion, cervicitis remains a condition yet to be fully characterized. It is common, often asymptomatic and may be associated with significant adverse outcomes for women. Research in NSC is a particular area of need. Wide variations of case definition, study populations and methods for pathogen isolation hinder the ability to draw conclusions on the aetiology, natural history and best management of cervicitis on a population basis. Certainly the evidence suggests it is a multifactorial condition. We suggest future research should combine a microscopic definition of >30PMNL/hpf, the more frequently cited criterion, with at least one of the accepted clinical signs, such as yellow mucopus.
Urinary-based NAAT methods of STI testing have revolutionized the process of STI testing, but ironically without internal genital examination, the diagnosis of cervicitis cannot be made. With the streamlining of clinical services and, in some practices, the replacement of genital examination with urinary NAAT testing, particularly for asymptomatic screening, we risk overlooking significant pathology in women with negative results from STI screening tests. In the meantime further research is needed to elucidate the contribution of new putative aetiological agents, such as M. genitalium and bacterial vaginosis, and their antibiotic susceptibility patterns and noninfectious factors implicated in the aetiology of cervicitis. This will improve diagnosis and management of this condition and thus ultimately improve health outcomes for women and their partners.

Acknowledgements
We thank the St George Hospital librarians for invaluable assistance in the literature search for this review.

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
• of outstanding interest
Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 107).

This is an excellent, up-to-date, expert discussion of the dilemmas currently faced in defining aetiology, clinical significance and management of cervicitis.
This study proposes streamlining of STI screening by potentially replacing internal cervical sampling with urinary NAAT testing, challenging current standards of practice.
This paper presents emerging evidence linking bacterial vaginosis and cervicitis. They characterize the risk factors for cervicitis among women with bacterial vaginosis, which included new male or female partner, lack of H2O2-producing lactobacilli, older age and recent oral sex.
This landmark paper examines the aetiology of the analogous condition of nongonococcal urethritis in men. It provides important data supporting the roles of M. genitalium, adenovirus and HSV-1 in the aetiology of nongonococcal urethritis and provides impetus for similar focus and investigations of the role of these organisms in cervicitis in women.
This is the latest version of widely accepted guidelines used by clinicians, which undergo continual review and evaluation.
This article provides a novel approach modelling three algorithms for management of cervicitis addressing the less-explored psychological morbidity of an STI diagnosis and the impact on health in young women. Although this analysis was based on the assumption that nonchlamydial cervicitis did not confer a risk of subsequent morbidity, the need for further clarification of the significance of a diagnosis of ‘cervicitis’ is highlighted.
This is a very clear, thorough review of the literature on Chlamydia, and the epidemiology, pathophysiology, management, serious reproductive sequelae and strategies for screening of this pathway.
This interesting study suggests that nonspecific endocervical inflammation (defined as >10 PMNL/hpf) in pregnant women in preterm labour may be a useful clinical marker for women who might benefit from antibiotic intervention.
This small cross-sectional study demonstrates an association between upper-genital tract infection (plasma cell endometritis) and increased HIV shedding, which has important implications for heterosexual transmission.
a previous population-based study, with thoughtful discussion of topical issues. This article reviews previous reported associations between cervical organisms and adverse pregnancy outcome. It focuses on the need for standardized testing kits and specific treatment.

In response to lack of a demonstratable link between trichomoniasis and HIV-1 acquisition in previous studies, the authors analysed data from a large 11-year prospective study of female sex workers in Nairobi. They demonstrated a 1.5-fold increased risk of HIV acquisition with trichomoniasis, which was also present in other studies. The researchers have pursued the aetiology of persistent nonspecific urethritis and widened the horizons of STI research.

In this study, the researchers have pursued the aetiology of persistent nonspecific urethritis and widened the horizons of STI research.

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Trichomonas vaginalis: underdiagnosis in urban Australia could facilitate re-emergence

M Josephine Lusk,1* Zin Naing,2 Ben Rayner,2 Nikolas Rismanto,2 Christopher J McIver,2,3 Robert G Cumming,4 Kevin McGeechan,4 William D Rawlinson,2,3 Pam Konecný1,3

ABSTRACT

Objectives Trichomonas vaginalis (TV) has a low profile in urban sexually transmitted infection (STI) clinics in many developed countries. The objective of this study was to determine the true prevalence of TV in an Australian urban sexual health setting using sensitive molecular diagnostic techniques.

Methods A cross-sectional study investigating the aetiology of cervicitis in women attending two urban sexual health clinics in Sydney, Australia, enrolled 356 consecutive eligible women from 2006 to 2008. The diagnostic yield from the standard clinical practice of discretionary high vaginal wet preparation microscopy in women with suspicious vaginal discharge was compared with universal use of nested PCR for TV of cervical samples.

Results TV was detected by PCR in 17/356 women (4.8%, 95% CI 2.8 to 7.5%), whereas only four cases (1.1%, 95% CI 0.3 to 2.8%) were detected by discretionary wet preparation microscopy. Eleven of the 17 women (p=0.003) were of culturally and linguistically diverse background. Additionally, cervicitis was found to be significantly associated with TV, RR 1.66 (1.14 to 2.42), p=0.034.

Conclusions Traditional TV-detection methods underestimate TV prevalence in urban Australia. The TV prevalence of 4.8% by PCR testing in this study exceeds previously reported urban Australian TV rates of <1%. An increase in trichomoniasis-associated adverse reproductive outcomes and enhanced HIV transmission poses a salient public health threat. Accordingly, TV warrants a higher profile in urban STI clinic settings and we suggest that priority be given to development of standardised molecular TV detection techniques and that these become part of routine STI testing.

INTRODUCTION

Trichomonas vaginalis (TV) is a sexually transmitted infection (STI) causing significant morbidity worldwide. Trichomonads are highly site-specific protozoan parasites. In women, TV infects the lower urogenital tract, causing superficial vaginal and cervical ulceration. Typical symptoms include frothy yellow discharge, itch, odour, dyspareunia and occasionally vaginal bleeding. Infection of the urethra and paraurethral glands causes dysuria and frequency.1 However, at least one-third of infected women may be asymptomatic.2 Trichomoniasis has been associated with premature rupture of membranes,3 pelvic inflammatory disease (PID),4 cervicitis5 and enhanced risk of HIV transmission.7 Men may present with balanitis, urethral discharge or dysuria, but again, high rates of asymptomatic carriage have been reported. A recent US STI clinic study detected TV in 72% of male partners of infected females, and of these men, 77% were asymptomatic.8 The duration of TV infection in women may be prolonged for up to 3–5 years but only about 4 months in men.9 The natural history of TV infection is not well defined.

Trichomoniasis is the most common curable STI. In 1999, WHO estimated 174 million new cases per year, more than double the number of Chlamydia trachomatis cases and treble the cases of gonorrhoea. The high TV prevalence worldwide is concentrated in developing countries and socio-economically disadvantaged groups, with a dramatic decline in TV rates in some developed countries in the past few decades.10 TV prevalence in urban Australia is reportedly low based upon routine wet preparation diagnostic methods.9 10 Australian rates of TV peaked in the 1950s at 20–30% and rapidly declined through the 1960s and 1970s to below 1% in 1990.10 This has been attributed to the combination of widespread use of the Nitroimidazoles, and increased surveillance through Papanicolaou smears.9 10 This decline and the fact that TV is not a notifiable disease in Australia have led to the present situation where testing for TV has assumed a very low priority in urban Australian settings. An audit of commercial sex workers (CSW) undergoing regular STI screening at a sexual health clinic in Melbourne in 2003 reported a very low incidence of TV at 0.11 per 100 person months.11 By contrast, TV prevalence in indigenous Australian women, in remote Northern Territory, was high at 25% in a self-sampling PCR-method-based study.12 The prevalence of TV by PCR among US women of reproductive age was recently found to be 3.1%, with an even lower prevalence of 1.3% in the subgroup of non-Hispanic white women.13

We are conducting a prospective study investigating the prevalence and aetiology of cervicitis using molecular diagnostic techniques in women attending two urban STI clinics in Sydney, Australia. In this paper, we report the prevalence of TV by PCR testing against traditional methods of detection, and compare our findings with previous data from other Australian urban STI clinics.

METHODS

This study was conducted in two urban Sydney STI clinics from July 2006 to December 2008. Ethics approval was granted by the South Eastern Health services research

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The information in this manuscript is not being considered elsewhere for publication. Some of the interim findings of the parent cervicitis study were recently presented by MJL at the Australasian Sexual Health Conference, Brisbane, 3 September 2009.

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Published Online First
1 November 2009
Subject selection
Women were eligible for this study if they were 18 years or older, had been sexually active in the previous 3 months and required an interim examination. The parent cervicitis study protocol excluded women if they had previously enrolled, had clinical PID, had received antibiotics or undergone gynaecological intervention in the previous month, had an intrauterine contraceptive device, were currently menstruating or pregnant, attending for sexual assault or unsuitable for enrolment due to psychosocial ill health or comprehension difficulties. Of a total of 957 consecutive first attendances, 561 women (58.6%) were ineligible, 40 (4.2%) declined, and 356 (37.2%) were enrolled. The most common reasons for ineligibility included examination not required (30.7%), antibiotics in previous month (14.2%), pregnancy (8.6%) and no sex in previous 3 months (8.3%). Women with clinical PID comprised 3.1% of ineligible women. This report is an interim analysis of 356 consecutive first attendances of women enrolled in the parent cervicitis study.

Sampling procedure
A sterile speculum was used to visualise the vagina and cervix. A Gram stain was performed on all cervical and high vaginal swabs (HVS). Due to the nature of the parent study, HVS for wet preparation microscopy was discretionary based upon clinical suspicion of TV infection, in keeping with usual clinical practice, and so not performed for all women. The endocervix was sampled in all women, with a sterile cotton swab (Cohan, Brescia, Italy) for Gram stain and bacterial agents. An additional endocervical sterile cotton swab was taken and placed in viral transport medium 199 (GIBCO Invitrogen, Grand Island, New York) and stored at −70°C for subsequent PCR testing for TV by the method described below. Swabs from all women were tested for TV by PCR.

Cervicitis was defined as >30 polymorphonuclear cells per high-powered field (PMN/hpf) in at least three non-adjacent fields of cervical mucus on Gram stain of the HVS.

Nucleic acid extraction and PCR amplification
Swabs were suspended in 500 μl of viral transport medium (above) before extraction of the total nucleic acid using a robotic extraction machine (MagNaPure LC, Roche, Germany) applying the Total NA protocol according to the manufacturer’s instructions (Roche, Germany). Extracts were stored at 4°C before testing within 48 h of collection.

Detection of TV was performed using a nested PCR. Briefly, the first-round reaction comprised 10 μl of template in a 50 μl of PCR reaction mixture containing 9 μl of nuclease-free water, 25 μl of 2 × iScript reaction mix (BioRad, Sydney, Australia), 0.5 μM each primer: TricV-IF (5′-CTTAATGCGAACATTGTTACCTGCCTC 3′) and TricV-IR (5′-CTGTTGGAGAGGACATGAACCTTGCGA 3′).14 Cycling conditions included denaturation and activation at 95°C for 5 min, 35 cycles of: 94°C for 20 s, 57°C for 20 s, 72°C for 20 s; a final extension at 72°C for 10 min and a 4°C hold. PCR products of 206 bp were expected for TV-positive and were visualised by gel electrophoresis. Using positive controls from either culture-proven or molecularly proven sources, sensitivity was assessed by measuring the limit of detection (10^2 copies per reaction) of plasmid constructs of the target sites as previously described14 and is estimated to be 95–98%. The specificity was determined as follows: confirmation by probe hybridisation following the PCR amplification, and DNA sequencing performed on PCR products of the first 10 TV positive samples from the study. Nucleotide BLAST on NCBI site (for all 10 samples) confirmed that all DNA sequences produced from sequencing were TV. The results from the above methods confirmed that the assay has 100% specificity.

Analysis
We report the prevalence with 95% CI of TV by traditional methods and PCR methods. Population characteristics of women with and without TV were compared using χ² testing. p Values <0.05 were considered statistically significant. Data were analysed with SAS software SAS Institute (Cary, North Carolina).

RESULTS
Prevalence of TV by PCR testing was 17/356 (4.8%, 95% CI 2.8 to 7.5%). Clinical suspicion prompting discretionary wet-mount preparation microscopy identified TV in only 4/356 women (1.1%, 95% CI 0.3 to 2.8%). PCR identified a higher percentage of women with TV (p=0.0008).

Of the 17 women positive for TV by PCR testing, only 11 had wet-preparation microscopy performed. The use of discretionary wet preparation was not significantly different in women with and without TV by PCR (p=0.498). Detection by Papnicolaou (Pap) smear occurred in only two of the 11 Pap smears done in women with TV. The mean age of the women with TV, 33.2 years, was not significantly different from the mean age of women without TV, 30.7 years (p=0.221) (table 1). Significantly, 11/17 women with TV (p=0.005) were of culturally and linguistically diverse background and identified consorts from populations of higher TV prevalence overseas (Africa, China, Sri Lanka, South America, Lebanon, Black American). Three women identified rural or ‘bush’ Australian contacts. Five women were CSW. No cases of TV were indigenous women. Six cases had BV, and three had concurrent STIs (two with Chlamydia and one with HIV and active genital herpes). Dysuria was significantly associated with women with TV (p=0.014). Prevalence of cervicitis in women without TV was 59%, compared with 68% (11/17) in women with TV, giving a RR of cervicitis in the presence of TV of 1.66 (95% CI 1.14 to 2.42) p=0.034.

DISCUSSION
These data show that traditional methods of detection greatly underestimate the prevalence of TV. We report a TV prevalence of 4.8% by PCR testing, whereas clinical suspicion prompting discretionary wet-preparation microscopy identified TV in only 4/356 women (1.1%) in the same study population. The lower
We report a higher than previously reported prevalence of *Trichomonas vaginalis* (TV) of 4.8% by PCR methods in an urban Australian sexually transmitted infection (STI) clinic setting.

TV is likely underdiagnosed in urban STI clinic settings using only traditional methods of detection. This may presage re-emergence with important Public Health consequences.

We find TV is significantly associated with women of culturally and linguistically diverse backgrounds, and cervicitis is significantly associated with TV (RR 1.66, p=0.034).

TV warrants a higher profile. Priority should be given to the development of standardised molecular TV detection techniques for inclusion in routine STI testing.

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### Table 1 Characteristics of women with and without *Trichomonas vaginalis* (TV) by PCR

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Women with TV N=17</th>
<th>Women without TV N=339</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>33.4</td>
<td>30.7</td>
<td>0.221</td>
</tr>
<tr>
<td>Culturally and linguistically diverse†</td>
<td>11 (65%)</td>
<td>104 (31%)</td>
<td>0.003</td>
</tr>
<tr>
<td>Condoms always</td>
<td>4 (24%)</td>
<td>75 (22%)</td>
<td>0.892</td>
</tr>
<tr>
<td>&gt;1 partner last 3 months</td>
<td>6 (35%)</td>
<td>95 (28%)</td>
<td>0.516</td>
</tr>
<tr>
<td>Commercial sex workers</td>
<td>5 (29%)</td>
<td>58 (17%)</td>
<td>0.195</td>
</tr>
<tr>
<td>Concomitant STI‡</td>
<td>2 (12%)</td>
<td>24 (7%)</td>
<td>0.469</td>
</tr>
<tr>
<td>Signs and symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dysuria</td>
<td>7 (41%)</td>
<td>59 (17%)</td>
<td>0.014</td>
</tr>
<tr>
<td>Vaginal discharge</td>
<td>11 (65%)</td>
<td>143 (42%)</td>
<td>0.067</td>
</tr>
<tr>
<td>Bacterial vaginosis§</td>
<td>6 (35%)</td>
<td>79 (23%)</td>
<td>0.258</td>
</tr>
</tbody>
</table>

* Values <0.05 are statistically significant.
† Defined as women identifying at clinic registration as preferring a language other than English, speaking a language other than English at home, or identifying a non-English ethnic background.
‡ Concomitant chlamydia or gonorrhoea.
§ Defined by Nugent score on Gram stain of high vaginal swab.

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**Key messages**

- We report a higher than previously reported prevalence of *Trichomonas vaginalis* (TV) of 4.8% by PCR methods in an urban Australian sexually transmitted infection (STI) clinic setting.
- TV is likely underdiagnosed in urban STI clinic settings using only traditional methods of detection. This may presage re-emergence with important Public Health consequences.
- We find TV is significantly associated with women of culturally and linguistically diverse backgrounds, and cervicitis is significantly associated with TV (RR 1.66, p=0.034).
- TV warrants a higher profile. Priority should be given to the development of standardised molecular TV detection techniques for inclusion in routine STI testing.
Funding  Financial support was provided in part by the HIV and AIDS Related Funding Program (HARP) South Eastern Sydney and Illawarra Area Health Service, and MJL was supported in part by a Novartis Research Scholarship.

Competing interests  None.

Patient consent  Obtained.

Ethics approval  Ethics approval was provided by the South Eastern Sydney and Illawarra Area Health Service Human Research Ethics Committee and the Sydney South West Area Health Service Ethics Review Committee (Royal Prince Alfred Hospital Zone).

Contributors  MJL and PK: designed and implemented the clinical research project, analysed the database management and statistical analysis and reviewed manuscript. JN, McClelland, MJ and SR: contributed to statistical methods, and contributed to reviewing the manuscript. KM: assisted with most of the manuscript (excluding lab methods section). RGC: assisted with the design of the study, choice of patients for the study, and sample and data collection, undertook data entry and statistical analysis and wrote most of the manuscript (excluding lab methods section). ZN, NR, B Rayner, CJM and WDR: assisted with design of the study, processing and storage of samples and undertook the PCR testing of the clinical samples and contributed to writing the manuscript (lab methods). RGC: assisted with the design of the study, choice of statistical methods, and contributed to reviewing the manuscript. KM: Assisted with the database management and statistical analysis and reviewed manuscript.

Provenance and peer review  Not commissioned; externally peer reviewed.

REFERENCES


**Mycoplasma genitalium** is associated with cervicitis and HIV infection in an urban Australian STI clinic population

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**ABSTRACT**

**Objectives** To investigate the prevalence of the genital mollicutes, *Mycoplasma genitalium* (MG), *Mycoplasma hominis* (MH), *Ureaplasma urealyticum* (UU) and *Ureaplasma parvum* (UP), and their associations with cervicitis in a sexually transmitted infection (STI) clinic population. Clinical correlates of MG infection were also assessed.

**Methods** 527 women were enrolled in a cross-sectional study at two STI clinics in Sydney between June 2006 and January 2010. Genital mollicutes were detected by multiplex PCR testing of cervical swabs, and associations with cervicitis were analysed. Cervicitis was defined as >30 polymorphonuclear cells per high-power field in at least three non-adjacent fields of cervical mucus on Gram stain.

**Results** MG was found in 4.0% of women, MH in 17.1%, UU in 14.1%, and UP in 51.8%. MG was the only mollicute associated with cervicitis (unadjusted prevalence ratio (PR) 1.85, 95% CI 1.52 to 2.26, p<0.0001), and this association remained after adjustment for *Chlamydia trachomatis* (CT) infection (adjusted PR 1.24 (95% CI 1.04 to 1.48), p=0.02). MG was significantly associated with women being HIV positive (p<0.03), but not with age, vaginal discharge, commercial sex work, being of culturally and linguistically diverse background, or concurrent CT infection. Two of the 21 women with MG had ectopic pregnancies.

**Conclusions** The authors recommend wider application of PCR testing for MG in STI services, particularly in high-risk women and those with cervicitis or HIV infection.

**INTRODUCTION**

Increasing evidence is emerging implicating *Mycoplasma genitalium* (MG) as a genital tract pathogen.1–5 However, the potentially pathogenic roles of other genital mollicutes, *Mycoplasma hominis* (MH), *Ureaplasma urealyticum* (UU) and *Ureaplasma parvum* (UP), are less well characterised.1 MG has been associated with cervicitis1–3 endometritis1–4 and pelvic inflammatory disease.1,5

The objectives of this study were to determine the population prevalence of MG, MH, UU and UP and their associations with cervicitis, and to examine clinical correlates of MG.

**METHODS**

A cross-sectional study was undertaken in two urban Sydney sexually transmitted infection (STI) clinics from June 2006 to January 2010. Ethics approval was granted by South Eastern Sydney and Illawarra Area Health Service human research ethics committee and Sydney South West Area Health Service ethics committee.

**Participant selection**

A total of 527 consecutive eligible consenting women were enrolled, with 85% recruited from a primary site. Both sites are part of a network of public STI clinics in urban Sydney servicing the same priority populations and are HIV referral centres. Inclusion and exclusion criteria and sample collection have previously been described. Of 570 eligible women, 527 consented (92.5%). All women had cervical swabs tested for MG, MH, UU, UP and *Chlamydia trachomatis* (CT). Cervicitis was defined as >30 polymorphonuclear cells per high-power field in at least three non-adjacent fields of cervical mucus on Gram stain. Clinicians’ cervical Gram stains for case definition of cervicitis were later read by a single laboratory scientist blinded to clinical diagnosis.

**Nucleic acid extraction and PCR amplification**

Specimens were extracted within 48 h of receipt, stored, and subsequently tested in batches. Nucleic acid extraction was carried out using a robotic extraction machine (MagNA Pure LC; Roche, Mannheim, Germany) applying the ‘Total NA protocol’. Detection of the mollicutes MG, MH, UP and UU involved a single-round multiplex PCR (mPCR) (VDL06) followed by PCR ELISA. The VDL06 mPCR was carried out as described,5 except reagents from the iScript One-Step RT-PCR kit (BioRad, Hercules, California, USA) were used for amplification. The sensitivity of VDL06 mPCR has been described previously.5 The specificity was determined by DNA sequencing of the first 10 positive samples of each mollicute from the study, and resulted in 100% specificity. *Chlamydia* testing was performed in a NATA-accredited diagnostic laboratory, using a commercial assay (COBAS Amplicor CT/NG multiplate ELISA; Roche) following the manufacturer’s instructions.

**Statistical methods**

Associations of mollicutes and CT with cervicitis were estimated using prevalence ratios (PRs), 95% CIs and p values. Given that the study was cross-sectional and cervicitis is common, we calculated PRs rather than ORs. Multivariate analysis in logistic regression models involved forward selection including only covariates with p<0.05. MG correlates were examined using χ² tests and t tests. All analyses were performed with SAS V9.2.
RESULTS

The prevalence of the mollicutes was: MG, 4.0%; MH, 17.1%; UU, 14.1%; UP, 51.8%. CT prevalence was 5.7%. MG was the only mollicute associated with cervicitis (unadjusted PR 1.85, 95% CI 1.52 to 2.26, p=0.0001), and this association remained after adjustment for CT (adjusted PR 1.24 (95% CI 1.04 to 1.48), p=0.02) (table 1).

MG was significantly associated with HIV positivity. Two of the 21 women with MG were HIV positive (9.5%) compared with 11 of 506 women without MG (2.2%) (p=0.03). There was no association between MG and age (p=0.28), vaginal discharge (p=0.52), commercial sex work (p=0.67), being culturally and linguistically diverse (p=0.95), or concurrent CT infection (p=0.44). Two of the 21 women with MG had an ectopic pregnancy.

Mollicutes were found in 346/527 (65.7%) women, and of these, 98 (27.5%) had more than one species. UP occurred significantly more often as a single infection (72% of UP infections) (p=0.0001) in contrast with the other mollicutes, which were more likely to coexist with other species.

The prevalence of cervicitis was 47.7%. Agreement between the clinician and laboratory scientists’ Gram stain diagnosis of cervicitis was 92.8% (κ estimate 85.4%).

DISCUSSION

In this study of an STI clinic female population, the prevalence of MG was 4.0%. This is similar to other high-risk populations: 6.3% in a Swedish STI population and 4.5% in a Norwegian STI clinic setting, but lower than 8.7% in women having abortions in New Zealand. Importantly, the prevalence of MG was similar to that of Chlamydia, also noted in other STI clinic populations.

MG was the only mollicute significantly associated with cervicitis in this study. Evidence concerning the role of MG in cervicitis is conflicting. Studies of associations of ureaplasmas and MH with cervicitis are also inconclusive. The prevalence of cervicitis in this study was high (47.7%), as noted in other STI clinic populations.

MG was significantly associated with HIV positivity (p=0.05). This association was also found in a study of West African commercial sex workers. As HIV shedding is associated with high MG organism burden, MG infection may facilitate HIV transmission. We did not find an association of MG with concurrent CT infection, consistent with other studies, but not with Huppert et al, who found an association in adolescent women.

Interestingly, two women with MG had ectopic pregnancies within 1 month of study enrolment. As these data were not specifically sought in all participants, we are unable to evaluate the significance of this. It may be a chance finding, but warrants further investigation.

UP was very common (52%) and was usually found as a single mollicute infection. This raises the possibility of a potential probiotic effect of UP with respect to other mollicutes.

PREVALENCE OF MOLLCUTES AND CERVICITIS

<table>
<thead>
<tr>
<th>Bacterial exposure</th>
<th>Prevalence (%) (95% CI)</th>
<th>PR of cervicitis, unadjusted (95% CI)</th>
<th>p Value</th>
<th>PR of cervicitis, adjusted* (95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>M genitalium</td>
<td>21/527 (4.0%) (2.5 to 6.0)</td>
<td>1.85 (1.52 to 2.26)</td>
<td>&lt;0.0001</td>
<td>1.24 (1.04 to 1.48)</td>
<td>0.02</td>
</tr>
<tr>
<td>M hominis</td>
<td>90/527 (17.1%) (14.0 to 20.6)</td>
<td>1.00 (0.79 to 1.28)</td>
<td>0.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U urealyticum</td>
<td>74/527 (14.1%) (11.2 to 17.3)</td>
<td>0.96 (0.73 to 1.25)</td>
<td>0.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U parvum</td>
<td>273/527 (51.8%) (47.4 to 56.1)</td>
<td>1.09 (0.91 to 1.31)</td>
<td>0.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlamydia</td>
<td>30/527 (5.7%) (3.9 to 8.0)</td>
<td>1.59 (1.26 to 2.01)</td>
<td>0.0001</td>
<td>1.16 (0.99 to 1.36)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

*Only covariates with p values <0.05 in unadjusted models were included in the adjusted model.

Key messages

- Prevalence in women of Mycoplasma genitalium (MG) was 4.0%, Mycoplasma hominis 17.1%, Ureaplasma urealyticum 14.1%, and Ureaplasma parvum 51.8%.
- MG was the only mollicute associated with cervicitis, and this association remained after adjustment for Chlamydia trachomatis infection.
- MG was associated with HIV positivity, but not with age, vaginal discharge, commercial sex work, culturally and linguistically diverse background or concurrent chlamydia.
- We recommend wider application of PCR testing for MG in STI services, particularly in high-risk women and those with cervicitis or HIV infection.

As this study involves a high-risk population, its applicability to general populations is limited. In addition, sample size was too small to detect weak associations. Strengths of the study include robust case definition for cervicitis and high participation rate in a consecutive series of women.

In conclusion, we found that MG was the only mollicute species significantly associated with cervicitis, and MG was associated with HIV positivity. We recommend wider application of PCR testing for MG in STI services.

REFERENCES


Clinical Study

Pharyngeal Gonorrhoea in Women: An Important Reservoir for Increasing Neisseria gonorrhoea Prevalence in Urban Australian Heterosexuals?

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We aim to characterize sexual behavioral aspects of heterosexual Neisseria gonorrhoea (NG) acquisition in two Sexually Transmitted Diseases clinics in Sydney, Australia, in 2008–2012. Of 167 NG cases, 102 were heterosexually acquired with a trend of increasing NG prevalence in heterosexuals from 1.1% (95% CI 0.6–2.1) in 2008 to 3.0% (95% CI 2.0–4.0) in 2012 (P = 0.027). Of heterosexual male cases, unprotected fellatio was the likely sexual activity for NG acquisition in 21/69 (30.4%) and commercial sex work (CSW) contact the likely source in 28/69 (40.6%). NG prevalence overall in CSW (2.2%) was not significantly higher than in non-CSW (1.2%) (P = 0.15), but in 2012 there was a significant increase in NG prevalence in CSW (8.6%) compared to non-CSW (1.6%) (P < 0.001). Pharyngeal NG was found in 9/33 (27.3%) female cases. Decreased susceptibility to ceftriaxone (MIC ≥ 0.03 mg/L) occurred in 2.5% NG isolates, none heterosexually acquired. All were azithromycin susceptible. A significant trend of increasing prevalence of heterosexual gonorrhoea in an urban Australian STD clinic setting is reported. We advocate maintenance of NG screening in women, including pharyngeal screening in all women with partner change who report fellatio, as pharyngeal NG may be an important reservoir for heterosexual transmission. Outreach to CSW should be enhanced.

1. Introduction

Latest surveillance indicates rising rates of Neisseria gonorrhoea (NG) in New South Wales, Australia [1]. The risk of HIV transmission is significantly enhanced by co-infection with NG [2, 3], and so the control of NG particularly in light of increasing minimum inhibitory concentration (MIC) values to ceftriaxone is a major public health concern [4, 5]. The predominance of gonorrhoea amongst Australian urban men who have sex with men (MSM) is well documented [1, 6] but heterosexual gonorrhoea in urban settings is less well characterised. NG is a notifiable disease in Australia but data is only collected by age, sex, and region of diagnosis and so heterosexual trends are poorly defined. Trends of increasing prevalence of heterosexually acquired NG and acquisition from fellatio and commercial sex worker (CSW) contact were noted in our suburban STD services in 2009, prompting this investigation specifically aimed at examining sexual behavioral aspects of heterosexual NG acquisition.

2. Methods

A case series was conducted from patient records at two STD services in South Eastern Sydney over a 5-year period, January 1, 2008 to December 31, 2012. Data was collected
Table 1: Summary of heterosexual patients and NG cases 2008–2012.

<table>
<thead>
<tr>
<th></th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. hetero patients (male and female)</td>
<td>875</td>
<td>1009</td>
<td>1102</td>
<td>1041</td>
<td>1091</td>
<td>5118</td>
</tr>
<tr>
<td>No. hetero male patients</td>
<td>473</td>
<td>529</td>
<td>577</td>
<td>539</td>
<td>599</td>
<td>2717</td>
</tr>
<tr>
<td>No. hetero female patients</td>
<td>402</td>
<td>480</td>
<td>525</td>
<td>502</td>
<td>492</td>
<td>2401</td>
</tr>
<tr>
<td>Total NG cases</td>
<td>18</td>
<td>35</td>
<td>27</td>
<td>33</td>
<td>54</td>
<td>167</td>
</tr>
<tr>
<td>Total hetero NG</td>
<td>10</td>
<td>26</td>
<td>13</td>
<td>20</td>
<td>33</td>
<td>102</td>
</tr>
<tr>
<td>Hetero male NG</td>
<td>5</td>
<td>19</td>
<td>11</td>
<td>13</td>
<td>21</td>
<td>69</td>
</tr>
<tr>
<td>Hetero female NG</td>
<td>5</td>
<td>7</td>
<td>2</td>
<td>7</td>
<td>12</td>
<td>33</td>
</tr>
<tr>
<td>Prevalence of NG in heterosexuals (%)</td>
<td>1.14</td>
<td>2.58</td>
<td>1.18</td>
<td>1.92</td>
<td>3.02</td>
<td>2.0</td>
</tr>
<tr>
<td>No. female CSW</td>
<td>50</td>
<td>63</td>
<td>76</td>
<td>75</td>
<td>58</td>
<td>322</td>
</tr>
<tr>
<td>Mean age (yrs) hetero males with NG**</td>
<td>32.4</td>
<td>33.1</td>
<td>38.2</td>
<td>44.3</td>
<td>37.4</td>
<td>37.3</td>
</tr>
<tr>
<td>Mean age (yrs) hetero female with NG***</td>
<td>32.4</td>
<td>39.0</td>
<td>44.0</td>
<td>25.7</td>
<td>32.8</td>
<td>33.2</td>
</tr>
</tbody>
</table>

* A significant trend of increasing NG prevalence in heterosexuals was noted over the 5 year period (P = 0.027).
** No significant age trend in heterosexual male NG cases (P = 0.122).
*** No significant age trend in heterosexual female cases (P = 0.387).

prospectively from late 2009 when the study started but retrospectively prior to this. These clinics operate in a culturally diverse suburban environment and offer free services by triage to high risk patients defined by "priority populations" specified in the 2010–2013 NSW Sexually Transmitted Infection (STI) strategy [7] (i.e., MSM, youth, CSW, multipartnered heterosexuals, intravenous drug users, HIV positive, indigenous) and other symptomatic patients, contacts of STDs, or those referred by General Practitioners (GPs).

NG cases were identified from the clinic database. All NG cases were included whether detected by routine screening or testing in symptomatic patients. Heterosexual acquisition was defined as sexual activity not involving any same sex contact in the preceding 12 months. Heterosexual patient numbers were derived from total client numbers, minus MSM and women who have sex with women (WSW). Likely acquisition source and activity was identified from detailed sexual histories, which routinely seek information on the nature and timing of recent sexual contacts including number and sex of consorts, type of sexual contact (oral, vaginal, anal, insertive/receptive), and condom use for each activity. In deciding the likely transmission mode and source of NG, we took into account onset of symptoms and NG disease incubation time.

Receipt of oral sex (fellatio) was considered the likely route of NG infection when this activity occurred in isolation without a condom or if this occurred concurrently with vaginal or anal sex where a condom was used for the latter activities but not for oral sex. A female commercial sex worker was defined as a woman who stated she was currently engaged in sex work. Local contact was defined as sexual contact with a person in Australia.

Clinic policy states that all symptomatic patients are tested for NG in the relevant anatomical site. MSM are screened for NG in the rectum, urine, and throat, CSW are screened in the throat and cervix or urine, heterosexual men in the urine, and heterosexual women in the cervix or urine. Cases diagnosed by PCR are also cultured where possible, in order to ascertain antimicrobial susceptibility data. NG was treated during the study with ceftriaxone 250 mg IMI, and this was increased to 500 mg IMI from early 2010 in keeping with local recommendations. Cefixime is not available in Australia.

NG was cultured on selective media of lysed horse blood agar containing vancomycin, colistin, nystatin, and trimethoprim (VCNT) inhibitors. Antimicrobial susceptibility testing was performed prospectively at the Neisseria Reference Laboratory, Randwick, Sydney, a WHO Collaborating Centre for STD, using published methodology [8]. Decreased susceptibility of NG to ceftriaxone in extragenital sites was reported when the MIC value was ≥0.03 mg/L and ≥0.06 mg/L in genital sites [8]. All samples positive for NG using Nucleic Acid Amplification Techniques (NAAT) (Roche Amplicor PCR from study commencement to June 2011 and then from July 2011 by Roche Cobas 4800) were confirmed by supplementary assays targeting porA and opa genes as required by the National Testing Guidelines [9].

Proportions were compared using Chi-Square tests and trends identified using a Mantel-Haenszel Chi-Square test with SAS (version 9.2; SAS Institute Inc., Cary, NC, USA). Ethics approval was granted by the South Eastern Illawarra Area Health Service Ethics Committee.

3. Results

During the study 6164 patients were seen of which 5118 (83.0%) were classified as heterosexual and 1046 (17.0%) MSM or WSW, with approximately equal numbers of male (53.1%) and female (46.9%) heterosexual patients (Table 1). There were 167 cases of NG, 102 (61.1%) heterosexually acquired (overall prevalence 2.0%) and 65 (38.9%) MSM acquired (overall prevalence 6.9%). Over the 5-year period there was a significant trend of increasing NG prevalence in heterosexuals rising from 1.1% (95% CI 0.6–2.1) in 2008 to 3.0% (95% CI 2.0–4.0) in 2012 (P = 0.027).
Of heterosexual cases, 69 were males and 33 were females (M:F ratio 2.1:1). 67/69 (97.1%) of heterosexual males and 25/33 (75.8%) of females had genital symptoms.

Receipt of unprotected fellatio was the likely source of acquisition for 21/69 (30.4%) of heterosexual males (11/21 CSW related and 10/21 non-CSW related). Commercial sex work (CSW) contact was the probable NG source for 28/69 (40.6%) of heterosexual males (18 CSW contacts local, and 10 whilst overseas). Only 7/33 (21.2%) of female cases reported current CSW. Importantly, NG prevalence during the study overall in CSW (2.2%) was not significantly higher than in non-CSW (1.2%) (\(P = 0.15\)), but in 2012 there was a significant increase in NG prevalence in CSW seen (8.6%) compared to non-CSW (1.6%), \(P < 0.001\).

Of female cases, 31/33 (93.9%) reported unprotected vaginal sex. Pharyngeal NG was found in 9/33 (27.3%) women, 5 of these CSW. NG was acquired locally in 24/33 (72.7%) of females and 47/69 (68.1%) of heterosexual males.

137/167 (82%) of NG cases were diagnosed by positive culture and 30/167 (18%) by positive PCR alone. Antimicrobial susceptibility data was available in 122/137 (89%) of NG cases diagnosed by culture. Decreased susceptibility to ceftriaxone was reported in 3/122 (2.5%), two pharyngeal and one rectal isolate all MSM related isolates, none heterosexually acquired. Of the 122 isolates with antibiotic susceptibility data, 55/122 (45.1%) were MSM related and 67/122 (54.9%) were heterosexually related. All NG isolates were azithromycin susceptible.

4. Discussion

This study found an increasing prevalence of heterosexual gonorrhoea in an urban Australian setting from 2008 to 2012, a trend which may be contributing significantly to rising NG notifications in Australia. At our services heterosexual acquisition accounted for 61.0% (102/167) of NG cases. The male:female ratio in heterosexual cases of 2:1 is comparable to the overall national Australian surveillance ratio of 2:1 [10]. The overall study male:female ratio including the MSM cases was 4.0:1, which is in marked contrast to our South East Sydney local health district reported ratio of 8:1 where NG detection predominates in the large MSM population [1,10]. This reflects the lower proportion of MSM attendances at our clinics (25.8%) compared to inner city services. The persistence of the 2:1 male:female case ratio in heterosexuals is interesting. Factors contributing to this male predominance in heterosexual NG case detection might include increased likelihood of symptomatic disease and therefore detection in males (and more asymptomatic disease in females), lack of pharyngeal screening in females, suboptimal screening of CSW and high risk females, and possibly some misclassification of “heterosexual” acquisition. Enhanced surveillance of NG might help to clarify this enigma.

Receipt of unprotected fellatio was the likely sexual activity resulting in NG acquisition for 30% of heterosexual men. Additionally the number of heterosexual male NG cases due to fellatio may be underestimated in this study if receipt of unprotected oral sex occurred in conjunction with unprotected vaginal or anal sex. The pharyngeal NG reservoir in MSM is well recognized [11,12], but this reservoir could also be important in all women with partner change who practice fellatio, not just CSW. This theory is supported by the high transmission rate in heterosexual men receiving fellatio and noting that equal numbers of fellatio-related transmissions occurred in men reporting contact with CSW and non-CSW females. Oral sex is frequent amongst heterosexuals and is typically unprotected. This combined with lack of awareness of the associated STD transmission risk, common perception that oral sex is not sex [13] and infrequent pharyngeal screening in women, may facilitate heterosexual NG transmission via this route. We isolated pharyngeal NG from 27% of female cases (by culture), but this is likely to be an underestimate due to clinic policy at the time of only undertaking pharyngeal screening in CSW and MSM. Increased uptake of more sensitive NAAT testing in the pharynx [14] is also likely to improve female NG detection. Our findings in both men and women suggest that an NG pharyngeal reservoir in women may be a common source of NG infection for heterosexual males. A recent UK study [15] also suggested that the pharynx may be an important NG reservoir in heterosexual women with a similar finding of 30% of female NG cases being pharyngeal. We found that NG infections in heterosexual men were almost always associated with genital symptoms (97.1%) but women less commonly so (75.8%). Hence, asymptomatic screening in women may be particularly important. Accordingly, our clinic guideline has changed to recommend the maintenance of NG screening in heterosexual women with additional pharyngeal NG screening in those women reporting partner change and fellatio. As female cases generally reported unprotected vaginal sex (93.9%), condom use is also reiterated.

CSW in Australia have low rates of STDs reflecting good condom use [16]. However, a recent study of CSWs providing fellatio in Sydney [17] found that Cantonese speaking women were significantly less likely to use condoms for this service than Thai-speaking and English-speaking CSW. Additionally, women who do not identify as being CSW (e.g., working in massage) may be less likely to engage in safe sex, including safe oral sex [17]. Our study found that 40% of heterosexual males reported CSW contact but only 21% of female cases were CSW, an inconsistency which could reflect suboptimal testing rates and outreach to CSW in our population. Importantly NG prevalence in female CSW overall was no different from that in female non-CSW, except for the significant rise noted in 2012. Of concern, however, was the finding that 2/3 of the NG infections related to CSW contact occurred locally in Sydney, the rest acquired from overseas contacts. This would suggest a need to enhance local educational and testing services available to women engaged in CSW.

Three quarters of all infections were acquired from local contact, reflecting the increasing local heterosexual NG prevalence noted in this study and from local surveillance [1]. Numbers of NG cases rose in 2012 which is also in keeping with the rise in local NG prevalence.

Antimicrobial susceptibility data was available for 89% of cultured isolates. Decreased susceptibility to ceftriaxone occurred in 3/122 (2.5%) isolates, all MSM related cases.
from extragenital sites. No decreased susceptibility was noted in heterosexually acquired isolates but numbers are too small to speculate on the significance of any difference in antibiotic susceptibilities between these populations at this time, but this should be the subject of ongoing monitoring and surveillance to inform treatment recommendations in these different populations. The finding of 3 isolates with decreased susceptibility to ceftriaxone is consistent with the right shift in MIC values to ceftriaxone reported locally [4] and globally [5, 18] and cause for concern for disease control in the absence of viable treatment alternatives if resistance to ceftriaxone develops. All isolates were sensitive to azithromycin. We recommend that cases positive by PCR should also be cultured where possible for purposes of monitoring NG isolate susceptibility. The widespread supplanting of culture methods with PCR has the advantages of greater sensitivity and no fuss specimen transport, but at the cost of antimicrobial surveillance. STD services are best placed to maintain this NG antimicrobial resistance surveillance role.

This study is limited by small numbers, reliance on patient sexual histories, and its partially retrospective nature in two triaged STD clinic populations within the same local health district. Inevitably some definitions particularly relating to transactional sex can become blurred and sexual histories are reliant on patient recall and propensity to disclose the exact nature of the contact. Some cases were unwilling or unable to identify the likely source of the infection. Clinical judgment was applied to determine the likely source of infection based on disease incubation, onset of symptoms, and detailed recent sexual history. Triage processes were unchanged over the study period and priority population groups remained relatively stable. Importantly antibiotic resistance testing was prospective and performed in a reference laboratory, a WHO Collaborating Centre for STD.

5. Conclusion
A significant trend of increasing prevalence of heterosexual gonorrhoea in an urban Australian STD clinic setting is reported. This study suggests that the pharynx may be an important reservoir for heterosexual NG transmission, and we advocate maintenance of NG screening in women, particularly inclusion of pharyngeal screening in women with partner change who practice fellatio. Case detection, enhanced surveillance, and health promotion are pivotal to NG control. Health promotion efforts should include messages concerning STD transmission risks associated with oral sex in heterosexuals, and we recommend enhanced CSW engagement with education and STD testing opportunities.

Conflict of Interests
The authors declare no conflict of interests.

Authors’ Contribution

Acknowledgments
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References


