MYCOPLASMA GENITALIUM

Detection of *Mycoplasma genitalium* in women with laparoscopically diagnosed acute salpingitis

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Objectives: *Mycoplasma genitalium* has been associated with cervicitis, endometritis, and tubal factor infertility. Because the ability of this bacterium to ascend and infect the fallopian tube remains undefined, we performed an investigation to determine the prevalence of *M genitalium* in fallopian tube, endometrial, and cervical specimens from women laparoscopically diagnosed with acute salpingitis in Nairobi, Kenya.
Methods: Women presenting with pelvic inflammatory disease were laparoscopically diagnosed with salpingitis. Infection with *M genitalium* in genital specimens was determined by polymerase chain reaction (PCR).
Results: Of 123 subjects with acute salpingitis, *M genitalium* was detected by PCR in the cervix and/or endometrium in nine (7%) participants, and in a single fallopian tube specimen. In addition, those infected with *M genitalium* were more often HIV infected than women not infected by *M genitalium* (seven of nine (78%) *v* 42 of 114 (37%), $p<0.03$).
Conclusions: *M genitalium* is able to ascend into the fallopian tube, but its association with tubal pathology requires further investigation.

Pelvic inflammatory disease (PID) is the most common serious gynaecological disorder diagnosed in women and can result in debilitating sequelae including infertility, chronic pelvic pain, and ectopic pregnancy. *Neisseria gonorrhoeae* and *Chlamydia trachomatis*, two well established PID aetiologies,[1 2] have declined over the past two decades in most regions and are found less commonly in HIV seropositive women with PID.[3] Other facultative and anaerobic bacteria have been cultured from the endometrium, fallopian tube and pelvic exudate from women with PID, but the role of these organisms as primary PID pathogens or secondary colonisers has not been well established.[2] Nevertheless, in studies of acute salpingitis, no microbial aetiology has been identified in 20%–70% of women.[1–3] The definitive association of a pathogen with salpingitis is dependent upon its detection in fallopian tube tissue. However, such specimens are difficult to obtain, and thus such studies are not frequently performed.

Mycoplasma genitalium has been detected in the female reproductive tract, but its association with disease has only recently been studied because of the difficulty in culturing this fastidious organism. Thus, although *M genitalium* was first cultured in 1981 from two of 13 men with non-gonococcal urethritis (NGU), the development and application of specific polymerase chain reaction (PCR) assays a decade later enabled studies to establish its association with NGU.[4–7] Applying these PCR assays to studies in women, *M genitalium* has been detected in vaginal, cervical, and endometrial specimens,[6] and has been associated with cervicitis,[8] clinical PID,[9] and histologically diagnosed endometritis in women with acute pelvic pain.[10] While early serological investigations designed to assess an association between PID and *M genitalium* were inconclusive,[11 12] *M genitalium* was shown to cause salpingitis experimentally in several non-human primate species[13] and more recent serological studies established an association between *M genitalium* and tubal factor infertility.[14] Although most of these studies suggest that *M genitalium* may be a significant reproductive tract pathogen in women, its ability to ascend to the female upper genital tract and cause tubal disease has not been firmly established.

In the present study *M genitalium* was assayed using PCR in fallopian tube, endometrial, and cervical samples from women with laparoscopically confirmed acute salpingitis. In addition, laboratory and clinical findings were measured to define factors associated with *M genitalium* infection in women with salpingitis.

METHODS

A prospective case-control study, defined by HIV serostatus, was utilised to investigate the aetiology of acute salpingitis. Women aged 18–40 presenting with clinically suspected PID at Kenyatta National Hospital (KNH) were screened for study participation between April 2000 and January 2003. Inclusion criteria, identical to those used for our earlier investigation,[1] included a complaint of low abdominal pelvic pain for 2 weeks or less in addition to one or more of the following signs or symptoms: temperature ⩾38°C, dysuria, and complaint of abnormal vaginal discharge. Women who reported pregnancy, abortion, or surgery within the past 6 weeks, or who used any antibiotic within the previous 2 weeks were excluded. Subjects were selected, examined, and underwent laparoscopy within 6 hours of hospital admission.

After giving written informed consent, study participants underwent HIV pretest counselling, a detailed questionnaire, and general and gynaecological examination. A clinical severity score (CSS) was used to assess the clinical severity[15] of PID. During pelvic examination, a single Dacron swab was used to obtain the cervical specimen for *M genitalium*, *N gonorrhoeae*, and *C trachomatis* PCR testing. Blood was obtained for HIV and CD4 lymphocyte counts (HIV seropositive women only).

Abbreviations: CSS, clinical severity score; NGU, non-gonococcal urethritis; PCR, polymerase chain reaction; PID, pelvic inflammatory disease; TOA, tubo-ovarian abscess

To confirm the clinical diagnosis of acute PID and visually stage the severity of acute salpingitis, laparoscopy was performed on all participants by one of two gynaecologists trained in the laparoscopic diagnosis of acute salpingitis. At surgery, the gynaecologist was blinded to laboratory findings, including HIV serostatus. After induction of anaesthesia, and following a full surgical preparation that included application of Betadine three times to the cervix, vagina, and vulva an endometrial biopsy was obtained with a pipelle suction curette (Unimar, Inc, Wilton, CT, USA) for *M genitalium*, *N gonorrhoeae*, and *C trachomatis* testing. The severity of acute salpingitis was graded as (1) mild (tubal erythema or oedema, mobile tubes, with or without spontaneous exudate), (2) moderate (marked tubal erythema and oedema, limited tubal mobility, questionable or no tubal patency, and gross exudate), and (3) severe (pyosalpinx or tubo-ovarian abscess (TOA)).[16] Pus obtained from a pyosalpinx-TOA aspirate or collected from the peritoneal cavity, or tubal ostia biopsy if pus was not present, was tested for *M genitalium*, *N gonorrhoeae*, and *C trachomatis* by PCR. For analysis, these specimens were treated collectively as fallopian tube specimens.

Subjects with laparoscopic evidence of acute salpingitis received treatment with cefotetan 2 g intravenously every 12 hours and doxycycline 100 mg orally every 12 hours until the CSS was reduced by 75% compared to baseline findings. If the CSS did not improve within 72 hours of antibiotic treatment, intravenous clindamycin 500 mg every 6 hours was added. At discharge, all patients were treated with an additional week of metronidazole 500 mg every 8 hours and doxycycline 100 mg every 12 hours. Women received HIV post-test counselling at time of hospital discharge. Participants were scheduled for re-evaluation at 1, 2, and 4 weeks after discharge.

All samples from the cervix, endometrium, and fallopian tubes, (including abscesses) were collected in a dry tube and 800–1000 µl of 2-SP buffer (0.2 M sucrose in phosphate buffer, pH 7.5) were added, and frozen at −20°C until tested for *N gonorrhoeae* and *C trachomatis* by PCR (Amplicor, Roche Diagnostic Systems, Branchburg, NJ, USA) in Kenya. Subsequently, the frozen specimens were shipped to Seattle and analysed for *M genitalium* using the MgPA-IMW PCR assay[12] after purification of DNA using the MasterPure DNA purification kit (Epicentre, Madison, WI, USA). All PCR assays were performed with an equivalent amount of preprocessed sample (12.5 µl). Among the specimens tested, endometrial (33), and to a lesser extent, fallopian tube (16) specimens were more often inhibited than cervical specimens (three). However, inhibition was always eliminated by a 1:5 or 1:10 dilution of the original sample. We repeated the MgPa-IMW PCR analysis on all initially positive specimens to confirm that they were true positives and not PCR contaminants. Among one, six, and 10 specimens initially positive from the fallopian tube, endometrium, and cervix, respectively, none, one, and four did not repeat as positive and therefore were given a negative final result.

Serum was tested for HIV antibodies by ELISA (Detect HIV, BioChem ImmunoSystems, Montreal, Canada), with positive results confirmed by a second ELISA (Recombigen, Cambridge Biotech, Ireland). CD4 cells from peripheral blood were enumerated using a Facscan (Beckton-Dickinson, Baltimore, MD, USA).

Data were analysed using SPSS for Windows 11.5 (SPSS Inc, Chicago, IL, USA). Univariate analyses included Pearson's χ^2 and Fisher's exact tests for categorical data; Mann-Whitney test and Student's *t* test for continuous variables.

RESULTS

Salpingitis was laparoscopically confirmed in 142 (90%) of the 158 women enrolled with a clinical diagnosis of acute PID; 16 (10%) women had no evidence of salpingitis and were excluded from the study. Sixteen (11%) of the 142 women with salpingitis did not have specimens sufficient for *M genitalium* PCR. Of the remaining 123 cases with specimens available for *M genitalium* testing, 53 (43%), 35 (28%), and 36 (29%), had mild, moderate, and severe salpingitis, respectively, and 50 (40%) were diagnosed with HIV infection. Age, marital status, sexual history and microbiological findings of women who did and did not have specimens tested for *M genitalium* did not differ significantly (data not shown). However, women who did not have specimens tested for *M genitalium* tended to have more mild salpingitis (12 (75%) *v* 56 (44%), p = 0.06) and were less likely to be HIV infected (two (13%) *v* 49 (40%), p = 0.05) than women with specimens available.

Table 1 depicts the prevalence of *M genitalium*, *C trachomatis*, and *N gonorrhoeae* detected in cervical, endometrial, and tubal specimens stratified by the severity of salpingitis. *M genitalium* was detected in the fallopian tube specimen from

Table 1 Prevalence of *Mycoplasma genitalium*, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in 126 women with confirmed acute salpingitis compared by laparoscopic severity and specimen site

	Mild salpingitis (n = 53)	Moderate salpingitis (n = 35)	Severe salpingitis (n = 36)
M genitalium (detected in 9 subjects)			
Cervix	1 (2%)	4* (11%)	2 (6%)
Endometrium	3 (5%)	2 (6%)	0
Tube	1 (2%)	0	0
Any site	3 (6%)	4 (11%)	2 (6%)
C trachomatis (detected in 8 subjects)			
Cervix	2 (4%)	4 (11%)	2 (6%)
Endometrium	1 (2%)	3 (9%)	2 (6%)
Tube	1 (2%)	2 (6%)	2 (6%)
Any site	2 (4%)	4 (11%)	2 (6%)
N gonorrhoeae (detected in 21 subjects)			
Cervix	6 (11%)	5 (14%)	8 (23%)
Endometrium	5 (9%)	5 (14%)	7 (20%)
Tube	1 (2%)	5 (14%)	6 (18%)
Any site	7 (13%)	5 (14%)	9 (26%)

*Co-infected with *C trachomatis*.

Table 2 Comparison of sociodemographic, clinical, and laboratory findings for 126 women diagnosed with acute salpingitis with and without *M genitalium* infection

	M genitalium positive (n = 9)	*M genitalium* negative (n = 114)	p Value
Sociodemographics			
Age (mean (SD))	26.9 (4.9)	28.1 (5.5)	0.52*
Marital status			
Married	3 (33%)	68 (60%)	
Single	3 (33%)	23 (20%)	
Divorced/separated,	3 (33%)	18 (16%)	
widowed	0	4 (4%)	0.32†
Number of sex partners (median, range)			
Last 3 months	1 (0–1)	1 (0–5)	0.09‡
Last year	1 (0–2)	1 (0–10)	0.70‡
Lifetime	4 (1→97)	3 (1→97)	0.39‡
Clinical findings			
Clinical severity score (mean (SD))	15.6 (7.1)	15.6 (7.6)	0.88*
Laparoscopic severity of salpingitis			
Mild	3 (33%)	50 (44%)	1.0¶
Moderate	4 (44%)	31 (27%)	0.47¶
Severe	2 (22%)	34 (30%)	1.0¶
Laboratory findings			
HIV seropositive	7 (78%)	42 (37%)	0.03¶
CD4 count $\times 10^6$/l (mean (SD))§	230 (151)	342 (229)	0.23*
C trachomatis (any site)	1 (11%)	7 (6%)	0.48¶
N gonorrhoeae (any site)	0	21 (19%)	0.36¶

*Student's *t* test.
†Pearson's χ^2 test.
‡Mann-Whitney test.
¶Fisher's exact test.
§For HIV seropositive subjects only.

a single, HIV infected woman with mild acute salpingitis. Overall, *M genitalium* was identified in nine (7%) of the 126 women: in the cervix alone in four (3%) women, the endometrium alone in a single woman (1%), both the cervix and endometrium in three women (3%), and in a single woman (1%) in both the endometrium and fallopian tube. In comparison, *C trachomatis* and *N gonorrhoeae* were detected from any genital tract site in eight (6%) and 21 (17%) women, and from the fallopian tube in three (2%) and 15 (12%) participants, respectively. The ratio for detecting *M genitalium* in the fallopian tube to that found in any genital tract site was 1:9 (11%) in comparison with 3:8 (38%) for *C trachomatis* ($p = 0.30$) and 15:21 (71%) for *N gonorrhoeae* ($p = 0.002$).

Age, marital status, and median number of sex partners in the previous 3 months and lifetime did not differ between women infected and those not infected with *M genitalium* (table 2). *M genitalium* infection was not associated with severity of disease based on CSS and laparoscopic findings. None of the women infected with *M genitalium* had a concomitant *N gonorrhoeae* infection, while *C trachomatis* was identified in a single woman infected with *M genitalium* (table 1). HIV infection was more commonly detected in women infected with *M genitalium* than those not infected with *M genitalium* (seven of nine (78%) *v* 42 of 114 (37%), $p = 0.03$).

DISCUSSION

In this study, *M genitalium* was detected in cervical and endometrial specimens from women with laparoscopically confirmed acute salpingitis, and in a single fallopian tube sample. Although this represents the first detection of *M genitalium* in the fallopian tube, the prevalence among women with salpingitis was low (1% overall and 11% of all *M genitalium* genital tract infections), similar to that of *C trachomatis* (6% overall and 38% of all *C trachomatis* infections), but statistically different from that of *N gonorrhoeae* (17% overall and 71% of *N gonorrhoeae* infections). Several theories could explain why *M genitalium*, although found in cervical and endometrial specimens, was rarely detected in fallopian tube samples of women with confirmed salpingitis in our sample set: (1) *M genitalium* may be associated with milder forms of PID,[10] and therefore would rarely infect our study population, most of whom were hospitalised with more severe disease; (2) cervical and/or endometrial *M genitalium* infection or colonisation may be associated with salpingitis caused by as yet unidentified pathogens rather than ascending into the fallopian tubes itself; (3) *M genitalium*, similar to *Ureaplasma urealyticum*[17] may not cause salpingitis, but rather may colonise the cervix and possibly the endometrium without causing significant tubal pathology, a possibility that cannot be excluded since our study did not contain a true control group for comparison; and (4) *M genitalium* may infect the fallopian tube in amounts that were undetectable by our PCR assay. Although the sensitivity of the *M genitalium* assay is similar to that of the Amplicor *C trachomatis* and *N gonorrhoeae* assays,[18] it is possible that several freeze-thaw cycles, by the time the samples were shipped to Seattle and tested for *M genitalium*, contributed to decreased sensitivity.

In our previous investigation of women with outpatient PID, all 10 *M genitalium* infected women with histologically diagnosed endometritis complained of mild abdominal pain in comparison to 68% of those with endometritis who did not have *M genitalium* detected ($p = 0.06$).[10] In the current study, *M genitalium* was not associated with severity of disease by either clinical criteria (CSS) or the well established laparoscopic scoring system. In fact, *M genitalium* was evenly distributed among women with mild and moderate salpingitis and was found in two women with severe disease. Nevertheless, the prevalence of *M genitalium* among a similar population of Kenyan women diagnosed with outpatient PID was slightly higher (16%) than we observed in the present study of hospitalised women (7%), consistent with the hypothesis that *M genitalium* causes less severe symptomatology.[10]

The increased prevalence of *M genitalium* in HIV seropositive women in comparison with HIV seronegative women in our study was remarkably similar to that demonstrated by

Key messages

(1) *Mycoplasma genitalium* was detected in cervical and endometrial specimens from women with laparoscopically confirmed acute salpingitis
(2) This study represents the first detection of *M genitalium* from the fallopian tube
(3) The prevalence of *M genitalium* in the fallopian tube among women with salpingitis was low (1% overall and 11% of all *M genitalium* genital tract infections), similar to that of *Clhlamydia trachomatis* (6% overall and 38% of all *C trachomatis* infections), but statistically different from that of *Neisseria gonorrhoeae* (17% overall and 71% of *N gonorrhoeae* infections)
(4) *M genitalium* detection was positively correlated with HIV infection in women with acute salpingitis

Irwin *et al*[19] in a US population, and in our earlier investigation.[10] In Nairobi women with outpatient PID, *N gonorrhoeae* and *C trachomatis* were more often detected in HIV seronegative women, whereas bacterial vaginosis was more often associated with PID in HIV seropositive women.[3] In addition, *Enterobacteriaceae* and anaerobic Gram negative rods isolated from the endometrium were positively correlated with HIV infection in women with salpingitis.[20] Whether HIV infection affects susceptibility to *M genitalium* infection and disease, or serves as a marker for high risk exposure will require further investigation.

Most evidence to date suggests that *M genitalium* has a role in PID and its sequelae.[9–12] Based on this and our earlier investigation demonstrating an association between *M genitalium* and endometritis, we expected to detect this organism in fallopian tube specimens from a higher proportion of women with salpingitis. Almost 25 years ago European and American researchers were involved in similar controversy regarding *C trachomatis* as a cause of salpingitis.[21] As a consequence, rather than thwart enthusiasm for *M genitalium* as a potential cause of female upper genital tract infection, these results should encourage further investigation to determine the role of *M genitalium* in PID, including salpingitis.

CONTRIBUTORS

CRC designed the study, supervised the collection of data and analysis, and wrote the paper; NRM supervised the field site, collection of data, and assisted with writing of the paper; SGA supervised the *M genitalium* assay, helped oversee the laboratory procedures in Nairobi, and assisted with writing the paper; RO performed many of the laboratory assays in Kenya, helped with quality control of the *M genitalium* assay, and assisted with writing of thepaper; LEM helped design the protocol and assisted with writing of the paper; JAK helped supervise the field site in Nairobi, oversaw local laboratory procedures, and helped write the paper; WES helped design the protocol, and assisted with writing the paper; PGW helped supervise the Kenyan laboratory and assisted with writing the paper; PAT designed the *M genitalium* component of the study, helped analyse and interpret results, and co-wrote the paper.

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The study protocol was approved by the institutional review board for human subjects at the University of Washington, and by the ethics review committee at Kenyatta National Hospital and the Kenya Medical Research Institute, Nairobi, Kenya. Procedures followed were in accordance with the ethical standards for human experimentation established by the Declaration of Helsinki of 1975, revised in 1983.
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