Prevalence Rates of *Chlamydia Trachomatis* and Other Sexually Transmitted Organisms in Infertile Couples Attending a Tertiary Medical Centre in Malaysia

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ABSTRACT

INTRODUCTION: *Chlamydia trachomatis (CT)*, *Neisseria gonorrhoeae (NG)*, *Trichomonas vaginalis (TV)* and *Mycoplasma genitalium (MG)* infections are well recognized and prevalent sexually transmitted infections (STIs). The role of *Mycoplasma spp* and *Ureaplasma spp* are still controversial as some are commensals of genitourinary tract. OBJECTIVES: To estimate the prevalence rate of 7 organisms: *Chlamydia trachomatis, Neisseria gonorrhoea, Mycoplasma genitalium, Trichomonas vaginalis, Mycoplasma hominis, Ureaplasma urealyticum* (UU) and *Ureaplasma parvum* (UP) in infertile married couples during infertility evaluation.

MATERIALS & METHODS: A total of 274 samples from all of the 137 couples who attended the reproductive center from June to December 2014 were collected. Detection of the organisms was performed using multiplex polymerase chain reaction.

RESULTS: STI-associated organisms were detected in 35.4% (97/274) of subjects. The prevalence rates of CT, MG, TV, UU, MH were 7.3%, 1.1%, 0.4%, 5.4% and 5.1% respectively. Twenty-one (7.7%) subjects were positive for more than one organism. 24/274 (8.8%) of subjects had history of urogenital tract-related symptoms and 50% (12/24) were tested positive to one or more organisms. The presence of symptoms in both male and female subjects were found to be 10% (2/20) in CT infection, 10% (7/67) in UP, 14% (2/14) in MH and 13% (2/15) in UU infections. CONCLUSION: Sexually transmitted organisms were detected in one third of subjects planning for fertility evaluation. The absence of symptom in most subjects particularly in CT infection emphasizes the need for microbiological screening during infertility evaluation. The presence of genital ureaplasmas and mycoplasmas in infertile couples should not be neglected. There is a growing need to clarify whether their roles are simply colonizers or pathogens implicated in infertility.

KEYWORDS: Infertility, sexually transmitted infections, multiplex polymerase chain reaction

INTRODUCTION

Sexually transmitted diseases were long recognized as one of the factors affecting human infertility. Infections such as *Chlamydia trachomatis* infection and gonorrhoea are known to cause alterations in female and male genital tracts. A few other organisms such as *Trichomonas vaginalis, Mycoplasma* spp and *Ureaplasma* spp are recently drawing interest of researchers in the field of infertility.

*Chlamydia trachomatis*, now the leading cause of bacterial sexually transmitted infections worldwide,
mostly causes asymptomatic infection in both males and females. Untreated infection may allow the bacteria to ascend to the upper part of female reproductive tract, causing functional and structural damage of the fallopian tube and tubal related infertility as a consequence.

*Neisseria gonorrhoeae*, discovered in 1879 by Albert Neisser is the causative agent of gonorrhoea, an ancient disease that persists until today. This obligate human pathogen causes genitourinary infections such as urethritis, orchitis and epididymitis in males, where the infections may cause urethral strictures leading to impairment of urogenital tract function. In females, most infections are silent or cause only mild symptoms. Thus, untreated, such infections may ascend to the upper genital tract causing pelvic inflammatory disease with infertility as a sequela.

Trichomoniasis is a genitourinary infection caused by the protozoa *Trichomonas vaginalis*. Although reported prevalence in some country such as Malaysia is low, it is a common non-viral STI in many countries. This disease remained poorly diagnosed and high in prevalence despite being curable. Ureaplasma and Mycoplasma on the other hand, are commonly found in healthy women. These species are now recognized as emerging sexually transmitted organisms as their role in early pregnancy loss, stillbirths and neonatal morbidity has been described.

With the exception of *N. gonorrhoeae*, most of the STIs organisms such as *C. trachomatis*, *T. vaginalis*, *Mycoplasmas* and *Ureaplasmas* are difficult to culture in the routine hospital laboratory. However, the availability of non-culture-based test such as nucleic acid amplification test (NAAT) has allowed further studies on these organisms. The investigations of infectious diseases during infertility evaluations include serology tests for human immunodeficiency virus (HIV), hepatitis B virus, hepatitis C virus, rubella and syphilis. Commonly, STIs such as *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections are looked for only in patients with symptoms of urogenital tract infection whereas ureaplasmas, mycoplasmas and *Trichomonas vaginalis* are commonly not evaluated.

This study was conducted with the aim to determine the percentage prevalence rates of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, and *Ureaplasma parvum* in couples with infertility using multiplex real-time polymerase chain reaction. Other sexually transmitted organisms such as HIV, *Treponema pallidum* and *Gardnarella vaginalis* (causing bacterial vaginosis) may have a role in infertility, however, the assessment of these organisms are beyond the scope of this study.

### MATERIALS AND METHOD

#### Study design and population

This is a descriptive, cross-sectional study conducted over a period of 7 months among the couples attending the Reproductive Centre for infertility evaluation from June to December 2014. During the study period, 137 couples attended and all were recruited. Each couple was counseled together by the attending doctor who explained about the seven sexually transmitted organisms and the infections they caused. The participants were also provided with fact sheets of information regarding each infection, its transmission, symptoms and signs, diagnosis and treatment. A written informed consent were taken for each participant. Data collection sheet in the form of questionnaire stating symptoms of STI, previous history or treatment of STIs as well as patients’ medical record were used to obtain demographic data and clinical information of STIs. Regular infectious disease screening tests such as hepatitis B surface antigen (HbsAg), rapid plasma
reagin (RPR) for syphilis and HIV antigen/antibody tests were performed in most female subjects but the information regarding these tests were not included in this study.

Sample size

Previous studies on prevalence of sexually transmitted organisms in Malaysia were mostly done on Chlamydia trachomatis in high risk groups or in patients attending genito-urinary clinic. Hence, we decided to use the prevalence of CT in female in Genito-urinary Clinic in Malaysia (Norashikin et al. 2007), with 17.5% prevalence.

Using the sample size calculation formula for prevalence study by Kish L. 1965, whereby sample size n = \( \left( Z_{\alpha/2} \right)^2 \left( P \times (1-P) / D^2 \right) \), with the prevalence of positive identification P= 0.175, and absolute precision (D) of 5%, ie D= 0.05, the total sample calculated is 221. Due to budget limitation, we collected 137 samples from female subjects and 137 samples from their male partners, which amounted to 274 samples.

Specimen processing

The swab specimens were equilibrated to room temperature and mixed by vortexing. The caps from the specimen tubes were removed carefully to avoid contamination. Excess mucus in the specimen was removed by collecting in on the swab. One ml specimen was transferred to 1.5-ml microcentrifuge tubes and the tubes were centrifuged at 5 000 x g (7,500 rpm) for 10 minutes. The supernatant was discarded and the pellet was re-suspended in 1000 µL of 1 x phosphate-buffered saline (PBS) by vortex.

The urine specimens were equilibrated to room temperature and 1 ml specimen was transferred to 1.5-ml microcentrifuge tubes. The urine specimens were then centrifuged at 15,000 x g (13,000 rpm) for 15 minutes, as per manufacturer’s protocol. Similar centrifugation speed has also been decribed. The urine samples required higher and longer centrifuge speed and time to ensure collection of as many cells possible in the samples. Afterwards, the supernatant was discarded and the pellet was re-suspended in 1 ml of 1 x PBS by vortexing.

DNA extraction

The DNA was extracted from the processed specimens (swab or urine) using the StarMag 96 tissue kit (Seegene, Seoul, Korea), in accordance with the manufacturer’s instructions in automated DNA extraction system called Nimbus IVD (Hamilton). The PCR mastermix was prepared by Nimbus, and sample and negative control were loaded into the mastermix by Nimbus. This system is used to prevent carry-over contamination in DNA extraction process. An internal control (IC) provided within the manufactured detection kit was added to the samples as an exogenous whole process control, immediately before the DNA extraction. The presence of internal control is to confirm the success of the extraction steps and also to monitor for any presence of inhibitor which can affect the PCR process.

Specimen collection

Endocervical swab specimens were collected from female patients by the attending doctors using a manufactured collection kit, eNat® (Copan Italia S.P.A, Italy). The kit contains a swab and guanidine thycianate based medium which stabilizes RNA and DNA of viruses, bacteria, Chlamydia, Trichomonas vaginalis and Mycoplasma and was validated for molecular assay formats. The male patients were asked to provide first-voided urine (at least 2 hours after previous urination) in a sterile 50ml screw-cap plastic container. After collection, the specimens were put immediately into a cooler box and transported to microbiology laboratory. Specimens received out of working hours were kept in 4°C in the refrigerator and processed the next working day.
**Multiplex real-time PCR (Anyplex™ II STI-7 Detection Kit)**

Real-time PCR amplification for seven microorganisms (C. trachomatis, N. gonorrhoeae, T. vaginalis, M. genitalium, M. hominis, U. urealyticum, and U. parvum) was performed using the Anyplex™ II STI-7 Detection Kit (Seegene, Seoul, Korea), in accordance to the manufacturer’s protocol. This assay utilizes the tagging oligonucleotide cleavage and extension technology (TOCE™) which has the ability to detect the seven microorganisms simultaneously in a single fluorescent channel on real-time PCR platform using melt-curve analysis. In melt-curve analysis, issues in accuracy and reproducibility may occur due to the temperature differences among DNA that has high sequence variation. The TOCE™ technology however was designed to be not affected by sequence variation, in which it was able to produce consistent melting temperature (Tm). The assay was performed in a CFX96™ Real-time thermocycler (Bio-Rad, Hercules, CA, USA). Each PCR was performed in 5µL of extracted DNA, 4x STI-7 TOM, and Anyplex PCR Mix in a 20 µL reaction. Five µL of RNase-free water and 5µL of STI-7 PC were used as negative control and positive control respectively. Results and analysis and interpretation were provided by Seegene Viewer software.

**STATISTICAL ANALYSIS**

Data were analysed using descriptive statistical methods (chi-square test) in SPSS software (version 22) and p < 0.05 was considered statistically significant. Kappa (k) statistics proposed by Landis and Koch (1977) was used to assess the agreement between the detection of C. trachomatis, U. urealyticum, U. parvum and M. hominis in husband and wife. Guidelines for the interpretation of k were as follows: k < 0.20 indicating poor agreement; k = 0.21-0.40 indicating fair agreement; k=0.41-0.60, moderate agreement; k = 0.61-0.80, good agreement; and k= 0.81-1.00 indicating very good agreement. The study protocol was approved by the Research Ethics Committee of the Universiti Kebangsaan Malaysia Medical Centre.

**RESULTS**

A total of 274 samples from all of the 137 couples who attended the Reproductive Centre from June 2014 to December 2014 for infertility evaluations were collected and analysed in this study. Organisms were detected in 35.4% (97/274) of subjects (Table I).

More than 60% of the subjects were Malay, followed by Chinese (26%), Indian (10%) and others (4%) who were mainly from Vietnam, Nigeria and Thailand. Only one female gave history of previous chlamydial infection, however, she was found to be negative for any of the tested organism. A statistically significant correlation was seen between female gender and infections (p =0.001). No statistical association was found between age group, race, symptoms of urogenital tract infection and history of STIs.

The general prevalence of all detected organisms was shown in Table II. The prevalence of C. trachomatis, M. genitalium, T. vaginalis, U. urealyticum, M. hominis were 7.3%, 1.1%, 0.4%, 5.4%, 5.1% respectively and Ureaplasma parvum showed the highest prevalence of 24.4%. C. trachomatis detection in both female and male subjects was 10.9% & 3.6% respectively. All detected organisms were found more prevalent in female genital tract than that of male. No M. genitalium was detected in male partners of infertile couples. No Neisseria gonorrhoea was detected in this study.

Among the coupled partners, four organisms were detected in both husband and wife. Ureaplasma parvum was detected in 18/137 couples (13%), C. trachomatis in 3/137 couples (2.2%), M. hominis in
**Table I: Characteristics of infertile couples attending reproductive centre**

<table>
<thead>
<tr>
<th>Categories</th>
<th>Positive(%) for tested organisms*</th>
<th>Negative(%) for tested organisms*</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age group</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.336</td>
</tr>
<tr>
<td>&lt; 35 years</td>
<td>62 (22.6%)</td>
<td>103 (37.5%)</td>
<td>165</td>
<td></td>
</tr>
<tr>
<td>≥ 35 years</td>
<td>35 (12.8%)</td>
<td>74 (27.1%)</td>
<td>109</td>
<td></td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.978</td>
</tr>
<tr>
<td>Malay</td>
<td>60 (21.9%)</td>
<td>107 (39.1%)</td>
<td>167</td>
<td></td>
</tr>
<tr>
<td>Chinese</td>
<td>24 (8.8%)</td>
<td>47 (17.2%)</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>Indians</td>
<td>9 (3.3%)</td>
<td>17 (6.2%)</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>4 (1.5%)</td>
<td>6 (2.2%)</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>Female</td>
<td>66 (48.1%)</td>
<td>71 (51.8%)</td>
<td>137</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>31 (22.6%)</td>
<td>106 (77.4%)</td>
<td>137</td>
<td></td>
</tr>
<tr>
<td><strong>Presence of Symptoms</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.117</td>
</tr>
<tr>
<td>Yes</td>
<td>12 (4.4%)</td>
<td>12 (4.4%)</td>
<td>24/274</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>85 (31%)</td>
<td>165 (60.2%)</td>
<td>250/274</td>
<td></td>
</tr>
<tr>
<td><strong>History of STIs</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.176</td>
</tr>
<tr>
<td>Yes</td>
<td>0</td>
<td>1 (0.36%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>97 (35.4%)</td>
<td>176 (64.23%)</td>
<td>273</td>
<td></td>
</tr>
</tbody>
</table>

* = C. trachomatis (CT), T. vaginalis (TV), M. genitalium (MG), U. urealyticum (UU), M. hominis (MH) and U. parvum (UP)

**Symptoms: pelvic pain, dysuria, per vaginal discharge (p/v discharge), pelvic pain with p/v discharge, dysuria with p/v discharge /pelvic pain

2/137 couples (1.5%), and U. urealyticum in 3/137 couples (2.2%). We calculated k value to see the agreement between positive individuals and their partners for detected organisms (reference in statistical analysis section). Fair agreement (k= 0.21 - 0.40) was noted between husband and wife for U. parvum (0.406), C. trachomatis (0.259), M. hominis (0.260) and U. urealyticum (0.369).

Table III shows the numbers of subjects harbouring more than one organisms. In total, 21/274 (7.66%) of subjects were positive for more than one organism. This finding was more common in females (6.9%) than in males (0.7%). Among the subjects harbouring more than one organisms, 2 different species were detected in 19 individuals and 3 different species were detected in the remaining two individuals. Dual detection of C. trachomatis and U. parvum was most common (2.2%) followed by M. hominis with U.parvum (1.8%) and U. urealyticum with U.parvum (1.1%). One woman was found positive for C. trachomatis with T. vaginalis. Dual detection of other organisms (UP+UU, MH+UU, MG+UP) were also seen. One male and 1 female subjects were positive for 3 organisms.

A total of 8.8% (24/274) of male and female subjects had history of symptoms. Among them, 50% (12/24) were found to be positive for organisms (Table IV). In female subjects, pelvic pain was reported by 29% (7/24) of the individuals and 5 of them were positive for organisms. Dysuria was reported in 1 male and 1 female with U. parvum detected in both. Other symptoms were per vaginal discharge (20%), pelvic pain with per vaginal discharge (12.5%), dysuria with per vaginal discharge, and dysuria with pelvic pain. U. parvum was the most prevalent organism in symptomatic individuals 41% (5/12). In general, in both male and female subjects, symptoms were reported in 10%(2/20) of CT infection, 10% (7/67) of UP, 14% (2/14) of MH and 13%(2/15) of UU infections.

Vice-versa, these findings showed that majority of the subjects with infections were asymptomatic.
Table II: Total distributions of detected organisms

<table>
<thead>
<tr>
<th></th>
<th>Female (n=137)</th>
<th>Male (n=137)</th>
<th>Total (n=274)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Detected (%)</td>
<td>Detected (%)</td>
<td>Detected (%)</td>
</tr>
<tr>
<td><em>U. parvum</em></td>
<td>44 (32.1)</td>
<td>23 (16.8)</td>
<td>67 (24.4)</td>
</tr>
<tr>
<td><em>C. trachomatis</em></td>
<td>15 (10.9)</td>
<td>5 (3.6)</td>
<td>20 (7.3)</td>
</tr>
<tr>
<td><em>U. urealyticum</em></td>
<td>10 (7.3)</td>
<td>5 (3.6)</td>
<td>15 (5.4)</td>
</tr>
<tr>
<td><em>M. hominis</em></td>
<td>11 (8)</td>
<td>3 (2.2)</td>
<td>14 (5.1)</td>
</tr>
<tr>
<td><em>M. genitalium</em></td>
<td>3 (2.2)</td>
<td>0</td>
<td>3 (1.1)</td>
</tr>
<tr>
<td><em>T. vaginalis</em></td>
<td>1 (0.7)</td>
<td>0</td>
<td>1 (0.4)</td>
</tr>
</tbody>
</table>

Total 84 (61.2) 36 (26.2) 120 (43.7)

DISCUSSION

By using the multiplex molecular method, we were able to demonstrate the prevalence rates of 10.9% for CT, 0.7% in TV, 2.2% in MG, 7.3% in UU, 8.0% in MH and 32.2% in UP among females with infertility. A few researchers have reported the prevalence rates among males and females in general population using the same platform (*Anyplex™ II STI-7 Detection Kit*).

In a study among 2735 endocervical specimens from women of childbearing age in Italy by Avolio et al (2016), lower prevalence rates of CT (2.6%), TV (0.9%), MG (0.8%), UU (6.3%), MH (6.5%) and UP (30.9%) were documented in comparison to our study.

However, in contrast to the female subjects, lower prevalence rates of CT (3.6%), UU (3.6%), MH (2.2%), and UP (16.8%) and 0% detection for TV, MG, and NG were found in infertile male subjects in our study. In a study by Choe et al (2013) who evaluated 510 urine samples from male volunteers in South Korea (using *Anyplex™ II STI-7 Detection Kit*), a higher prevalence of CT (11%), TV (1.0%), NG (5.9%) MG (3.1%), UU (13.9%), MH (3.1%) and UP (17.1%) were reported.

The prevalent rates of CT and NG in male cohorts in the present study is also lower in comparison to male populations who are engaged in high risk sexual behaviour and to those attending genitourinary clinic in Malaysia.

Among the seven sexually transmitted organisms, studies on *Chlamydia trachomatis* and *Neisseria gonorrhoeae* have consistently demonstrated the pathogenic effects of these two organisms leading to infertility. Meanwhile, there is limited evidence in the literature to implicate *Trichomonas vaginalis*, *Mycoplasma* spp and *Ureaplasma* spp in infertility. However, the availability of NAATs have paved a new approach to evaluate the role of these organisms in infertility.

**Chlamydia trachomatis**

The reported prevalence rates for *Chlamydia trachomatis* (CT) in those with infertility varies widely depending on the cohort of patients studied, the detection methods used and the geographical region in which the study is conducted. In women, CT...
Table IV: Distribution of symptoms among symptomatic individuals with and without detected organisms

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Total n=24 (%)</th>
<th>Female n=20</th>
<th>Male n=4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Pelvic pain only</td>
<td>7(29%)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5 (CT,UP,MH,MG,UP)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dysuria only</td>
<td>7(29%)</td>
<td>2</td>
<td>1 (UP)</td>
</tr>
<tr>
<td></td>
<td>1(UP)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Per vaginal discharge</td>
<td>5(20%)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4(UP,UU+MH,UP+CT,UP)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pelvic pain with p/v discharge</td>
<td>3(12.5%)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Dysuria with p/v discharge</td>
<td>1(4%)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Dysuria with pelvic pain</td>
<td>1(4%)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

CT - C. trachomatis, MG - M. genitalium, TV - T. vaginalis, UU-U. urealyticum, MH-M. hominis, UP - U. parvum, p/v - per vaginal

is a recognised and established cause of tubal infertility. In Malaysia, earlier study in infertile female by Hazlina et al. (2005) using direct immunofluorescence and Chlamydia IgM reported a low prevalence of 4%.15 Yeow et al. (2016) used PCR and reported a significantly higher rate of 82% in infertile women suspected to have bacterial infection.16 The present study found 10.9% (15/137) prevalence rate in Malaysian women with infertility. The same prevalence rate (10.9%) was also reported by Fernandes et al. (2014) when they study 340 women undergoing assisted reproduction in a public reference service in Brazil.17

The low prevalence in comparison to Yeow et al. was most likely due to the difference in inclusion criteria of the subjects. Worldwide, studies in infertile females reported rates ranging from 2.2% in USA to 32% in Iran.18-19 In male subjects in this present study, the positive rates for CT was 3.6%; almost similar to rates in Kuwait (3.9%)20 and slightly higher than rates reported in China (2.58-2.92%).21 Higher prevalence rate among infertile males were reported in Jordan (4.3%)22 and very high rate in Tunisia (43.3%).23 Other study in Tunisia, Sellami et al. (2014) also reported widespread Chlamydia trachomatis infection among male partners of infertile couple albeit at lower rate (15.2% vs 43.3%). Although both study employed molecular method for detection, the use of quantitative real time PCR in Sellami et al. was most likely more specific than in-house PCR-microtiter plate hybridization method used in earlier study by Gdoura et al. (2008), thus yielding lower detection of CT.23,24

The role of Chlamydia trachomatis in male infertility was studied by many and had yielded conflicting results. Some works showed evidence that CT caused reduction in sperm quality and increased sperm DNA fragmentation in comparison to fertile male24 while others report no association between CT infection and male infertility.25 Thus, its role as a direct aetiological agent in male infertility is controversial. Nevertheless, CT infection in male still poses a significant risk as it can be sexually transmitted to their female partners, giving rise to unwanted sequelae as discussed above.

Neisseria gonorrhoea

Similar to Chlamydia trachomatis, Neisseria gonorrhoeae (NG) infection also leads to pelvic inflammatory disease which resulted in tubal infertility, ectopic pregnancy and chronic pelvic pain. Reported prevalence rate in infertile male subjects ranged from 2% to 6.5% in various regions.26
In infertile females, lower prevalence rates of 0.4% to 2% were observed. In this present study we did not find any subjects with *N. gonorrhoeae*, similar to the findings by Hazlina et al. (2005).

In Malaysia, prevalence rates of gonorrhoea were mostly reported among subjects at higher risk for HIV and other STIs such as commercial sex workers (5.8%) and male attendees of genitourinary clinic (24.2%). Prevalence rates data among specific population such as infertile groups are scarce and mostly in female subjects. The reason for non-detection of NG in our cohorts could not be ascertain likely because the risk factors for gonorrhoea and other STIs were not fully assessed in our questionnaire. The participants were only required to state history of STIs and any treatment received. Information on sexual history, sex partner(s) and sexual practices were not attained. Only one female subject gave a history of previous CT infection and was treated for it. Hence we postulated that non-detection was likely due to reason that the infertile couples were regular lifetime partner, thus risk of STI particularly gonorrhoea is relatively lower. Furthermore, this study was conducted at a tertiary care centre, hence prior testing and treatment may have occurred in the patients, which possibly leads to non-detection of NG. Nevertheless, it remains an important aetiological agent of STI that should be excluded and treated in any sexually active individual.

**Trichomonas vaginalis**

Unlike gonorrhoea and Chlamydia infections, trichomoniasis is still considered a sexually transmitted infection of minor importance. Nevertheless, there is growing body of evidence that incriminate its role in increased HIV transmission, development of cervical intraepithelial neoplasia (CIN) and infertility in women. In males, evidence for the role of trichomoniasis in infertility was conflicting. Daly et al. (1989) reported that *Trichomonas vaginalis* has no effect in sperm motility and numbers. In contrast, Gopalakrishnan et al. (1990) described abnormal sperm and decreased sperm motility with reversible effects following metronidazole treatment. Although *T. vaginalis* was not singly isolated in this study, it was detected together with *C. trachomatis* in one woman (0.8%) while no man was positive for this organism. Prevalence rates of TV in infertile groups varies among countries. Casari et al (2010) studied 952 female subjects in Italy and found a low prevalence rate in infertile vs fertile women (0.25% vs 0.54%). However higher prevalence rates were reported in Egypt (14.5 vs 2.5%) and Turkey (18% vs 0%). In Malaysia, prevalence rates *T. vaginalis* of 9.5%-19.2% were reported among high risk groups such as commercial sex workers and female drug abuser. However, in low risk females, zero prevalence were reported in two studies; among females attending obstetrics and gynaecology clinic (Norhayati et al 2016) and among female attendees of STI clinic (Amal, 2010). Amal et al. also reported 0.36% prevalence were seen in female attending family planning clinic. Norhayati et al. postulated that low prevalence in Malaysia is likely because the participants enrolled were in a low-risk group, whereby most were married and had one stable sexual partner, also financially stable as housewives or had secure occupation. Another explanation for the low prevalence was the possibility of the women using vaginal washings and antiseptics after coitus with their partners. These reasons could also explain the low prevalence in this present study.

**Mycoplasma genitalium**

*Mycoplasma genitalium* (MG) is an etiological agent of non-gonococcal urethritis in men. Its role in male infertility was reviewed by Huang et al., (2015) in a meta-analysis from several studies involving 307 men.
with infertility, and they suggested a minimal role for MG in male infertility.\textsuperscript{38} Prevalence studies from Tunisia and Kuwait mostly showed low prevalence from 1.3% to 4.7%.\textsuperscript{39,20} No male was found positive for this organism in this present study. In female, the role of MG in female infertility is under much scrutiny. Some reports associated it with cervicitis, pelvic inflammatory disease, preterm birth, spontaneous abortion and infertility.\textsuperscript{40} However, a study among women with tubal infertility did not isolate MG.\textsuperscript{41} A meta-analysis looking at role of MG in female infertility found an estimated 2.5-fold increased risk of infertility associated with MG, however this increased risk was not statistically significant. The same study on the other hand, reports a statistically significant role of MG in cervicitis and PID.\textsuperscript{42} Regardless, this organism was detected more often in cervical swabs (19.6%) and in peritoneal cavity (5.8%) of infertile women compared to healthy fertile women.\textsuperscript{43} The present study found a low prevalence of M. genitalium in infertile female (2.2%). Although prevalence in this study is low, it is an important finding as treatment of individuals with MG urogenital infections will prevent sexual transmissions and likely to reduce the risk of complications including tubal factor infertility.\textsuperscript{44} Concerning M. hominis infection found a significant relationship between MH and male infertility.\textsuperscript{38} In addition, Ahmadi et al., (2017) noted higher detection of MH in infertile male (14.5%) compared to fertile male (3.6%), improvement in semen quality after antibiotic treatment and 58% of the female partners of the infertile group were reported to have successfully become pregnant 4 months after completion of treatment. They concluded that antibiotic therapy improved semen quality and fairly treated the male infertility.\textsuperscript{47} Although these evidences are encouraging, these should be interpreted carefully. Most studies did not include other STIs such as gonorrhoea, and Chlamydia trachomatis and also did not demonstrated that by treating MH, fertility was restored.\textsuperscript{44} Studies of pathogenicity of MH in females are generally complicated due to its high prevalent in sexually active asymptomatic females,\textsuperscript{45} and also the fact that MH were often recovered or detected together with other recognized STI organisms such as CT and NG.\textsuperscript{48} Hence, in comparison to male counterparts, literatures studying the role of MH in female infertility did not found conclusive evidence incriminating MH in female infertility.\textsuperscript{41} Ureaplasma urealyticum \& Ureaplasma parvum In healthy sexually active adults, Ureaplasma urealyticum (UU) and Ureaplasma parvum (UP) are both highly prevalent and are thus considered as commensals of the genitourinary tract.\textsuperscript{46} Due to high colonization rates among both men (3-56%) and women (8.5-77%)\textsuperscript{45}, these ureaplasmas have also been evaluated for its relationships with infertility. Many investigators have attempted to determine these organisms as a cause of infertility. Previous studies among infertile males have demonstrated that UU may have an aetiological role in male infertility. It has been shown to adhere to sperm cells, resulting in reduction of sperm motility and.

**Mycoplasma hominis**

*Mycoplasma hominis* and ureaplasmas can be transmitted horizontally and sexually. The colonization rates of MH in both sexually active men and women varied from zero to 13% in males and zero to 31% in females.\textsuperscript{45} Among infertile males, reported prevalence varies from 5% to 21% depending on geographical region.\textsuperscript{20,39} In infertile females, reported prevalence ranges from zero to 8.1%.\textsuperscript{41,46}

As compared to *M. genitalium*, the evidence of *Mycoplasma hominis’* (MH) role in male infertility is increasing. A large meta-analysis looking at nine case-control studies with 2410 cases and 1223 controls...
alterations in cell membrane. A significant role of UU but not UP was also demonstrated in male infertility. Other studies however, reported no correlation between male infertility and UU. The prevalence among infertile males for UU were reported from 10.8-24%. and 2.9-9.6% for UP. Our findings of 3.6% prevalence rate for UU is lower than others, while for UP, we report a higher prevalence rate of 16.8%.

Remarkably, both organisms were more commonly isolated from infertile female subjects as compared to fertile women as described by Fenkci et al, 2002 and Kasprzykowska et al., (2013). Kasprzykowska et al. studied the potential pathogenicity of these organisms and demonstrated that the presence of UP and UU in the pouch of Douglas may produce chronic inflammation of upper genital tract of women. This finding showed that colonization in cervicovaginal region can lead to ascending inflammation and infection in the sterile upper reproductive tract subsequently adversely affecting it. Their study also indicated that UP can cause similar asymptomatic infection like UU. Hence, the presence of Ureaplasma spp calls for further scrutiny. In the present study, UP and UU were demonstrated in 32.2% and 7.3% respectively. This is comparable to rates of detection in cervical swab reported in Italy (33.2% UP & 4.7% UU) and in Poland (22.2% UP, 25.9% UU).

Detection of multiple organisms

Infertility afflicts males and females worldwide. Hence, identifying preventable causes such as sexually transmitted infections should be a priority. The utilization of multiplex PCR method allows rapid detection of culturable and difficult-to-culture sexually transmitted organisms with high sensitivity and throughput. It also has the advantage of detecting co-infection, which was not possible using conventional PCR. In this study, co-infections with two or three organisms were noted. Approximately almost 14% of women and 2.2% of men were found to have more than one microorganism. The most common dual detection organisms were between C. trachomatis with U. parvum (2.2%) followed by M. hominis with U. parvum (1.8%). Ureaplasma urealyticum and M. hominis were both detected in 0.7% of test subjects, while C. trachomatis and T. vaginalis were observed in 0.4%. Co-infections of the genital tract have been frequently reported by other researchers.

The silent nature of infection by many sexually transmitted organisms was apparent in this study. We found that up to 90% of the CT, MH, UU and UP infections were asymptomatic. If these infections remain undiagnosed, they might eventually cause adverse effects to the individual, their sexual partners and the public health. Detection of well-established STIs such as CT and NG in patients during evaluation for fertility treatment may assist the reproductive doctors to look for tubal and pelvic abnormalities as the possible causes of female infertility. Meanwhile although the evidence for the role of mycoplasmas and ureaplasma in the aetiology of infertility is still inconclusive, the presence of these organisms should not be indeterminately ignored. Further studies showing independent association of genital mycoplasmas and ureaplasmas with PID and infertility (by excluding CT, NG, TV and MG infections in the studied populations) will be valuable to guide the diagnostic approach of sexually transmitted diseases in sexually active adults including the infertile individuals.

LIMITATIONS

The main limitation of this study is the lack of the control group in fertile subjects to serve as comparison, thus the statistics presented were only descriptive in nature. We also acknowledge the limitation in the method of study by using only one commercial kit, with no comparison to other diagnostic approaches such as culture and/or other
NAAT-based test available. Nevertheless, the method in this study was chosen after consideration that NAAT in general has been established as superior in performance compared to culture and other nonculture diagnostic methods.  

The prevalence of studied organisms in couples with primary and secondary infertility were also not explored in this study due to limitation in data collection. Therefore, future research should address the diagnostics issues in sexually transmitted infections in infertility and its’ clinical impact on patient care and management.

CONCLUSION

Sexually transmitted organisms were detected in one third of men and women planning for fertility evaluation. *Chlamydia trachomatis*, *Mycoplasma hominis*, *Ureaplasma parvum* and *Ureaplasma urealyticum* were mostly detected in asymptomatic patients. The findings emphasize the need for screening particularly for *Chlamydia trachomatis* in couples undergoing infertility evaluation. High prevalence rate of *Ureaplasma parvum* among infertile couples and detection of *Ureaplasma urealyticum*, *Mycoplasma genitalium* and *Mycoplasma hominis* should be scrutinized further to clarify their role whether simply being innocent colonizers or actual pathogens in causing male and female infertility.

CONFLICT OF INTEREST

We declare that we do not have any conflict of interest when performing this study.

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