Evaluation of Culturing Method and Mycoplasma System Plus Kit in Diagnosis of Genital *Mycoplasma* Isolated from Infertile Women

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Abstract
The goal: This study aimed in part to evaluate the diagnostic value of culturing and the commercial system plus kit in diagnosis of genital *Mycoplasma* isolated from infertile women in Al-Diwanyia governorate, Iraq. Subjects and specimens: A total of 83 endocervical swabs were collected from infertile women who admitted to AL-Diwanyia Maternity and Pediatric Teaching Hospital through a period of seven months (from October 2012 to April 2013) in an attempt to detect the genital *Mycoplasma* represented by *Mycoplasma hominis* and *Ureaplasma urealyticum*. Experimental work: Two techniques were used; M4 modified medium and Mycoplasma System Plus Kit (Italy). Mycoplasma System Plus Kit was used for identification and differentiation of *Mycoplasma hominis* and *Ureaplasma urealyticum*. Results and discussion: The results indicated that *M. hominis* and *U. urealyticum* isolated on M4 medium in a frequency of 25 (30.1 %) and 36 (43.4 %) respectively, while both of them together on the same medium resulted in relatively low frequency which accounted for 14(16.8 %). The results by Mycoplasma System Plus Kit were high compared with M4 medium since *M. hominis* alone accounted for 29.0 (34.9 %), *U. urealyticum* alone 42.0 (50.6 %) and 21.0 (25.3 %) for both of them together. Conclusion: Both; culturing and commercial Mycoplasma System Plus Kit are valuable techniques in diagnosis of genital Mycoplasma. Genital Mycoplasma represented by *Mycoplasma hominis* and *Ureaplasma urealyticum* are frequently colonizing the vaginal niche of infertile women which can be a risk factor of women infertility. Future prospects: Some other maternity problems, e.g. abortion, preterm birth, neonatal sepsis and death of fetus and/or mothers can be suggested to investigate the role of genital *Mycoplasma* in such clinical cases.

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1. Introduction

1.1 Definition
*Mycoplasmas* are described as the smallest self-replicative organisms lack the cell wall. These organisms are commensals of the lower genitourinary tract of sexually active women (Kilic et al., 2004; Krohn et al., 1995) resulting in certain pathogenic conditions and pregnancy complications, e.g. bacterial vaginosis, pelvic inflammatory disease, postpartum fever, postpartum sepsicemia,
infertility, premature rupture of the membranes, preterm labor, preterm birth, and systemic neonatal infections (Waites et al., 1988).

1.2 Diseases
Mycoplasma hominis and Ureaplasma urealyticum has been implicated as a contributory cause of chronic lung disease of prematurity and in association with meningitis, hydrocephalus and intraventricular haemorrhage among neonates (Sanchez, 1993; Waites et al., 2008). Urogenital tract infections are the major cause of morbidity in sexually active individuals all around the world, since World Health Organism (WHO) has stated that sexually transmitted diseases (STDs) rank the second in importance after cancer as treatable disease in women (Yamazaki et al., 2012). Most of mycoplasmal diseases are underdiagnosed because the specific laboratory diagnostic strategies are quite difficult compared with those of fast cultivable bacteria (Waites, 2006).

1.3 Diagnosis
Commercially culture media are currently available depend upon the arginine and urea metabolism (hydrolysis) for M. hominis and U. urealyticum respectively (Ekiel et al., 2009), with diagnostic test kits for antibiotic susceptibility based on the rate of urea or arginine hydrolysis which is proportional to the number of cells contained in the sample and identitiobi by identification based on the sensitivity of the organisms to multiple antibiotics (Zhu et al., 2012).

1.4 Objective
According to our knowledges, clinical studies on Mycoplasmas and their role in human urogenital infections in Iraqi population are rare; therefore this study was suggested and designed to detect the incidences of M. hominis and U. urealyticum in women infertility using Mycoplasma System Plus Kit and conventional method (culture on modified M4 medium).

2. Materials and Methods
2.1 Specimens
The specimens investigated in this study included double endocervical swabs being taken from 83 infertile women who admitted to AL-Diwanyia Maternity and Pediatric Teaching Hospital, Infertility Unit. The age of women ranged from 18-45 years during a period of seven months (from February 2012 to August 2013). The swabs were immersed in 2 ml Mycoplasma transport medium (LioFilChem, Italy) to maintain the swabs wet and directly transported to the laboratory for bacteriological investigation (Díaz et al., 2013).

2.2 Culture medium
An inoculum of 300 µl from Mycoplasma transport medium was inoculated in modified M4 medium and incubated at 37° C under anaerobic condition for 5-7 days (Al-Azawi, 2012).

2.3 Mycoplasma system plus kit
Commercial Kit supplied by (LioFilChem, Italy) which is supplied by a microtray of 24 wells as illustrated in figure-1. Wells from 1-3 represent the growth density, wells from 4-5 represent identification value of M. hominis and U. urealyticum while wells from 7-24 are designed for antibiotic susceptibility. Well number 6 is recommended for detection of Candida, therefore it was ignored in this study.

2.4 Statistical analysis
T-Test recommended by (Danial, 1988) was used for statistical analysis to show if there is any significant differences between results.

3. Results and Discussion
The results obtained by M4 medium shown in
Table 1 indicate that out of the 83 samples, 25 (30.1 %) were positive for *M. Hominis* (alone), 36 (43.4 %) were positive for *U. urealyticum* (alone) while both of them together accounted for 14 (16.9 %) were positive. Genital *Mycoplasmas* have been detected more frequently in women of age range from 29-38 tears. The results indicated that *M. hominis* and *U. urealyticum* strongly reside and colonize the genital tract of infertile women (Imudia et al., 2008). The presence of *Mycoplasma* in the endocervical niche is considered as a potential risk factor for infertility (Al-Azawi, 2012). Incidence of genital *Mycoplasma* with high rate in cervix and uterus has been reported as high as 80% for *U. urealyticum* and 50 % for *M. hominis* (Peerayeh and Sattari, 2006). Diagnosis of genital *Mycoplasma* mainly depends on colonial morphology and some other diagnostic characters (Al-Azawi, 2012). Colonies resembling a fried egg appearance indicate for *M. Hominis* as shown in figure-2. This characteristic is due to the penetration and proliferation of *M. hominis* deeply in to the agar in the central region of the colony, while tiny and brownish colonies indicate for *U. urealyticum* as shown in figure-3 due to precipitation and accumulation of manganese oxide contained in media within colony. The results obtained by Mycoplasma System Plus revealed that out of the 83 patients included in this study 29 (34.9 %) *M. hominis* (alone) were positive, 42 (50.6 %) *U. urealyticum* (alone) were positive, while both of them together accounted for 21 (25.3 %). Mycoplasma System Plus Kit also provides valuable indicative parameters for cell density of organisms under test, as indicated by manufacturer, since a titer range 103-106 CFU/ml is considered as an evidence for disease case (Díaz et al., 2013). Accordingly, results obtained by Mycoplasma System Plus Kit revealed that out of the 83 samples investigated in this study; 11 (13.3 %), 46 (55.4 %) and 15 (18.1 %) which evaluates semi-quantitative count. The results documented in this study, both by culturing and commercial kits are in compatible with those results reported (Clegg et al., 1997; Al-Hammadani et al., 2014). Moreover the distribution of the genital *Mycoplasmas* in accordance to patient’s age is presented in table 1. There are a significant difference ($p < 0.01$) was found between the age of patients whose sample was positive by culture (positive group) and that of other patient (negative

<table>
<thead>
<tr>
<th>Age( years)</th>
<th>18-28</th>
<th>29-38</th>
<th>39-45</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. hominis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13 (52 %)</td>
<td>8 (32 %)</td>
<td>4 (16 %)</td>
<td>25 (30.1)</td>
<td></td>
</tr>
<tr>
<td><em>U. urealyticum</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 (55.5 %)</td>
<td>10 (27.7 %)</td>
<td>6 (16.6 %)</td>
<td>36 (43.4)</td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 (57.1 %)</td>
<td>4 (28.5 %)</td>
<td>2 (14.2 %)</td>
<td>14 (40.9)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1:** Frequency of *M. hominis* and *U. urealyticum* according to the age of patients

**Figure 2:** Colonies of *M. hominis* on Modified medium

**Figure 3:** Colonies of *U. urealyticum* in Modified M4 medium
group). Forty healthy productive married women were randomly selected and investigated as a control for infertility related to genital Mycoplasma. The isolation rate of genital Mycoplasma from fertile to infertile women was 1:8 respectively.

**Research Highlights**

1- To evaluate the diagnostic value of culturing and commercial kits.
2- To determine the companionship of *M. hominis* and *U. urealyticum* in vaginal environment.
3- To determine the risks factors associated with prosperity of genital Mycoplasmas.
4- To highlight the role of genital Mycoplasmas in maternal clinical problems, e.g. nongonococcal urethritis in men, chorioamnionitis, habitual spontaneous abortion, low-weight infants, pelvic inflammatory diseases such as tubo-ovarian abscess or salpingitis, as this study is the first serious research dealing with these microorganisms in Iraq.

**Limitations**

There are some limitations encounters the authors during this study. These are represented by difficulty in obtaining samples from women properly, delay in arrival the necessary equipments and kits at the appropriate time in addition to the Lab. conditions particularly stability of electricity. Furthermore, growth nature of Mycoplasma is a difficult matter per se.

**Recommendations**

Research on Mycoplasma is quite difficult and needs separated Lab. supply with all the necessary culture media, equipments, reagents and others apparatus. Work on Mycoplasmas always should be under aseptic conditions.

**Justification of Research**

The work is an academic research to improve the community heath and to highlight the role of genital Mycoplasma in maternal clinical cases, e.g. abortion, infertility, premature delivery, etc.

**Conclusion**

Both; culturing and commercial Mycoplasma System Plus Kit are valuable techniques in diagnosis of genital Mycoplasma. Genital Mycoplasma represented by *Mycoplasma hominis* and *Ureaplasma urealyticum* are frequently colonizing the vaginal niche of infertile women which can be a risk factor of women infertility.

**References**


