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Detection of Genital Mycoplasmas Infections among Infertile Females

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Objective: Detection of Genital *Mycoplasma hominis* and *Ureaplasma urealyticum* among infertile female patients attending gynecological clinic.

Design: Prospective study.

Setting: Medical Center Gynecological Clinic, Khamis Mushayt City, Saudi Arabia.

Method: Duplicate genital Swabs for genital mycoplasmas were taken from two hundred and sixty-three infertile female patients between March 2011 and August 2012; the age range was 21 to 45 years. Agar plates were examined every 24-72 hours for the characteristic *Ureaplasma* and *Mycoplasma hominis (M. hominis)* colonies. Isolates were identified serologically as *M. hominis* or *Ureaplasma urealyticum (U. urealyticum)* by growth inhibition test (disc method).

Result: Two genital mycoplasmas were detected out of 263 specimens by culture method. The positive specimens were identified as *M. hominis.*

Conclusion: The result demonstrates lower values and further investigations for rapid detection of genital mycoplasmas in infertile female patients using PCR could be important and necessary for the detection of mycoplasmas infections.

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Mycoplasmas are the small commensals, some of them are considered normal flora of the respiratory or genitourinary tract[1]. *Mycoplasma genitalium, Mycoplasma hominis* and *Ureaplasma spp* (collectively, genital mycoplasmas) are most often implicated in genital or reproductive health conditions[2]. *Ureaplasma urealyticum* and *Mycoplasma hominis* can be found in the cervix or vagina of sexually mature women[3-7]. The possible role of genital mycoplasmas in infertility is still debatable[8].

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Mycoplasma genitalium, *Mycoplasma hominis* and *Ureaplasma urealyticum* could cause pelvic inflammatory disease, vaginitis, cervicitis, chorioamnionitis, pyelonephritis, postpartum, postabortion fever and infertility in women. In men, it could lead to non-gonococcal urethritis, acute epididymitis and in newborn, septicemia, neonatal pneumonia, neonatal conjunctivitis, meningitis, low birth-weight infants, premature delivery and premature rupture of membranes[9-11].

The aim of this study is to detect *M. hominis* and *U. urealyticum* in genital tract specimens from infertile female patients.

METHOD

Duplicate genital swabs for genital mycoplasmas were taken from two hundred and sixty-three infertile female patients between March 2011 and August 2012; the age range was 21 to 45 years. A detailed history including infertility was obtained. One swab was inoculated into mycoplasma transport medium. The other swab was placed in phosphate buffer saline solution (PBS) and frozen at -70°C for further PCR assays study. The inoculated transport media were inoculated onto Shepard's growth medium for recovery of *M. hominis* and *U. urealyticum*[12].

All inoculated broths were incubated aerobically at 37°C and cultures showing pH shift were sub-cultured onto agar plates, which then incubated anaerobically in a candle jar at 37°C up to 7 days. Agar plates were examined every 24-72 hours under stereomicroscope for the characteristic of *Ureaplasma* and *M. hominis* colonies. Isolates were identified serologically as *M. hominis* or *U. urealyticum* by growth inhibition test (disc method), as described by Clyde[13].

Data were analyzed using the SPSS version 13.0 (SPSS software, Inc., Chicago, USA).

RESULT

The result of the conventional culture system for the detection of *M. hominis* and *U. urealyticum* are shown in table 1. Two specimens out of 263 infertile female patients specimens screened for *M. hominis* and *U. urealyticum* were positive and identified as *M. hominis* by growth inhibition test (disc method). Zones of inhibition less than 1.5 mm in diameter were considered negative, see figures 1 and 2.

Table 1: Detection of Genital Mycoplasmas by Using the Culture Method

	Positive	**Negative**	**Total**
M. hominis (N=263)	2	261	263
U. urealyticum (N=263)	0	263	263

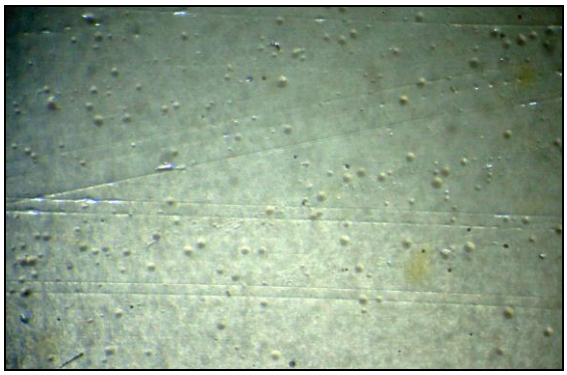

Figure 1: Colonies of the Recovered *Mycoplasma Hominis* Growing on A8 Agar Medium after 72 Hours of Incubation

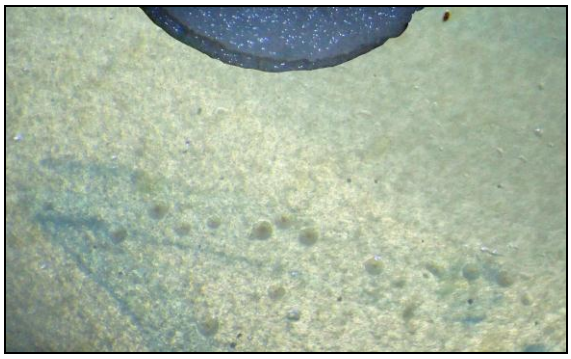

Figure 2: A Recovery of *Mycoplasma Hominis* from Genital Tract Specimens as Tested by GI Test (Disc Method) Exhibiting a Zone Diameter of Growth Inhibition (Zone Size Measured in Millimeters from Rim of Disc Previously Saturated with Antiserum of *M. hominis* Type Strain PG21, to Edge of Growth)

DISCUSSION

Culture techniques are the current diagnostic methods of choice. Detection of mycoplasmas in the genital tract usually depends on culturing specimens on appropriate media and identifying the isolates, which was found to be the most sensitive method for the isolation of both *M. hominis* and *U. urealyticum*[12,14]. Furthermore, isolation of the mycoplasma through culture will remain a worthy goal since quantitative results are more difficult to achieve with the PCR technique and it does not permit an assessment of antibiotic sensitivity or other biological features[10].

However, the use of other diagnostic method, such as, PCR could be necessary because it was found to be higher compared with culture in detection of genital mycoplasmas infections[15]. PCR has an advantage of detecting DNA of dead organisms[16].

The result of this study had lower values in detection of genital mycoplasmas in comparison with other studies[12,16].

Although studies have demonstrated the association of genital mycoplasmas with variety of genital infections, its possible role in infertility is not clear[2,8].

In this study, the detection rate of *M. hominis* is low, which might be due to high sensitivity of mycoplasma (pH, temperature, and materials present in culture media and clinical specimens) as well as loss of viability during specimen collection and/or transport[10,15].

Mycoplasma antibody response is one of the criteria for diagnosing the disease[10]. Antibiotic inhibition is sometimes difficult to deterimine[10].

Further research study is to determine the role of mycoplasma genital infection and infertility and its association with urethritis, vaginitis, cervicitis, pelvic inflammatory disease, and pathology of pregnancy and newborns[15].

CONCLUSION

This study showed low detection rate of *Mycoplasma hominis* by culture method. Further study using PCR for detecting genital mycoplasma is needed to determin the possible implication of those organisms in various genital infections.

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