

*Full Length Research Paper*

# **Prevalence of *Mycoplasma hominis* and *Ureaplasma urealyticum* among women with unexplained infertility, with and without vaginitis and cervicitis**

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The main objective of this study was to find the incidence of urogenital mycoplasma such as *Mycoplasma hominis* and *Ureaplasma urealyticum* among patients with history of infertility of unexplained origin and to ascertain their implications in non specific cervicitis and non specific vaginitis. The total number of (n = 337) female patients with history of un-explained vaginitis and cervicitis were screened to observe the prevalence of urogenital mycoplasmas in the disease. Both the high vaginal swabs (HVS) and mid-stream urine were cultured on the liquid media, U-9, and the solid differential mycoplasma agar A-7. The differentiation of the implicated organisms were made on the basis of metabolic characteristics of the species. Amongst the total (n = 337) samples, the n = 89 (26.40%) were positive for *M. hominis* whereas n = 162 (48.07%) isolates determined the *U. urealyticum*. Upon the outcomes of disease, the cases of non-specific cervicitis yielded the growth of *M. hominis* and *Ureaplasma urealyticum* as 32.58% and 59.87% respectively, whereas, among patients of non specific vaginitis, the *M. hominis* and *U. urealyticum* were isolated in ratio of 59.55 and 21.60% likewise. From the obtained data in both categories, no solid and direct etiological evidence could be linked to a definite disease outcome of infertility. However, it is certain that these mycoplasmas do cause the urogenital problems as indicated by percents of isolates obtained exclusively. Consequently, after the infection, these organisms could possibly associate for their role in the reproductive failure due to the resulting pathological complications in the urogenital tract of women.

**Key words:** *Mycoplasma hominis*, *Ureaplasma urealyticum*, urogenital infections.

## **INTRODUCTION**

The first reported culture of mycoplasma from human was from an abscess of a Bartholin's gland. Five years later, there were indications that mycoplasmas were commonly found in the female genital tract (Stray-Pedersen et al., 1982; Miettinen, 1987). Difficulties with medium formulations, bacterial contamination problems and uncertainties over the relationship of L- forms of bacteria, delayed the recognition of mycoplasmas as resident flora of the urogenital tract. Using improved culture media and relative inhibitors to minimize bacterial contamination, these difficulties of isolation and culturing

of mycoplasmas was overcome. The tiny strains of mycoplasmas or *Ureaplasma urealyticum* have the unique ability to metabolize urea, therefore, they were placed in separate genus *Ureaplasma* (Stray-Pedersen et al., 1982; Miettinen, 1987; Jagielski and Biologiczne, 1987). *Mycoplasma hominis* and other classical mycoplasmas form large "Fried-egg" type colonies and are usually recognized by the agar growth inhibition technique, and the serotyping for strains identification (Stray-Pedersen et al., 1982). Substantial evidence has indicated that both the species of mycoplasmas can cause urogenital problems. The *M. hominis* is implicated in a variety of infections whereas, the *U. urealyticum* is associated with non-specific cervicitis and non-specific vaginitis leading possibly to, the non-specific conditions of infertility (Taylor-Robinson et al., 2002). The aim of the

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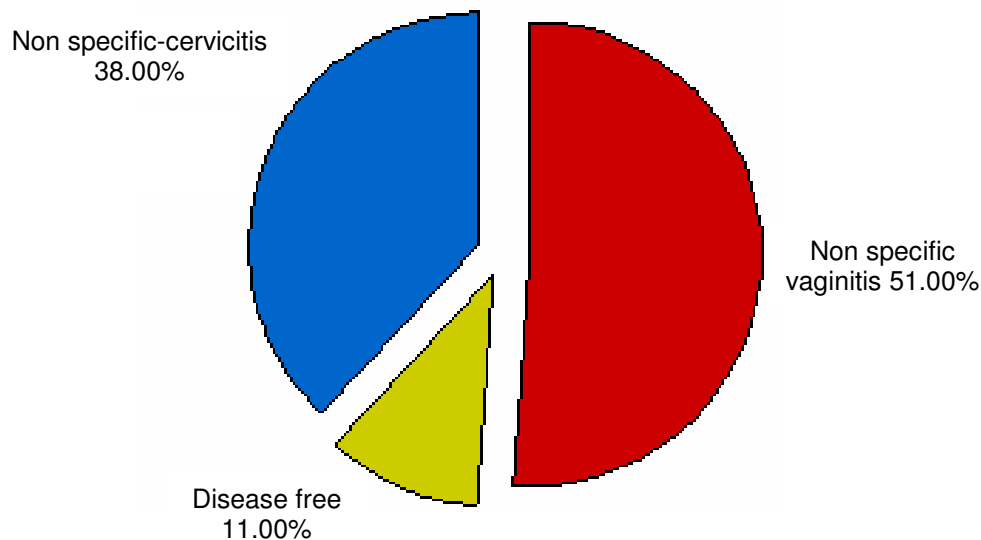


Figure 1. Percentage of disease and disease free cases (an overview).

study was to see the pathogenic role of *M. hominis* and *U. urealyticum* with clinical manifestation of the genital urinary inflammatory conditions among women and to observe their incidence in non specific vaginitis and cervicitis.

## MATERIALS AND METHODS

A total of 337 specimens, including both high vaginal swabs (HVS) and urine were obtained from women with history of infertility, due to unexplained reasons with chronic non specific vaginitis and non specific cervicitis. The study was conducted on the regularly attending women patients of Gynecology and Obstetrics departments at the tertiary care hospitals of Rawalpindi/Islamabad Pakistan. A written informed consent was also obtained in this regard for further investigations and to meet the ethical standards. High vaginal swabs were collected on sterile plain cotton wool swabs. "First Catch" and "Midstream" urine samples were obtained under sterile conditions and avoiding contact with antiseptics. The specimens were transported immediately to laboratory at 4°C in ice box and were cultured in liquid and solid mycoplasma media, especially U-9 broth and A-7 differential agar. In order to detect mycoplasma growth in broth medium, advantage was taken of the metabolic activities of these mycoplasma species. The clinical material (for example, urine, urine deposit, expressed swab were diluted in serial tenfold steps, for example, 0.2 ml of specimen in 1.8 ml of the medium) up to a dilution of at least  $10^{-3}$  in screw capped vials, containing medium supplemented with phenol red and urea solution. Similarly, the specimens were diluted in medium containing arginine solution and again, in medium containing the glucose. The caps of vials were screw tight and the vials were incubated at 37°C under atmospheric conditions to observe the difference in pH by the change in color of liquid medium. Aliquots of medium from culture, showing the color changes were sub cultured to fresh broth medium and on agar medium. It is essential to subculture from the medium, when the change in color just starts, that is, from the bottom of the tube. The differential agar medium A-7 and U-9 broth at pH 6 were incubated at 36°C, when inoculated with color changing broth media. The incubation was under aerobic and microaerophilic conditions. Tissue culture incubators for the

atmosphere of 5 to 10% of CO<sub>2</sub> were also used for this purpose. After 72 h of incubation, the plates were first scanned, unopened, under reflected light of the microscope, using low power magnification. After that, the agar portion, having the colony, were cut into 1 cm<sup>2</sup> blocks with a flamed surgical scalpel and placed on glass slide for Dienes staining. The micrometer in the eyepiece was calibrated to measure the diameter of the colonies at 72 h of the incubation. Besides the staining and size differences of the colonies, the mycoplasma isolates were also further characterized biochemically, serologically and also using growth inhibition test (GIT) / invitro antibiotic sensitivity.

## RESULTS

Three hundred and thirty seven (337) samples comprising both urine and high vaginal swabs from infertile women were investigated for presence of mycoplasmas. Eighty nine (26.40%) cases yielded *M. hominis* and the number of isolates positive for *U. urealyticum* were 162 (48.07%) but no other mycoplasma type was recovered. Among the total infertile women who yielded both the species of mycoplasma (*M. hominis* and *U. urealyticum*), 38% were those who had nonspecific cervicitis and 51% were the cases of non specific vaginitis. The infertile women, who were infected by both of these species of mycoplasma, showing no apparent signs of urogenital disease, were 11% as shown in Figure 1.

In cases of non specific cervicitis, *M. hominis* and *U. urealyticum* were recovered as 32.58 and 59.87% respectively (Table 1). The percentage recovery of *M. hominis* and Ureaplasmas in respective cases of non specific vaginitis was 59.55 and 21.60% each. The infertile women without the other cause of urogenital disease were found infected with *M. hominis* as 7.86% and *U. urealyticum* as 18.51% of the cases (Table 2).

**Table 1.** The total incidence pattern of Urogenital mycoplasma infection among infertile women.

Type of cases under investigation	Total No. of samples	Types of samples taken	Total No. of isolates +ve for <i>M. hominis</i> and percentage	Total No. of isolates +ve for <i>U. urealyticum</i> and percentage	Other mycoplasma species isolated	Percentage of disease and disease free cases
Infertility of unexplained origin	337	Urine/HVS (Random)	89 (26.40%)	162 (48.07%)	Nil	Females with I) Non- specific cervicitis= 38% II) Non specific vaginitis = 51% III) Disease free= 11%

**Table 2.** Number and percentage of *Mycoplasma hominis* and *Ureaplasma urealyticum* isolates from disease and disease free female subjects.

Total No. and percentage of Mycoplasma isolates	Patients with clinical manifestation	Age of patients (%)	No. and percentage of isolates:	
			<i>M. hominis</i>	<i>U. urealyticum</i>
<i>M. hominis</i> 89 26.40 (%)	Non specific cervicitis	38	29 (32.58%)	97 (59.87%)
<i>U. urealyticum</i> 162 48.07 (%)	Non specific vaginitis	51	53 (59.55%)	35 (21.60%)
----	Disease free	11	7 (7.86 %)	30 (18.51 %)

## DISCUSSION

Mycoplasmas constitute a unique and enigmatic group of microorganisms, whose pathogenicity in man was first demonstrated for mycoplasma pneumonia, Eaton's agent, in relation to primary atypical pneumonia. However, the earliest isolation of mycoplasmas from a human source was reported by Dienes and Edsa, who recovered such organisms from a Bartholinian abscess. Since then, sporadic reports have implicated mycoplasmas in diseases of the female genitalia

and in complications of pregnancy. Such infections might not give rise to symptoms until and unless, infertility or reproductive wastage was to occur. In this connection, the clinical manifestations might be either minimal or even absent (Stray-Pedersen et al., 1982; Miettinen, 1987; Jagielski and Biologiczne, 1987). Infertility and genital tract infection have been produced experimentally in cattle by local implantation of classical types of bovine mycoplasmas, since human is not readily available for such experimentation, and the strains of mycoplasmas

are also specific to pathogenicity. The pathogenic role of human strains can however be demonstrated only indirectly by epidemiological studies. Additional supportive evidence could be provided, if antimicrobial therapy were shown effective to eliminate both the organisms and the complaints of the patients from whom they had been isolated (Taylor-Robinson et al., 2002). Earlier isolation was done for a group of 222 with the similar symptoms, and found *U. urealyticum* in 31.8% and *M. hominis* in 3% of the cases (Elias et al., 2005).

McCormack also found a higher prevalence of *U. urealyticum* in 54% of students with abnormal urogenital findings (McCormack, 2001). The low detection of *M. hominis* may be due to the fact that there were no women with bacterial vaginitis in our study group. Other researchers have also seen that these pathogens are significantly detected more often in women with bacterial vaginitis than in those without (Paavonen et al., 1983; Shafer et al., 1985). The association of *M. hominis* with bacterial vaginitis was further observed with the genital carriage of these microorganisms in 53% of women with bacterial vaginosis than in none without the disease (Keane et al., 2000). We found the highest percentage of positive cultures for *U. urealyticum* and *M. hominis* respectively, which is consistent with the studies of high prevalence of these organisms when cultured from the vaginal fluid of the STD patients (Koch et al., 1997; Van Belkum et al., 2001). Among infertility patients, *U. urealyticum* in cervical swabs was detected in as many as 37.2% of cases, while *M. hominis*, only in 2.3%. Another similar studies in past, also revealed that in the cervical samples; *U. urealyticum* was present in about 50% of infertile women (Stray-Pedersen et al., 1982).

In a study related to infertility, the *Ureaplasmas* were obtained in 23.5% and *M. hominis* in 4.8% from the specimens of the cervical swabs of the examined infertile women (Rodriguez et al., 2001). Similar incidence rates were observed of 33.9 and 11 % respectively for *U. urealyticum* and *M. hominis* in women with vaginal discharge (Yavuzdemir et al., 1992). The incidence of both the microorganisms was 61.4 and 16.5% for *U. urealyticum* and *M. hominis* respectively, which resulted out of a similar work (Di Bartolomeo et al., 2002). In patients with cervical inflammation, with or without erosion, *U. urealyticum* was isolated in 28.7% and *M. hominis*, 3.8% of cases. The presence of *U. urealyticum* in 56% of chronic cervicitis in women was also reported in one of the investigation (Bhandari et al., 2000). Whereas, *U. urealyticum* and *M. hominis*, were the most common microorganisms in women with abnormal colposcopic findings (Pisani et al., 1999; Zdrodowska et al., 2006).

The present study suggests that *Ureaplasmas* are more significant in isolation from the urogenital tract of women with symptoms of reproductive failure. The *M. hominis* also holds similar importance, within these specific disease groups of women, with chronic non specific vaginitis and cervicitis. The exact mechanisms relating to the reproductive casualty by the presence of mycoplasmas in the lower part of the genital tracts of gravid women may become evident further with a detailed futuristic study of selected groups of such cases and also, appropriate controls.

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