Rate of *Chlamydia trachomatis*, *Mycoplasma hominis* and *Ureaplasma urealyticum* in Infertile Females and Control Group

*N Badami*  
MH Salari

1Dept. of Pathobiology, School of Public Health and Institute of Public Health Research,  
Tehran University of Medical Sciences, P.O.Box 14155-6446, Tehran, Iran.

**Key Words:** Chlamydia trachomatis, Mycoplasma hominis, Ureaplasma urealyticum, infertile females

**ABSTRACT**
Infertility in females is one of the most important sequela of genital infection with *Chlamydia trachomatis*, *Mycoplasma hominis* and *Ureaplasma urealyticum*. In the present study the frequency of these bacteria was studied in 125 infertile female by direct and indirect immunofluorescence tests and culture method and compared with 250 normal population. *Mycoplasma hominis* was isolated from 32 (35.6%) of infertile females compare with 18 (7.2%) of normal population. *Ureaplasma urealyticum* was isolated from 41 (32.8%) of infertile females compare to 48 (19.2%) of normal population. *Chlamydia trachomatis* was detected by direct IF in 11 (8.8%) of infertile and 2 (0.8%) control group. The antibody titer against D-K serotypes of *Chlamydia trachomatis* was also measured in both groups of infertile and normal population and a positive titer of 1/16 and above was detected in 26 (20.8%) of infertile cases and in 8 (3.2%) of control group. The rate of *Chlamydia trachomatis*, *Mycoplasma hominis* and *Ureaplasma urealyticum* in case and control groups was significant (respectively P<0.0001, P<0.0001, p= 0.0018).

**INTRODUCTION**
Female genital tract is a suitable environment for growth of various pathogenic and nonpathogenic microorganisms. Some of the organisms such as Chlamydia trachomatis, Mycoplasma hominis, Ureaplasma urealyticum, Gardnerella vaginalis, Listeria monocytogenes and Neisseria gonorrhoeae upon localization and colonization at the appropriate anatomical site may cause various pathological disorders like cervicitis, vaginitis, urethritis, endometritis, salpingitis and Bartholinitis. The pathological disorders might lead to pelvisitis, ectopic pregnancy and infertility. It was reported that some of these isolated microorganisms produce noramindase like substances which showed to induce certain disorders in blastocyst of murine model, and many cause a similar pathological disorders in human (1, 6, 10, 11-14). The following hypothesis are proposed in regard to attachment of the organisms to sperm in genital tract:

1. The attachment of bacteria to sperm might cause unusual metabolism in sperm.
2. Attachment of bacteria to sperm might cover the region of sperm membrane, which is essential for sperm identification and attachment.
3. Attachment of bacteria to sperm might inhibit sperm penetration to the ovum.

The above mentioned hypothesis would initiate the concept that some of the microorganisms present in vagina cause disorder in sperm function which might lead to infertility in females. The role of infectious agent in infertility is not only due to creation of certain disorder in sperm function, but also infection in different parts of the female genital tract might induce infertility due to variable reasons (4, 7, 9, 17, 20).

The purpose of this study was to investigate the rate of Chlamydia trachomatis, Mycoplasma hominis and Ureaplasma urealyticum in infertile females and comparison with control group.

A serial dilution of each serum sample was prepared and added to different acetone fixed antigens, after 30 minutes of incubationin 37°C in moist condition, slides were washed with PBS, for 10 minutes and then dried at room temperature. Then the labeled anti-human FITC conjugate was added to each slide and incubated at 37°C in moist condition for 30 minutes. The slides were washed with PBS and PBS containing Evans blue, slides were dried at ambient temperature and a drop of buffered glycerol solution was added to each. Slides were overlaid with a cover slip, and were observed under florescence microscope (2, 3, 16).

RESULTS
In this study 125 samples form infertile females and 250 samples form control group (Healthy volunteers) were collected during the years 1996-1998. The samples were processed by culture and serological test. The results are as follow:

Table 1 shows frequency distribution of females on the basis of age, signs of disease and level of education in case and control groups. In regard of the profession of the subjects under investigation, out of 125 cases, 107 (85.6%) were housewives and 18 (22.4%) were employed and in 250 control group, 93 (37.2%) were housewives and 157 (62.8%) were employed.

Table 2 and 3 shows rate of Chlamydia trachomatis, Mycoplasma hominis, Ureaplasma urealyticum and antibody titers against D-K serotypes of Chlamydia trachomatis in case and control groups.

DISCUSSION
The rate of Mycoplasma hominis, Ureaplasma urealyticum and Chlamydia trachomatis in infertile females and control group are shown in Tables 1-3 and indicated as follow:

Table 1. Frequency distribution of females on the basis of age, signs of disease and level of education in case and control groups

<table>
<thead>
<tr>
<th>Age</th>
<th>Case group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>12 (9.6)</td>
<td>13 (5.2)</td>
</tr>
<tr>
<td>21-24</td>
<td>27 (21.6)</td>
<td>61 (24.4)</td>
</tr>
<tr>
<td>25-29</td>
<td>38 (30.4)</td>
<td>70 (28)</td>
</tr>
<tr>
<td>30-34</td>
<td>29 (23.2)</td>
<td>59 (23.6)</td>
</tr>
<tr>
<td>35-39</td>
<td>17 (13.6)</td>
<td>37 (14.7)</td>
</tr>
<tr>
<td>≥40</td>
<td>2 (1.6)</td>
<td>10 (4)</td>
</tr>
</tbody>
</table>

Table: Signs of disease

- Discharge
- Itching and burning
- Urine frequency
- Pain beneath abdomen
- Painful intercourse
- Painful menstrual cycle
- Spitting after sexual contact
- STD of sex partner
- Self STD
- Level of education
- Blister
- Primary education
- High school education
- Higher education

Samples
By using speculum, 4 sterile cotton swabs were used to collect specimens. One of the swabs was transferred to PPLO Broth medium, the two other were used for prepare samples for Gram stain and direct fluorescence test, the forth swab was kept at 70°C for further validation. Then the samples were transported to laboratory for further tests. Five ml of blood sample was collected and antichlamydial antibodies.

The media containing through membrane filter inoculated to urea and at 37°C with 5% CO2 and sample was transferred to PPLO agar medium for further confirmation (2, 5, 15).

Serum samples were checked for chlamydia antigens:

1. A, B, Bl, C;
3. L1, L2, L3.
It was reported the rate of these bacteria in healthy persons 39.5% and 45%, respectively (17,20).

The obtained results from the study of the samples for detection of *Chlamydia trachomatis* particles by the method of direct immunofluorescence indicates that in infertile females 11 (8.8%) and in control group 2 (0.8%) were positive (P<0.0001). It was reported the rate of *Chlamydia trachomatis* by direct immuno fluorescence in infertile females 18.8% (3).

In this study specific antibody titer equal or more than 1/16 against serotypes D to K of *Chlamydia trachomatis* was observed in 26 (20.8%) of infertile females and 8 (3.2%) of control group (P<0.0001). Our results is nearly the same as reported (18, 19).

Z statistical analysis test with 99% confidence showed that the rate of *Chlamydia trachomatis*, *Mycoplasma hominis* and *Ureaplasma urealyticum* case and control group was significant.

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Table 3. Frequency distribution of females on the basis of specific antibody titers against *Chlamydia trachomatis* in case and control groups

<table>
<thead>
<tr>
<th>Antibody titers</th>
<th>Case group</th>
<th>Control group</th>
<th>Z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Against chlamydia</td>
<td>N = 125</td>
<td>N = 250</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive n = 26</td>
<td>1/128</td>
<td>1/64</td>
<td>1/32</td>
<td>1/16</td>
</tr>
<tr>
<td>Number</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Percentage</td>
<td>8.8</td>
<td>4.8</td>
<td>8.8</td>
<td>0.8</td>
</tr>
</tbody>
</table>

REFERENCES


