International Journal of Research
Available at https://edupediapublications.org/journals
p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 04 Issue 02
February 2017

Urogenital Mycoplasma and Mucopurulent Cervicitis

Dr. Amal. Sayed,.M.D.* Dr. Afaf El-Banna.,M.D* ,Dr. Ahmed A.El-Megid,.M.D **And Dr.Reham Hamed ,.M.D*

* clinical and chemical pathology department

** Gynecology and Obstetrics department.

Faculty of Medicine, Cairo University

Abstract

The incidence of cervical *Mycoplasma hominis* (Mh) and *Ureaplasma urealyticum* (Uu) was investigated in 40 female patients with suspected cervicitis who had attended the gynecologic out-patient clinic in Cairo University Hospital (Kasr Al-Aini), faculty of Medicine, Cairo, Egypt, between September 2006 and June 2007. Age ranged from 18 to 54 years (mean 31 years). *Chlamydia trachomatis* was investigated in 20 randomly selected patients out of the 40 studied group. Mh was found in 12 (30 %) women while Uu was found in 23 (57.5 %). Women co-infected with both Mh and Uu were 11 (27.5 %). Women found to be positive for Mh and / or Uu were 24 (60 %) while those who were negative for both were 16 (40 %). Chlamydia was found in 14 (70 %) of the Chlamydia-tested group, 2 out of 14 had pure Chlamydia infection. Mh infection was significantly associated with history of spontaneous abortion, (*P*= .04). And with MPC (P= .05). Mh infection was significantly found more common among women with history of previous gynecologic operations (*P*=.01). Women with Mh complaining of resistant infection were significantly having a high titre. (*P*= .02). There was a significant relationship between Uu infection and the use of IUD as a contraception method. (*P*= .03).

Key words:

Mucopurulent cervicitis, mycoplasma hominis, Ureaplasma urealyticum, Chlamydia trachomatis.

Introduction

Mycoplasmas are the smallest free-living micro-organisms[.1]. In the urogenital tract, the relevant species are M. genitalium, Ureaplasma urealyticum, U. parvum, and M. hominis.

.

In women, several studies have demonstrated the association between M.

genitalium and urethritis, cervicitis, endometritis, and pelvic inflammatory M.genitalium leads to persistent or recurrent disease which necitate optimal diagnosis and treatment .[2].

Transmission is primarily by direct genital-genital mucosal contact. Mother-to-

child transmission at birth has not been systematically studied, but M. genitalium has been detected in the respiratory

tract of newborn children[3]. The risk of contracting M. genitalium per sexual

encounter has not been determined, but because M. genitalium is

present in lower concentration in genital tract specimens than C. trachomatis, it could be considered slightly less contagious

than chlamydia.[4] In women Urogenital infections may be Complicated by PID

(endometritis, salpingitis) Tubal factor infertility (probably)and Sexually acquired

reactive arthritis (SARA) may occur[5&6]. To diagnose M.genitalium It is difficult to make accurate recommendations regardingthe optimal sample type. First void

urine (FVU) from women provide a good diagnostic specimen which may be self-obtained.
No data regarding the importance of holding urine for a certain time are available, so

disease (PID).. Failure to eradicate

rocedures already in place for C. trachomatis sampling can be followed. Vaginal swab (physician or self-
collected) also provide an appropriate sensitivity.[7]

Mucopurulent cervicitis (MPC)

(MPC) is characterized by a purulent or mucopurulent endocervical exudate visible in the endocervical canal or in endocervical swab specimen. Some specialists also diagnose MPC on the basis of easily induced cervical bleeding. Although some specialists consider an increased number of leukocytes on endocervical Gram stain as being useful in the diagnosis of MPC, this criterion has not been standardized, has a low positive-predictive value (PPV), and is not available in some settings. Some women with MPC have an abnormal vaginal discharge and vaginal bleeding (e.g., after sexual intercourse). MPC can be caused by C. trachomatis or N. gonorrhoeae; however, in most cases neither organism can be isolated and MPC can persist despite repeated courses of antimicrobial therapy. [8]
The aim of this study is to characterize the association of urogenital mycoplasma with mucopurulent cervicitis (MPC) and to identify correlates of urogenital mycoplasma infection in women.

Patients and methods

The study population consists of 40 female patients presenting with signs and symptoms of cervicitis who attended the gynecologic out patient clinic in Cairo University Hospital (Kasr Al-Aini), faculty of Medicine, Cairo, Egypt, between September 2006 and june 2007. Age ranges from 18 to 54 years old (mean 31 years). All women participating in the study were sexually active. The local hospital chairman approval was obtained. Chlamydial antigen detection was done to 20 randomly selected patients out of the 40 studied group.

Clinical assessment

A routine medical history was taken and recorded on a standardized form by a single clinician. Data were collected regarding the reasons for attendance, age, symptoms (abnormal vaginal discharge, lower abdominal pain, back pain, intermenstrual, or postcoital bleeding), current contraception method, day of menstrual cycle at the time of examination , frequency of sexual intercourse per month, history of spontaneous abortion, smoking, douching >2 times per month. Female patients who received antibiotics within the previous 3 weeks were excluded.

"Mucopurulent cervicitis "is defined as the presence of either visible yellow mucopus or of > 30 polymorphnuclear leukocytes (PMNL) / HPF on a Gram-stained smear of cervical mucus.External genital inspection, pelvic speculum, and bimanual examinations were carried out. Clinical signs potentially indicating cervicitis were mucopurulent yellowish discharge in endocervical canal, pus on the cervical swab, bleeding after cervical sampling. The presence of cervical motion tenderness was also recorded.

Collection, transportation and preparation of samples for microbiological analysis

The speculum was moistened with warm water since the lubricants contain antibacterial agents. The cervix was visualized and excess mucus was removed from the exocervix with a separate swab or cotton ball and discarded. A cervical swab was taken with the cytobrush (BIO-BRUSH, Bio-Optica, Milano, code 14-360). The cytobrush was inserted approximately 2 cm into the cervix, rotated and moved from side to side several times for 30 seconds before it was removed. It was immediately soaked in a vial containing 2 ml of suspension / transport media (BIO-RAD, Mycoplasma Duo suspension medium code 62739). The tip of the cytobrush was left inside the vial which was kept at 4-8 C during transport. It is essential to obtain cells by scraping the mucosa with the cytobrush because mycoplasmas adhere to cells. Another 2 cervical swabs were taken with ordinary sterile cotton swabs, and one Dacron-tipped swab for Chlamydia. All the swabs were inserted into the endocervix and rotated against the surface of the cervical canal from 10 to 30 seconds without touching any vaginal surface when withdrawing. Both the vials and swabs were sent to the department of clinical pathology, Cairo University Hospitals, Kasr Al-Aini for further analysis. The vials containing the cytobrush were

stored at -20 C, while other swabs were analyzed at once.

Microbiological analysis:

• The first cotton swab was used to examine the pH of cervical discharge using pH strips, and for wet smear examination using 0.9% NaCl. All smears were examined with a light microscope. Wet smears were examined using 400 x magnification for T vaginalis with its characteristic motility. The second cotton swab was used to culture the cervical secretions on blood, chocolate and Mackonkey agar. It was also used to perfom direct Gram staining. The Gram stain demonstrates the presence and the number of PMNLs (pus cells), epithelial cells, and yeast cells using 400x and the presence of any organisms (diplo-cocci) using 1000x.

• The Dacron-tipped swab was used to detect Chlamydia trachomatis using Clearview chlamydia MF kit, Unipath limited, UK.

• Vials containing Mycoplasma suspension media were used to detect Ureaplasma urealyticum (Uu) and Mycoplasma hominis (Mh) using BIO-RAD MYCOPLASMA DUO KIT for identification and differential titration of genital mycoplasmas code 62740.

Detection of Ureaplasma urealyticum and Mycoplasma hominis

Principle

Identification / titration of urogenital mycoplasmas are based on the specific metabolic properties of each organism, i.e., hydrolysis of urea by Ureaplasma urealyticum (Uu), and hydrolysis of arginine by Mycoplasma hominis with release of ammonia and alkalinization of the medium (without clouding the medium).The reaction is visualized by a change in colour of pH indicator from yellow to red. i.e., yellow microwell indicates no mycoplasmas and red microwell indicates presence of mycoplasmas.Titration is based on the principle of dilutions in liquid medium. By successively diluting in the suspension medium, in well D and in wells U≥10^4 and H≥ 10^4 , the titre of the strain can be determined. The X microwell by selective enrichment of the specimen using an antifungal agent and a mixture of antibiotics, allows preparation of a standard inoculum for antibiotic susceptibility testing after incubation for 24 hours.

Mycoplasma Duo (code 62740) consists of:•

20 microplates: each microplate includes 6 microwells containing dehydrated substrates for identification, Mycoplasma growth factors, and agents inhibiting the concomitant flora .

• Wells U and U ≥10^4 : identification and titration of UU (contains urea). Wells H and H≥ 10^4 : identification and titration of MH (contain arginine).

D: dilution well. Well X: selective mycoplasma enrichment preparation of a standard inoculum for antibiotic susceptibility testing.

• 20 vials, each containing 2 ml of suspension medium (suspension / transport medium).

• 1 dropper bottle containing 15 ml diluent.

• 40 plastic micropipettes (2 are neded for each specimen).• 20 adhesive sheets.

The kit is stored at 2 - 8C. Its shelf-life is estimated until the expiry date printed on the

kit.

Procedure

A. Microplate seeding: Using the dropper bottle, 4 drops (200 µl) of diluent were transferred to each of the three wells of the lower row of the microplate, i.e., U ≥ 10^4 , D, and H ≥ 10^4.

The suspension medium seeded with the specimen was distributed using a micropipette as follows:

4 drops (100 µl) were added to each of the three wells of the upper row of the microplate, i.e., U, X, H and 1 drop (25 µl) was added to well D.

B. Dilution: The contents of well D were thoroughly homogenized by drawing up the contents three times using a different pipette. It is essential to use a different pipette for the dilutions since mycoplasmas adhere to plastic. Then the suspension was aspirated and one drop (25 µl) was transferred to well U ≥ 10^4 and one drop (25 µl) was transferred to well H ≥ 10^4.

C. Incubation: the microplate was covered with the adhesive sheet then incubated for 24 hours in a 37 C incubator and if needed this incubation was prolonged for another 24 hours.

Reading - interpretation

1) Reading

Results were first read after 24 hours which provided the definitive result in high titre specimens (≥ 10^4 CCU/ ml). A second reading performed after 48 hours incubation was needed to confirm negative results, to detect strains present in low titres (≤ 10^3 CCU/ ml), and to detect strains present in high titres (≥ 10^4 CCU/ ml) but characterized by a slow metabolic rate.

2) Interpretation

A change in colour from yellow to red of urea and/ or arginine microwells, without clouding the medium, indicates the presence of mycoplasmas.

The reading and interpretation diagram is identical for both mycoplasma species,i.e., read U and U >10^4 for UU , H and H> 10^4 for MH. MYCOPLASMA DUO allows differential titration of the two mycoplasma species which may both be present in the same specimen.

Specimens are classified as:

- Negative: no change in colour (yellow medium)
- Positive for UU: the change in colour occurs only in the U well(s).
- Positive for MH: the change in colour occurs only in the H well(s).
- Positive for UU and MH: a change in colour in both U and H well(s) indicates that both species are present in the specimen; the titre for each species was read separately.

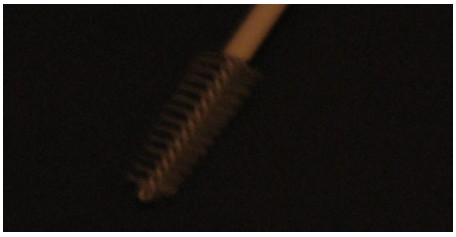

Figure 1: The cytobrush.

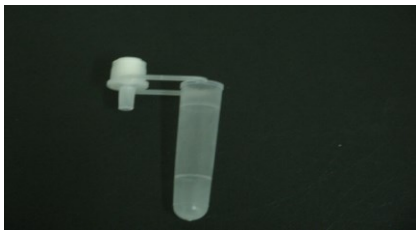

Figure 2: Clearview Chlamydia MFextraction tube

Detection of chlamydia :Clearview Chlamydia MF kit was used. It is a rapid immunoassay for the direct qualitative detection of *Chlamydia trachomatis* antigen in either female endocervical swab specimen or male urine specimens. [9].

Test principle

The absorbent pad contains coloured microspheres attached to the genus-specific anti-chlamydia monoclonal antibodies. The extract mobilizes these microspheres, and moves up the attached test strip. The test strip contains a region of immobilized monoclonal anti-chlamydia antibody in the result window (B). If the extract contains chlamydial antigen, it will complex with the antibodies attached to the coloured microspheres, and the immobilized antibodies in the result window. Therefore a line will form in the result window if chlamydial antigen is present in the extract. If no antigen is present the result window will remain clear. Clearview Chlamydia MF also provides an integral control feature; the appearance of a line in the control window shows the test has been carried out correctly.

Each kit contains:

- 3 × 5ml R1 (extraction reagent)
- 1 × 1ml R2 (positive control containing non infective chlamydial antigen)
- 20 individually foil-wrapped devices
- 20 extraction tubes. The kit should be stored at 2-8 C.

Procedure

The heating apparatus was ensured to be at 80 C, and all reagents, devices and specimens were at 18- 30 C before beginning the assay.

- **Extraction:** a clean extraction tube was filled to the line (0.6 ml) with R1. the swab was immersed in R1 and agitated for at least 5 seconds. The extraction tube with the swab was placed inside the heating apparatus and left for 10- 12 minutes. The extraction tube was then removed from the heating apparatus. The swab was rotated in the extraction tube for at least 5 seconds. Liquid from the swab was removed by pinching the rim of the extraction tube between thumb and finger with gently removing the swab from the tube then discarding it. The swab extract was allowed to cool for at least 5 minutes at 18- 30 C.
- **Test procedure:** A device was removed from the foil wrapper and placed on a level surface. The extraction tube was capped with the attached dropper, and 5 drops of the extract were applied to the sample window (A).
- **Interpretation of results:** the test should be read 15 minutes after applying the extract to the sample window (A). A line appearing in the control window (C) within 15 minutes shows that the test has worked correctly. If no line appears in the control window within 15 minutes the test must be repeated with a new Clearview Chlamydia MF device. A positive result is indicated by a line in the result window (B) at 15 minutes. A difference in intensity may occur between the lines in the result and the control window but this does not affect the interpretations of the results. A negative result is indicated if no line has formed in

the result window (B) at 15 minutes read time.

Positive control: Five drops of R2 (a positive antigen control provided with the kit) were added to a clean extraction tube which was filled to the line with R1. The tube was then agitated for at least 5 seconds to allow mixing then placed in the heating apparatus (pre-heated to 80C) for 10-12 minutes. The tube was capped with the attached dropper and the test procedure was completed as for an extracted specimen.

Negative control:

It was performed by following the female specimen extraction and test procedure steps but without the addition of a swab.

Methodology: Data was coded and entered using the statistics package SPSS version 12. Data was summarized using mean, standard deviation and range for quantitative variables and percent for qualitative variables. Comparisons between groups were done independent 84

sample T Test and ANOVA test (analysis of variance) for quantitative data and Chi-square and Fisher's exact test for qualitative variables. P values ≤ 0.05 were considered as statistically significan

Results

The incidence of cervical Mycoplasma hominis (Mh) and Ureaplasma urealyticum (Uu) was investigated in 40 female patients with suspected cervicitis who had attended the gynecologic out-patient clinic in Cairo University Hospital (Kasr Al-Aini), faculty of Medicine, Cairo, Egypt, between September 2006 and June 2007. Age ranged from 18 to 54 years (mean 31 years). Chlamydia trachomatis was investigated in 20 randomly selected patients out of the 40 studied group.

Mh was found in 12 women while Uu was found in 23 women. Women co-infected with both Mh and Uu were 11 (27.5 %). Women found to be positive for Mh and / or Uu were 24 (60 %) while those who were negative for both were 16 (40%).Out of the 12 (30 %) women who were positive for Mh 6 (15 %) had a high titre ≥ 10^4 CCU / ml specimen and 6 (15 %) had low titre ≤ 10^3 CCU / ml specimen, while 28 (70%) were Mh negative, i.e., didn't have Mh infection(table1). Out of the 23 (57.5 %) women who were Uu positive 12 (30 %) had a high titre ≥ 10^4 CCU / ml specimen and 11 (27.5 %) had low titre ≤ 10^3 CCU / ml specimen, while 17 (42.5 %) were Uu negative, i.e., didn't have Uu infection (table2). Chlamydia was investigated in 20 randomly selected patients out of the 40 studied group and it was found in 14 (70 %) of this group.N. gonorrhea and T. vaginalis couldn't be found in the studied group.

Table 1. Titration of Mh infection in 40 female patients.

Mh			
Frequency(40)			Percent%
	negative	28	70
vaild	Low titre	6	15
	High titre	6	15
	total	40	100

Table 2. Titration of Uu infection in 40 female patients.

Uu			
		Frequancy(40)	Percentag%
	Negative	17	42.5
Valid	Low titre	11	27.5
	High titre	12	30
	Total	40	100

Out of the 12 women who were positive for Mh, 83.3% have had a spontaneous abortion vs. 46.4% of women without Mh infection. (*P*= 0.04). These 2 groups were similar with respect to age, the use of any current contraceptionanddouching practices(table3). MPC was present in 100 % of women with and in 75 % of women without Mh infection. (*P*=0.05). Women with Mh infection were negatively associated with abdominal pain (*P*=0.07). There was no association with cervical PMNL counts or having easily induced cervical bleeding. There was no statistically significant relationship between Mh infection and abnormal vaginal discharge, back pain or phase of menstrual cycle. About 50 % of women infected with Mh had an infection described as being resistant to treatment. Women infected with Mh were more likely to have had previous gynecologic operation, with highly significantPvalue. (*P*=0.01(table4).

table 3. Demographic, gynecologic, and behavioral characteristics of 40 female patients 203. 0with and without Mh infection who attended the gynecologic out-patient clinic.

Characteristics	**Subjects with Mh n=12**	**subjects without Mh n=28**	***P* value**
Demographic			
Mean age in years	32 (18 - 45)	30 (20 - 54)	[a]0.69
Gynecologic			
Current contraception			
None	4 (33.3)	11 (39.2)	[c]1
any			

OCP	0	1(5.8)	[b]0.4
IUD	8 (100.0)	12 (70.5)	
Others	0	4 (23.5)	
spontanous abortion	10 (83.3)	13 (46.4)	[c]**0.04**
characteristics	**Subjects with Mh n=12**	**subjects without Mh n=28**	***P* value**
Behavioral			
>2 douches each month	5 (41.6)	11 (39.2)	[c]1

Note. Data are no. (%) of subjects, unless otherwise indicated. OCP, oral contraceptive pills. IUD, intrauterine device. Statistical significant value is considered if *P* value ≤ 0.05.
[a] By T- Test.
[b] By Pearson's Chi Square. [c] By Fisher' s Exact Test.

Table 4. Clinical findings associated with detection of Mh infection in 40 female patients who attended the gynecologic out-patient clinic.

Charcteristics	Subjects with Mh no=12	Subjects without MhNo=28	Pvalue
MPC [a]	12 (100.0)	21 (75.0)	[b]**0.05**
Cervical PMNL, mean (range)	16 (0 - 30)	18 (0 -30)	[d]0.63
Easily induced cervical bleeding	7 (58.3)	14 (50.0)	[c]0.73
Complaint			
Abnormal vaginal discharge	11 (91.6)	22 (78.5)	[c]0.65
Abdominal pain	5 (41.6)	21 (75.0)	[c]0.07
Low back pain	8 (66.6)	15 (53.5)	[c]0.5
Resistant infection	6 (50.0)	8 (28.5)	[c]0.28
Previous gynecologic operation	9 (75.0)	9 (32.1)	[c]**0.01**
Cervical erosion	2 (16.6)	8 (28.5)	[c]0.69

Note. Data are no. (%) of subjects, unless otherwise indicated. PMNL, polymorphonuclear leukocytes. Statistical significant value is considered if *P* value ≤ 0.05.
[a] MPC " mucopurulent cervicitis " was defined as either the presence of visible yellow mucopus or ≥ 30 PMNL / HPF in cervical mucus.
[b] By Pearson's Chi Square.
[c] By Fisher' s Exact Test.
[d] By T-Test.

93 % of contraception users out of the 23 Uu positive women have been using an IUD, with a significant relationship (*P*=0.03). The 23 women who were infected with Uu were similar to the group without Uu infection with respect to age, having a spontaneous abortion ever before, having douched at least 2 times each month(table5). MPC was found in 91.3 % of women with Uu and in 70.6 % of women without. (*P*=0.08). i.e., women infected with Uu were morelikely to have MPC than those without Uu. There was no association between Uu infection with cervical PMNL counts, having easily induced cervical bleeding, abnormal vaginal discharge, abdominal pain and. There was also no association between Uu and having had previous gynecologic operation or having cervical erosion. Women with Uu infection were negatively associated with low back pain. (*P*=0.05) (table6).

The associations between Mh infection and demographic, gynecologic, and behavioral characteristics were similar in magnitude and statistical significance regarding being of high titre or of low titre. The same applies also to Uu infection with the exception of the age. Uu high titre was found more often in younger age (mean years 28) while Uu low titre was found more often in older age (mean years 33), although that difference was not statistically significant. (*P*= 0.09) (table7). The association between clinical characteristics with both Mh and Uu infection were similar in magnitude and statistical significance regarding being of high titre or of low titre with few exceptions. Women with Mh high titre were more often in the proliferative phase of their menstrual cycle (days 6 –14), whereas women with Mh low titre were more often in their secretory phase (days 15 – 28) and there was a statistically significant relationship according to these results. (*P*= 0.04). Also 83.3 % of women with Mh high titre had a resistant infection vs. 16.7% of women with Mh low titre. i.e., Women with Mh complaining of resistant infection were significantly having a titre. (*P*= 0.02). Also, 54.5 % of women with Uu low titre had cervical erosion vs. 16.7 % of women with Uu high titre. i.e., women with Uu low titre were significantly more common to have cervical erosions than women with Uu high titre. (*P*= 0.05)(table8).

Uu infection was found in 91.7 % of women with Mh vs 42.9% of women without Mh (*P*= .005) whereas Mh infection was found in 47.8% of women with Uu vs 5.9% of women without Uu (*P*= 0.005). i.e., there is strong association between the 2 infections with a high statistical significant *P* value. Chlamydia was found in 75% of women with and in 62.5% of women without Uu infection in the group where the Chlamydia was investigated . The difference between those 2 groups was not statistically significant. (*P*=0.64).i.e., there is no association between Chlamydia and Uu infection(table9). Chlamydia was found in 60% of women with and in 73.3% of women without Mh infection. The difference between those 2 groups was not statistically significant. (*P*= 0.61). i.e., there is no association between Chlamydia and Mh infection(table10). the group tested for Chlamydia, 14 out of 20 were Chlamydia positive. Chlamydia + Uu were found in 6 women, Chlamydia + Mh +Uu were found in 3 women and Chlamydia alone was found in 5 women. Chlamydia + Mh were not found.

Table 5: Demographic, gynecologic, and behavioral characteristics of 40 female patients with and without Uu infection who attended the gynecologic out-patient clinic.

Characteristic	subjects withUu no= 23	subjects without Uu no= 17	Pvalue
Demographic			
Mean age in year	30.8(18—54)	31.5(21- 50)	[a] 0.78
Gynacological			
Current contraception			
Non	7(30.4)	8(47.1)	
Any	16(69.6)	9(52.9)	[b] 0.03
OCP	0	1(11.1)	
IUD	15(93.7)	5(55.5)	
Others	1(6.2)	3(33.4)	
Spontous abortion	13(56.5)	10(58.8)	[c]1.0
Behaviarol			
>2douches/month	7(30.4)	9(52.9)	[c]0.19

Note. Data are no. (%) of subjects, unless otherwise indicated. OCP, oral contraceptive pills. IUD, intrauterine device. Statistical significant value is considered if *P* value ≤ 0.05.
[a] By T-Test. [b] By Pearson's Chi Square. [c] By Fisher' s Exact Test.

Table 6: Clinical findings associated with detection of Uu infection in 40 female patients who attended the gynecologic out-patient clinic.

charcteristic	Subjectswith Uu no ==23	Subjects without Uu no =17	Pvalue
MPC	21 (91.3)	12(70.6)	[b] 0.08
CervicalPMNL(mean&range)	16(0 -30)	20(0 30)	[d]0.22
Complain			
Abnormal vaginal discharge	20(86.9)	13(76.5)	[c]0.43
Abdominal pain	13(56.5)	13(76.5)	[c]0.31
Low back pain	10(43.3)	13(76.5)	[c]0.05
Resistant infection	7(30.4)	7(41.2)	[c]0.52
Previous gynecological operation	11(47.8)	7(41.2)	[c]0.75
Cervical erostion	8(34.8)	2(11.8)	[c]0.14

Note. Data are no. (%) of subjects, unless otherwise indicated. PMNL, polymorphonuclear leukocytes.

[a] MPC " mucopurulent cervicitis " was defined as either the presence of visible yellow mucopus or ≥ 30 PMNL / HPF in cervical mucus. Statistical significant value is considered if *P* value ≤ 0.05.

[b] By Pearson's Chi Square. [c] By Fisher' s Exact Test. [d] By T-Test.

Table 7: Demographic, gynecologic, and behavioral characteristics of women infected with Mh high titre, Mh low titre, Uu high titre, Uu low titre:

Chercteristic	Subjects with Mh high titre n=6	subjects without Mh low titre n=6	Pvalue	subjectswith Uh high titre n=12	subjects withoutUh low titre n=11	pvalue
Demographic						
Mean age in year	32(18-45)	32(25-43)	[a]0.93	28(18-44)	33 (22 - 54)	[a]0.09
Gynacological						
Current contraception	2 (33.3)	2 (33.3)	[b]1	3(25.0)		
None	4 (66.7)			9(75.0)		
Any		4 (66.7)	[b]1	6(50.0)	4(36.4)	
Spontanous abortion ever	5 (83.3)	5 (83.3)		3(50.0)	7(63.6)	[b]0.36
Behaviarol >2douches/month	3 (50.0)	2 (33.3)	[b]1		7(63.6)	[b]0.68
					4(36.4)	[b]0.66

Table8: Clinical characteristics of women infected with Mh high titre, Mh low titre, Uu high titre, Uu low titre:

Charcterstics	Mh high titre n=6	Mh low titre n=6	pvalue	Uu high titre n=12	Uu low titre n=11	pvalue
MPC[a]	6(100)	6(100)	[C]1	12(100)	9(81.8)	[C]0.21
cervicaPMNLmean(range)	20(5-30)	13(0-30)	[d]0.48	19(5-30)	12(0-30)	[d]0.11
easily induced cervical bleeding	4(66.7)	3(50.0)	[c]1	6(50.0)	8(72.8)	[c]0.4
complain						
abnormal vaginal discharge	6(100)	5(83.3)	[c]1	11(91.7)	9(81.8)	[c]0.59
abdominal pain	2(33.0)	3(50.0)	c1	7(58.3)	6(50.0)	[c]1
low back pain	3(50.0)	5(83.3)	[c]0.54	5(41.7)	5(41.7)	[c]1
resistant infection	5(83.3)	1(16.7)	[b]0.02	5(41.7)	2(18.2)	[b]0.37
previousgynecological operation	5(83.3)	4(66.7)	[c]1	6(50.0)	5(41.7)	[c]1.0
cervical erostion	2(33.0)		[c]0.45	2(18.2)	6(50.0)	[b]0.05

[b] By Pearson's Chi Square.
[c] By Fisher' s Exact Test. [d] ByMann-Whitney test.

Table 9: The relation between Chlamydia and Uu infection.

			Uu		total
			negative	positive	
Chlamydia	positive	count	5	9	14
		%withUu	62.5	75.0	70.0
Total cases tested for chlamydia		count	8	12	20
					100%

Table 10: The relation between Chlamydia and Mh infection.

		Mh		total
		negative	positive	
Chlamydia positive	count	11	3	14
	%Mh	73.3	60.0	70.0
Total cases tested for chlamydia	count	15	5	20
				100%

Figure 4: A mycoplasma Duo Kit microplate showing negative Uu and positive Mh high titre(left) And positive Uu high titre and negative Mh.(right).

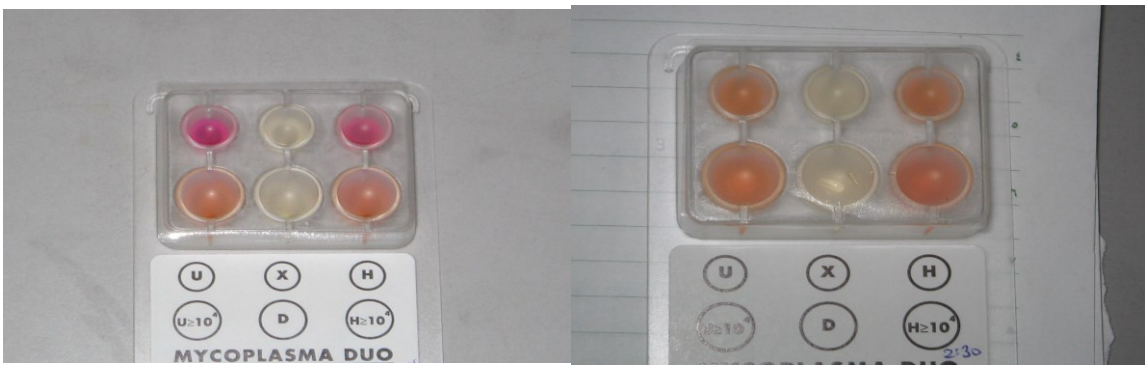

figure 5. A mycoplasma Duo Kit microplate showing positive Uu low titre and positive Mh low titre. (left) and Uu and Mh are both negative.(right).

Discussion

Cervicitis is characterized by inflammation of the cervix and most commonly is caused by sexually transmitted diseases (STDs). *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) are the causative agents of the most common STDs and are the most frequent causes of cervicitis. However, in many cases of cervicitis the etiology is unknown or unclear[10]. In those cases, *Trichomonas vaginalis* (TV), *Mycoplasma genitalium* (MG), and others (such as those associated with bacterial vaginosis (BV), herpes simplex virus Type 1, and herpes simplex virus Type 2) have been implicated as potential causative pathogens of nonchlamydial, nongonococcal cervicitis. herpes simplex virus Type 2) have been implicated as potential causative pathogens of nonchlamydial, nongonococcal cervicitis.[11] In models using multiple logistic regression, MG was the only pathogen to be associated with cervicitis. Others have reported an association of MG with cervicitis.[10]. Cervicitis may be persistent due to lack of clear understanding of the infectious etiology or noninfectious

origin of disease. It is essential to determine the extent to which the various microorganisms contribute to cervicitis so that appropriate patient diagnosis and treatment can occur. Asymptomatic cases of cervicitis also represent a continuing challenge in the chain of transmission and persistence in the population because etiologic agents are often not detected or treated.[10]. However, the association of Mycoplasma Gentalium(MG) with cervicitis is not as clear. Recently, Hjorth et al. provided evidence that MG is sexually transmitted using DNA-based typing. Advances in nucleic acid amplification techniques (NAATs) now allow for the testing of all organisms with high sensitvity and specificity.[12].

The incidence of cervical *Mycoplasma hominis* (Mh) and *Ureaplasma urealyticum* (Uu) was investigated in 40 female patients with suspected cervicitis who had attended the gynecologic out-patient clinic in Cairo University Hospital (Kasr Al-Aini), faculty of Medicine, Cairo, Egypt, between September 2006 and June 2007. Age ranged from 18 to 54 years (mean 31 years). *Chlamydia trachomatis* was investigated in 20 randomly selected patients out of the 40 studied group. Mh was found in 12 (30 %) women while Uu was found in 23 (57.5 %). Women co-infected with both Mh and Uu were 11 (27.5 %). Women found to be positive for Mh and / or Uu were 24 (60 %) while those who were negative for both were 16 (40 %). Mean age was 32.0 years for Mh and 30.8 years for Uu infected patients respectively. Supporting our results is the opinion adopted during the 1st Meeting of the Scientific network on genital mycoplasmas, they found that *U. urealyticum* was more frequently isolated than *M. hominis* both in women with cervicitis (39%) and in men with NGU (9.7%). They concluded that U. urealyticum was strongly associated with cervicitis and endometritis and serologically with salpingitis and tubal factor infertility. [13]. One study in Spain found that 23% of cases were positive for *M. hominis* and 19% for *U. urealyticum* (*P* = NS). [14]. In one study by Gyado etal they found that the overall prevalence for MG was(19.2%) In all cases, the prevalence for the organism was greater in those participants with cervicitis (28.6%) than those without cervicitis (12.7%).[15]. Another Polish study that investigated the prevalence of Mh and Uu showed that Uu was found in 31.8% of the tested group (42 of 132 women) while Mh was found in 3% (4 of 132) of patients. i.e., *Ureaplasma urealyticum* was found most often, Average age was 32.1 ±7.7 years with no statistically significant correlation was found between the age of the patients and the incidence of mycoplasmas. [16]. This also goes with a study done on an open population in Mexico City which showed that there was no association between colonization with *Ureaplasma urealyticum* and age. [17] A Portuguese study showed that 57.4% of women were positive for *M. hominis* and/or *U. urealyticum*. *M. hominis* and *U. urealyticum* separately isolated from infected women yielded frequencies of 31.5 and 27.8%, respectively, the remainder were

infected with both species. [18]. Our study shows that out of the 12 (30 %) women who were positive for Mh, 6 (15 %) had a high titre ≥ 10^4 CCU / ml specimen, and 6 (15 %) had low titre ≤ 10^3 CCU / ml specimen, while out of the 23 (57.5 %) women who were Uu positive, 12 (30 %) had a high titre ≥ 10^4 CCU / ml specimen, and 11 (27.5 %) had low titre ≤ 10^3 CCU / ml specimen. Uu high titre was found more often in younger age (mean years 28) while Uu low titre was found more often in older age (mean years 33, P= .09). Differential titration of Mh was not associated with age difference. A Chinese study showed that the high titre colony counting(≥10^4 cfu/ml) in *Ureaplasma urealyticum* infection patients accounted for 76.7%, while *Mycoplasma hominis* infection represented only 18.2%. [19]. A German study that investigated the influence of quantitative *Ureaplasma urealyticum* colonization level concluded that the degree of colonization with Uu correlates strongly with an adverse effect on pregnancy outcome. As high-density Uu colonization was an independent risk factor for chorioamnionitis and preterm delivery, whereas low colonization levels had no effect on an adverse outcome of pregnancy[20] Pathogenicity of Mh requires further study (regarding the titre) as shown in a study done in Côte d'Ivoire which found that among women colonized with Mh, HIV seroprevalence was 21% in women with high titre of *M. hominis* (≥ 10^4 CCU / ml) versus 7% in women with lower titre (*P*=0.01). [21].

Our study shows that Mh infection was significantly associated with history of spontaneous abortion, as 83.3 % of women with Mh have had a spontaneous abortion ever before vs. 46.4 % of women without Mh infection (*P*= .04). Uu infection was not associated with history of spontaneous abortion neither was the differential titration of Mh or Uu.

A Spanish study showed that genital mycoplasma (Mh and Uu) have no influence on spontaneous abortion[14]. On the other hand, ***Cernescu 2004*** stated that Uu role in the etiology of pregnancy complications is suggestive, inducing pre-term labor and spontaneous abortion[22]. A Japanese study demonstrated that *U. parvum*, but not *U. urealyticum*, is an independent risk factor for late abortion or early preterm birth, [23]. The same Japanese study showed that Mh was not a risk factor for late abortion or preterm birth. [23]. On the contrary, a study done in South Africa showed that positive culture for *M. hominis* was associated with more preterm deliveries. [24].

Our study showed that there was no association between Mh infection and the use of any contraception method, as 66.6 % of women with Mh confirmed the use of any current contraception (all of them had been using an IUD) vs. 60.7 % of women without Mh (*P* = NS). Women with Uu were more likely to use any current contraception method than those without Uu infection although the difference between groups was not statistically significant (*P*=.33), as 69.6 % of women with Uu infection did use any contraception method vs. 52.9 % of women

without Uu infection taking into consideration that 93 % of contraception users have been using an IUD, which was statistically significant (*P*= .03). i.e., there was a significant relationship between Uu infection and the use of IUD as a contraception method. Differential titration of Mh and Uu was not associated with the use of any current contraception.

Unlike our study a Mexican study showed that there was association between colonization with *Ureaplasma urealyticum* and no birth control method use. [25]. A Chinese study showed that the incidence rate of the genital mycoplasmas was significantly affected by the type of contraception and the number of sexual partners[26].

Our study showed that Mh and Uu infection were not associated with factors related to alterations in the vaginal / cervical environment, such as douching, as 41.6 % of women with Mh had douched at least 2 times each month vs. 39.2 % of women without Mh infection (P=NS) and 30.3% only of women with Uu infection reported douching. Differential titration of Mh and Uu didn't have an association with douching. What pushed us to see the association between douching and Mh and Uu was the presence of previous studies that report associations between douching and adverse outcomes including pelvic inflammatory disease, bacterial vaginosis, cervical cancer, low birth weight, preterm birth, human immunodeficiency virus transmission, sexually transmitted diseases, ectopic pregnancy, recurrent vulvovaginal candidiasis, and infertility. Studies conflict, however, and the strength of association vary enormously between studies. [27].

A study by Scholes et al. found results that support the hypothesis that vaginal douching predisposes to acquisition of cervical chlamydial infection. Fonck et al. found that, in female sex workers in Nairobi, Kenya, douching in general and douching with soap and water were significantly associated with bacterial vaginosis with a significant trend for increased frequency of douching and higher prevalence of bacterial vaginosis. [28]. In our study Mh was negatively associated with abdominal pain, a potential manifestation of upper reproductive tract infection (*P*=.07) while Uu was negatively associated with low back pain, a potential manifestation of long standing infection (*P*=.05).The role of Mh and Uu in MPC was investigated. Mh was significantly associated with MPC as MPC was present in 100 % of women with and in 75 % of women without Mh infection. (*P*= 0.05). Women with Uu infection were more likely to have MPC than women without Uu infection as MPC was present in 91.3% of women with vs. 70.6 % of women without Uu infection (*P*= 0.08). Differential titration for both Mh and Uu didn't have a role. Many cases of mucopurulent cervicitis (MPC) are idiopathic and cannot be attributed to the known cervical pathogens *Neisseria gonorrhoeae, Chlamydia trachomatis, or herpes simplex virus*. [29]. In a review by McGowin and Anderson-Smits,39 of 14 studies of lower genital tract inflammation, seven had M. genitalium associated with urethritis, vaginal discharge, microscopic cervicitis and mucopurulent

cervical discharge. This study also revealed that in three of six studies from 2002 to 2010 there was a significant association between M. genitalium and vaginal discharge[30]. One of the studies adjusted for bacterial vaginosis but still found asignificant association between M. genitalium and vaginal discharge. In contrast to this, Thurman et al.[31] in a similar population,found no significant association. These inconsistencies may be due to the definition of pathologicalvaginal discharge and/or the subjectivity of patient symptoms.The relationship between M. genitalium and cervicitis also requires further investigation. Manhart et al.[29]. conducted a study examining 719 cervical secretions archived from 1984 to 1986. The authors concluded that M. genitalium infection was an independent cause of cervicitis .This conclusion was established after a multivariate logistic regression analysis correcting for confoundingfactors and excluding participants with C. trachomatis and/or N. gonorrhoeae. The study had an attributable risk of 70% suggesting that among the women with cervicitis and M. genitalium, 70% of the cervicitis was caused by independently.2 Nevertheless, there are difficulties in comparing studies because of the inconsistency in defining cervicitis. A standard definition is required for comparison. Two previous studies comparing *Mycoplasma* prevalence among women with and without cervicitis produced conflicting results. ***Uno, et al., 1997*** found an association between *M. genitalium* and cervicitis while ***Casin, et al., 2002*** found no association between *M. genitalium* and cervicitis among women presenting with symptoms to an STD clinic.[32&33].

In our study PMNL infiltration of the cervix didn't provide a clue that Mh or Uu may cause cervicitis, as mean PMNL counts in cervical mucus was always lower in case of Mh or Uu infections. Cervical PMNL count mean was16 in women with Mh vs. 18 in women without Mh, while it was 16 in women with Uu vs. 20 in women without Uu (*P*=NS). It is to be noted that within the positive groups, cervical PMNL counts may be an indicator to the high titre cases. This indicator is stronger in Uu infection than in Mh infection. Cervical PMNL count mean was19.6 in women with Mh high titre vs. 13.3 in women with Mh low titre (*P*=.48), while it was 19.4 in women with Uu high titre vs. 12.2 in women with Uu low titre (*P*=.11). The small sample size of the Mh positive group (12) compared to the Uu positive group (23) may account for this difference.

A previous study showed that PMNL infiltration of the lower genital tract provided the first clue that *M. genitalium* may cause cervicitis in female primates inoculated with Mycoplasma genitalium[29].

In our study 50 % of women infected with Mh had an infection described as being resistant to treatment, while 30.4 % only of women infected with Uu had a resistant infection (*P*=NS). Women with Mh complaining of resistant infection were

significantly having a high titre. (*P*= .02). i.e., 83.3 % of women with Mh high titre had a resistant infection vs. 16.7% of women with Mh low titre. Differential titration for Uu didn't have a role.

Mh infection was significantly found more common among women with history of previous gynecologic operations (*P*=.01), as 75% of women with Mh infection had previous gynecologic operations vs. 32.1 % of women without Mh infection. Uu infection was not associated with history of previous gynecologic operations neither was the differential titration of Mh or Uu. Mycoplasma hominis. It also showed that U. urealyticum is an important pathogen in post-caesarean delivery endometritis. [34]. Another study was done to investigate the frequency and clinical significance of bacteremia with urogenital mycoplasmas in immunocompetent patients following gynecological surgery found that in 4 of the 11 patients colonized by Ureaplasma urealyticum the pathogen was also detected from the postoperative blood samples, while no case of bacteremia with Mycoplasma hominis was detected. The postoperative course was uncomplicated in all patients. [35].

Our study showed that cervical erosions were present in 34.8 % of women infected with Uu vs. 11.8 % of women without Uu (P=.14). women with Uu low titre were significantly more common to had them, i.e., 54.5 % of women with Uu low titre had cervical erosion vs. 16.7 % of women with Uu high titre (*P*= .05). Mh infection was not associated with the presence of cervical erosions neither did its differential titration.

Chlamydia trachomatis was found in 14 (70 %) of the Chlamydia-tested group. *N. gonorrhea, T. vaginalis* couldn't be found in the whole tested group. i.e., our study shows that there was no association found between Mh , Uu and *Chlamydia trachomatis with N. gonorrhea* or *T. vaginalis*. A Portuguese study demonstrated that the genital mycoplasmas appear to be associated with trichomonosis. [36] A study done in South Africa showed that Mh was associated with a higher frequency of *C. trachomatis, U. urealyticum* and bacterial vaginosis. [37].Another study showed that *U. urealyticum* was associated with *T. vaginalis, M. hominis*, and bacterial vaginosis. [38] On the other hand, one study clearly confirms the association between *T. vaginalis* and *M. hominis* infection while *U. urealyticum* infection and infection by *T. vaginalis* are independent. [39] .

Our study showed that there is a strong association between Mh and Uu infection with a high statistical significant *P* value (*P*=.005). As Uu infection was found in 91.7 % of women with Mh vs. 42.9% of women without Mh whereas Mh infection was found in 47.8% of women with Uu vs. 5.9% of women without Uu. Supporting our opinion is a Chinese study which came to a conclusion that *Ureaplasma urealyticum* and *Mycoplasma hominis* should be detected simultaneously. [19].

Recommendations

- The incidence of genital mycoplasma should be investigated among healthy normal individuals, males and females to demonstrate its prevalence among normal Egyptian population.
- Antibiotic susceptibility for both Mh and Uu should be investigated to detect their sensitivity patterns and to detect the presence of antibiotic resistance if any, in order to put an end to the problem of resistant infections.
- The sample size of the study population should be larger so that the near significant statistical values have a better chance to appear significant if they really are.
- The role of Mh and Uu should be investigated in cases of pre-term labor, pre mature rupture of membranes, infertility, puerperal fever and Pelvic Inflammatory Disease, bacterial vaginosis and respiratory tract diseases in neonates.
- Other types of Mycoplasmas should be also investigated, as Mycoplasma genitalium .And their role in various diseases should be assessed.
- Patients with history of spontaneous abortion, previous gynecologic operations or suffering from cervicitis resistant to usual treatment should be tested for Mh.
- Patients suffering from cervicitis while using an IUD or having cervical erosions should be tested for Uu.

References

[1]Taylor-Robinson D, Gilroy CB, Jensen JS. The biology of *Mycoplasma genitalium*. *Venereology* 2000;**13**:119-27.

[2]. Jensen JS, Bradshaw C. Management of *Mycoplasma genitalium* infections - can we hit a moving target? *BMC Infect Dis* 2015;**15**(1):343. 12.

[3]. Deguchi T, Yasuda M, Yokoi S, et al. Failure to detect *Mycoplasma genitalium* in the pharynges of female sex workers in Japan. *J Infect Chemother* 2009;**15**(6):410-3.

[4]Walker J, Fairley CK, Bradshaw CS, et al. The difference in determinants of *Chlamydia trachomatis*

and *Mycoplasma genitalium* in a sample of young Australian women. *BMC Infect Dis* 2011;**11**:35.

[5]Lis R, Rowhani-Rahbar A, Manhart LE. *Mycoplasma genitalium* infection and female reproductive tract disease: A meta-analysis. *Clin Infect Dis* 2015.

[6]Taylor-Robinson D, Gilroy CB, Horowitz S, Horowitz J. *Mycoplasma genitalium* in the joints of two patients with arthritis. *Eur J*

Clin Microbiol Infect Dis 1994;**13**(12):1066-9.

[7]Jensen JS, Björnelius E, Dohn B, Lidbrink P. Comparison of first void urine and urogenital swab specimens for detection of *Mycoplasma genitalium* and *Chlamydia trachomatis* by polymerase chain reaction in patients attending a sexually transmitted disease clinic. *Sex Transm Dis* 2004;**31**(8):499-507.

[8]Sexually transmitted diseases treatment guidelines 2002. Centers for Disease Control and Prevention. Diseases characterized by urethritis and cervicitis. May; 51(RR-6):30-42.

[9]Matthews R, Ridgeway G, Carder C, Hook E, Pate M, Radcliffe K, Robinson A, Gleason B, Davidson l and Sheard P. (1996). Evaluation of a new rapid immunoassay (Clearview Chlamydia MF) for the detection of Chlamydia trachomatis in female endocervical swabs and in male urine samples. ASM.

[10] Marrazzo JM. Mucopurulent cervicitis: No longer ignored, but still misunderstood. Infect Dis Clin North Am. 2005;19:333–349. [PubMed]

[11]Cohen CR, Manhart LE, Bukusi EA, et al. Association between *Mycoplasma genitalium* and acute endometritis. Lancet. 2002;359:765–766. [PubMed] .

[12] Hjorth SV, Bjornelius E, Lidbrink P, et al. Sequence-based typing of *Mycoplasma genitalium* reveals sexual transmission. J Clin Microbiol. 2006;44:2078–2083.[PMC free article] [PubMed]

[13]Cernescu C. Urinary tract infections and antibiotic resistant patterns of ureaplasma urealyticum and Mycoplasma hominis in Romanian patients. 1st Meeting of the Scientific network on genital mycoplasmas, Athens, Greece, 12-14 October 2004.

[14]Núñez-TroconisJT.(1999).Mycoplasma hominis and Ureaplasma urealyticum in different gynecologic diseases. Invest Clin. Mar; 40(1):9-24.

[15] Gaydos,CA . Maldeis,N , Hardick,A Hardick.,J *Mycoplasma genitalium* as a Contributor to the Multiple Etiologies of Cervicitis in Women Attending Sexually Transmitted Disease Clini. Sex Transm Dis. 2009 Oct; 36(10): 598–606.

[16]Elias M, Grześko J, Siejkowski R, Nowicka J, Maczyńska B, Goluda M, St Gabryś M. The presence of Mycoplasma hominis and Ureaplasma urealyticum in the cervical canal of uterus. Ginekol Pol. Jan;2005. 76(1):28-32.

[17]González PA, Catalina MA, Inzunza-Montiel AE, Ponce-Rosas ER.. Frequency of isolation of Ureaplasma urealyticum in an open population from southern Mexico City. Rev Latinoam Microbiol. 1995 Apr-Jun; 37(2):79-86.

[18]Domingues D, Távora TL, Duarte A, Sanca A, Prieto E, Exposto F. Genital mycoplasmas in women attending a family planning clinic in Guiné-Bissau and their susceptibility to antimicrobial agents. Acta Trop. Apr; *2003.* 86(1):19-24.

[19]Yan ZH, Zhou M, Zhang W, Zhang DL. Prevalence and drug tolerance of mycoplasma in patients with urogenital inflammation. Zhonghua Nan Ke Xue = National journal of andrology. 2003. Nov;9(8):599-600, 603.

[20]Abele-Horn M, Scholz M, Wolff C, Kolben M. High-density vaginal Ureaplasma urealyticum colonization as a risk factor for chorioamnionitis and preterm delivery. Acta Obstet Gynecol Scand. 2000. Nov; 79(11):973-978.

[21]Faye-Ketté H, La Ruche G, Ali-Napo L, Messou N, Viho I, Welffens-Ekra C, Dosso M, Msellati *P.* Genital mycoplasmas among pregnant women in Cóte d'Ivoire, West Africa: prevalence and risk factors. Int J STD AIDS. Sep; *2000.* 11(9):599-602.

[22]Cernescu C. Urinary tract infections and antibiotic resistant patterns of ureaplasma urealyticum and Mycoplasma hominis in Romanian patients. 1st Meeting of the Scientific network on genital mycoplasmas, Athens, Greece, 2004. 12-14 October 2004.

[23]Kataoka S, Yamada T, Chou K, Nishida R, Morikawa M, Minami M, Yamada H, Sakuragi N, Minakami H. (2006). Association between preterm birth and vaginal colonization by mycoplasmas in early pregnancy. J Clin Microbiol.;2006. 44(1):51-55.

[24]Odendaal HJ, Popov I, Schoeman J, Grové D. Preterm labour--is Mycoplasma hominis involved? S Afr Med J. Mar; 2002. 92(3):235-237.

[25]González PA, Catalina MA, Inzunza-Montiel AE, Ponce-Rosas ER. Frequency of isolation of Ureaplasma urealyticum in an open population from southern Mexico City. Rev Latinoam Microbiol. (1995). Apr-Jun; 37(2):79-86.

[26]Wang N. (1992*)*. Epidemiological study on mycoplasmas colonization and infection in the female genital tract. Zhonghua Liu Xing Bing Xue Za Zhi.Dec;13(6):368-371.

[27]Martino J and Vermund S. Vaginal Douching: Evidence for Risks or Benefits to Women's Health. Epidemiologic Reviews; (2002*)*. 24:109-124.

[28]Fonck K, Kaul R, Keli F, Bwayo JJ, Ngugi EN, Moses S, Temmerman M.*)*. Sexually transmitted infections and vaginal douching in a population of female sex workers in Nairobi, Kenya. Sex Transm Infect. 2001 Aug; 77(4):271-275.

[29]Manhart LE, Critchlow CW, Holmes KK, Dutro SM, Eschenbach DA, Stevens

CE, and Totten PA. Mucopurulent Cervicitis and Mycoplasma genitalium. The Journal of Infectious Diseases;2003. 187:650–657.

[30]Falk L, Fredlund H, Jensen JS, et al. Signs and symptoms of urethritis and cervicitis among women with or without Mycoplasma genitalium or Chlamydia trachomatis infeciton.Sex Transm Dis 2005; 81: 73–78.

[31]Thurman AR, Musatovova O, Perdue S, et al. Mycoplasma genitalium symptoms, concordance andtreatment in high-risk sexual dyads. Int J STD AIDS2010; 21: 177–183.

[32]Uno M, Deguchi T, Komeda H. *Mycoplasma genitalium* in the cervices of Japanese women. Sex Transm Dis; 1997. 24:284–28.

[33]Casin I, Vexiau-Robert D, De La Salmoniere P, Eche A, Grandry B, Janier M. . High prevalence of *Mycoplasma genitalium* in the lower genitourinary tract 113of women attending a sexually transmitted disease clinic in Paris, France. Sex2002.

[34]Patai K, Füzi M, Kanjo AH, Sipos M, Paulin F.*).* Severe genital mycoplasma infection following cesarean section. Orv Hetil. Mar 15; 139(11):641-643. (1998

[35]Daxboeck F, Iro E, Tamussino K, Krause R, Assadian O, Wenisch C. Bacteremia with Mycoplasma hominis and Ureaplasma urealyticum in patients undergoing hysterectomy. Eur J Clin Microbiol Infect Dis. 2003.; 22(10):608-611.

[36]Domingues D, Távora TL, Duarte A, Sanca A, Prieto E, Exposto F. Genital mycoplasmas in women attending a family planning clinic in Guiné-Bissau and their susceptibility to antimicrobial agents. 2003. Acta Trop. Apr; 86(1):19-24.

[37]Odendaal HJ, Popov I, Schoeman J, Grové DPreterm labour--is Mycoplasma hominis involved? S Afr Med J. . 2002. Mar; 92(3):235-237.

[38]Carey JC, Blackwelder WC, Nugent RP, Matteson MA, Rao AV, Eschenbach DA, Lee ML, Rettig PJ, Regan JA, Geromanos KL, et al. . Antepartum cultures for Ureaplasma urealyticum are not useful in predicting pregnancy outcome. The Vaginal Infections and Prematurity Study Group. Am J Obstet Gynecol. 1991Mar; 164(3):728-733.

[39]van der Schee C, Sluiters HJ, van der Meijden WI, van Beek P, Peerbooms P, Verbrugh H, van Belkum A. . Host and pathogen interaction during vaginal infection by Trichomonas vaginalis and Mycoplasma hominis or Ureaplasma urealyticum. J Microbiol Methods. 2001; 45(1):61-67.