

## Association of *Mycoplasma hominis* and *Ureaplasma urealyticum* with Some Indicators of Nonspecific Vaginitis

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**ABSTRACT.** The purpose of this study was to determine the isolation rates of *Mycoplasma hominis* and *Ureaplasma urealyticum* from three populations of women and also to relate the presence of these microorganisms with some indicators of nonspecific vaginitis. Three hundred vaginal swabs were taken from delivery, pregnant and control (not pregnant) women. Cultures were done in E broth supplemented with arginine or urea. *M. hominis* was isolated in 5% at delivery, 12% from pregnant and 5% from control women and *U. urealyticum* was isolated in 21%, 31% and 28% respectively. There was statistical difference in the isolation rate of *M. hominis* in pregnant women respect to the other groups. Both microorganisms were more frequently isolated in women with acid vaginal pH, amine-like odor in KOH test, clue cells and leucorrhea. *M. hominis* was isolated in 17% and *U. urealyticum* in 52% from women with nonspecific vaginitis. *M. hominis* was isolated in 2% and *U. urealyticum* in 13% from women without nonspecific vaginitis. Although the presence of clue cells and amine-like odor in KOH test have relationship with *Gardnerella vaginalis*, these tests could also suggest the presence of these mycoplasmas.  
**Key words:** *Mycoplasma hominis*, *Ureaplasma urealyticum*, nonspecific vaginitis.

**RESUMEN.** El objetivo del presente trabajo fue determinar el porcentaje de aislamiento de *Mycoplasma hominis* y *Ureaplasma urealyticum* del aparato genitourinario de 3 poblaciones de mujeres y relacionar la presencia de estos microorganismos con los criterios empleados para diagnosticar una vaginitis inespecífica. Se procesaron 300 exudados vaginales de 3 grupos de mujeres clínicamente sanas: a) embarazadas; b) durante el trabajo de parto y c) testigos (no embarazadas). Los cultivos se realizaron en caldo E suplementado con arginina o urea. *M. hominis* se aisló en un 5% de mujeres durante el trabajo de parto, 12% en embarazadas y 5% en el grupo testigo. *U. urealyticum* se aisló en 21%, 31% y 28% respectivamente. Hubo diferencias estadísticamente significativas en el porcentaje de aislamiento de *M. hominis* en las mujeres embarazadas con respecto a los otros grupos. Ambos microorganismos fueron más frecuentemente aislados en mujeres con pH vaginal ácido, leucorrea, desprendimiento de aminas y presencia de células guía. En mujeres con vaginitis inespecífica se aisló a *M. hominis* en 17% y a *U. Urealyticum* en 52% mientras que en mujeres sin vaginitis *M. hominis* se aisló en 2% y *U. Urealyticum* en 13%. Aunque el desprendimiento de aminas y células guía están asociadas con *Gardnerella vaginalis*, una prueba positiva nos podría sugerir también la presencia de estos micoplasmas.

**Palabras clave:** *Mycoplasma hominis*, *U. urealyticum*, vaginitis inespecífica

### INTRODUCTION

Mycoplasmas are the smallest cell free-life microorganisms.<sup>9</sup> They can be isolated as commensals or pathogens from plants, insects, animals and humans,<sup>10</sup> some of them are considered normal flora of the respiratory or genitourinary tract. Seven species of mycoplasmas can be isolated from genitourinary tract but only *Mycoplasma hominis* and *Ureaplasma urealyticum* have been implicated in human diseases.<sup>2,5</sup> *U. urealyticum* can be found in the cervix or vagina of 40-80% of sexually mature, asymp-

tomatic women and *M. hominis* in 21-53%.<sup>2,12</sup> In women, colonization of *M. hominis* and *U. urealyticum* are linked to younger age, lower socioeconomic status, sexual activity with multiple partners, black ethnicity and oral contraceptive use.<sup>7</sup> Diseases for which they have been suspected to play an etiological role are: acute pyelonephritis, bacterial vaginosis, pelvic inflammatory disease, chorioamnionitis, postabortion and postpartum fever, respiratory distress syndrome and pneumonia in newborns, wound and joint infections, nongonococcal urethritis, prostatitis and epididymitis.<sup>5,14</sup>



In healthy women vaginal flora may be different according to pH of secretions and level of glucogen in the vaginal epithelium, these factors depend on the ovary function. The production of cervical mucus as response to estrogenic stimulus is the normal secretion of vagina. The normal status of vagina is in function of estrogen levels, acid pH and Doderlein rods, if there is a change in these relationships, this could predispose to infection and nonspecific vaginitis could appear.<sup>13</sup> Bacterial vaginosis or nonspecific vaginitis is a defined syndrome, the hallmark of which is an excessive malodorous vaginal discharge associated with many microorganisms. The diagnosis for bacterial vaginosis requires at least three of the following signs: excessive vaginal discharge, vaginal pH > 4.5, clue cells and a fishy, amine-like odor in the KOH test.<sup>1</sup>

Bacterial vaginosis is a clinical syndrome resulting from replacement of the normal H<sub>2</sub>O<sub>2</sub> producing *Lactobacillus* spp. in the vagina with high concentration of anaerobic bacteria, *Gardnerella vaginalis* and *M. hominis*. Bacterial vaginosis previously known as nonspecific vaginitis is the most common cause of vaginal discharge. It may be the cause of up to one half of cases of vaginitis in all women and the cause of from 10-30% of cases in pregnant women.<sup>6</sup>

Normal levels of estrogen and progesterone in healthy not pregnant women protect them against an infection, but during pregnancy and contraceptive use, the levels of hormones are higher, which produce changes in vagina that could predispose to infection by mycoplasmas.<sup>13</sup>

In Mexico there are few studies that report the frequency of *M. hominis* and *U. urealyticum* but micoplasmas may produce 25% of cervical infection.<sup>3,4</sup> The purpose of this study was to determine the frequency of *M. hominis* and *U. urealyticum* in pregnant, delivery and control women and to relate the presence of these microorganisms with some indicators of nonspecific vaginitis.

## MATERIALS AND METHODS

**Population.** Women between 19–40 years old admitted to the gynecology service of the University Hospital of Puebla were included in the study and they were divided in three groups: a) 100 pregnant women; b) 100 women at delivery and c) 100 control women (non-pregnant) from which vaginal swabs were taken.

**Collection of specimens.** Vaginal discharge was collected with cotton-tipped swabs. Two swabs were used for *M. hominis* and *U. urealyticum* isolation. One swab was used to prepare a Gram stained smear and a wet mount. The vaginal pH was measured with pH paper strips, but care should be taken not to touch the endocervix. Amine-like odor in KOH was done according to Amsel *et al.*<sup>1</sup>

**Culture and identification.** Vaginal swabs were dipped inoculated into 1 ml of E broth supplemented with arginine (0.1%) or urea (0.05%). E broth composition (100

ml) is: Mycoplasma broth base 2.0 g, 0.5 ml of phenol red solution (0.4%), 25 % horse serum, 10 ml of yeast dialysate and 200 000 UI of penicillin G. Cultures were incubated at 37°C until the phenol red indicator changed to a purple color suggesting the hydrolysis of arginine or urea.

*M. hominis* was identified by arginine hydrolysis, colonial morphology and sensitivity to lincomycin and *U. urealyticum* was identified by urea hydrolysis, colonial morphology and sensitivity to erythromycin. Antibiotic sensitivity tests were done using paper disks with lincomycin (15 µg/ml) or erythromycin (100 µg/ml) and layered on E agar with arginine or urea previously inoculated with *M. hominis* or *U. urealyticum*.

**Statistical analysis.** Chi square was done in order to associate the presence of *M. hominis* or *U. urealyticum* from different groups of women with the indicators of nonspecific vaginitis.

## RESULTS

Three hundred women were included in the study and divided in three groups (Table 1). There was an association between the presence of *M. hominis* and pregnant women ( $p < 0.0001$ ).

Table 2 shows the association of the presence of *M. hominis* and *U. urealyticum* and age of the women. *M. hominis* and *U. urealyticum* were more frequently isolated 19-29 years old ( $p = 0.0118$ ).

There was an association between the presence of *M. hominis* and *U. urealyticum* and vaginal pH (Table 3). *M. hominis* was isolated at a higher percentage at pH between 5-6. Both mycoplasmas were more frequently isolated at pH less than 7 ( $p < 0.0001$ ).

Table 4 shows the association of the presence of *M. hominis* and *U. urealyticum* with the production of amine-like odor. Both microorganisms were positive for the production of amine-like test ( $p < 0.0001$ ).

There was also an association between the isolation *M. hominis* and *U. urealyticum* from women with clue cells ( $p < 0.0001$ ) (Table 5).

Two hundred and two women had leucorrhea at the moment the samples were taken. All the women from whom *M. hominis* was cultured showed leucorrhea and seventy four women from whom *U. urealyticum* was cultured had leucorrhea ( $p < 0.0001$ ) showing an association between the presence of mycoplasmas and leucorrhea (Table 6).

Amsel criteria (leucorrhea, vaginal pH > 4.5, odor amine-like test and presence of clue cells) were used to determine bacterial vaginosis or nonspecific vaginitis.<sup>1</sup> A bacterial vaginosis was diagnosed if three of these criteria were filled. Table 7 shows the association of *M. hominis* and *U. urealyticum* with nonspecific vaginitis ( $p < 0.0001$ ). Eighteen of twenty two women from whom *M. hominis* was isolated showed nonspecific vaginitis and fifty five of eighty women from whom *U. urealyticum* was isolated

Table 1. Isolation of *M. hominis* and *U. urealyticum* from different groups of women.

	Pregnant women		Delivery women		Control women		Total
	Positive F (%)	Negative F(%)	Positive F(%)	Negative F(%)	Positive F(%)	Negative F(%)	
<i>M. hominis</i>	12 (12)	88 (88)	5 (5)	95 (95)	5 (5)	95 (95)	300
<i>U. urealyticum</i>	31 (31)	69 (69)	21 (21)	79 (79)	28 (28)	72 (72)	300

% = Percentages were calculated from the total samples for each group. F = Frequency  
There was statistical difference between isolation rates ( $X^2$   $p < 0.0001$ )

Table 2. Isolation of *M. hominis* and *U. urealyticum* from different groups of women according to age.

	AGE (YEARS)					
	19 - 29		30 - 39		> 40	
Microorganism isolated	A	B	A	B	A	B
Pregnant women	11	24	1	7	0	0
Delivery women	4	15	1	5	0	1
Control women	2	11	2	10	1	7
Total	17	50	4	22	1	8

A. Number of positive *M. hominis* cultures.

B. Number of positive *U. urealyticum* cultures.

There was an association between the presence of microorganism and age ( $X^2$   $p = 0.0118$ )

Table 3. Association of the *M. hominis* and *U. urealyticum* with different vaginal pH.

	pH < 5		5 < pH < 6		6 < pH < 7		7 < pH < 8		pH > 8	
	A	B	A	B	A	B	A	B	A	B
Pregnant women	4	12	8	12	0	3	0	1	0	3
Delivery women	0	0	0	0	4	8	0	6	1	7
Control women	0	8	2	10	3	8	0	1	0	1
Total	4	20	10	22	7	19	0	8	1	11

A. Number of positive *M. hominis* cultures.

B. Number of positive *U. urealyticum* cultures.

There was an association between the presence of mycoplasmas and vaginal pH ( $X^2$   $p < 0.0001$ )

Table 4. Isolation of the *M. hominis* and *U. urealyticum* in women with positive odor amine-like test.

Microorganism isolated	Positive odor amine-like test	Negative amine-like test	Total
<i>M. hominis</i>	20	2	22
<i>U. urealyticum</i>	68	12	80
Without mycoplasmas	77	121	198

There was an association between the presence of mycoplasmas and positive odor amine like test ( $p < 0.0001$ )

Table 5. Isolation of the *M. hominis* and *U. urealyticum* in women with clue cells.

Microorganism isolated	With clue cells	Without clue cells	Total
<i>M. hominis</i>	18	4	22
<i>U. urealyticum</i>	68	12	88
Without mycoplasmas	35	163	198

There was an association between the presence of mycoplasmas and production of clue cells ( $p < 0.0001$ )

Table 6. Isolation of the *M. hominis* and *U. urealyticum* in women with leucorrhea.

Microorganism isolated	With leucorrhea	Without leucorrhea	Total
<i>M. hominis</i>	22	0	22
<i>U. urealyticum</i>	74	6	80
Without mycoplasmas	106	92	198
	202	98	300

There was an association between the presence of mycoplasmas and leucorrhea ( $p < 0.0001$ )

Table 7. Isolation of *M. hominis* and *U. urealyticum* in women with nonspecific vaginitis.

Microorganism isolated	With nonspecific vaginitis	Without nonspecific vagini-	Total
<i>M. hominis</i>	18 (17%)*	4 (2%)	22
<i>U. urealyticum</i>	55 (52%)	25 (13%)	80
Without mycoplasmas	32	166	198
Total	105	195	300

Percentages were calculated with respect to the total of each group.

There was an association between the presence of mycoplasmas and nonspecific vaginitis ( $p < 0.0001$ )

showed nonspecific vaginitis.

## DISCUSSION

*M. hominis* and *U. urealyticum* are part of the microbial flora of the genitourinary tract in asymptomatic sexually active women, however only *U. urealyticum* has been implicated in diseases of humans at this level.<sup>2</sup> The isolation rates of these microorganisms in the world are diverse and controversial because these bacteria are generally isolated together with other pathogens,<sup>2</sup> so it is very difficult to know if they are the cause of the diseases. The purpose of this study was to determine the presence of *M. hominis* and *U. urealyticum* in three groups of women and to relate the presence of these microorganisms with the criteria used to diagnose nonspecific vaginitis.

Although there was only statistical difference in the isolation rate of *M. hominis* in pregnant women respect to the other groups, the percentages of isolation are lower according to those reported in other studies,<sup>7,8,11</sup> however these results could be due to the group of study because colonization of these microorganisms in humans are linked to younger age, lower socioeconomic status, sexual activity with multiple partners, black ethnicity and oral contraceptive use,<sup>7</sup> in the population that we studied it is difficult to know which factor is associated with the presence of *M. hominis*, the isolation of mycoplasmas was lower at the moment of delivery probably because by the end of pregnancy mother synthesizes more antibodies against mycoplasmas and these could decrease the presence of viable mycoplasmas.

*M. hominis* and *U. urealyticum* were more frequently isolated from younger women according to McCormack,<sup>7</sup> *M. hominis* and *U. urealyticum* were recovered more frequently from women with vaginal acid pH, it was reasonable in the case of *U. urealyticum* because its optimum pH for growth is 5-6, while *M. hominis* grows better at neutral pH.<sup>8</sup> The absence of lactobacilli could produce a light increase in pH and favor the proliferation of mycoplasmas. The isolation rate of mycoplasmas was higher in women with positive test for amine-like odor in KOH and women with clue cells in the different groups. During bacterial vaginosis some amines are produced because of decarboxylation of some amino acids and this is the cause of the fishy odor (amine odor).<sup>3</sup> *M. hominis* is able to decarboxylate arginine while *U. urealyticum* hydrolyzes urea. Mycoplasmas could adhere to the epithelial cells exfoliated by the alkaline pH producing clue cells. During pregnancy there is an increase in the cases of bacterial vaginosis probably because of the changes in the vagina produced by the increase of levels of sexual hormones.<sup>3</sup> These results are according to that reported by Paavonen. He showed that changes in the vaginal pH could favor the overgrowth of some microorganisms including mycoplasmas.<sup>8</sup> The pathogenesis of bacterial vaginosis is still unclear, al-

though it is frequently associated with *G. vaginalis* and anaerobes.<sup>1</sup> Mycoplasmas have been associated with bacterial vaginosis a condition in which vaginal lactobacilli diminish but other bacteria proliferate (*G. vaginalis* and anaerobes).<sup>13</sup> When it became clear that *G. vaginalis* was probably not the sole agent involved in the etiology of bacterial vaginosis, a number of workers investigated the possible role of *M. hominis*. While there seems no doubt that *M. hominis* is associated with bacterial vaginosis, whether it makes a real contribution to the pathological process is still unclear.<sup>5,14</sup> The main contribution of this study was the strong association of *M. hominis* and *U. urealyticum* with some indicators of nonspecific vaginitis. The tests used to detect these indicators are easy to perform by any physician and these could be very helpful in the diagnosis.

## CONCLUSION

*Mycoplasma hominis* and *Ureaplasma urealyticum* were more frequently isolated from women with positive indicators of nonspecific vaginitis (positive amine-like odor in KOH test, positive clue cells and leucorrhea) than in women without bacterial vaginosis. Although the role of these microorganisms in nonspecific vaginitis is not clear, we suggest to isolate them together with *G. vaginalis* and anaerobes in cases of nonspecific vaginitis.

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