Signs and symptoms of urethritis and cervicitis among women with or without genital mycoplasma infection in governorate of Basrah

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Abstract:

Due to the absence of antisera in Iraq, thereupon, in this search, the isolated species which belonged to genus Mycoplasma are presumptive (probably) species of genital mycoplasma, the characteristic and biochemical properties of them were fit with those of *M.hominis*, *U.urealyticum*, *M.fermentans*, *M.genitalium and M.penetrans* in dependence on Bergey's Manual of determinative bacteriology (Holt *et al.*, 1994).

This study was current on 120 women attending the outpatient clinic of the obstetric and gynecology department of Basrah General Hospital during the period from February to July, 2007. Comprised women who suffered from some obstetric and gynecological conditions, for that purpose urethral swabs and the other from endocervix region were cultured then handled and processed with a Monophasic-diphasic culture setup (MDCS), a statistically significant difference at the level of (P< 0.01) was noted in the isolation of both presumptive mycoplasmal species *M.fermentans* and *M.hominis* from urethral region in comparison with endocervix region while, the isolation rates of *U.urealyticum* and *M.genitalium* were highest from endocervix region.

Also, the results show, presumptive (probably) mycoplasmal species: *M.fermentans*, *U.urealyticum*, *M.hominis* and *M.penetrans* were more frequently distributed in women who were complaining of vaginal discharge followed by urethral abnormal and itching. Statistically, the associated significance was noted only in case of *M.hominis* at level of (P< 0.05). The genital mycoplasmas were recovered 15.0 percent in 18 cases as a single infection and 20.8 percent in 25 cases as a mixed infection with other causative agents (bacterial other than mycoplasmas), so this study showed the conjunction of *U.urealyticum* with *M.hominis* in 5 cases (4.1 %) and the *M.genitalium* with *M.fermentans* in 6 cases (5.0 %). Finally, the *E.coli* was found as a single infection in 5 cases and conjuncted with *E.faecalis* in 3 cases (2.5 %) while, *P.aeruginosa* found as a single infection in 4 cases and recovered 0.8 percent in 1 case only as a mixed infection with *S.epidermidis*.

Keywords: Genital mycoplasma, Gynecological condition, urethritis, vaginitis, cervicitis, Basrah governorate.

Introduction

The mycoplasmas are spherical to filamentous cells with no cell walls, and they are consequently placed in a separate class Mollicutes (mollis, soft, cutis, skin), comprising more than 150 species [1-2]. At least five species of mycoplasmas can be isolated from genitourinary tract as a major importance to human health (causative agents of disease):*Mycoplasma penetrans* was first reported in 1993 as an emerging infectious agent which is linked to the urogenital tract disease as sexually transmitted agents and isolated from patients with a severe immunodeficiency [3-4,18,27].*M.hominis* is a case of pelvic inflammatory disease, septic

abortion, salpingitis and occasionally causes postpartum fever [7-8,22,26]; both *M.genitalium & M.fermentans* are associated with non-gonococcal urethritis in men and cervicitis in women. Also, are linked to urethral infections [6,9,19,21,24], and *Ureaplasma urealyticum* is a case of nongonococcal urethritis in adults and lung disease in premature infants [3,10,20,25].One of the most useful distinguishing features of mycoplasmas is their peculiar fried-egg colony shape, consisting of a central zone of growth embedded in the agar and a peripheral one on the agar surface [1,3,20]. Nutritionally, mycoplasmas are very exacting ; many require cholesterol (a unique property among prokaryotes). Ureaplasmas require urea for growth , arginine and the usual carbohydrates are not metabolized . Generally, mycoplasmas require a specialized complex media for growth (PPLO, Soy Peptone , SP_4) [1,19-20] . Various serological tests have been developed and are useful in classification [22-23] . Most of these tests (or methods) are very expensive or unavailable in many developing countries. **The aim of this study therefore, was to 1-** adopt the simple and inexpensive method for the

rapid diagnosis of the genital mycoplasmas namely the monophasic-diphasic culture setup MDCS [24]; **because** the possible role of mycoplasmas in conditions like bacterial vaginitis, symptoms of urethritis and cervicitis has been indicated by many researchers [14-15,19,32-33] while, the others not [9,18,31] **thereupon, 2-**In this study an attempt was made to relate the isolation of genital mycoplasmas from cases with some urogenital symptoms as predictor for mycoplasmal colonization of the genital tract of women.

Methodology

Study population :The population under study was among women attending the outpatient of obstetric and gynecology department of Basrah General Hospital . The samples obtained consisted of women with genital tract symptoms or signs (vaginal discharge, urethral pain during micturition, urethral discharge) . A total of (120) women were investigated during the period extending from February to July of 2007, their ages ranged from 15-54 years.

Collection of specimens:

Two swabs from urethra and the other from endocervix were obtained from each women by a doctor, and each was inoculated onto a suitable medium (the modified PPLO agar / broth) according to the MDCS . A sterile speculum was used . All swabs were transported to the laboratory within one hour for culture [3,24].

Media for isolation and identification species of genital mycoplasmas (Applied according to Marmion and Harris, 1996):

1- Liquid medium (liquid-phase)

PPLO broth	
	70ml
Horse serum	20ml
Yeast extract	10 1
Thallium acetate 1 in 80 (w/v)	10ml
Penicillin (50000 L.U./ml)	1111
Glucose solution 10% (w/v)	0.2ml
$C_{rocol} = rod 0.20/(m/m)$	IOml
Cresor red 0.2% (w/V)	1 ml
DNA 0.2 (w/v)	1 ml
K_2 HPO ₄ 1.0 (mol/liter)	1111
$\mathbf{T}_{\mathbf{k}} = \left\{ \begin{array}{c} \mathbf{r}_{\mathbf{k}} \\ \mathbf{r}_{$	2ml
I ne final pH snould $De(/.8)$.	

1-a. Base Mycoplasms (PPLO) broth.

 Beef heart infusion Peptone Sodium chloride (NaCl) 	50gm 10gm 5gm
2- <u>Solid media</u> (Solid-phase)	
PPLO agar	70ml
Horse serum	20ml
Yeast extract	10ml

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Thallium acetate 1 in 80 (w/v)	1 ml
Penicillin (50000 I. U./ml)	0.2ml
Glucose 10% (w/v)	10ml
DNA 0.2 (w/v)	1 ml
K ₂ HPO ₄ 1.0 (mol/liter)	2ml

2-b. Base Mycoplasma (PPLO) agar.

1- Beef heart infusion	50gm
2- Peptone	10gm
3- Sodium chloride (NaCl)	5gm
4- Agar	14gm

Cultivation and isolation of mycoplasmas (genital mycoplasmas) and other bacteria:

Urethral and endocervix swabs were taken from the women under study, then each specimen was directly inoculated into the liquid phase of the MDCS, mixed up well and then tilted once or twice to cover the upper portion of the slant for a while prior to incubation [24]. All inoculated media were incubated aerobically at 37 C⁰ and observed daily for colour change from red to yellow in the liquid phase after 24 hrs. Isolated colonies appeared after that on the slanted solid phase. Sheep erythrocytes (7 %) and the suspension of egg-yolk (15 ml) were added to the solid phase of the MDCS and standard PPLO agar to detect both ; blood haemolysis of *M.fermentans*, *U.urealyticum* and lipolytic ability by *M.fermentans* only [3,24]. Also, other same swabs (specimens) were obtained from the women under study for bacterial isolation other than mycoplasmas, then; each specimen (swab) was directly cultured by the streaking method onto MacConkey and Blood agar then incubation after transported to the laboratory within (one) hour [1,25]

Tests for identification of genital mycoplasmas (**species**) were applied according to Bergey's manual of determinative bacteriology (1994), as shown in Table (1).

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Results:

In table (2) the difference noted was significant for both mycoplasmal species *M.fermentans* and *M.hominis* with a higher percentage from the urethra region than endocervix region (x^2 =40.96; p<0.01), respectively, $(x^2 = 16.00;$ p<0.01) whereas, U.urealyticum and M.genitalium were isolated in higher percentage from the endocervix region than the urethra region with a significant difference (p<0.01) for only M.genitalium. In case of *M.penetrans* the freedom degrees correspond to zero therefore we can't calculate the x^2 value (CNC). The relation between genital mycoplasmas and some urogenital complaints is shown in table (3). A difference was found to be just significant for *M.hominis* $(x^2=6.63; p<0.05)$ and *M.penetrans* $(x^2=23.33; p<0.01)$ that's evident, from the higher isolation rates of these microbes which associated with women complaining of vaginal discharge followed by urethral discharge and itching, generally, four mycoplasmal species: M.fermentans, U.urealyticum, M.hominis and M.penetrans tend to be frequently distributed in women complaining of

vaginal discharge except *M.genitalium* tends to be frequently distributed in women complaining of urethral discharge followed by vaginal discharge and itching. The total isolates identified as genital mycoplasmas were 43 of 120 urethral and endocervix swabs, which correspond to 15.0 percent distributed in 18 cases as a single infection with genital mycoplasmas and 20.8 percent in 25 cases as a mixed infection with other causative agents, as shown in Table (4).

Concerning figure (1) it shows *U.urealyticum* as a single causative agent in 7 cases and associated with *M.hominis* in 5 cases, which corresponds to 4.1 percent, whereas*M.genitalium* was found as a single causative agent in 3 cases and associated with *M.fermentans* in 6 cases, with a rate of 5.0 percent. Figure (2) shows *E.coli* as a single causative agent in 5 cases and associated with *E.faecalis* in 3 cases, with a rate of 2.5 percent. While *P.aeruginosa* was found as a single causative agent in 4 cases and associated with *S.epidermidis* in 1 case, which corresponds to 0.8 percent.

	No. of isolates (+ve)	No. of tested women 120						
Presumptive Species of genital mycoplasma		Sources of isolation						
		Urethra	%	Endocervix	%	\mathbf{X}^2	Р	
Mycoplasma fermentans	11	9	81.8	2	18.1	40.96	0.01	
M.genitalium	9	3	33.3	6	66.6	11	0.01	
Ureaplasma urealyticum	12	5	41.6	7	58.3	2.56	NS	
M.hominis	10	7	70	3	30	16	0.01	
M.penetrans	1	1	100	0	0	CNC		
Total	43		•		•			

Table (2): Association between genital mycoplasmas and sources of isolation

		No.and (%) of women +ve in						
Urogenital complaints	No.of tested women	M.fermentans	M.genitalium	U.urealyticum	M.hominis	M.penetrans		
Vaginal discharge	55	6(10.9)	4(7.2)	8(14.5)	7(12.7)	1(1.8)		
Itching	25	1(4.0)	0	0	1(4.0)	0		
Urethral discharge	40	4(10.0)	5(12.5)	4(10.0)	2(5.0)	0		
Total	120	11	9	12	10	1		
X ²		3.44	1.8	1	6.633	23.33		
Р		NS	NS	NS	0.05	0.01		

Table (3): Association of genital mycoplasmas with some urogenital complaints

Table (4):presence of genital mycoplasmas alone or in conjunction with other bacteria

No. of tested women 120										
Total of in conjunction with										
genital mycoplasmas	Alone	E.coli	P.a	aeruginosa	K.p	oneumonia	E.faecalis	S.aureus	P.mirabilis	S.epidermidis
43	18	8		5		5	3	2	1	1



Figure(1): Probably four species of genital mycoplasmas alone or in association



Figure(2): Bacterial isolates (other than mycoplasmas) alone or in association

Discussion:

Mycoplasmas have been implicated as an etiological factor (as pathogens) of the human genitourinary tract [26]. Their isolation rates of these microorganisms in the world are diverse and controversial because these bacteria are generally isolated together with other pathogens,

so it is very difficult to know if they are the cause of the disease [28]. Various laboratory tests have been designed to diagnose mycoplasma infections [2,20-27,23-24,9]. The diagnostic value and applicability of any method depends on its simplicity, rapidity, reproducibility and cost thereupon, the method of monophasic-diphasic culture setup MDCS was used in this recent search for the rapid isolation and identification of genital mycoplasmas from clinical specimens [3,24].

Statistically the difference was significant (P<0.01), in the isolation rates of *M.fermentans* and M.hominis from the urethra region than endocervix region, whereas both U.urealyticum were isolated in higher and *M*.genitalium proportion from endocervix region than urethra region with a significant difference (X2=11.00; P<0.01) for *M.genitalium* Table (2). Graber *et al* (30) found a significant reduction in the sperm counts and the mean percentage of progressive sperm motility in the Ureaplasma positive group. And in the study of Friberg (29) the presence of U.urealyticum was significantly higher in the infertile group than in a group of pregnant women. Also, Clausen et al [31] found that the mycoplasmas especially M.genitalium may be an independent risk factor in the development of an inflammatory process leading to the scarring of the uterine tubes in women and thereby causing infertility. Thereupon, the results of the present search indicated that both U.urealyticum & M.genitalium were recovered in higher proportion rates from endocervix than urethra of infected women, may be associated with inflammation disease leading to infertility. Furthermore, in the present study, it is evident that the highest isolation rates of M.fermentans, U.urealyticum, M.hominis and M.penetrans were associated with women complaining of vaginal discharge followed by urethral discharge and itching with a significant difference for both M.hominis & M. penetrans wheres, M. genitalium was isolated in a higher rate from women complaining of

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3- B. Marmion., and R. Harris. *Mycoplasma pneumoniae* and other medically important members of the family mycoplasmataceae In:Mackie & Macrtiney Practical medical microbiology, Vol.30,pp.591-595; 600-601, urethral discharge with no significant difference Table (3). The same results were reported by several investigators [14-15,19,32-33].

While the others found that the presence of the vaginal discharge, itching and moniliasis is not a sufficient indicator for mycoplasmal infection [18,9,34]. Besides, among the suggestions that might be taken into consideration are the possibilities that strain variations or a threshold number of microorganisms may determine the outcome of the association of mycoplasmas as normal microbial flora of the host genitalia or as pathogens. On the other hand, in this study, the occurrence of genital mycoplasmas as a single causative agent corresponds to (15.0 %) and in mixed infection with other bacteria is (20.8 %), as shown in Table (4). In the study of Horner et al [18] they found that the rate of mixed infection caused by at least two pathogens was (15.8 %). Also, the current study shows that the associated rate of U.urealyticum with M.hominis was (4.1 %) and that of , *M.genitalium* associated with *M.fermentans* in (5.0 %), as

appeared in Figure (1). Concerning Al-Bahli [35] study. the association between both (U.urealyticum and *M.hominis*) was also detected. Finally, the present research shows that, the associated rate of E.coli with E.faecalis was (2.5 %) and that of , P.aeruginosa associated with S.epidermidis in (0.8 %) only, Figure (2). Al-Ali [36] noted that the percentages of most of the bacterial isolates from women using IUCDs were higher than women not-using IUCDs or any other contraceptive method with a rate of the mixed infection caused by at least two pathogens was (17.5 %).

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أعراض وعلامات التهاب الاحليل وعنق الرحم المرتبطة أو غير المرتبطة بالإصابة بالمايكوبلازما التناسلية للنساء في محافظة البصرة

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الخلاصة:

لعدم وجود المضاد المصلى في العراق فان الانواع المعزولة في هذا البحث والعائدة لجنس المايكوبلازما هي الانواع المحتملة من المفطور إت التناسلية, وكانت مميز إنها وخو اصبها البايو كيميائية مطابقة لكل من M.hominis و U.urealyticum و M.fermentans و M.genitalium و M.genitalium من المصدر المعتمد (Holt et من المصدر المعتمد M.genitalium) al., 1994) . أجربت هذه الدر اسة على 120 أمر آة من المر اجعات إلى العيادة الاستشارية الخارجية في قسم النسائية والتوليد في مستشفى البصرة العام للفترة من شباط إلى تموز 2007. حيث شملت النساء اللواتي يعانين من بعض الحالات النسائية الخاصة ولهذا الغريض زرعت مسحات من الاحليل وأخرى من منطقة عنق الرحم وعوملت بتقنية نظام الزرع أحادي الطور وثنائيه MDCS حيث لوحظ وجود اختلاف مهم إحصائيا وبفارق معنوى عال عند مستوى الأهمية (p< 0.01) في عـزل كـلا مـن. M.fermentans و M.fermentans المحتملة من منطقة الاحليل مقارنة بمنطقة عنق الرحم بينما كانت معدلات عزل الـ U.urealyticum و M.genitalium أعلى من منطقة عنق الرحم. كما وأظهرت النتائج بان كل من الانواع المايكوبلازمية المحتملة (الافتراظية) وهي M.fermentans و U.urealyticum و M.hominis و M.penetrans منتشرة بكثافة في النساء اللواتي يعانين من الإفرازات المهبلية المفرطة تتبعها إفرازات الاحليل غير الطبيعية والحكة والارتباط المهم إحصائيا لوحظ فقط في حالة M.hominis عند مستوى الأهمية (P< 0.05). كما و غطت المايكوبلازما التناسلية 15.0 % كإصابة مفردة في 18 حالة و 20.8 % متداخلة مع مسببات مرضية أخرى غير المفطورات (بكتريا أخرى غير المايكوبلازما) في 25 حالة على التوالي. . كذلك أظهرت تلك الدراسة تداخل إل U.urealyticum مع M.hominis في 5 حالة وبنسبة (4.1 %) إضافة إلى تداخل مفطورة M.genitalium مع M.fermentans في 6 حالة بنسبة (5.0 %) , وأخيرا وجدت جرثومةُ E.coli كإصابة منفردة في 5 حالة ومتداخلة مع E.faecalis في 3 حالة بنسبة (2.5 %) بينما جرثومة P.aeruginosa وجدت كإصابة منفردة في 4 حالة ومتداخلة مع S.epidermidis في 1 حالة فقط وبنسبة (0.8 %) . Journal of Basrah Researches ((Sciences)) Vol. 35, No.3, 15 June ((2009))