Pre valence of Genital Mycoplasma, *Mycoplasma hominis* and *Ureaplasma urealyticum* in Women in Al-Qadisiya Province. A New Record

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الخلاصة

استهدفت هذه الدراسة الكشف عن دور بكتريا *Ureaplasma urealyticum* و *Mycoplasma hominis* في اصابة الجهاز البولي والتناسلي حيث تم جمع 735 عينه من نساء متزوجات 530/735 وبنسبة (72.1%) ونساء عازبات 205/735 وبنسبة (27.9%) اللواتي يراجعن مستشفى النسائيه والاطفال التعليمي في مدينه الديوانيه ومستشفى الديوانيه التعليمي العام وعيادات خاصه وذلك خلال فتره(12 شهرا من نيسان 2011 الى نيسان 2012) شملت العينات مسحات من عنق الرحم، الاحليل، اعلى المهبل والادرار.

تم زرع النماذج على اوساط سا ئلة واخرى صلبة على ضوء هذه النتائج تم اعتماد هذين الوسطين في تنميه البكتريا حيث تم عزل *Mycoplasma hominis* و *Urea plasma urealyticum* من عينات الادرار، عنق الرحم،الاحليل، اعلى المهبل .

وبالنسبة لنوع البكتريا المسببة للإصابة أوضحت النتائج أن النوع *Ureaplasma urealyticum* كان أعلى من النوع *Mycoplasma hominis* بنسبة 1:3.9 بنسبة 79.4 % إلى 20.6 % على التوالي.

Abstract

Seven hundred and thirty five samples included endocervical, high vaginal, urethral and urine, were collected from married 530/735 (72.1%) and unmarried women 205/735 (27.9%) who admitted to Al-Diwaniya Maternity and Pediatric Teaching Hospital, Al-Diwaniya Teaching Hospital, and private clinics in Al-Qadisyia province, through a period of twelve months (from April 2011 to April 2012) in an attempt to isolate these bacteria.

Through the study two types of media were used for cultivation of the genital Mycoplasma.

The incidence rate of genitourinary infection caused by *U.urealyticum* was significantly higher (79.4%) while the incidence caused by *M.hominis* which a The main goal of this study was to detect and isolate the *M.hominis* and *U.urealyticum* from genitourinary tract of women Al-Diwaniya city.
ccounted (20.6%).

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Introduction

Mycoplasmas are unique types of bacteria. They are the smallest free living organism known on the planet able to multiply autonomously. Mycoplasmas do not have cell-wall, so can take many different shapes which make them difficult to identify. There is an attachment organelle at the tip of filamentous. Fried –egg-shaped colonies are seen on agar. It is so difficult to cultivate them in the laboratory and is often missed as pathogenic causes of disease for this reason. Moreover, they are completely depending on host sterols and wide range of biosynthetic precursors (amino acids, nucleotides, and fatty acids) (1).

The lack of atypical cell wall containing peptidoglycan renders these organisms insensitive to cell wall-active antimicrobial agents, such as penicillins and cephalosporins (2).

Mycoplasmas have adapted to wide varity of hosts and can colonize many other animals and plants. The colonizing organisms are host specific in human, since they colonize mainly the upper respiratory tract and the genitourinary tract causing atypical pneumonia, pyelonephritis, pelvic inflammatory disease, abortion, infertility, postpartum fever, bacterial vaginosis, neonatal bacteremia, meningitis, and abscesses (3).

They usually reside extracellular and rarely penetrate the sub mucosa, except in the case of immunosuppression or instrumentation, since they invade the blood stream and disseminate to numerous organs and tissues. Some species also occure as intracellular pathogens. *Mycoplasma* have very little DNA of its own ,but are capable of using DNA from the host cell leading to malfunction cell or can cause DNA mutation of host cell (4 ، 5).

Mycoplasmas are classified in the class (Mollicutes) which mean (soft skin) due to their lacking a rigid bacterial cell wall. The Mollicutes are Eubacteria that have probably derived from Lacobacilli, Streptococci and Closteridia (6).

Direct culture is the most common way to reliably detect *Mycoplasma* but there is no single medium formulation is adequate for all Mollicutes species due to their different nutritional requirements. Previously most Mycoplasmas were cultured on media composed in part of cell extracts and animal sera. Shepard and Lunceford (1976) by regressive evolution and genome (7).

Materials and Methods

Specimen's collection:

A total of 735 specimens were collected from women. Admitted to Al-Diwaniya Maternity and Pediatric Teaching Hospital, Al-Diwaniya Teaching Hospital, and private clinics in Al-Qadisyia Province during the period from April 2011 to April 2012; these specimens obtained from married women (530) either pregnant or non pregnant including: high vaginal (264), urethral

(216), endocervical swabs (42) and urine (8) samples, and unmarried (205) urine samples only.

Swabs were placed in tube containing special transport media as indicated to maintain the swab wet. Each specimen was put in ice bag until be taken to the laboratory for bacterial investigation. Specimens were incubated at 37℃ for 30 minutes, loopful were transferred to (Arginine broth) for *Mycoplasma hominis* and (A7 broth) for *Ureaplasma urealyticum*. All tubes were incubated aerobically at 37℃ for 24-72 hours, when the broth became an alkaline (arginine and urea changes) a small inoculums have been spreading on agar (Arginine agar medium) (4) for *M.hominis* and (A7 agar) (4) for *U. urealyticum* , incubated at 37℃ in candle jar with small wet cotton to provide a little moisture. Incubation for 3- 12 days. Colonies were investigated directly, since the colonies of *M.hominis* as fried-egg appearance, while the colony of *U. urealyticum* appear granular colonies and dark brown color due to accumulation of manganese oxide (8).

Biochemical tests were also used to detect genital mycoplaosma. Arginine hydrolysis, glucose fermentation, tetrazolium reduction, and urea hydrolysis (9).

Results

1- Colonial morphology:

light microscope on low power was used to exam bacterial colony. On agar medium (A7) *U.urealyticum* was identified as dark golden-brown or rich deep-brown colonies moreover, it was identified by its granular appearance (figure 1).

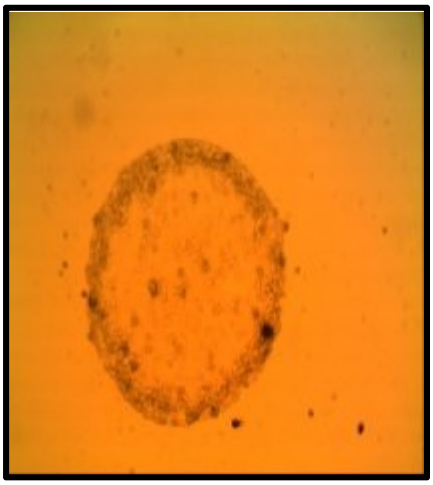

Figure (1) *U.urealyticum* colonies x40

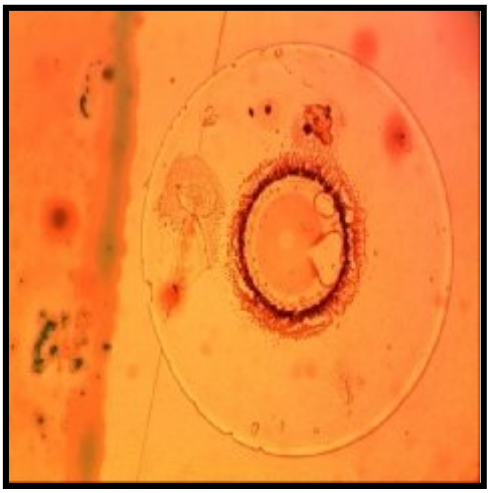

Figure (2) Fried –egg colony of *M.hominis* x40

M.hominis variable but takes a characteristic fried-egg aspect because the organisms pentrate deeply into the agar in the central region of the colony (figure 2).

Also, dissecting microscope was used to identify the *Mycoplasma* colony (figure 3)

Figure (3) *M. hominis* colony by disecting microscope

2- Biochemical testes:

All *M.hominis* isolates revealed positive arginine hydrolysis and negative for glucose fermentation, tetrazolium reduction and urea hydrolysis.These were similar results because of similar species. While *U.urealyticum* isolates reveled positive urea hydrolysis and negative glucose fermentation, arginine hydrolysis and tetrazolium reduction (9) .

Frequency of Isolation and identification of *M.hominis* and *U.urealyticum:*

The positive results of culture revealed that 443/735 (60.2%) for *M.hominis* and *U.urealyticum* versus 292 (39.8%) negative as shown in (figure 4).

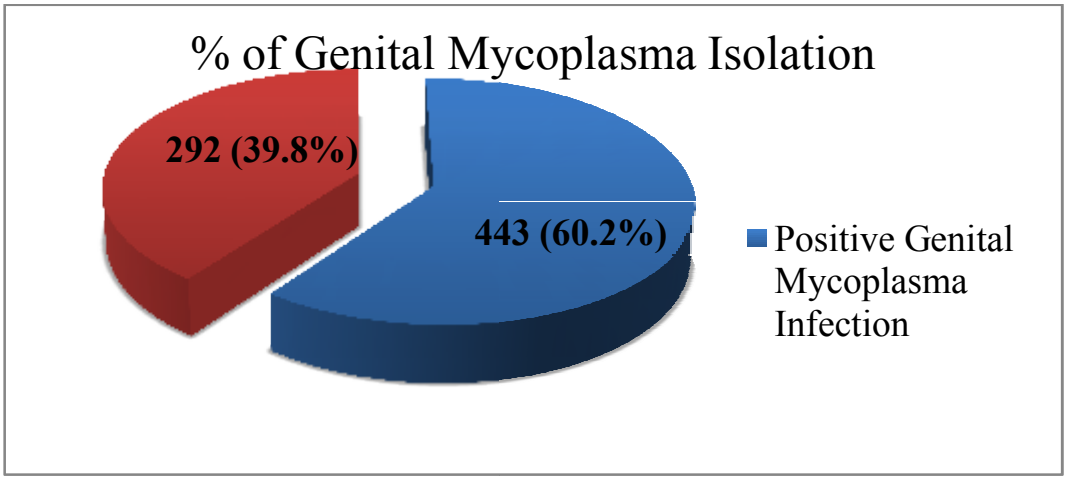

Figure (4) Percentage of isolation of genital mycoplasma

Regarding the social status of women, a total of 530 (72.0%), specimens were collected from married women, while 205 (28.0%) specimens were collected from unmarried women.

Relationship between the isolation of genital mycoplasmas and site of specimens.

Figure (5) shows the distribution of bacterial isolate of genital mycoplasmas according to the clinical specimens . The results exhibited that urine given high percentage of isolation 206/213 (97%) followed by high vaginal swab 128/264 (48.4%) while endocervical swab and urethral swab 19/42 (45.2%) and 90/216(41.6%) respectively.

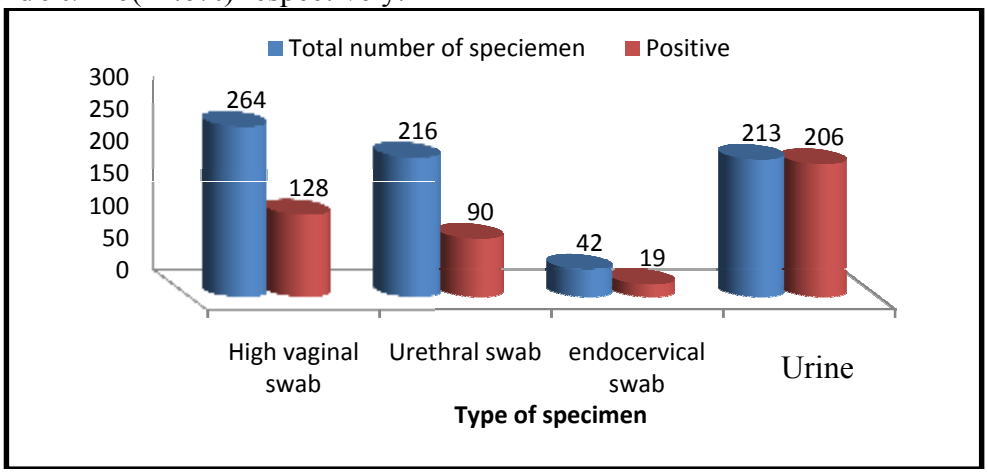

Figure (5) Distribution of genital mycoplasma isolates among clinical specimens

Prevalence of *M.hominis* and *U.urealyticum* in women

The results showed in figure (6) represent the incidence rate of genitourinary infection caused by *U.urealyticum* since it accounted for 352/443 (79.5%) of positive samples.

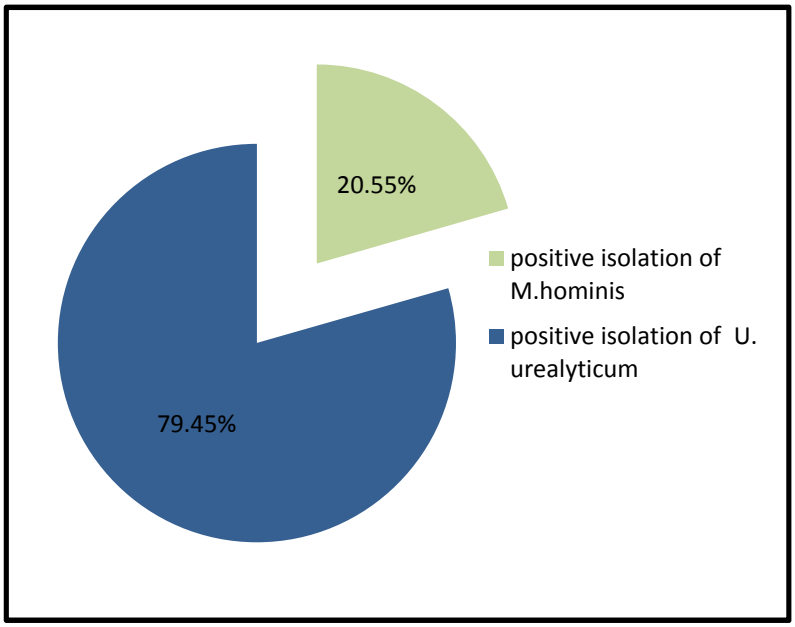

Figure (6) Percentage of isolation of *M.hominis* and *U.urealyticum*

It is high frequency compared with *M.hominis* which account for 91 (20.5%). Hence the infection ratio is (3.9:1)respectively.

Discussion

The results of the present study agreed with other studies that both married and unmarried women are encountered for *Mycoplasma* infection (10). Moreover, in a survey on women from a sexual health clinic in Australia, the rate of colonization with genital mycoplasmas were (57.0%). Another study pointed out that these bacteria were the most predominante microorganisms in female urogenital tract (11).

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Finding of this study showed that urine is the best sample for cultivation of *M.hominis* and *U.urealyticum*, and could be thought that these microorganisms colonise numerously in urogenital tract of women but could not be detected due to less sensitivity of general methods of microbiological cultivation methods.

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It was found that *U.urealyticum* seems to be is a common commensal of urogenital tract in both men and women (12). It is generally difficult to determine whether these agents cause colonization or infection. The incidence of infection is affected by some factors, such as: menstrual cycle and pregnancy, use of vaginal contraceptive, bacterial and protozoan infection (co-infections), and socio-economic conditions like poverty, and multiple of sexual partners (13, 14).

Conclusions

The incidence rate of genitourinary infection due to genital mycoplasma is considerably higher as compared with other cause of bacteria. The frequency of *U.urealyticum* in urogenital infection is higher than *M.hominis.*

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