

Rapid Detection and Antibiotic Susceptibility of Genital Mycoplasma Isolated from Male with Urethritis and Prostatitis, Iraq

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Ureaplasma urealyticum and *Mycoplasma hominis* are most important genital mycoplasma responsible for many genitourinary tract infections. Nongonococcal urethritis (NGU) and bacterial prostatitis are most clinically significant infections that affect a wide spectrum of male age groups. The incorrect or inappropriate diagnosis and susceptibility testing leads to unsuitable medication and as a results delay of recovery and bad squeal like infertility. During a period of six months, twenty two expressed prostate secretion (EPS) samples and eighteen urethral swabs were collected from patients with age (44.55±21.22 years). All samples were inoculated to strip and after 24 hours at 37° C the results recorded. The results of detection of genital mycoplasma revealed that only 3 (16.7%) of expressed prostate secretion samples give positive results for both *Ureaplasma urealyticum* and *Mycoplasma hominis* while 10 (45.5%) urethral swab were positive for both *Ureaplasma urealyticum* and *Mycoplasma hominis*. Common age group of prostatitis infected with genital mycoplasma is (41-60 years) while urethritis was detected among all three groups. The results of antibiotic susceptibility revealed that all isolates were fully sensitive (100%) to all antibiotics incorporated within the well except for erythromycin, roxithromycin, tetracyclin and levofloxacin in which one isolate (7.7%) was exhibit resistance in both *Ureaplasma urealyticum* and *Mycoplasma hominis*. The current study conclude the genital mycoplasmas (*Ureaplasma urealyticum* and *Mycoplasma hominis*) are predominant among non gonococcal urethritis and the macrolides still the choice for treatment while prescription of quinolones and tetracyclin must be under control due to emergence of resistance isolates.

Keywords: *U. urealyticum*, *M. hominis*, Prostatitis, EPS.

Mycoplasmas are cell wall lacking, small bacteria that not effected by cell wall synthesis inhibiting antibiotics. Clinically significant Mycoplasmas includes *Ureaplasma urealyticum*, *Mycoplasma pneumoniae*, *Mycoplasma genitalium*,

and *Mycoplasma hominis*¹. As a causative agents of non-gonococcal, con-chlamydial urethritis and prostatitis *Ureaplasma urealyticum* and *Mycoplasma hominis* were recovered from many males and females undergoing sexually transmitted disease with variable percentage^{2,3}.

Inflammation of urethra called urethritis and it is may be due to bacterial, viral and

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trichomonal infection. Bacteria urethritis is most common and be on two form: gonococcal and nongonococcal urethritis. Nongonococcal urethritis is emerge due to bacterial infection other than those caused by gonococci. Commonly bacterial causes of nongonococcal urethritis comprises: *CHYPERLINK* "https://en.wikipedia.org/wiki/Chlamydia_trachomatis".*HYPERLINK* "https://en.wikipedia.org/wiki/Chlamydia_trachomatis" *trachomatis*, *U. urealyticum* and *M. hominis*^{4,5}. Bacterial prostatitis is the infection of prostate gland and regards hard-treated urologic illness that need 2-12 weeks of antibiotics treatment to be cured effectively. Genitourinary mycoplasmas (*U. urealyticum* and *M. hominis*) can cause prostatitis and may implicated in the prostate cancer development^{6,7}.

Both of *U. urealyticum* and *M. hominis* were associated with sexually transmitted diseases and have the ability to destroy genitourinary epithelial cells^{8,9}. Three species have been isolated from the surface of the genitourinary tract mucosa: *M. hominis*, *U. urealyticum* and recently discovered *Mycoplasma genitalium*^{10,11,12}. *M. hominis* colonizes the genito-urinary tract and behave as an opportunistic pathogen, causing urethritis, cervicitis, pelvic inflammatory disease or chorioamnionitis. *U. urealyticum* regarded the common pathogen among men with urethritis and its complications^{13,14}.

Abuse and misuse of antibiotics leads to emerging of antibiotics resistance which push a risk for human health. Due to that they are targeting cell wall, the β -lactam antibiotics and vancomycin are not active against *Mycoplasmas* and their susceptibility be conferred to only agents that inhibit protein synthesis, including aminoglycosides, macrolides, tetracycline chloramphenicol. They are also sensitive to that anti-topoisomerases antibiotics like fluoroquinolones¹⁵. Spreading of resistance to tetracycline and erythromycin leading to the use of clindamycin, fluoroquinolones or other macrolides following the failure of treatment with tetracycline or erythromycin^{16,17,18}.

The current study aimed to detect genital mycoplasma (*U. urealyticum* and *M. hominis*) and their antibiotic susceptibility among nongonococcal urethritis and prostatitis patients in Hilla city-Iraq.

MATERIALS AND METHODS

Samples

Twenty two expressed prostate secretion (EPS) samples and eighteen urethral swabs were collected from patients with age (44.55±21.22 years) during a period of 6 months. All samples were transfereed directly to transport media supplied with kit^{19,20}.

Mycoplasma IES Plus Kit Principle

It is Dehydrated culture medium based assay for the screening, indicative enumeration and the identification of UU (*U. urealyticum*) and MH (*M. hominis*) in genitourinary tract of human. In the case of positive screening result, Mycoplasma IES Plus provides an extra strip for further susceptibility testing, which is partially based on CLSI (Clinical and Laboratory Standards Institute) recommendations. The mixed medium is prepared by mixing the freeze-dried powder and the diluent. After Mycoplasma has been cultivated, urease in UU will breakdown the urea and release NH₃. Release of NH₃ also occur by MH via breakdown of arginine by arginase leading to increases the pH of the liquid medium, the result is judged according to the color change of the indicator. The strip contains 11 antibiotics. Changing the color to red meaning growth of UU, MH or both although presence of antibiotics which reveal resistance to the specific antibiotic in the well²¹.

Mycoplasma IES Plus Kit Component

The kit components includes:
1-10 strips (with 30 wells have dehydrated culture medium and another supplied with antibiotics with different concentration).
2- 10 vial of Freeze-dried Powder
3- 10 vial transport medium
4- 1 vial of mineral oil

The scheme of strip and component of each well were mentioned below (figure 1):

Culture and identification and Enumeration (wells no. 1, 2, 3, 4, 5)

Well no. 1 (UU): identification of *U. urealyticum*, Well no. 2 (MH): identification of *M. hominis*, Well no. 3 (UU e⁺ 10⁴): enumeration of *U. urealyticum*, Well no. 4 (MHe⁺ 10⁴): enumeration of *M. hominis* and Well no. 5 (C+): positive control (table 1).

Susceptibility tests (wells no. 6 to 30)

These wells are used to test the susceptibility of the strain with 11 antibiotics (table 2).

Strip Inoculation

The sample transferred directly to the transport medium, mixed well and transferred completely to the freeze-dried powder, and shake to

mix completely. After the sample is inoculated, 0.5 ml of the inoculated medium incubated at 36-38 °C for 24 hours and if the color of the culture medium does not turn to red or peachblow, the sample could be deemed to be negative and no further operations are needed. When the color of the culture medium turns to red or peachblow, a 100 µl of the culture medium will dispensed to the wells of strip, the strip was shaken to dissolve the coated materials and then one drop of the mineral oil will added to prevent evaporation during incubation. The cover the strip placed and the strip will incubated at 36-38 °C for 24 hours. The yellow color indicate negative results while peachblow to red indicate positive results.

Table 1. Show the wells for identification and enumeration

Wells #	Tests	Principal Substrate
No.1	UU	Lincomycin
No.2	MH	Erythromycin
No.3	UU≥104	Lincomycin and inhibition agent
No.4	MH≥104	Erythromycin and inhibition agent
No.5	C+	N/A

RESULTS AND DISCUSSIONS

The results of detection of genital mycoplasma revealed that only 3 (16.7%) of

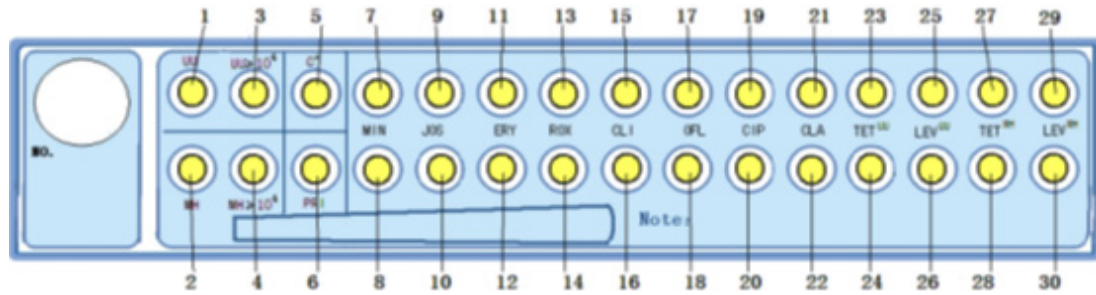


Fig. 1. Show the Mycoplasma IES Plus strip

Table 2. Show the wells for antibiotic susceptibility

Concentrations mg/l	Abbreviations	Antibiotics and	Wells
	2	PRI	Pristinamycin No.6
8	2	MIN	Minocycline No.7 and 8
8	2	JOS	Josamycin No.9 and 10
16	8	ERY	Erythromycin No.11 and 12
4	1	ROX	Roxithromycin No.13 and 14
0.5	0.25	CLI	Clindamycin No.15 and 16
4	1	OFL	Ofloxacin No.17 and 18
2	1	CIP	Ciprofloxacin No.19 and 20
4	1	CLA	Clarithromycin No.21 and 22
2	1	TETUU	Tetracycline No.23 and 24
4	2	LEVUU	Levofloxacin No.25 and 26
8	4	TETMH	Tetracycline No.27 and 28
2	1	LEVMH	Levofloxacin No.29 and 30

expressed prostate secretion samples give positive results for both *Ureaplasma urealyticum* and *Mycoplasma hominis* while 10 (45.5%) urethral swab were positive for both. The common age group of prostatitis infected with genital mycoplasma is (41-60 years) while urethritis was detected among all three groups (table 3). The positive and negative results for both *Ureaplasma urealyticum* and *Mycoplasma hominis* were showed in figure (2) and (3) respectively.

Many studies stated that the coexistence of *Ureaplasma urealyticum* and *Mycoplasma hominis* was common and the affected age group 30-50 years. *M. hominis* is found as a colonizer of the genito-urinary tract in sexually active, and is also associated with bacterial vaginosis^{22,23,24,25}.

U. urealyticum and *M. hominis* are prominent pathogens among men with urethritis and its complications and occur more frequently in the semen of prostatitis patients^{11,26}.

lacking of cell wall, target of beta-lactam antibiotics and vancomycin, the most common and appropriate medication for urogenital infections caused by mycoplasmas depends upon tetracyclines, macrolides, and quinolones^[15,27]. The results of antibiotic susceptibility revealed that all isolates were fully sensitive (100%) to all antibiotics incorporated within the well except for erythromycin, roxithromycin, tetracyclin and levofloxacin in which one isolate (7.7%) was exhibit resistance in both *Ureaplasma urealyticum* and *Mycoplasma hominis*.



Fig. 2. Show the positive results of culture for both *U. urealyticum* and *M. hominis*

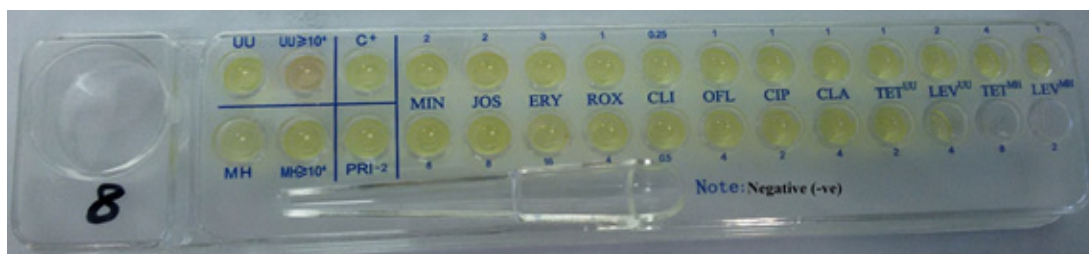


Fig. 3. Show the negative results of culture for both *U. urealyticum* and *Mycoplasma hominis*

Table 3. Distribution of genital mycoplasma among different age groups

Age group (Year)	Prostatitis n=18		Urethritis n=22	
	Positive (%) For both UU and MH	Negative(%) For both UU and MH	Positive(%) For both UU and MH	Negative(%) For both UU and MH
1-20	0	1	2	3
21-40	0	0	4	1
41-60	3	14	4	8
Total	3 (16.7)	15 (83.3)	10 (45.5)	12 (54.5)

Table 4. Antibiotics susceptibility among *Ureaplasma urealyticum* and *Mycoplasma hominis*

Antibiotics (mg/l)	Antibiotic Susceptibility of <i>U. urealyticum</i> n=13		Antibiotic Susceptibility of <i>M. hominis</i> n=13	
	Sensitive	Resistance	Sensitive	Resistance
Pristinamycin (2)	13 (100%)	0(0.00%)	13 (100%)	0(0.00%)
Minocycline (2)	13 (100%)	0(0.00%)	13 (100%)	0(0.00%)
Minocycline (8)	13 (100%)	0(0.00%)	13 (100%)	0(0.00%)
Josamycin (2)	13 (100%)	0(0.00%)	13 (100%)	0(0.00%)
Josamycin (8)	13 (100%)	0(0.00%)	13 (100%)	0(0.00%)
Erythromycin (8)	12 (92.3%)	1(7.7%)	12 (92.3%)	1(7.7%)
Erythromycin(16)	12 (92.3%)	1(7.7%)	12 (92.3%)	1(7.7%)
Roxithromycin (1)	12 (92.3%)	1(7.7%)	12 (92.3%)	1(7.7%)
Roxithromycin(4)	12 (92.3%)	1(7.7%)	12 (92.3%)	1(7.7%)
Clindamycin (0.25)	13 (100%)	0(0.00%)	13 (100%)	0(0.00%)
Clindamycin (0.5)	13 (100%)	0(0.00%)	13 (100%)	0(0.00%)
Ofloxacin (1)	13 (100%)	0(0.00%)	13 (100%)	0(0.00%)
Ofloxacin(4)	13 (100%)	0(0.00%)	13 (100%)	0(0.00%)
Ciprofloxacin (1)	13 (100%)	0(0.00%)	13 (100%)	0(0.00%)
Ciprofloxacin (2)	13 (100%)	0(0.00%)	13 (100%)	0(0.00%)
Clarithromycin (1)	12 (92.3%)	1(7.7%)	12 (92.3%)	1(7.7%)
Clarithromycin (4)	12 (92.3%)	1(7.7%)	12 (92.3%)	1(7.7%)
Tetracycline (1) UU	12 (92.3%)	1(7.7%)	-	-
Tetracycline (2) UU	12 (92.3%)	1(7.7%)	-	-
Levofloxacin (2) UU	13 (100%)	0(0.00%)	-	-
Levofloxacin (4) UU	13 (100%)	0(0.00%)	-	-
Tetracycline (4) MH	-	-	13 (100%)	0(0.00%)
Tetracycline (8) MH	-	-	13 (100%)	0(0.00%)
Levofloxacin (1) MH	-	-	13 (100%)	0(0.00%)
Levofloxacin (2) MH	-	-	13 (100%)	0(0.00%)

Our data was highly similar with those gathered from many studies which found that all genital mycoplasma were fully sensitive to doxycycline, josamycin, ofloxacin and pristinamycin^{27,28,29,30,31}. Resistance to erythromycin were recorder for both of *Ureaplasma urealyticum* and *Mycoplasma hominis* isolated from genital samples of sexually active individuals. Resistance to erythromycin among mycoplasmas is naturally occur while resistance to tetracyclin and quinolones can be attributed to antibiotic targets mutation and it may be related to higher pathogenicity^{15,32,33}.

The current study conclude that the genital mycoplasmas (*Ureaplasma urealyticum* and *Mycoplasma hominis*) are common among non gonococcal urethritis and the macrolides still the choice for treatment while prescription of quinolones and tetracyclin must be under control due to emergence of resistance isolates.

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