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Prevalence and antibiotic susceptibility profile of *Mycoplasma hominis* and *Ureaplasma urealyticum* as well detection other vaginal pathogens in some aborted Iraqi women by using two specific Kits.

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ABSTRACT:

Mycoplasma hominis and Ureaplasma urealyticum are significant opportunistic pathogens that cause complications during pregnancy. Finding out how common those pathogens were in miscarriage cases among women was the study's main goal. Detection by using rapid and direct identification kits A.F. Genital System and Urogen Well D-one, which determined other Genital Infections in abortion in a group of Iraqi women. The study was a descriptive cross-sectional study done in the period from November 2019 to December 2020. Two hundred (200) clinical specimens were collected; it was comprised only of swabs from vaginal or cervical of 176 specimens with miscarriages and 24 controls in public maternity hospitals. The positive results were 17 (9.7%) of Mycoplasma hominis in both kits. In contrast, for Ureaplasma urealyticum, positive results were 26 (14.8%) in the A.F. Genital System Kit and 27 (15.3%) in the Urogen Well D-one kit from 176 miscarriages specimens. All 24 control women revealed negative results for M. hominis and U. urealyticum in both Kits. Most of the isolates were identified as resistant to levofloxacin, Erythromycin, and Clarithromycin. At the same time, the highest sensitivity was recorded for moxifloxacin and doxycycline as on the A.F. Genital System and Urogen Well D-one Kits. In conclusion, with a 24-hour turnaround time, both kits have the potential to enable quick identification of the common organisms that lead to miscarriages in women and direct antibiotic treatment. Patients benefit greatly from this, and the need for empirical infection treatment is decreased.

Keywords: *Mycoplasma hominis, Ureaplasma urealyticum, aborted women, Urogen Well D-one, A.F. Genital System*.

INTRODUCTION:

Species belonging to the Mollicutes class, Mycoplasma hominis and Ureaplasma urealyticum/ parvum, which are classified as opportunistic pathogens, they are frequently isolated from the urogenital tract. Research has indicated a clear connection between the isolation of these microorganisms and a number of illnesses, including preterm labor, infections during pregnancy, pelvic inflammatory disease, and neonatal infections (16, 9). The risk of miscarriage increased sevenfold when Mollicutes were found in placental tissue (14). The current rapid diagnosis methods often rely on costly tests that may not be available in all institutions. Furthermore,

these tests fail to provide essential information regarding the antimicrobial susceptibility of a particular treatment, which is crucial for treatment implementation and epidemiological research. The presence of other bacteria responsible for genitourinary infections is frequently related to Urogenital Mycoplasma positive, including Streptococcus agalactiae, Neisseria spp., Staphylococcus aureus, Candida spp., Escherichia coli, Trichomonas vaginalis, Gardnerella vaginalis, and Enterococcus spp. The concurrent detection *of these agents can direct the course of action to be taken with patients, including the clinical diagnosis* (10, 8).

Antimicrobial agent resistance is on the rise, and infections caused by *M. hominis* and *U. urealyticum* are common in expectant mothers. As a result, it's critical to recognize the isolates and start using the proper empirical antibiotics right away in order to preserve a safe pregnancy (11). The pathogenesis and chronicity of those two pathogens involve their ability to evade the host's immune response locally. *M. hominis* may disrupt embryonic implantation, particularly in early pregnancy, and can be isolated from the endometrial tissue of healthy, non-pregnant women (2). Pregnant women's health is thought to be at risk from genital mycoplasmas, which include *M. hominis* and *U. urealyticum*. These are remarkable emergent bacterial pathogens that are transmitted sexually and can induce chronic, asymptomatic, and long-lasting infections in the genitourinary tract (12).). Many local studies revealed that the high prevalence of bacterial species among Iraqi women with vaginitis with miscarrage cases and high antibiotic resistance (13, 14, 15). Because of this, accurate treatment is frequently delayed because routine testing procedures currently used to determine the antibiotic sensitivity of pathogens causing genital and urinary tract infections can take two to three days to complete. Treatment is delayed as a result, which may increase morbidity. Studying the frequency and antibiotic resistance patterns of genital mycoplasmas is crucial for choosing the most effective medications to treat these microorganisms. The objective of this study was to assess the occurrence and antibiotic resistance patterns of *M. hominis* and *U. urealyticum* strains obtained from women who experienced abortion and were admitted to hospitals in Baghdad.

MATERIALS AND METHODS

Swab Sampling:

Two clinical specimens Swabs were taken carefully from the Vaginal or Cervical for each woman from all patients and control groups at public maternity hospitals like Al-Elweya Maternity Teaching Hospital, Al-Yarmouk Teaching Hospital, Fatima Al- Zahra'a Maternity, Pediatric Teaching Hospital, and Al-Karkh Maternity Hospital. Those two swab specimens were collected and directly worked in both A.F. Genital System and Urogen Well D-one kits for the detection of both *Mycoplasma hominis* and *Ureaplasma urealyticum* with its Susceptibility test and identification of other sexually transmitted diseases (STD) vaginal microorganisms in the same specimen for aborted women.

First kit (A.F. Genital System) Principle of the method:

The 24-well A.F. Genital System (Liofilchem/Italy) is used to detect, identify, and test for antibiotic susceptibility of microorganisms from urogenital specimens. It contains desiccated biochemical and antibiotic substrates. *Mycoplasma hominis* and *Ureaplasma urealyticum*, the two urogenital mycoplasmas, are detected by the system using a semi-quantitative method. The clinical specimen suspension is used to inoculate the system, and it is then incubated for 18 to 24 hours at 36±1 °C. Microorganisms that are frequently obtained from vaginal swabs include *Proteus spp. / Providencia spp., Escherichia coli, Pseudomonas spp., Gardnerella vaginalis, Trichomonas vaginalis, Enterococcus faecalis, Staphylococcus aureus, Streptococcus agalactiae (Group B), Neisseria gonorrhoeae, and Candida spp.* The susceptibility of the urogenital mycoplasmas in the specimen is assessed by measuring

the color change in different wells, followed by a microscopic examination.

Kit Contents:

The kit includes twenty vials of physiological solution (7.0 mL per vial) and twenty A.F. genital systems, 1 Test results form, and an Instruction sheet. The Physiological Solution contains (g/L) 9 g of sodium chloride, 1000 mL of distilled water, and a pH of 6.8 ± 0.2.

Test procedure:

The endocervical exudate and vagina were used to obtain the specimens in accordance with the protocols set forth by each laboratory. After obtaining the clinical material, the swab was submerged in the vial of physiological solution, and five minutes were allowed to pass. Subsequently, the swabs were compressed against the vial inside wall, and 0.2 milliliters of the clinical specimen suspension were added to each system well. One drop of Vaseline Oil was applied to wells 1 through 5, 7 through 15, 19, and 24. The system was then covered with the included lid and incubated about 37 °C for 18 to 24 hours. Then a drop of liquid from well 6-TR/YE was removed and placed on a glass slide; where it was inspected under a 40x microscope to check for detect of *Candida* spp and *Trichomonas vaginalis*. An Oxidase Test Stick was filled with a drop of liquid from well 22-NES, and a positive oxidase test resulted in the quick (10 seconds or so) development of a blue color. The incubation lasted between 24 and 48 hours at 36±1 °C. Either a human reader or an automatic one read and interpreted the test results. Quality control is applied to each batch using multiple reference microorganisms.

Second kit (Urogen Well D-one) Principle of the method:

A 32-well polypropylene plate is ideal for observing the colorimetric responses produced by microorganisms grown in a medium designed for the selective cultivation of *M. hominis, Mycoplasma spp.,* and *U. urealyticum / parvum*. The Urogen Well D-one system (Ridacom/Italy) is used to determine the antibiotic susceptibility and presumed identity of urogenital mycoplasmas. Additionally permits the presumed identification of *Gardnerella vaginalis, Trichomonas vaginalis, Staphylococcus aureus, Neisseria spp., Streptococcus agalactiae* (group B), *Escherichia coli, and Candida* spp. The system enables the performance of microscopic observation, sowing in selective media, molecular and serological testing, all directly from the contents of the wells.

Kit Contents:

Urogen well D-one® KIT, 10 Identification panels, 10 x 10 mL sterile physiological Saline Solution, and 10 Sterile Swabs.

Test Procedure:

For the urethral, vaginal, and endocervical exudates: The specimens were collected as swabs in accordance with the protocols set forth in each laboratory. After resuspending the swabs with the specimen in the saline solution provided in the kit, the swabs were submerged for duration of three to five minutes. Afterward, the swabs were mixed and pressed up against the tube walls until a homogenous suspension was achieved. Each system panel well-received 150 µL of the obtained suspension, two drops of sterile paraffin were added to wells 1 through 25, and the panels were then incubated for 24 to 48 hours at 36±1 °C. Every batch of Urogen well D-one undergoes stringent quality control testing using numerous reference strains of bacteria. Prior to any nonspecific reactions, the control strains are used for both positive

reactions from separate wells to ensure that the media formulations in each well are operating as intended.

RESULTS AND DISCUSSION:

Results Detection of pathogenic microorganisms

1. **First Kit A.F. Genital System:**

This study investigated 176 specimens from patients to detect *Mycoplasma hominis*, *Ureaplasma urealyticum,* and other pathogenic microorganisms using A.F. Genital System Kit. Positive findings for *M. hominis* overall (176 specimens) were 17 (9.7%) and 159 (90.3%) negative, while for *Ureaplasma urealyticum* positive 26 (14.8%) and 150 (85.2%) negative, shown in **Figure: 1 and 2.**

Figure 1: Positive results of *M. hominis*, *U. urealyticum,* and other pathogenic microorganisms in A.F. Genital System Kit after incubating at 36±1 °C for 18 - 24 hours.

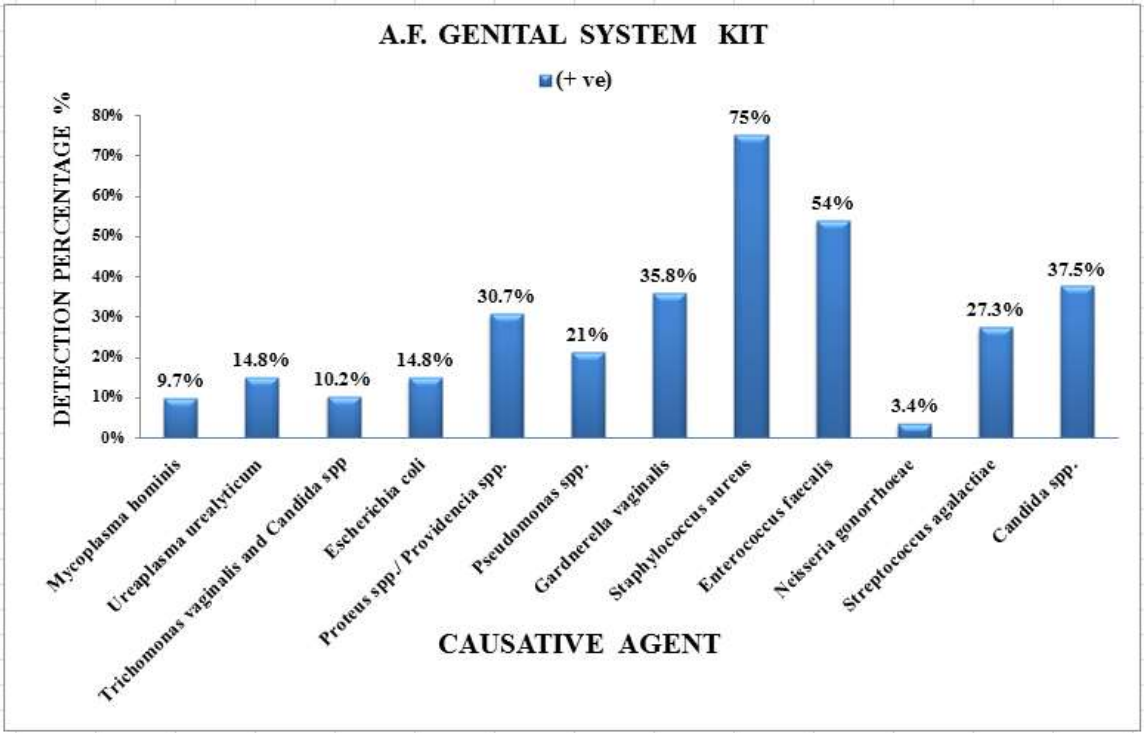

Figure 2: Percentages for detection and diagnosis of the causative agents from vaginal swabs using A.F. Genital System.

The prevalence of *M. hominis* and *U. urealyticum* in miscarriages utilizing the AF Genital System for this study was 9.7% and 14.8%, respectively. This kit also revealed the prevalence of other pathogenic bacteria and other microorganisms from genital infections of pregnant and aborted women, which included a high prevalence of *Staphylococcus aureus* (75%), followed by *Enterococcus* spp. (54%). Also, (37.5%) of *Candida* spp., while the lowest prevalence of pathogenic agent was *Neisseria* spp. (3.4%).

According to Tjoa *et al*. (2021) (16), Compared to PCR, the AF Genital System® demonstrated lower specificities for *M. hominis* (82.9% specificity) and *U. urealyticum* (86.5% specificity) in vaginal swab specimens. However, the system's specificity was still quite good human species), and 82.3% and 84.8% (*U. urealyticum*) (17). The meta-analysis indicated that the prevalence of *M. genitalium*, and *M. hominis* was 11.33 and 9.68%, respectively, and that the prevalence of *U. urealyticum* was 17.53%. Additionally, this study found that the prevalence of *M. genitalium, U. urealyticum,* and *M. hominis* among infertile women in Iran was higher than that of fertile women (3%, 10.85%, and 4.35%) (12). among the patients, twelve women had spontaneous abortions before reaching 36 weeks of gestation. Out of these instances, eight (66.7%) were found to have been infected with both *M. hominis* and *U. urealyticum* (18). This study, conducted in Turkey, examined the frequency of *M. hominis* and *U. urealyticum* in a sample of 100 pregnant women.

2. **Second Kit Urogen Well D-one:**

In *M. hominis* detection overall, 176 clinical specimens by Urogen Well D-one kit found the same positive result in A.F. Genital System, 17 (9.7%) Positive and 159 (90.3%) negative for *M. hominis*, while for *Ureaplasma urealyticum* positive result was 27 (15.3%) and 149 (84.7%) negative, shown in **Figure: 3 and 4.**

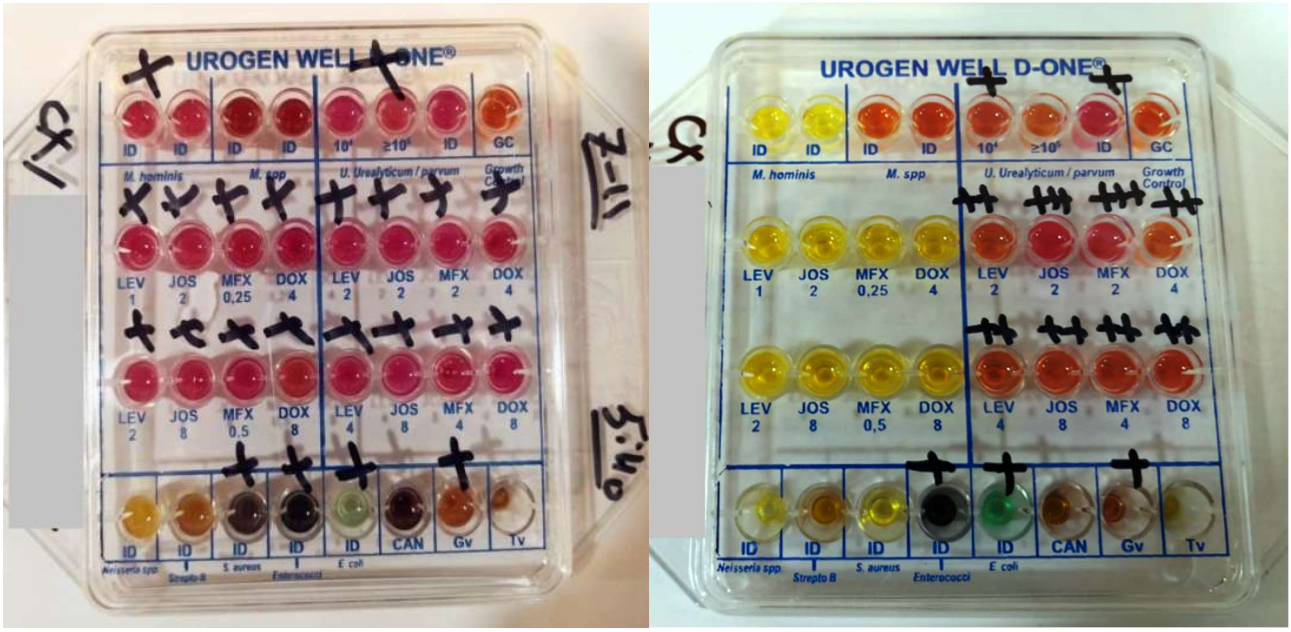

Figure 3: Positive results of *M. hominis*, *U. urealyticum,* and other pathogenic microorganisms in Urogen well D-one kit after incubated at 36±1 °C for 24 – 48 hours.

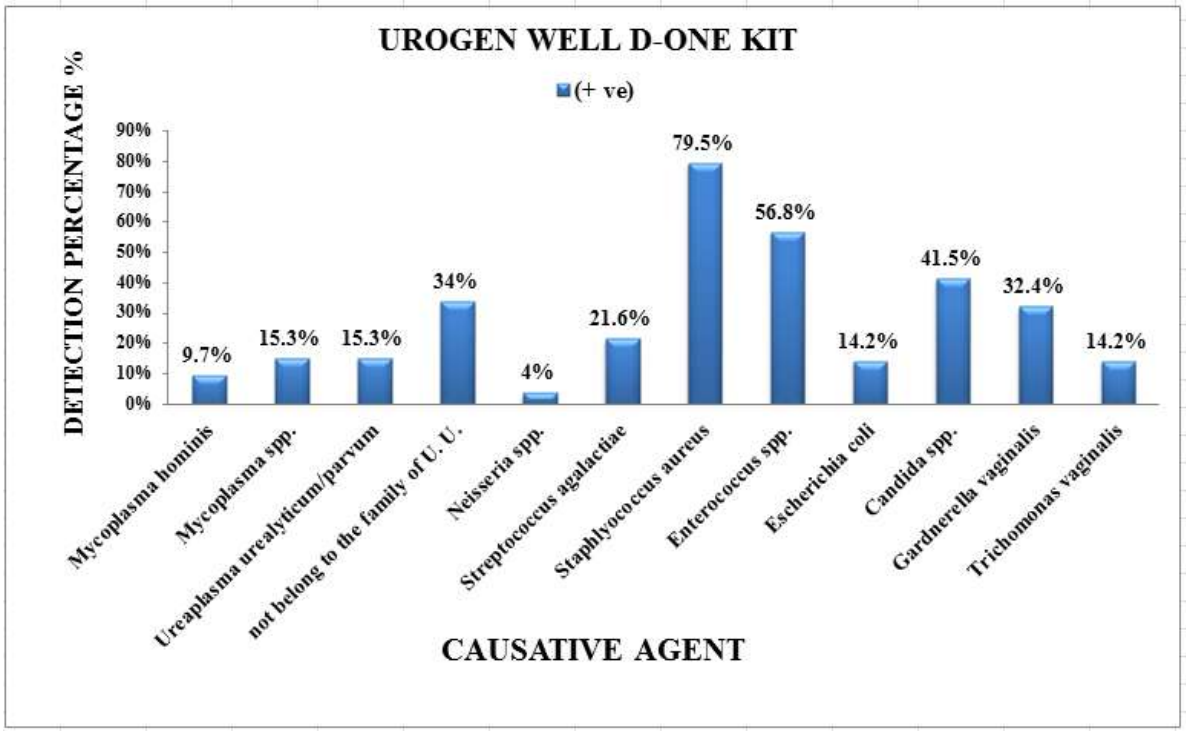

Figure 4: Percentages for detection and diagnosis of the causative agents from vaginal swabs using Urogen Well D-one.

The prevalence of *M. hominis* and *U. urealyticum* in miscarriages women using the Urogen well D-one kit for this study was 9.7% and 15.3%, respectively. This kit also revealed the prevalence of other pathogenic bacteria and other microorganisms from genital infections of pregnant and aborted women, which included a high prevalence of *Staphylococcus aureus* (79.5%), followed by *Enterococcus* spp. (56.8%). Also, (41.5%) of *Candida* spp., while the lowest prevalence of pathogenic agent was *Neisseria* spp. (4%).

Urogen Well D-one is a quick benchtop diagnostic for urogenital infection detection that is based on culture. It identifies the causing organisms and ascertains whether they are resistant to antibiotics. The new test can identify bacterial infections that include *Gardnerella vaginalis*, the protozoa *Trichomonas vaginalis*, *Candida albicans, E. coli, Staphylococcus* spp*., Enterococcus,* Group B *Streptococci, Mycoplasma* spp*., Ureaplasma* spp*., Neisseria* spp., and others. Because of this, this diagnostic could be a very useful tool for detecting infections, identifying the causative organism, and guiding the treatment of these infections based on the assessment of antibiotic susceptibility (5, 13). The results of the current investigation demonstrated the high sensitivity and specificity of Urogen Well D-one when compared to the results of the assay, which revealed that *Ureaplasma* spp. *M. hominis* sensitivity of 78.23% and specificity of 98.84%, and sensitivity of 91.98% and 96.44%, respectively (13).

Results Antibiotic Susceptibility Testing

1. **First Kit A.F. Genital System:**

A total of 17 *M. hominis* and 26 *U. urealyticum* isolates on the AF Genital System were also screened for one or more antibiotic resistance, like Tetracycline, Doxycycline, Minocycline, Pefloxacin, Ofloxacin, Erythromycin, Clarithromycin, Josamycin, and Clindamycin. For *M. hominis and U. urealyticum,* most of the isolates were identified as Erythromycin (40%) and Clarithromycin (40%) resistant, while the highest sensitivity was recorded for Doxycycline (20%) as shown in **Table: 1 and Figure: 5**

Antibiotic (µg/mL)	**Single agent *M. hominis* (n=4)**		**Single agent *U. urealyticum* (n=13)**		**Double agent *M. hominis* and *U. urealyticum* (n=13)**		**Total (R) From 30 sample**
	S	**R**	**S**	**R**	**S**	**R**	
Tetracycline 8	3(75.0%)	1(25.0%)	10(76.92%)	3(23.08%)	9(69.23%)	4(30.76%)	**8 (27%)**
Pefloxacin 16	4(100.0%)	0(0.0%)	9(69.23%)	4(30.76%)	8(61.54%)	5(38.46%)	**9 (30%)**
Ofloxacin 4	2(50.0%)	2(50.0%)	10(76.92%)	3(23.08%)	8(61.54%)	5(38.46%)	**10(33%)**
Doxycycline 8	3(75.0%)	1(25.0%)	11(84.61%)	2(15.39%)	10(76.92%)	3(23.08%)	**6 (20%)**
Erythromycin 16	2(50.0%)	2(50.0%)	9(69.23%)	4(30.76%)	7(53.85%)	6(46.15%)	**12(40%)**
Clarithromycin 16	3(75.0%)	1(25.0%)	8(61.54%)	5(38.46%)	7(53.85%)	6(46.15%)	**12(40%)**
Minocycline 8	2(50.0%)	2(50.0%)	9(69.23%)	4(30.76%)	9(69.23%)	4(30.76%)	**10(33%)**
Josamycin 8	3(75.0%)	1(25.0%)	9(69.23%)	4(30.76%)	9(69.23%)	4(30.76%)	**9 (30%)**
Clindamycin 8	3(75.0%)	1(25.0%)	10(76.92%)	3(23.08%)	6(46.15%)	7(53.85%)	**11(37%)**

* S: susceptible, R: resistant.

Table 1: Antibiotic Susceptibility Testing shows single and double agents for *M. hominis* and *U. urealyticum* in the A.F. Genital System.

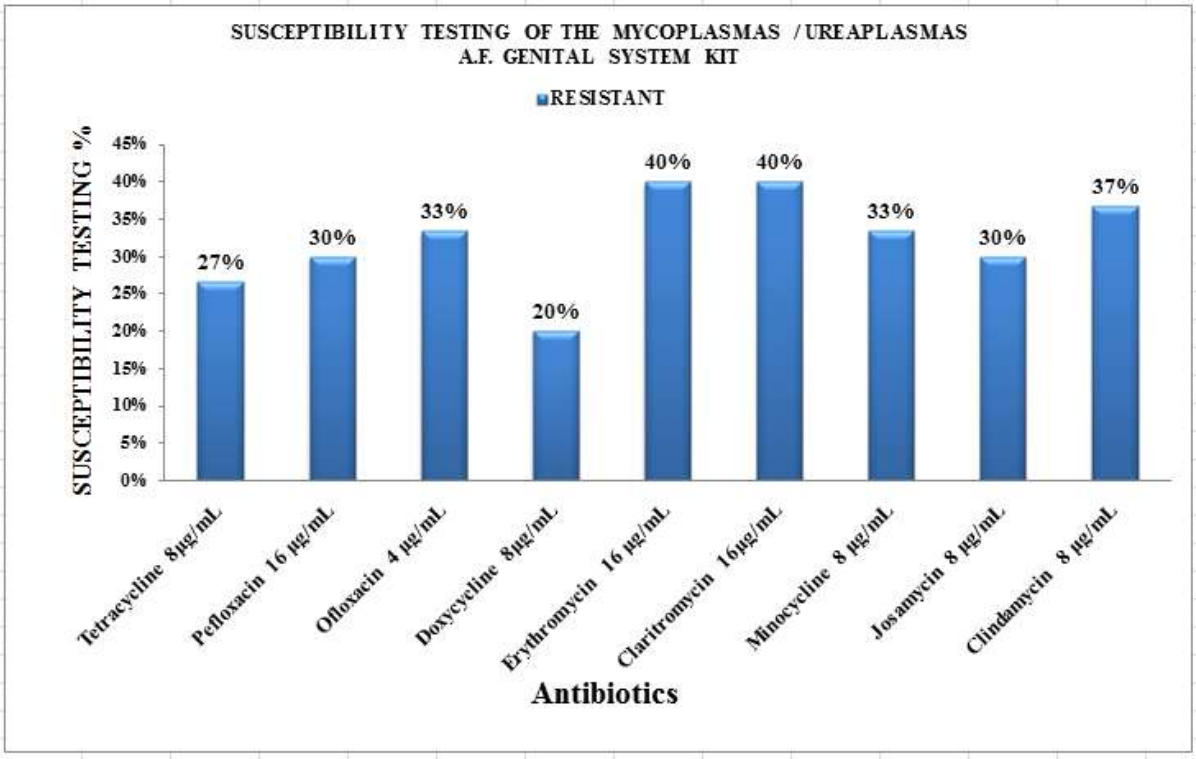

Figure 5: Antimicrobial Susceptibilities percentages of *M. hominis* and *U. urealyticum* from vaginal swabs according to A.F. Genital System **Table 1** result.

2. Second Kit Urogen Well D-one:

A total of 17 *M. hominis* and 27 *U. urealyticum* isolates on the Urogen Well D-one assay were also screened for one or more antibiotic resistance, like levofloxacin, josamycin, moxifloxacin and doxycycline. For *M. hominis and U. urealyticum*, most of the positive resistance wells isolates were identified as levofloxacin (41%) and josamycin (34%), while the highest sensitivity was recorded for moxifloxacin (16%) as shown in **Table: 2 and Figure: 6**

Antibiotic Susceptibility Testing (µg/mL)		Single agent *M. hominis* (n=3)%		Single agent *U. urealyticum* (n=13)%		Double agent *M. hominis* and *U. urealyticum* (n=14)%		Total (R) (n=)%
		S	R	S	R	S	R	
For *Mycoplasma hominis*	Levofloxacin 1	0(0.0%)	3(100.0%)	-	-	4(28.57%)	10(71.42 %)	**13 (41%)**
	Josamycin 2	1(33.33%)	2(66.67%)	-	-	5(35.71%)	9(64.28%)	**11 (34%)**
	Moxifloxacin 0,25	2(66.67%)	1(33.33%)	-	-	8(57.14%)	6(42.85%)	**7 (22%)**
	Doxycycline 4	1(33.33%)	2(66.67%)	-	-	8(57.14%)	6(42.85%)	**8 (25%)**
	Levofloxacin 2	1(33.33%)	2(66.67%)	-	-	6(42.85%)	8(57.14%)	**10 (31%)**
	Josamycin 8	2(66.67%)	1(33.33%)	-	-	5(35.71%)	9(64.28%)	**10 (31%)**
	Moxifloxacin 0,5	0(0.0%)	0(0.0%)	-	-	9(64.28%)	5(35.71%)	**5 (16%)**
	Doxycycline 8	0(0.0%)	0(0.0%)	-	-	8(57.14%)	6(42.85%)	**6 (19%)**
For *Ureaplasma urealyticum*	Levofloxacin 2	-	-	9(69.23%)	4(30.76%)	6(42.85%)	8(57.14%)	**12 (38%)**
	Josamycin 2	-	-	8(61.54%)	5(38.46%)	8(57.14%)	6(42.85%)	**11 (34%)**
	Moxifloxacin 2	-	-	8(61.54%)	5(38.46%)	11(78.57 %)	3(21.42%)	**8 (25%)**
	Doxycycline 4	-	-	10(76.92 %)	3(23.08%)	10(71.42 %)	4(28.57%)	**7 (22%)**
	Levofloxacin 4	-	-	11(84.61 %)	2(15.39%)	9(64.28%)	5(35.71%)	**7 (22%)**
	Josamycin 8	-	-	9(69.23%)	4(30.76%)	9(64.28%)	5(35.71%)	**9 (28%)**
	Moxifloxacin 4	-	-	10(76.92 %)	3(23.08%)	12(85.71 %)	2(14.28%)	**5 (16%)**
	Doxycycline 8	-	-	10(76.92 %)	3(23.08%)	11(78.57 %)	3(21.42%)	**6 (19%)**

*S: susceptible, R: resistant.

Table 2: Antibiotic Susceptibility Testing shows single and double agents for *M. hominis* and *U. urealyticum* in Urogen Well D-one.

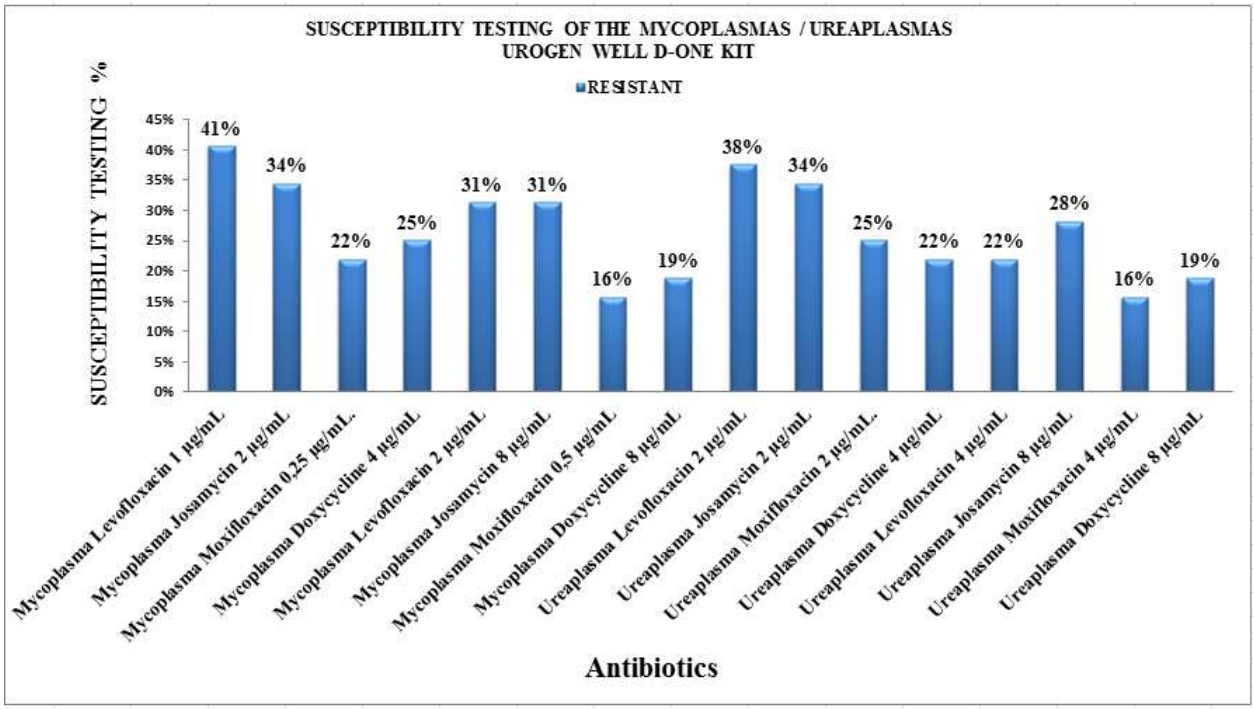

Figure 6: Antimicrobial Susceptibilities percentages of *M. hominis* and *U. urealyticum* from vaginal swabs according to Urogen Well D-one **Table2** results.

Levofloxacin resistance for *Ureaplasma* spp. and *Mycoplasma* spp. was greater than the rates reported in other research. 250 clinically isolated *Ureaplasma* spp. were found in Minnesota. MIC testing revealed that the rates of levofloxacin resistance for *U. parvum* and *U. urealyticum* were 6.4% and 5.2%, respectively. (6). Moxifloxacin, in comparison with levofloxacin and ciprofloxacin, has emerged as a potential treatment option based on the reported susceptibility of *M. hominis* to this agent, and it has a bactericidal activity (7).

Pristinamycin and Josamycin were the most effective antibiotics in eliminating Mycoplasma hominis and Ureaplasma urealyticum, according to a study conducted in Romania on the prevalence of these infections in infertile patients. A number of other antibiotics also demonstrated high efficacy, including Doxycycline (98.23%) and Minocycline (96.00%). Levofloxacin (82.00%) and azithromycin (78.94%) can be used in addition to one another to treat such infections based on antibiograms (4). Using the *Mycoplasma* IST-2 kit, antimicrobial susceptibilities were ascertained in Turkey. Tetracycline and doxycycline were found to be effective against *M. hominis* and *U. urealyticum*, respectively, and could be used as empirical therapy for infected individuals (1). *The local study conducted on blood samples of women with recurrent pregnancy loss revealed that M. hominis* could be detected in 7.5% of women who had miscarried. Still, it wasn't seen in the control group. In 40% of the patient group and 4% of the control group, *U. urealyticum* was found. It may be crucial and important to quickly detect *M. hominin* and *U. urealyticum* by PCR in pregnant women who have lost their pregnancies (3).Also, the local study conducted by Mohamed and. Al- Thwani, A. (2024), (20) demonstrated that the bacterial pathogens which isolated from Iraqi women with vaginitis were *Escherichia coli*, *Pseudomonas aeruginosa*, *Gardnerella viginals* , *Klebsiella oxytoca* , *Staphylococcus aureus* and *Proteus mirabilis*.

CONCLUSION:

Urogen Well D-one Kits and the AF Genital System both have the ability to quickly identify common organisms that cause urethritis, UTIs, and abortions, as well as urogenital mycoplasmas. With a 24-hour turnaround time, it decreases the empirical treatment of infection and has highly significant positive outcomes for patients. *Mycoplasma hominis* and *Ureaplasma urealyticum* are detected as secondary infections that worsen after infection, and they can occur by themselves or in conjunction with other bacterial vaginosis, candidiasis, and trichomoniasis. As an alternative to doxycycline and moxifloxacin, these antibiotics may be useful in treating *M. hominis* and *Ureaplasma* spp. infections, as evidenced by its extremely sensitive antibacterial activity.

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