

The Antimicrobial Susceptibility of *Ureaplasma Urealyticum*, the Primary Causative agent in Intramaneiotic Infection, in Urogenital Fluid Sample

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Abstract:

Background: *Ureaplasma* is a genus of bacteria belonging to the Mycoplasmataceae family, found in the genitals of up to 70% of men and women. Although it usually acts as a commensal organism, it sometimes grows excessively and is associated with pathological conditions of the reproductive system.

Objective: to assess the antimicrobial susceptibility of *Ureaplasma urealyticum* obtained from urogenital samples collected from women within the reproductive age group, an evaluation was conducted.

Patients and method: 390 patients were included in the current study, 197 of them were positive for *Ureaplasma urealyticum*. From all patients we investigated the culture of urine and vaginal fluids for *Ureaplasma urealyticum* microne, in those within the age between 18 and 40 years old, in the period from April 2018 to January 2020.

Results: A total of 426 samples, comprising both urine and vaginal discharge, were collected from a cohort of 390 patients. A total of 197 patients were found to have tested positive for *Ureaplasma urealyticum*. The given value is expressed as a percentage, specifically 50.5%. The susceptibility rates for Erythromycin, Clarithromycin, and Azithromycin were found to be 88.4% (174 participants), 87.9% (173 participants), and 91.9% (181 participants), respectively. These results were not statistically significant. Out of a total of 197 patients, a subset of 15 individuals (7.6%) shown resistance to all three macrolide antibiotics. The susceptibility rates for Quinolones were found to be 55.3% for Ciprofloxacin and 94% for Ofloxacin. All individuals in the study shown susceptibility to tetracycline.

Conclusion: Approximately 10% of *Ureaplasma urealyticum* strains identified in our study exhibit resistance to macrolide antibiotics, hence playing a role in the failure to completely eliminate the infection during empirical treatment approaches. Among the available options, azithromycin demonstrates the highest efficacy.

Keywords: Antimicrobial susceptibility, urogenital samples, *Ureaplasma urealyticum*, intramaneiotic infection

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1. INTRODUCTION

Sexually transmitted diseases (STDs) and sexually transmitted infections (STIs) are a growing problem at a general level. Chlamydia, gonorrhea, syphilis and trichomoniasis are some of the most common STIs, although there are more than 20 pathogens capable of affecting humans in this area. Ureaplasma infection is one of the least known, but it is interesting to take its symptoms into account to make a differential diagnosis (1). The bacteria *Ureaplasma urealyticum* is found in the genitals of up to 70% of men and women. Although it usually acts as a commensal organism, it sometimes grows excessively and is associated with pathological conditions of the reproductive system (2). *Ureaplasma* is a genus of bacteria belonging to the Mycoplasmataceae family. This group of microscopic organisms inhabits both the respiratory and genital environments of humans, but they behave as commensals in normal situations (3). Even so, certain situations cause them to grow excessively at the colony level, which generates inflammation in the tissues that are affected. As a differential feature, it should be noted that *Ureaplasma* lacks a cell wall (4). It is estimated that the bacteria *Ureaplasma urealyticum* is found in the genital tract of up to 40-80% of healthy women. The percentage of colonization in the vagina of pregnant women ranges from 29-42%, which can pose a danger to the baby during childbirth. This infection occurs in both the adult and pediatric populations (5). Urogenital colonization by genital mycoplasmas, including *Mycoplasma hominis* and *Ureaplasma urealyticum*, depends on the sex, age, sexual activity and hormonal level of the person studied (6). They can be frequently isolated in vaginal secretion samples (60 – 80%) in sexually active women (7). The pathogenic effects of these bacteria are found mainly in the obstetric/perinatal area (intra-amniotic infection, premature rupture of membranes, premature birth). The most frequent, *Ureaplasma urealyticum* is capable of invading the amniotic cavity, being one of the most frequently isolated agents (42%) in amniocentesis in pre-term pregnancies (8). The origin of *U. urealyticum* that invades the amniotic fluid is the maternal urogenital tract. Screening women of childbearing age will allow monitoring prevalence and monitoring their susceptibility to antimicrobials. This is essential in situations such as premature rupture of membranes, given that the initial management of these patients will generally include empirical antimicrobial regimens (9). From a therapeutic point of view, the use of beta-lactams is not feasible given that these microorganisms do not have a cell wall, which is the site of action of these drugs. The antimicrobials of choice are those that interfere with

protein synthesis (macrolides and tetracyclines) or that act on the enzymes that control the supercoiling and unwinding of bacterial DNA (quinolones). In recent years, the increase in resistance by *Ureaplasma urealyticum* to the three categories of antimicrobials has been described in various parts of the world, which reduces the therapeutic arsenal for this type of infectious agents (10-12).

2. PATIENTS AND METHODS

The present study had a total of 390 participants, of whom 197 tested positive for *Ureaplasma urealyticum*. The study involved the examination of urine and vaginal fluid samples obtained from a cohort of individuals aged 18 to 40 years, with the aim of culturing *Ureaplasma urealyticum*, in the period from April 2018 to January 2020. The vaginal fluid samples were taken with Sterile Dacron or Polyester swabs, and urine swabs, from the first jet sample. The samples are processed with a specialized kit, which allows the diagnosis of *Ureaplasma urealyticum* and *Mycoplasma hominis* (Culture, identification, indicative count, and antimicrobial susceptibility test). This kit uses a selective culture broth, adapted for the optimal growth of mycoplasmas (pH, substrates and association of various growth factors), added to the presence of specific substrates and an indicator, which in the case of positive cultures allows the visualization of a change in color of the broth linked to an increase in pH. This kit is validated for use in urine and cervicovaginal secretion, but not for amniotic fluid. Those who tested positive for *Ureaplasma urealyticum* were studied for susceptibility to macrolides, tetracycline and quinolones.

3. RESULTS

The urine samples (131) and vaginal fluid (259) correspond to 390 patients. Of them, 197 (50.5) were presented with urea plasma u., and they were susceptible to Azithromycin was 181 (91.9%), to Erythromycin was 174 (88.4%), to Clarithromycin was 173 (87.9%). While 16 (8.1%) was resistance to Azithromycin, 23 (11.6%) to Erythromycin and 24 (12.1%) resistance to Clarithromycin (**Table 1**). Of the 197 positive samples, 100% were susceptible to Tetracyclines (Doxycilcine and Tetracycline). Susceptibility to Ciprofloxacin was more than half 109 (55.3), and to Ofloxacin was 185 (94%) (**Figure 1**).

Table 1. The urine samples and vaginal fluid correspond to the patients

Macrolides	Azithromycin		Erythromycin		Clarithromycin	
	No.	%	No.	%	No.	%
Susceptibility	181	91.9	174	88.4	173	87.9
Resistance	16	8.1	23	11.6	24	12.1
Total	197	100.0	197	100.0	197	100.0

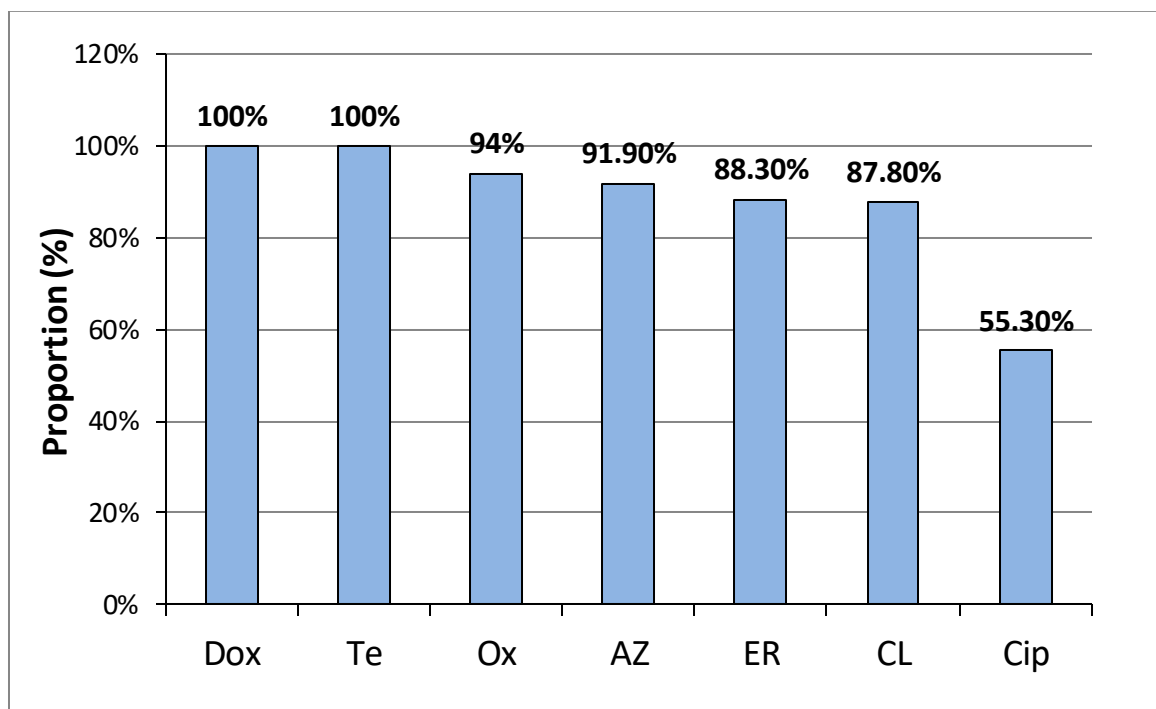


Figure 1. Susceptibility of *Ureaplasma urealyticum* to macrolides, tetracyclines and quinolones in 197 women (Dox: Doxycycline, Te: Tetracycline, Ox: Oxfloraxine, Az: Azithromycin, ER: Erythromycine, CL: Clarithromycine, Cip: Ciprofloxacin).

4. DISCUSSION

The infiltration of microorganisms into the amniotic cavity is a significant determinant of adverse outcomes for both mothers and newborns, as well as the potential for lasting health issues in children who were exposed to infectious agents during their time in the womb (13).

It is present in at least 15% of premature births with intact membranes and reaches 30-40% of PROM. Prematurity, in turn, is the main cause of perinatal mortality, so its prevention and management constitutes an important concern for Maternal Fetal Medicine (14). In this

sense, PROM in preterm pregnancies presents a great clinical challenge. It is frequently associated with intra-amniotic infection, and the main agent involved is *Ureaplasma urealyticum*, as mentioned above. The use of antimicrobials, to date, has been associated with a significant reduction in the risk of chorioamnionitis, prolongation of the interval between rupture and delivery, reduction of neonatal morbidity, such as neonatal infection, need for surfactant use, oxygen therapy, or the risk of having an abnormal brain ultrasound prior to surgery. discharge from the neonatal intensive care unit (15). Therefore, the use of empiric antibiotics in any patient who presents this clinical picture is strongly recommended. The possibility of isolating the causal agent(s) of intra-amniotic infection in the context of premature rupture of membranes is made difficult by the frequent association with Oligohydramnios secondary to the loss of amniotic fluid. Therefore, the vast majority of the time, broad-spectrum regimens are used that cover most of the bacterial agents that have been described as causing early fetal and neonatal infection. Among them, *Ureaplasma urealyticum* is commonly identified as the microbe with the highest frequency of isolation. Knowing this, macrolides are part of the first-line empirical therapeutic regimen for this clinical condition (16, 17). In current study, more than 50% of women analyzed were positive for *Ureaplasma* spp. A somewhat lower percentage than what is reported in most other series, with figures greater than 60%, and up to 80% of sexually active women. However, regardless of the technique used for its detection (including the polymerase chain reaction, PCR), the variability in the detection rate is very wide, even with figures close to 31%. In relation to therapy, to date in pregnant women in our country, Erythromycin has been the antimicrobial of first choice in conditions involving this agent.

The increase in resistance to macrolides by *Ureaplasma urealyticum* has been reported in various parts of the world, which has generated concern regarding the reduction of the therapeutic arsenal in the case of pregnant women, in whom the use of tetracyclines is contraindicated. and in relation to quinolones (category C of the Food and Drugs Administration, FDA) they are contraindicated by many authors, and a secondary alternative by some “when the benefit outweighs the risk” (10-12, 18-20). In our experience, resistance to erythromycin is 11.6%, similar to Clarithromycin (12.1%), and slightly higher than Azithromycin (8.1%) (Difference was not significant) (**Table 1**). It is very important to take into consideration that 7.6% were resistant to all macrolides, which has enormous

significance, as it strongly limits the therapeutic options against this agent during pregnancy. These elements may contribute to failure to eradicate the agent from the amniotic fluid, and impact complications for the fetus/newborn. Failure to eradicate *Ureaplasma urealyticum* from the amniotic fluid has been described (9), worsening the perinatal prognosis. The increase in resistance to macrolides may be one of the important reasons why it is not possible to eradicate, although it is not the only one. Limitations on transplacental passage, duration of antibiotic treatment, dose used, route of administration, etc. also influence.

Fortunately, the resistance found in our study has not yet reached the alarm levels that have been described in other countries, such as studies in China, which describe resistance to erythromycin close to 40% (21), or in Poland, close to 30% (22), (**Table 2**), which practically disables the empirical use of said antimicrobial. It can only be used with demonstration of susceptibility of the previously isolated microorganism. In this scenario, although levofloxacin (a quinolone) which is considered in FDA category C, and is usually contraindicated, has been proposed in case of failure to eradicate *Ureaplasma urealyticum* with the use of macrolides, as an exception treatment, and having explained risks and benefits to the patient. There is very little national information regarding the susceptibility of *Ureaplasma* spp. to macrolides. And we believe that periodic monitoring of susceptibility is essential to choose the best alternative to treat this agent responsible for important fetal/neonatal morbidity. It is probably time that in our country we consider azithromycin as a first-line antimicrobial instead of erythromycin, given that the former has better passage into the amniotic fluid, better gastric tolerance, and there are reports in humans that show eradication of *Ureaplasma urealyticum* from the amniotic fluid in intra-amniotic infection (18). In our study it is the macrolide that demonstrates the greatest susceptibility. Finally, research in the coming years in relation to other antimicrobial alternatives that can be used in pregnancy to treat conditions linked to *Ureaplasma* will be of vital importance.

Table 3. Antimicrobial susceptibility of *Ureaplasma urealyticum* to Azithromycin, Erythromycin and Clarithromycin in different countries. Review by the authors.

Study	Antibiotic susceptibility (%)		
	Azithromycin	Erythromycin	Clarithromycin
Abarzúa F et al. (2019)	91.9	88.4	87.9
Foschi C et al. (2018)	96.8	–	–
Kasprzykowska U et al. (2018)	87.2	71.9	86.7
Zhang Y et al. (2018)	47.8	-	64.0
Maraki S et al. (2017)	96.6	81.9	–
D'Inzeo T et al. (2017)	–	96.8	–
Beeton M et al. (2016)	100.0	100.0	–
Huang C et al. (2016)	61.0	46.0	69.0

5. CONCLUSION

About 10% of *U. urealyticum* isolated in our study are resistant to some of macrolides, contributing to the non-eradication of the infection in empirical treatments. Within them, azithromycin appears most effectively. The escalation of resistance will impose constraints on therapeutic alternatives, hence exerting significant prenatal ramifications in the forthcoming period. The monitoring of susceptibility within each institution is crucial for making informed decisions regarding therapy selection.

Ethical Issues: All ethical issues were approved by the authors from the Iraqi Ministry of Health. Verbal and signed informed consents were obtained from all patients who included in the study during their first visit.

Conflict of interest: None

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