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Detection of Urogenital Mycoplasmas in Cuban Women with Infertility Antecedents

N. Rodríguez-Preval[1]*, A. Rivera-Tapia[2], C. Fernández-Molina[1], B. Mondeja-Rodríguez[1], E. Echevarria-Pérez[1] and D. Verdasquera-Corcho[1]

[1]Tropical Medicine Institute "Pedro Kouri", Havana, Cuba.
[2]Mycoplasma laboratory, Research Center Microbiological Sciences.
Universidad Autónoma de Puebla, Mexico.

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Mycoplasmas and ureaplasmas species have been associated with genitourinary tract infections including infertility, a complex medical problem whose causes may be diverse. These species are susceptible to protein synthesis-inhibiting tetracyclines, fluoroquinolones and macrolides. The objectives of the study were to determine the frequency of *M. hominis* and *U. urealyticum* in infertility women, to correlate such bacterial infection and risk/predisposing factors, and to study the antimicrobial susceptibilities to some antimicrobials. Endocervical swabs from 134 women were analyzed by the "*Mycofast Evolution 2*" Diagnosis Kit for the identification of *M. hominis* and *U. urealyticum,* and the antimicrobial susceptibility. It was obtained 48.5% (65/134) positive samples to *U. urealyticum*, 21.6% (29/134) to *M. hominis* and 17.2% (23/134) show coinfection with both species. The positive samples to *U. urealyticum* showed resistance to doxycycline (40%), ofloxacin (36.9%) and roxithromycin (29.3%), while the *M. hominis* positive samples showed resistance to roxithromycin (51.7%), ofloxacin (41.3%) and doxycycline (34.5%). In the coinfected samples the higher resistance was to roxithromycin (65.2%). Statistical association was not demonstrated between the mycoplasmas or ureaplasmas infection and the risk/predisposing factors in this women group.

Key words: Mycoplasmas, Ureaplasma, Infertility, Antimicrobial resistance.

Infertility is a complex medical problem whose causes may be diverse. Mycoplasmas are the microorganisms that by causing chronic, oligosymptomatic genital infectious may have a negative impact on fertility[1]. The role of these microorganisms, particularly *Ureaplasma urealyticum*, in the etiology of infertility has been very controversial.

Genital colonization by mycoplasmas can be associated to some risk factors such as: age, sexual activity, social economic conditions and the use of contraceptives. Such genital infection, pregnancy losses and ectopic pregnancy are predisposing factors associated to infertility[2].

Mycoplasma and *Ureaplasma* species have been associated with increased risk of genitourinary tract infections which includes pyelonephritis, pelvic inflammatory disease, chorioamnionitis, post-partum and postabortal fever. Whether these organisms causes involuntary infertility through fertilization or implantation impairment remains speculative[3].

Mycoplasmas are susceptible to bacteriostatic agents such as protein synthesis-inhibiting tetracyclines and macrolides, as well as bactericidal agents, including fluoroquinolones. Besides lincosamide and streptogramin antibiotics (MLSs) are others antimicrobials commonly used for the treatment of these infections[4-6].

* To whom all correspondence should be addressed.
Tel.: (537) 255 3530; Fax: (537) 202-0633;
E-mail: nadia@ipk.sld.cu

The objectives of the present investigation were to determine the frequency of *Mycoplasma hominis* and *Ureaplasma urealyticum* in women with infertility, to correlate such bacterial infection and risk/predisposing factors, and to study the antimicrobial susceptibilities to doxycyline, ofloxacin and roxithromycin.

MATERIALS AND METHODS

Subjects

Endocervical swabs were obtained from 134 women with infertility antecedents who were attended at the clinical laboratory of the Tropical Medicine Institute "Pedro Kouri" (IPK), since 2006 to 2009.

The taken samples were transported to the Mycoplasma Laboratory of the IPK within 24 hours, and inoculated in the "*Mycofast Evolution 2"* Diagnosis Kit, for the identification of *Mycoplasma hominis* and *Ureaplasma urealyticum*, and to detect the antimicrobial susceptibility to doxycycline, ofloxacin and roxithromycin.

The research protocol was approved by the hospital's ethics committee, and all patients gave informed consent in writing prior to participation in this study.

Mycofast Evolution 2

The samples were analyzed by the diagnosis kit. Briefly, the endocervical sample, inoculated in the transport media UMMt and homogenized, was used to restore the UMMlyo lyophilized culture media until their total dilution. The gallery was inoculated with 0,1 mL of the UMMlyo reconstituted media and was added 0,05 mL of *M. hominis* supplement to the sumps 9 and 10 corresponding to the identification of this specie. Each sump was covered with 2 drops of sterile oil mineral for microbiological use and the gallery was incubated during 24-48 hours at 37ºC. The reading was realized by visualization of colour change of the sumps of yellow to red, in the case of positive growth. For the antimicrobial susceptibility, the resistance was demostrated by a colour change of the corresponding sumps of yellow to red.

Patients information

Women antecedent data were obtained from patients in a survey.

Statistical analysis

Statistical analysis was performed by the software SPSS 13.0 version, $p < 0.5$ was considered to be significant.

RESULTS

In the analyzed samples by the "*Mycofast Evolution 2*" diagnosis kit, we obtained 48.5% (65/134) positive to *U. urealyticum*, 21.6% (29/134) positive to *M. hominis* and 17.2% (23/134) show coinfection with both species.

The evaluation of the antimicrobial susceptibility showed a bigger percent of resistance to doxycycline in the *U. urealyticum* positive samples (40%), continuing with ofloxacin (36.9%) and roxithromycin (29.3%). In the samples positive to *M. hominis*, the bigger resistance was found to roxithromycin (51.7%), followed by ofloxacin (41.3%) and doxycycline (34.5%). In the coinfected samples the higher resistance was found to roxithromycin (65.2%).

The mean age of the studied patients was 30.9 +/- 5 years, and the mean infertility years were 2.9 +/- 3 years.

It was found that 21.5 % (14/65) of the women with positive samples to *U. urealyticum* and 20.7% (6/29) of the women with positive samples to *M. hominis* had spontaneous abortion before the study. Ectopic pregnancies was documented in 2 (6.9 %) of the 29 positive women to *U. urealyticum*, and in 7 (10.8%) of the 65 positive women to *M. hominis*.

Statistical association was not demonstrated between the mycoplasmas or ureaplasmas infection and the spontaneous abortion and ectopic pregnancies.

DISCUSSION

The exact role of *Ureaplasma* spp. and *Mycoplasma* spp. in patients with infertility dysfunctions is not completely understood. Some studies have found *U. urealyticum* but not *M. hominis* present in the cervices of many culture-negative women, other authors found in their infertile population study group positive for at least one of the microorganisms, showing that *U. urealyticum* was related to infertility, while others failed to demonstrate any association between

genital mycoplasmas and infertility[6-10].

In this study we obtained a higher percent of positive samples to *U. urealyticum* in the study group. Many studies indicate that about a 40 % of the infertile women be positive for at least one of these microorganisms, being *U. urealyticum* more frequent in this women group[11].

Guven *et al*., found a higher positivity to *U. urealyticum* in a study realized to a group of unexplained infertile women, although the importance of detection of *U. urealyticum* positivity in the cervix of infertile is unclear. No case with proven *U. urealyticum* cervicitis had a positive PCR test in the Douglas peritoneum[12]. In a study using a micro-liquid culture method, Mycofast (IM, France) was found more positive samples to *U. urealyticum* than *M. hominis* in the infertile group. Besides, it have been described in many studies that the isolation rate of *U. urealyticum* from the endometrium or lower genital tract is significantly high in infertile women[13].

In our study we found between 29-51% of resistance to the analyzed antimicrobials. Results from previous reports, regarding the antimicrobial susceptibilities of genital mycoplasmas, originating from various countries, are very controversial[14,15].

Mycoplasmas lack a cell wall, the target of beta-lactam antibiotics and vancomycin, that's why tetracyclines, macrolides, and quinolones are the major antibiotics used in the treatment of urogenital infections caused by mycoplasmas. However, their therapeutic efficacy may be unpredictable due to increasing resistance[16].

Krausse *et al.*, found resistance to doxycycline in the 3% of the *Ureaplasma* spp. isolations and the 13% in the *M. hominis*, suggesting that doxycycline is still the drug of first-choice for the treatment of ureaplasmal infections and may be used for co-infection with *M. hominis*[17].

Karabay *et al.*, also conclude that doxycycline may be used in empirical treatment of genital tract infections in sexually active women, based on their results showing only a 1.6% and 5.9 % of resistance to the antimicrobial in their positive samples to *Ureaplasma* spp and *M. hominis* respectively. They also found between 41-58 % of resistance to ofloxacin[18]. However in this study we obtained about a 40% of resistance to doxycycline and ofloxacin. The extent of resistance varies geographically according to different antimicrobial therapy policies and the history of prior antimicrobial exposure in different populations, which lead to the emergence of resistance to one or other antimicrobial group [19]. The significant difference related to susceptibility to macrolides and quinolones has been reported before. Bayraktar *et al* found resistance to quinolones such as ofloxacin and ciprofloxacin in their clinical isolates of *U. urealyticum*, and suggest that the high resistance of mycoplasmas to antimicrobials could be due to mutations in antibiotic targets and may suggest their relation to higher pathogenicity[14,20].

The association between the genital mycoplasmas and ectopic pregnancy has been analyzed. Previous pelvic inflammatory disease (PID), particularly if this has resulted in damage to the fallopian tubes, often leads to an ectopic pregnancy. Whereas *Neisseria gonorrhoeae* and *Chlamydia trachomatis* are the most likely causative organisms, the genital mycoplasmas may be responsible. There is no evidence that ureaplasmas cause PID, but there is a little evidence to suggest that *M. hominis* does, and more evidence that *M. genitalium* may be implicated. Thus, there is a small chance that an ectopic pregnancy could have a mycoplasmal aetiology, but formal studies to establish this need to be conducted[21-23].

Some researchers have been unable to find a relationship between the presence of ureaplasmas and fetal loss, while in other studies, the isolation of these organisms was much more common among preterm birth, miscarriages and stillbirths than from healthy infants born at term or following therapeutic abortion[24,25].

The higher-than-expected prevalence of mycoplasmas and ureaplasmas, and the impact that they have in female reproduction, suggests a role for routine screening and treatment before undergoing infertility treatment.

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