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Mollicutes Isolates and their Relationship to Infertility in Women

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Genital mollicutes are considered a human pathogen of great importance as sexually transmitted agents and are involved in a variety of infectious such as urethritis, prostatitis, bacterial vaginitis and other inflammatory processes which leads to infertility. The objective was the isolation of mollicutes from vaginal swabs and establish its relationship to infertility in women. Two hundred fifty vaginal swabs were included, processing by microbiological culture and PCR, and data from the clinical history of the patients were analyzed in order to confirm their relationship of isolates with infertility. Of the 250 women examined, the total positive rates of ureaplasma and mycoplasma species for the 28-32 years old were the 20 %, while for the elder female being 48-52 year sold. The assay PCR-amplified products from urea plasma and mycoplasma of 429 bp and 301 bp, respectively. Some damage mechanisms involved in the infections by pathogenic organism such as genital mollicutes that affect or interrupt the fertilization process are oxidative stress, damage mechanism via receptor, membrane enzymes and DNA fragmentation. *Ureaplasma spp*., and *Mycoplasma spp*., is associated with several diseases of women of reproductive age, as urethritis, urinary tract, infections, chorioamnionitis, spontaneous abortions, pelvic inflammatory disease and infertility. In Mexico, are recognized as two of the major microbial genera isolated from patients diagnosed with in fertility.

Key words: Diagnostics, mycoplasma, ureaplasma, infertility, women.

The mollicutes are the smallest and simplest self-replicating organisms, being built of a plasma membrane, ribosomes, and a circular double-stranded DNA molecule-the typical prokaryotic genome. *Mycoplasmatales* are associated with infection of the genitourinary tract, reproductive failure, and neonatal morbidity and mortality. Infection with genital mycoplasmas has been linked with infertility. *Ureaplasma spp*. are the main cause of nonchlamydial, nongonococcal urethritis and acute prostatitis. In pregnancy, ureaplasma can cause chorioamnionitis and preterm delivery. Mycoplasma has also been associated with urethritis, acute endometritis, pyelonephritis, pelvic inflammatory disease, and postpartum septicemia[1-3].

The role of mycoplasmas and urea plasmas in acute nongonococcal urethritis in humans is not yet established. The pathogenetic role of mycoplasma has been suggested because it is isolated with remarkable frequency from the urogenital tract of patients with nongonococcal urethritis. Similarly, ureaplasma is considered a pathogen of the urogenital tract because its isolation is more prevalent in patients with nongonococcal urethritis than in asymptomatic subjects[4].

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Studies in women suggest that colonization with *Ureaplasma urealyticum* and *Mycoplasma hominis* may favor the development of bacterial vaginosis, pelvic inflammatory disease and postpartum sepsis[5].Being reported as flora in 40% of asymptomatic population, have been isolated frequently in patients with different diseases such as cervicitis, sepsis, pneumonia, meningitis, septic arthritis, prostatitis, pyelonephritis, kidney stones and infertility, and also in obstetric pathologies, such as prematurity, premature rupture of membranes, abortions, chorioamnionitis and neonatal infections.In adults, these organisms are acquired through sexual transmission, and its presence in semen is related to infertility[6].

It has also been established that virus infected acquired immunodeficiency (HIV-I) patients there are alterations of T cells and decreased CD4+ lymphocytes favoring in the later stages of the disease the frequency of opportunistic infections occur, highlighted ones caused by *Ureaplasma urealyticum*[7].*Mycoplasma genitalium* was initially isolated from the urogenital tract, but some work reported the isolation from the throat and identify the oral cavity colonization and other site[8].

Analyzed reports from European countries and the United States, which begs the question of the frequency of isolates in developing countries as socioeconomic factor plays an important role in the transmission of potentially pathogenic microorganisms.The aim was the isolation of mollicutes from vaginal swabs and establish its relationship to infertility in women.

MATERIALS AND METHODS

Clinical laboratories Puebla city in Mexico provided 250 vaginal swabs, samples were from patients attending the request of the doctor, but no indication of mycoplasma diagnosis and/or ureaplasmas,the age range of patients considered for this study was 18 to 52 years.

Microbiological culture

Each of the 250 vaginal swabs were resuspended in 2 ml of broth urea and simultaneously in 2 ml of broth Eaton, for isolating ureaplasma and mycoplasma, respectively, and incubated at 37° C for 15 days or vire of pH indicator. Then reseeding of 5 µl were performed in their respective agars Urea and Eaton, and incubated at 37° C for 7 days in order to confirm their isolation.

DNA preparation

Cultures obtained were resuspended in 1 ml of lysis buffer containing (10 mMTrisHCl pH 8.5, 100 mMKCl, 2.5 mM $MgCl_2$, 1% Tween-20, 1% Triton X-100 and 120 µg ml^{-1} of proteinase K. The cell suspension were incubated for 1 h at 60° C to lyse and deproteinize the cells. Finally, proteinase K was inactived at 95° C for 10 min and samples were allowed to cool at room temperature.

PCR primers and amplification

Primers AR1 and AR2 were used for amplification of a 301 bp fragment from mycoplasma DNA. The sequences are: AR1 (5´ ATG RGG RTG CGG CGT ATT AG 3´) and AR2 (5´ CKG CTG GCA CAT AGT TAG CCRT 3´). The thermal profile (TECHNE TC-412)included an initial denaturation at 95° C for 5 min followed by 40 cycles of denaturation at 95° C for 1 min, primer annealing at 50° C for 1 min and extension at 72° C for 1 min. A final extension was performed at 72° C for 5 min[9].

The primers used for detection of ureaplasma are: U5 (5´ CAA TCT GCT CGT GAA GTA TTA C 3´) and U4 (5´ ACG ACG TCC ATA AGC AAC T 3´) for amplification of a 429 bp fragment.The thermal profile (TECHNE TC-412)involved an initial denaturation step at 94° C for 3 min followed by 30 cycles of denaturation at 94° C for 1 min, primer annealing at 52° C for 1 min, and extension at 72° C for 1 min. The cycling was followed by a final extension step at 72° C for 10 min[10].

Aliquots of amplified samples (10 µl) were analyzed by electrophoresis on a 2% agarose gel and DNA bands were visualized by UV fluorescence after ethidium bromide staining.

Data from the clinical history of the patients were analyzed in order to confirm their relationship of isolates with infertility.

RESULTS

Of the 250 detected specimens, 160 were positive for containing *Ureaplasma* spp., and *Mycoplasma* spp., (64%) and 76 for both (30%).Samples were analyzed for microbiological assay and the PCR technique, the results were

Table 1. Number of specimens positive for mycoplasma and ureaplasma

Genus	Culture positive (%)	PCR positive (%)
Mycoplasma	60/250 (24)	71/250 (28)
Ureaplasma	77/250 (30)	89/250 (36)
Mycoplasma and Ureaplasma	65/250 (26)	76/250 (30)

variable, obtaining higher percentage of positive samples with PCR(Table 1).

The study by stereoscopic microscopy showed variations in colonial morphology isolates mollicutes (ureaplasma and mycoplasma species) (Figure 1).

Of the 250 women examined, the total positive rates of ureaplasma and mycoplasma species for the 28-32 years old were the 20%, while for the elder female being 48-52 years old. The positive rates were lower (4% and 8%), as show in figure 2.

The assay PCR-amplified products from urea plasma (429 bp) and mycoplasma(301 bp) is presented in the figures 3 and 4. PCR was shown to be more sensitive compared to microbiological culture, improving the diagnosis in an average of 5%(Table 1).

The analyzed information the medical histories of patients showed that 53% of patients with infertility, besides other genital disorders less frequently referred, such as: pelvic inflammatory disease, cervicitis and urethritis. Genital infections acquired via sexual transmission and caused by *Ureaplasma* spp., and *Mycoplasma* spp., are some the most important causes of reproductive disorders in recent years.

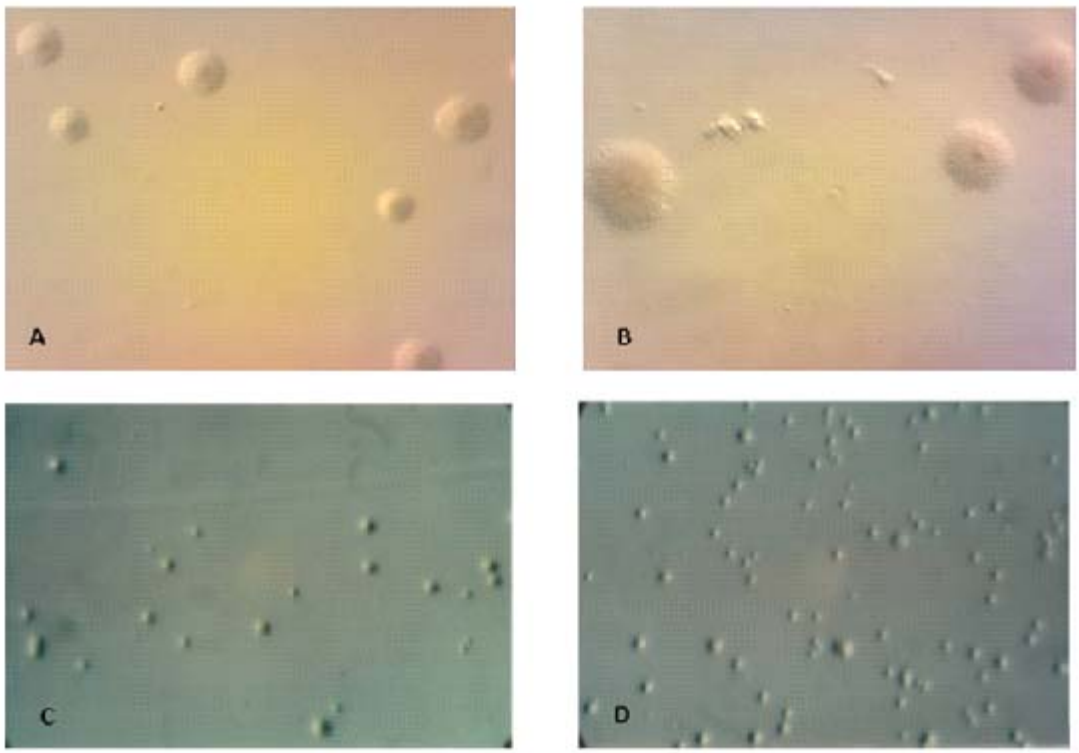

Fig. 1. Stereoscopic microscopy showing mycoplasma (A-B)and ureaplasma (C-D) isolates from vaginal swabs (40X)

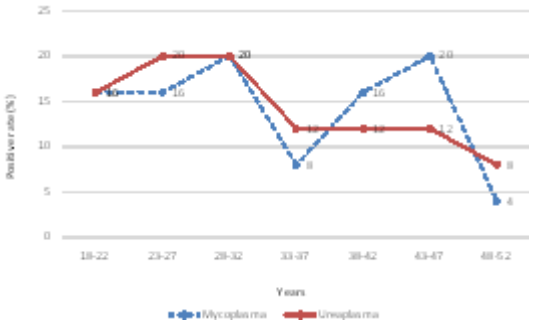

Fig. 2. Detection of mycoplasma and ureaplasma in vaginal swabs from female by ages

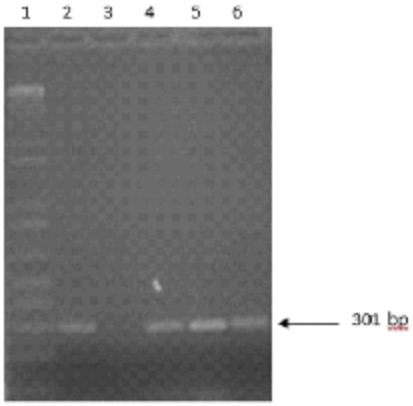

Fig. 3. Electrophoretic analysis of PCR products for mycoplasma from vaginal swabs. Lane 1: 100 bp size marker; lane 2: positive control (301 bp); lane 3: negative control; line 4-6 positive samples

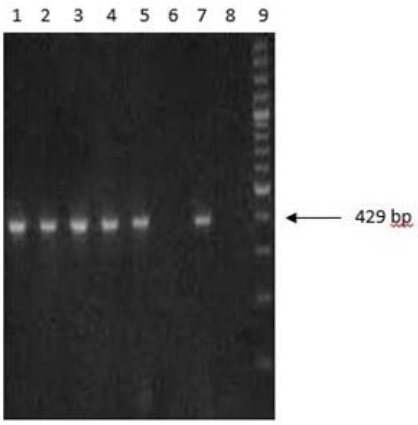

Fig. 4. Electrophoretic analysis of PCR products for ureaplasma from vaginal swabs. Lane 1-5 positive samples; lane 6: negative sample; lane 7: positive control (429 bp); lane 8: negative control; lane 9: 100 bp size marker

DISCUSSION

The genital mollicutes are associated with infection of the genito urinary tract, reproductive failure, and nenonatal morbidity and mortality. Infection with genital *Ureaplasmas* spp., and *Mycoplasmas* spp., has been linked with infertility[3].

Ureaplasma spp., and *Mycoplasma* spp., were commonly detected in vaginal swabs of patients with genital manifestation. The total positive rates of mollicutes reached to as high as 64%, the most common infection pattern was *Ureaplasma* spp., mono infection followed by *Ureaplasma* spp., and *Mycoplasma* spp., coinfection. These results were consisten with the results reported in China[11], but the positive rates were higher than those reported in Europe[12,13], this discrepancy might be due to the variation in socio economics conditions and living standards. The positive rates decreased drastically with patients over 50 years, it is perhaps due the obvious reduction of sexual activities in menopausal women.

Mollicutes lacks a cell wall, it can adhere to the membranes, there by potentially causing gamete dysfunctions. Adherence to the membranes may also enhance the adverse effects of superoxide and hydrogen peroxide produced by the organism, with subsequent hyper production of reactive oxigen species[14], also possess enzymes like phospholipases A1, A2 and C of *Ureaplasma urealyticum*, which the host cell membrane, allowing the microorganism to obtain the nutritional requirements necessary for their survival. *Ureaplasma urealyticum* is isolated significatly more often from women with infertility, miscarriages, and pelvicin flammatory disease[15-19].

The genital mollicutes represent a complex group of microrganisms that have been associated with a wide array of infectious diseases in adults and infants. In this work an incidence of 94% was obtained, trend I corroborate with other studies reported recently[20-22]. In conclusion, the prevalence of *Ureaplasma* spp., and *Mycoplasma* spp., was significantly associated with age and pelvic inflammatory disease and other sexually transmitted infection. Therefore it is recommended to performed the test in women of childbearing age to avoid posible complications in pregnancy.

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