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RESEARCH ARTICLE

Detection of *Ureaplasma Urealyticum* in Clinical Samples from Infertile Women by Polymerase Chain Reaction

SHAHIN NAJAR PEERAYEH and ROGHAYEH SAMIMI

For author affiliations, see end of text.

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ABSTRACT

Genital Ureaplasma urealyticum infection is considered to be a sexually transmitted infection. The bacterium has been found to be involved in PID, chorioamnionitis, urethritis, respiratory distress syndrome and pneumonia in newborn, abortion and infertility. U. urealyticum infections not only jeopardize fertility but also pose a risk for infertility treatment and resulting pregnancies. The purpose of this study was to determine the prevalence of U. urealyticum in specimens from infertile women by polymerase chain reaction (PCR). 377 endocervical swab samples were taken from infertile women. Mycoplasma genus and U. urealyticum were detected by means of the polymerase chain reaction with specific primers. Mycoplasma genus DNA was detected in specimens from 141(37.4%) of 377 patients. Of these 141 patients 85(22.5% of total specimens) were PCR positive with urease gene primers for U. urealyticum. The isolation rate of U. urealyticum in young women (<30 age) was higher than the others. Because of the potential adverse effects of U. urealyticum on the success rate of highly specialized infertility treatment, and its causal roles in several maternal complications of pregnancy and in neonatal morbidity and mortality, the rapid detection of U. urealyticum by PCR in infertile women could be important and necessary. The increased sensitivity and shorter time requirement of PCR support its further development for the diagnosis of U. urealyticum infections.

Keywords: *Ureaplasma urealyticum, Mycoplasma, Infertility, PCR*

Mycoplasmas are the smallest cell free-life microorganisms. They can be isolated as commensals or pathogens from plants, insects, animals and humans. Some of them are considered normal flora of the respiratory or genitourinary tract [1]. U. urealyticum can be found in the cervix or vagina of 40-80% of sexually mature, asymptomatic women [2-6]. The presence of U.urealyticum in a large proportion of healthy women complicates the assessment of the pathogenic roles of this organism, but several studies have indicated that genital colonization of the U.urealyticum can be associated with an increased risk of developing certain pathogenic conditions and pregnancy abnormalities, e.g., pelvic inflammatory disease, premature rupture of membranes, chorioamnionitis, and preterm labor and birth. In addition, it may be acquired by neonates either in utero or by vertical transmission at birth and can cause pneumonia, pulmonary hypertension, chronic lung disease, and meningitis of the newborn [7-12].

During the past decade, evidence has accumulated of causative role of *U. urealyticum* in human infertility. U.urealyticum was detected at a higher frequency in infertile women [13-15]. Colonization of the upper female genital tract with *U. urealyticum* was found to be associated with adverse pregnancy out comes [16]. In human in vitro fertilization systems, the presence of *U. urealyticum* in either semen or the female genital tract resulted in a decrease in pregnancy rate per embryo transfer [17].

Diagnosis of mycoplasm is usually made by serological determination or in vitro isolation of the organism. However, serological procedures are often hampered by interspecies cross-reaction, while cultivation is time-consuming and hard to for some fastidious mycoplasmas. Use of mycoplasma species-specific DNA probes made it possible to discriminate between different species, this method proved to be rapid and specific [18].In this study we determined the prevalence of *U.*

Table 1. Detection of Mycoplasma genus and U. urealyticum by PCR

PCR result	Number of specimens		Total number of specimens
	PCR positive	*PCR negative*	
Mycoplasma genus	141(37.4%)	236	377
U. urealyticum	85(22.5%)	292	377

Table 2. Detection of U. urealyticum from patients according to age

Age (years)	17-27	28-37	38-47	Total
Number of specimen	181	164	32	377
PCR positive	45(24.8%)	34(20.7%)	6(18.7%)	85(22.5%)

urealyticum in endocervical samples from infertile women by polymerase chain reaction (PCR).

METHODS

Clinical specimens

Endocervical swab samples were taken from a total of three hundred and seventy seven infertile women, ranging in age from 17 to 45 years. Swab samples were placed immediately in sterilized container with 2ml of PBS (0.1M Nacl, 2.5mM Kcl, 10mM Na_2HPo_4, 1.5 mM KH_2Po_4, PH 7.4) for subsequent PCR.

DNA extraction from specimens

DNA was extracted from standard strain *U.urealyticum* serotype VIII (type strain) and clinical samples as described previously by Cadieux et al [19]. Briefly, 1ml of the sample was centrifuged at 12000 ×g for 10 min. The pellet was washed in PBS and resuspended in 30µl of distilled water. After boiling for 10 min, an aliquot of 7µl was used directly in PCR experiments.

PCRs

Mycoplasmas have been detected previously with genus-specific primers, followed by amplification of positive samples with species-specific primers for *U. urealyticum. Mycoplasma*-specific 16S rRNA fragments amplified by use of the published [20] *Mycoplasma* genus specific primers GSO (5-GGGAGCAAACAGGAT TAG ATACCCT-3) and MGSO (5–TGCACCATCTGTCACTCTGTTAACCTC-3). The PCR assay was performed in 50µl of reaction mixture containing 10µl of 10× PCR buffer, 2.5 mM $MgCl_2$, 200 µM dNTP, 1.25 units of Taq polymerase, 20 pmol of each primer and 7µl of sample DNA. The reaction mixtures were placed in thermal cycler (Eppendorf, USA). The thermal profile involved an initial denaturation step at 94°C for 3 minutes followed by 30 cycles of denaturation at 94°C for 1 minute, primer annealing at 64°C for 1 minute, and primer elongation at 72°C for 1 minute. The cycling was followed by a final extension step at 72°C for 10 minutes. The primers published by Blanchard et al [21] were used for identification of *U. urealyticum*: primers U5 (5-CAATCTGCTCGTGAA GT ATTAC-3) and U4 (5-ACGACGT CCATAAGCAACT-3). The samples were placed in the same thermal cycler and heated to 94°C for 3 minutes. The cycling profile consisted of 30 cycles of 94°C(denaturation), 52°C(annealing), and 72°C(elongation) for 1 minute. The cycling was followed by a final extension step at 72°C for 10 minute. Aliquots of amplified samples (10µl) were analyzed by electrophoresis on a 1% agarose gel stained with ethidium bromide.

Statistical analysis

Chi-square (X^2) test was used for the generation of $p<0.05$ values.

RESULTS

The DNA from each sample was subjected to two PCRs. The first PCR with primers GSO and GMSO, were based on genus-specific 16S rRNA gene sequences. The genus-specific primers reacted with all mycoplasmal species investigated as well as with members of the genera *Ureaplasma, Spiroplasma, and Acholeplasma* [20]. Of the 377 patients studied, 141(37.4%) were positive with genus-specific primers (Table 1). Of these 141 patients, 85 (22.54% of total samples) were PCR positive with species-specific primers for *U. urealyticum* (second PCR).

A photograph of electrophoresis based on bromide-stained agarose gel for PCR-amplified products from the *Mycoplasma* and *Ureaplasma* strains is presented in figure 1. DNA from the 16S rRNA sequences that is amplified by the PCR primers used in this study shows at 270bp [20]. A 429bp fragment of the urease gene was amplified for identification of *U. urealyticum*. They have been shown previously to be highly specific for *U. urealyticum* and under optimal conditions, to allow detection of <10CFU of each serotype the organism [21].

The age of the patients from who were PCR positive varied from 17-45 with a mean age of 31 years. Distribution of the genital *U. urealyticum* in accordance to patient's age is presented in table 2. NO significant difference was found between the age of patients whose sample was PCR positive (positive group) and that of the other patients (negative group). There was also no difference regarding the duration of infertility, vaginal discharge, cervicitis, and abortion, between the positive and the negative group.

DISCUSSION

In this study, 22.54% of 377 infertile women were colonized with *U. urealyticum* as detected by PCR. Other studies using PCR for detecting *U. urealyticum* in endocervical specimens have reported prevalence rate as high as 40 to 80%.

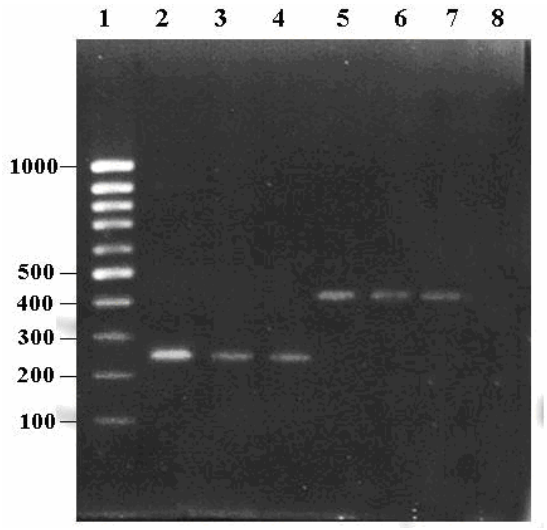

Fig 1. Electrophoretic analysis of PCR products for Mycoplasma genus and U.urealyticum from endocervical samples; Lane 1: 100bp size marker, lane 2: standard strain of Mycoplasma genus (270bp), lane 3&4: patients positive samples for Mycoplasma genus, lane 5: standard strain of *U.urealyticum* (429bp), lane 6&7: patients' positive samples for *U.urealyticum*, lane 8: negative control (distilled water).

Since *U. urealyticum* has been found significantly associated with low socioeconomic background, such as poverty, number of sexual partners, and use of contraceptive drug [2-6], it is not surprising that the rate of *U. urealyticum* was lower in our study. Although differences were not statistically significant, but the isolation rate of *U. urealyticum* was higher in women under 30 years of age, that is consistent with that previously described by others [2,3,5,22].

At present, the main method of detecting *U. urealyticum* is by culture, but the cultivation of *U. urealyticum* is laborious, time consuming, and requires specific expertise. PCR is revolutionizing the diagnosis of many infectious diseases, particularly those caused by organisms that are difficult to cultivate. PCR is a more sensitive and reliable means of detecting *U. urealyticum* in the endocervical specimens; its results can be available within a day, compared with 2-5 days for culture [23].

Although the precise role of *U. urealyticum* in human infertility has not firmly established, there is strong support in the literature for causal role in the cases of women and men infertility [12-17,24-29]. In addition, *U. urealyticum* has been implicated in several maternal complications of pregnancy and in neonatal morbidity and mortality. It may plays roles in endometraitis, chorioamnionitis, premature rupture of membranes, prematurity, low-birth-weight infants, postpartum fever, and it is important causes of pneumonia and meningitis in very low-birth-weight infants. Therefore the specificity, the exquisite sensitivity, and the rapidity of PCR make this technique most valuable in the diagnosis of genital *U. urealyticum* infections.

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CURRENT AUTHOR ADDRESSES

Shahin Najar Peerayeh, Department of Microbiology, School of Medical Sciences, Tarbiat Modarres University, Tehran, Iran. E-mail: NAJARP_S@modares.ac.ir (Corresponding author)

Roghayeh Samimi, Department of Microbiology, School of Medical Sciences, Tarbiat Modarres University, Tehran, Iran.