
Molecular Detection of *Ureaplasma Urealyticum* in Pregnant Woman Suffering from Urinary Tract Infection

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Abstract

Mycoplasma is a genus of bacteria that lack a cell wall, The present study was designed to identify the *Ureaplasma urelyticum* which causes the pregnant women suffering uti. The study included 200 samples of 150 pregnant women and 50 controls from the Bint Al-Huda teaching Hospital and Al-Haboubi teaching Hospital in the Thi-Qar provainc. The presence of the GPO gene in urine samples by using the PCR used as an indicator for mycoplasma colonization of urinary tract of pregnant, then the species was detected by DNA sequencing. in this study Regarding the GPO gene most of the cases were carry mycoplasmas and majority of the control groups expressing the positive results so that there was no significant statistical differences where (p value = 0.069). The present results recorded that the presence of about 94% homology between studied samples with *Ureaplasma urelyticum* sequences. Twenty-one, 12 and 6 genetic variations of 16SrRNA gene were identified in *Mycoplasma hominis*, *Mycoplasma*,



ureaplasma urelyticum. Some variations were variably distributed in the majority of the studied samples. The study showed that the ureaplasma urelyticum is an important cause of UTI in pregnant women in Iraqi population.

Keywords: pregnancy, urinary tract infection, mycoplasma, Ureaplasma urelyticum.

Introduction

Pregnancy is defined as a process and series of changes that take place in a woman's organs and tissues as a result of a developing fetus, and the entire process from fertilization to birth takes an average of 266–270 days, or about nine months. A condition of relative immunological impairment is pregnancy. Pregnancy causes the growing fetus's uterus to expand, which leads to pressure on the bladder and ureters. Low urine acidity, which results from the pregnancy's increased concentration of proteins, carbohydrates, and hormones, also raises the risk of UTIs [1, 2, 3].

Pregnancy-related alterations to the immune system and urinary tract, in particular, accelerate the spread of bacteriuria. [4] The International Classification of Diseases (ICD-10) defines urinary tract infections as infections that affect the kidneys, ureters, bladder, and urethra, among other structures involved in the secretion and excretion of urine. These infections can have serious consequences if they occur during pregnancy. Adverse maternal and neonatal outcomes, Urinary tract infections are a severe public health problem and are caused by a range of pathogens, but most commonly by Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterococcus faecalis and Staphylococcus saprophyticus [5,6].

Mycoplasma is a genus of bacteria that lack a cell wall around their cell membranes that can infect different parts of the body, which body part is affected such as lungs, skin, or urinary tract, depending on which type of mycoplasma bacteria is causing infection, and there are about 200 types of mycoplasma bacteria, but most common of cause uti in

genital tract: *Mycoplasma hominis*, *Mycoplasma genitalium*, *Urea plasma urealyticum* [7].

Urea plasma is a group of tiny bacteria that inhabit the respiratory and urogenital (urinary and reproductive) tract and can't be seen through a microscope. [8] Most healthy women have these bacteria in their cervix or vagina, and they don't cause any problems, also *Ureaplasma* can spread during sex, and pass the bacteria to the baby in the womb or during childbirth. [9] During pregnancy, the bacteria can lead to infections in both the mother and the baby, and the problems in newborn babies can include: Low birth weight, Pneumonia, and bacteria in the blood, called septicemia. [10] Advantages and limitations of serology, culture and molecular methods for the diagnosis of mycoplasma infections have recently been reviewed by Waites et al. [11].

Aims of the study

1. Detect the frequency of *Mycoplasma*.
2. Determine strains of *Urea plasma urealyticum* by DNA sequencing, with UTI in pregnant women Iraqi population.

Methods

The study included pregnant women suffering from UTI, by using a case-control study. A total of 200 urine samples were divided into three groups: a cases group of 150 women, a second group of 25 control pregnant women, and a third group of 25 control non-pregnant. The data were collected from Al-Habbobi teaching hospital and Bint Al-Huda teaching hospital in Thi_Qar Province/Iraq in the period from November 2022 to January 2023.

The research ethics committee board of Thi_Qar health directorate approved this protocol. The informed consent was applied from all participants. The data collected

from pregnant women include (age, address, trimester, Abortion), the specimen was collected in a sterile plan tube and transferred immediately to the laboratory to perform urine analysis in sterile condition. A urine sample containing 10 ml was centrifuged at 4000 rpm for 10minutes, after which the supernatant was discarded one drop of the sediment was put on clean slide and examined by a light microscope (omax /India) to detect the presence of R.B.Cs, pus cells and bacteria at low power (10x), then at high power (40x).

Detection of Mycoplasma in Urine Samples:

1- DNA Extraction

The extraction of the genomic DNA from urine samples was done by using the Human DNA-sorb-B Extraction Kit (sacace/ Italy) according to the manufacturer's instructions protocol.

2- The Amplification of the GPO Gene:

This test was done using the primer of GPO-3 and MGSO amplifying (270bp) size regional of the mycoplasma 16srRNA gene designed by (Van kuppveld et al,1992 forward: 5- F: TGCACCATCTGTC ACTCTGTTAACCTC -3 Reverse: 5- R* GGGAGCAAACAGGATrAGATACCCT -3. The final volume of reaction tubes is 20µl, consisting of 5µl Master Mix (Geneaid/Taiwan) 1µl of each forward and reverse of the primer for this gene, 3µl of DNA template and the volume was completed by adding nuclease-free water. The amplified DNA samples were electrophoresis (Biotech/ Germany) by mixing 5µl from DNA with loading dye (Bioner /Korea) and loaded into the dedicated wells, then exposed to an electric field (70V for 60 min). The thermocycling programs of PCR (Agilent /Germany) include: Initial denaturation at 94c° for 10 min, A 40 cycles of denaturation 94 c° for 30 sec. Anneling 60c° for 30 seconds and extension at 72c° for 35 sec. final



extension was 72 c° for 5 min. Finally, PCR products were examined under the UV light (optima INC/Japan, 300nm) transmitted through the gel (13).

DNA Sequencing of PCR Amplicons

The PCR amplicons were sequenced from the forward according to instruction manuals of the sequencing company (Macrogen Inc. Geumchen, Seoul, South Korea). Only clear chromatographs obtained from ABI sequence files were further analyzed, ensuring that the annotation and variations were not because of PCR or sequencing artifacts. By comparing the observed nucleic acid sequences of bacterial samples with the retrieved reference sequences of the bacterial database, the virtual positions, and other details of the retrieved PCR fragments were identified.

Interpretation of Sequencing Data

The sequencing results of the PCR products were edited, aligned, and analyzed with the respective sequences in the reference database using BioEdit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA). The observed nucleic acids were numbered in PCR amplicons as well as in their corresponding positions within the referring genome.

Ethical Approval: The study was carried out in conformity with the ethical standards outlined in the Helsinki Declaration. Before taking the sample, the patient's verbal and analytical consent were obtained. The research ethics committee board of Thi_Qar health directorate approved the study protocol, subject information, and consent form using document number 319 /2021(containing the number and date on October 20 / 2022).

Results

In this study regarding the GPO gene, most of the cases carried mycoplasmas and the majority of the control groups expressed positive results so there were no significant statistical differences where (p value = 0.069).

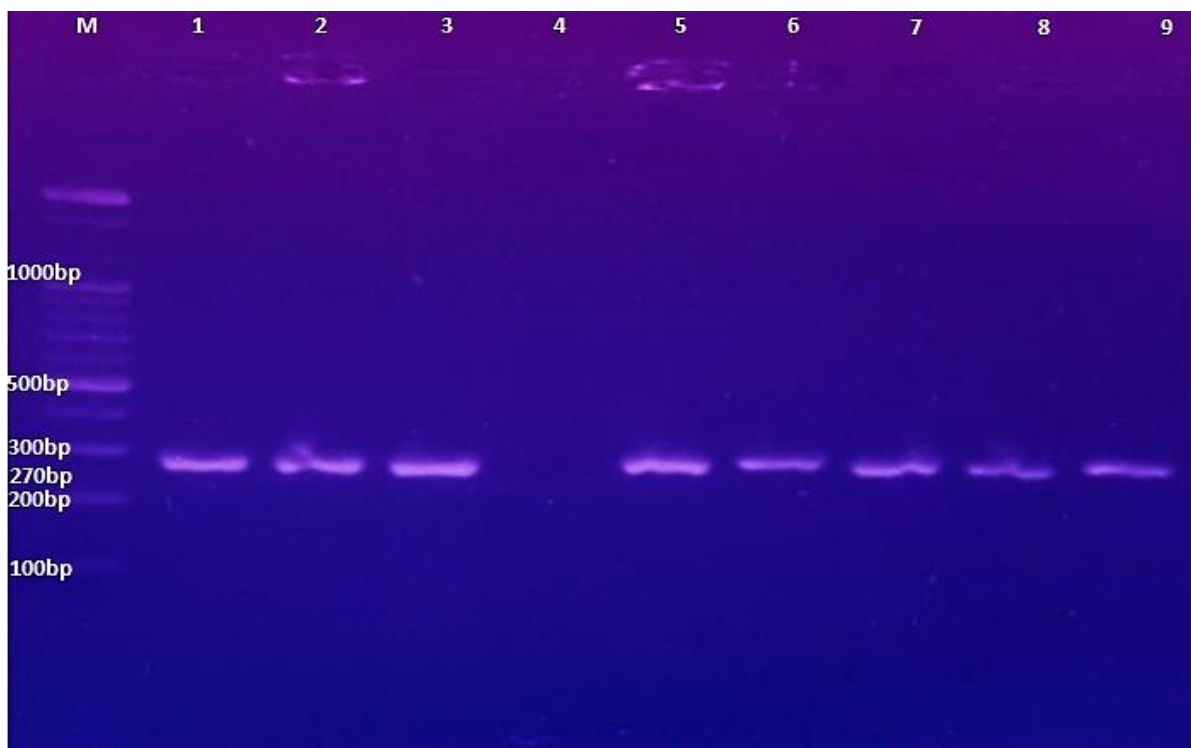


Figure (1): PCR product primer of GPO-3 and MGSO amplifying (270bp) size regional of the mycoplasma 16srRNA gene. Where M: marker the lans (1,2,3,5,6,7,8,9) give a positive PCR product. While lane for 4 was negative for GPO gene

Table (1): show distribution of gpo gene in study population

Sample	Result	Positive	Negative	Total
Control		42 (84%)	8(16%)	50
Patients		100 (66.7%)	50 (33.3%)	150



Results of *Ureaplasma Urelyticium* 16S rRNA Gene Sequence

To detect the variation of 16S rRNA gene sequence, 10 samples were included, which showed approximately 270bp amplicons length. Before sending these amplicons to sequencing, it was made sure that all the amplified amplicons had shown sharp, specific, and clean bands.

The sequencing reactions indicated the confirmed identity of the amplified products by performing NCBI blastn. Concerning the 270 bp PCR amplicons of the currently targeted 16S rRNA sequences, the NCBI BLASTn engine showed a high sequence similarity between the sequenced samples and *Ureaplasma urelyticium*

sequences. NCBI BLASTn engine indicated the presence of about 97% of homology with the expected target that partially covered the coding portion of the 16S rRNA gene sequences. By comparing the observed DNA sequences of the currently investigated samples with the retrieved DNA sequences (GenBank: MZ363965.1).

The alignment results of the 270bp samples revealed the detection of 6 nucleic acid variations compared with the corresponding *Ureaplasma urelyticium* referring sequences (Fig. 2). These sequences were prepared by aligning the investigated samples with the most relative sequences deposited in the NCBI database (GenBank acc. MZ363965.1).



	10	20	30	40	50	60	70	
80							
Ref.	GGGAGCAAATAGGATTAGATACCCCTAGTAGTCCACACCGTAAACAATG-GTTATTAAGTGTCCGGCACGAACGTGTCGGCG							
s1	-----							
s2	-----							
s3	-----							
s4	-----							
s5	-----							
s6	-----							
s7	-----							
s8	-----							
s9	-----							
s10	-----							
	90	100	110	120	130	140	150	
160							
Ref.	CTGCAGCTAACGCATTAAATAACCTGCCTGGGTAGTACATTCGCAAGAATGAAACTCAAACGGAATTGACGGGGACCCGC							
s1	T.....T.....							
s2	T.....T.....							
s3	T.....T.....							
s4	T.....T.....							
s5	T.....T.....							
s6	T.....T.....							
s7	T.....T.....							
s8	T.....T.....							
s9	T.....T.....							
s10	T.....T.....							
	170	180	190	200	210	220	230	240
170							
Ref.	ACAAGTGGTGGAGCATGTTGCTTAAATTTGACGATACACGTAAAACTTACCTAGGTTTGACATCCCCCTGCAAAGCTATAG							
s1C.....							
s2C.....							
s3C.....							
s4C.....							
s5C.....							
s6C.....							
s7C.....							
s8C.....							
s9C.....							
s10C.....							

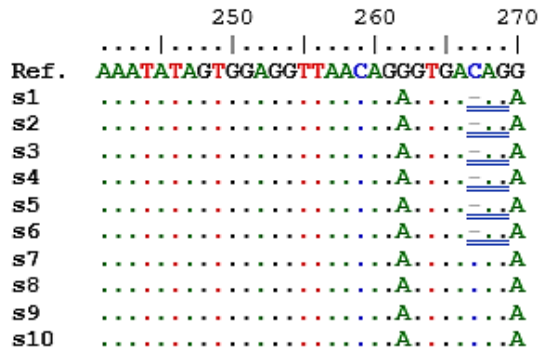


Figure (2): Nucleic acid sequence alignment of 10 bacterial samples with its corresponding reference sequences of the 16S rRNA within the *Ureaplasma urelyticum* genomic DNA sequences. The symbol ref: refers to the NCBI reference sequences, while “S: refers to sample numbers

To summarize all the results obtained from the sequenced 270bp fragments, the exact positions of the observed variations were described in Table 2.

Table (2): The pattern of the observed variations in the 270bp of the 16S rRNA amplicons in comparison with the NCBI referring sequences (GenBank acc. MZ363965.1)

Sample	Variant	Position in the PCR fragment
S1,S2,S3,S4,S5,S6,S7, S8,S9,S10	C81T	81
S1,S2,S3,S4,S5,S6,S7, S8,S9,S10	C103T	103
S1,S2,S3,S4,S5,S6,S7, S8,S9,S10	T178C	178
S1,S2,S3,S4,S5,S6,S7, S8,S9,S10	G262A	262
S1,S2,S3,S4,S5,S6,S7, S8,S9,S10	G270A	270
S1,S2,S3,S4,S5,S6	C267 del	267

A phylogenetic tree was generated based on the investigated 16SrRNA sequences in the studied bacterial samples. Along with the other deposited DNA sequences, this phylogenetic tree contained the 10 investigated samples aligned with its highly related sequences in Tamura-Nei mode. In the currently constructed tree, the total number of aligned nucleic acid sequences was 17 sequences. This tree entailed the presence of *Ureaplasma urelyticum*, which represents the incorporated nucleic acid sequences



within the tree. Based on the analyzed genetic sequences of *Ureaplasma urelyticum*, the analyzed 16SrRNA sequences were clustered into some adjacent phylogenetic branches, which entailed a wide range of diversity of this organism concerning the analyzed 16SrRNA sequences (Fig. 1).

The infer of phylogenetic tree the most relative sequences to studied samples (S1-S10) were found in separated branch belonged to *Ureaplasma urelyticum* with distance (0.036), while other compared isolates that had closely relation with *Ureaplasma urelyticum*, such as (MZ363965.1, MZ363963.1, MZ363964.1, CP001184) were positioned in other branch of this tree with distance (0.037).

Phylogenetic Tree

A specific phylogenetic tree was constructed in this study. The observed variants were compared with their neighbor homologous reference sequences using the NCBI-BLASTn server (Zhang et al. 2000). Then, an inclusive tree, including the observed variant, was built by the neighbor-joining method and visualized using iTOL suit to generate a traditional tool of branches construction (Letunic & Bork, 2019). The sequences of each classified phylogenetic species-group in the phylogenetic tree were annotated accordingly.

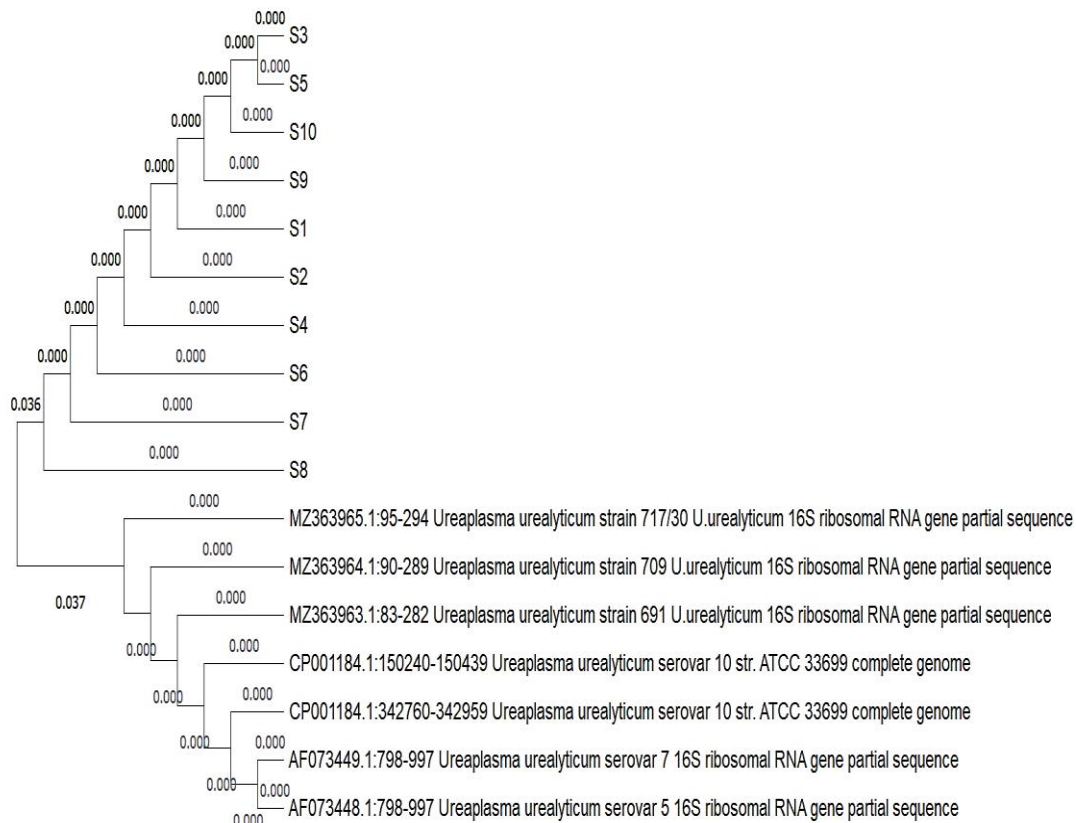


Fig (3): The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree is shown. (next to the branches). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004), and are in the units of the number of base substitutions per site. This analysis involved 17 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 222 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021).

Discussion

The current study aimed to detect the genetic variations of 10 samples of *Ureaplasma urelyticum*. To do so, the sequences of the 16S rRNA gene were screened in all bacterial samples that were taken from were isolated from pregnant woman suffering from UTI.

Genital mycoplasmas represent a group of microorganisms that are commonly found in the GU tract of pregnant and non-pregnant women. They are most commonly present in the genital tract of sexually active women (Bayraktar et al., 2010).

The identified nucleoids variations of 16S rRNA gene in *Ureaplasma urelyticum* were observed in the majority of samples such as: (C81T, C103T, T178C), while the deletion mutation (C267 del) were only detected in (S1, S2, S3, S4, S5, S6). All bacteria contain the 16S rRNA gene, thereby making the sequencing-based bacterial identification plausible (Clarridge, 2004). In addition, the 16S rRNA gene comprises variable regions interspersed with nucleotide sequences, which provide a species-specific signature sequence that is the hallmark of bacterial identification. The obtained sequences are compared with the known sequences in the database (Woese, 1987).

The similarity of bacterial species in investigated samples such as: *Mycoplasma hominis*, *Mycoplasma*, and *Ureaplasma urealyticum* to other strains was measured by BLASTn analysis of the 16S rRNA gene, there were recorded in the National Center for Biotechnology Information (NCBI) are in concordance of 91.30% homology with uncultured *Ureaplasma* sp. that isolated from many countries like: China (JN792306.1, JN792308.1), which isolated from feces. Also, the studied samples had 91.30% homology with *Ureaplasma urealyticum* that isolated from infertile male semen in China, as: (CP039963, and CP041200).

The importance of *Mycoplasmas* is obscured by the presence of *Mycoplasma hominis* and *Ureaplasma urelyticum* in many asymptomatic persons from whom these bacteria



could be isolated (Al-Daghistani and Abdel-Dayem, 2010). Latthe et al., 2008 recorded that 7.6% of the UTI cases are caused by both Mycoplasma and Ureaplasma (Latthe et al., 2008). The deduce of phylogenetic tree the most relative sequences to studied samples were found the Ureaplasma urelyticium presented in separated branch while other compared isolates that had closely relation with Ureaplasma urelyticium, such as (MZ363965.1, MZ363963.1, MZ363964.1, CP001184) were positioned in other branch of this tree. Furthermore, Musatovova and Baseman (2009) identified common and distinct sequences among the Texas clinical strains of Mycoplasma genitalium and obtained 18 DNA sequence variants, which were compared with all the other available clinical sequences.

The present results recorded closed relation among M. hominis in studied samples; this results disagreed with results of Jamalizadeh (2013) studied Mycoplasma hominis in infertile men referred to five separate lineages. It was pointed out that there was a rare similarity between the five lineages, the two lineages presented a significant genetic similarity with those available in the GenBank of Japan, Denmark, Russia and the United Kingdom and were arranged into one ancestry. The other three lineages were arranged into ancestry independent of the whole lineages and those available in GenBank. These lineages could be recorded in GenBank as native Iranian ones. Although a significant genetic similarity had been indicated between seven other lineages, there was no similarity between those available in GenBank and the five individual lineages.

In another study, Mohseni, (2013) examined Mycoplasma in infertile men and classified this bacterium into four different ancestries, with a rare similarity between them. In addition, there was a significant similarity between the four lineages and those available in GenBank of Denmark and the USA and were thus classified into one ancestry. This lineage could be recorded in GenBank as the native Iranian one. The

other two lineages were placed in two ancestries; however, there was a rare similarity between them and the rest of the lineages as well as those available in GenBank.

The results of alignment set by Bahaabadi et al., (2014) recorded that *M. hominis* isolates were genetically classified in five distinct lineages with little similarity between isolates of the five lineages; while other isolates have a high genetic similarity with isolated in the gene bank, which have been geographically isolated in Japan, Denmark, Russia, and Britain, and were placed in a line, and whom concluded that *M. hominis* could be considered as the most important bacterial factor of infertility in males and females (Bahaabadi et al., 2014).

Conclusion

There are high percent of mycoplasma colonization and infection among women in Iraqi population and This study showed the similarity of local isolates to global isolates, and these isolates were registered in the GenBank (LC779012, LC779013).

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Nil.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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