DOI: 10.7860/JCDR/2024/73501.20389

Original Article

Microbiology Section

Genomic Identification of Genital *Ureaplasma urealyticum* and *Ureaplasma parvum* Infection by Duplex Polymerase Chain Reaction in Symptomatic Women Attending a Tertiary Care Hospital: A Cross-sectional Study

P LAVANYA[1], N VIVEK MANDAL[2], G VINOJ[3], R RAMYA[4], SAMAL RUPAL[5], M BALASUBRAMANIAN[6], AN UMA[7], J PRADEEP[8]

ABSTRACT

Introduction: *Ureaplasma urealyticum* (*U. urealyticum*) and *Ureaplasma parvum* (*U. parvum*) are the two causative agents for Sexually Transmittable Diseases (STDs), they are often accompanied by Pelvic Inflammatory Disease (PID), vaginitis, endometriosis, etc.

Aim: To detect the prevalent genotypes/biovars of *Ureaplasma* species by using Duplex Polymerase Chain Reaction (PCR) in symptomatic women attending the Obstetrics and Gynaecology clinic of a tertiary care hospital.

Materials and Methods: This was a cross-sectional study, comprised of 200 symptomatic women aged from 18 to 45 years. This study was carried out in the Department of Microbiology, School of Allied Health Sciences, Department of Obstetrics and Gynaecology, Mahatma Gandhi Medical College and Research Institute, and Mahatma Gandhi Medical Advanced Research Institute, Sri Balaji Vidyapeeth, Puducherry, India for the period of January 2021 to January 2024. Duplex PCR was performed for the presence of *U. urealyticum* and *U. parvum* in endocervical samples. Amplified PCR products were analysed and the positive PCR products were sent for sequencing and the sequenced results were compared with GenBank and phylogenetic analysis was constructed. Data was analysed using Chi-square tests to test level of significance between the two groups (p-value≤0.05).

Results: The overall results found that 48 (24%) of symptomatic women were positive for *Ureaplasma* species. In Duplex PCR, 24 (12.0%) were positive for both urease and upv genes; followed by 15 (7.5%) for urease and 9 (4.5%) for upv genes only. The phylogenetic tree concluded that the genetic sequences of *Ureaplasma* species are closely related to various isolates within a species.

Conclusion: The study concludes that *U. parvum* is the most common pathogen in symptomatic women with predominant age of 26-35-year-old. The duplex PCR, increase the sensitivity and specificity by the application of two different genes in a single amplification reaction. Since the research will focus on different gene targets of *Ureaplasma* species by the application of multiplex in future study.

Keywords: Pelvic inflammatory disease, Sexually transmitted diseases, Urease gene, Upv gene, Vaginal discharge

INTRODUCTION

Ureaplasma urealyticum and Ureaplasma parvum are two significant bacterial agents which are characterised by lack of a cell wall can cause Sexually Transmitted Infections (STI) [1,2]. *Ureaplasma* species belong to the Mycoplasmatacae family, which consists of small and simplest bacteria. They are frequently discovered in the genitourinary system [1,3]. Symptomatic women are mostly diagnosed with PID, vaginitis or endometriosis, cervicitis and urethritis in men. In case of asymptomatic, presence of infections are mild to severe and may cause infertility more in females compared to males [3,4]. In pregnant women, the infection can lead to complications such as premature labour, miscarriage, stillbirth or Intrauterine Death (IUD) [5,6]. *U. parvum* is a commensal organism found in the lower urogenital tract. It is frequently linked to unfavourable pregnancy outcomes [5]. The presence of *U. urealyticum and U. parvum* in placenta is associated with chorioamnionitis, can cause a higher rate of spontaneous abortion, prematurity, perinatal morbidity and mortality [5]. Microscopic Haematuria (MH) has also been linked to *U. parvum* serovar-3 or serovar-14 infection [7]. Several research studies have documented the risk of *U. parvum* which may be confused with other infections like *Mycoplasma hominis, Chlamydia trachomatis* and other urinary tract infections [7-9]. *U. parvum* has been found to be involved in premature delivery [10,11]. The clinical presentation of *U. urealyticum* infection differs in males and females. *U. urealyticum* infection is highly associated with males having multiple female sex partners [12].

In earlier, the detection methods for *Ureaplasma* spp. was solely relied on selective culture methods, like Shepard's A7 agar, modified phenol red-urea, SP-4-urea, modified phenol red-arginine, and SP-4-arginine broths and now the demonstration of antibodies/antigens/DNA of these pathogens in the clinical specimens is simpler and less time-consuming than the culture [1]. The clinical sensitivity of culture methods is also unclear. Many studies have explored various clinical materials in which PCR exhibits greater sensitivity when compared to traditional culture techniques [13].

Diagnosing infections in different body systems requires a quick and accurate detection of *U. parvum* for prompt treatment as well as to avoid some clinical complications like infertility, stillbirth, miscarriages and recurrent abortions [5]. Development of Real Time PCR and Loop Mediated Isothermal Amplification (LAMP) is highly helpful to detect sensitivity and specificity for the diagnosis of *Ureaplasma* infection in respiratory tract of preterm infants [14,15]. The study employs the efficacy of duplex PCR technique to identify both *U. urealyticum and U. parvum* in single amplification and potentially setting a new standard for diagnosing genital infections, to reduce the cost and time. These infections are not frequently recognised

due to the constraints of conventional diagnostic techniques, which lacks in proper treatment. The study mainly focuses on a non invasive method for the detection of *Ureaplasma* species in symptomatic women, to improve the diagnostic sensitivity and accuracy. Identifying *Ureaplasma* genotypes/biovars can help healthcare providers to ensure that the patients may receive the most appropriate antibiotics and reducing the risk of resistance. The main aim of the study was to detect the prevalent genotypes/biovars of genital infections caused by *Ureaplasma* species using duplex PCR in symptomatic women attending the Obstetrics and Gynaecology clinic of a tertiary care hospital.

MATERIALS AND METHODS

This cross-sectional study was carried out in the Department of Microbiology, School of Allied Health Sciences, Department of Obstetrics and Gynaecology, Mahatma Gandhi Medical College and Research Institute, and Mahatma Gandhi Medical Advanced Research Institute, Sri Balaji Vidyapeeth, Puducherry, India for the period of January 2021 to January 2024. The ethical clearance from the committee member's MGMCRI/RAC/2021/IHEC/12 dated on 07.06.2021. After getting a written informed consent, specimen was collected from 200 symptomatic women based on the inclusion criteria.

Inclusion criteria: Patients presenting to Gynaecology OPD with complaints of Pelvic Inflammatory Disease (PID), abnormal vaginal discharge, burning micturition, cervical fragility, cervical erosions, genital ulcers and lower abdominal pain and age >18 years or <45 years were included in the study.

Exclusion criteria: Antenatal women with any known pregnancy complications. Patients who have received macro bile/tetracyclines during the past 10 days. Women with cancerous or precancerous lesions of the cervix detected by clinical examination and/or Pap smear, antenatal women in the third trimester were excluded from the study.

Sample size: The sample size was calculated by using proportionate formula based on the national prevalence of symptomatic cases of *Ureaplasma* was 25.8% which was rounded up to 26% [3]. The ethical clearance from the committee member's MGMCRI/RAC/2021/IHEC/12 dated on 07.06.2021. After getting a written informed consent, specimen was collected from 200 symptomatic women based on the inclusion criteria.

Study Procedure

Endocervical swabs were collected from the symptomatic women and transferred to the sterile phosphate buffered saline solution. Specimens were transported to the research laboratory for molecular testing and the DNA was extracted by using QIAmp Blood mini kit, QIAGEN, Germany. Molecular detection was performed by using Duplex PCR. The amplified PCR positive samples were sequenced for *Ureaplasma* species. Phylogenetic analysis was made for sequenced products using MEGA software version 10.0. After genomic extraction, the remaining clinical specimen will be autoclaved and disposed as per the biomedical waste management rules, 2016 [16].

Molecular diagnosis of *Ureaplasma* species: The endocervical swab was collected in Department of Obstetrics and Gynaecology, Mahatma Gandhi Medical College and Research Institute, Puducherry, India. Genomic DNA was extracted from the endocervical swab using the commercial extraction kit "QIAGEN Blood Mini DNA, Germany" [17]. The genomic DNA was extracted according to their manufacturer's instructions and the concentration of the extracted DNA was measured by spectrophotometry (NanoDrop 2000 Spectrophotometer, Thermo Scientific). The extracted DNA was preserved at -20°C for molecular testing.

Duplex PCR: Prepared mix of 25 µL contains 12.5 µL of the Taq DNA Polymerase 2x Master Mix RED 1.5 mM $MgCl_2$ (Ampliqon, Denmark), followed by final concentration 1 µL of 25 µM of each forward and reverse primers of *U. urealyticum and U. parvum* [Table/Fig-1]. Addition of 5.5 µL sterile nuclease-free water and finally 3.0 µL of genomic DNA to the reaction mix. The sample positive for *U. urealyticum and U. parvum* (size 424 bp and 153 bp) is utilised as a positive control and nuclease free water was added as No Template Control (NTC) for each run. The Duplex PCR was carried out using Applied Biosystem, VeritiDx 96-wells Thermal cycler (Thermo Fisher Scientific, Singapore). Primer sequences were confirmed with NCBI- BLAST (www.ncbi.nlm.nih.gov/blast/Blast.cgi) to show the high specificity for the known sequences available in the public domain. About 2 µL of 100-bp DNA ladder (HiMedia, BioSciences, India) was added to the well for determination of amplicon size for the specific target. About 8 µL of the amplified PCR products were analysed by 2% Agarose gel electrophoresis stained with Ethidium bromide solution. The voltage 80ºC for 30 minutes was applied by using a Power Pac 300 (Bio- 30 Rad, USA) power supply. The gel was visualised using Gel+

Specific Gene target primers	Amplicon size	References
***Ureaplasma urealyticum* (Urease gene)**		
Uu-1-5'- ACGACGTCCATAAGCAACT -3'	424 bp	[1]
Uu-2-5'- CAATCTGCTCGTGAAGTATTAC'-3'		
***Ureaplasma parvum* (upv gene)**		
Forward :5'–TGCGGTGTTTGTGAACT- 3'	153 bp	[1]
Reverse :5'TGATCAAACTGATATCGCAATT ATAGA- 3'		

[Table/Fig-1]: Details of Duplex PCR primers for Urease and upv gene targets.

Documentation system (Bio-Rad, USA). Amplicon PCR products of both *U. urealyticum* and *U. parvum* showed 424 bp and 153 bp were considered as positive, respectively.

Phylogenetic analysis of *Ureaplasma* species: For phylogenetic investigations, the PCR amplified products was purified using QIAmp PCR Purification kit (QIAGEN, Germany) and sent for sequencing to Mediomix Healthcare Private Limited, Bengaluru and they were sequenced by using Applied Biosystems ABI3730XL. The sequenced data was analysed for further determination using Nucleotide Basic Local Alignment Search Tool (BLAST) program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (National Centre for Biotechnology Information -NCBI database). These sequenced data were aligned with corresponding nucleotides from GenBank by using ClustalW (https://www.ebi.ac.uk/Tools/msa/clustalo/) and Maximum Likelihood (ML) method. Phylogenetic tree generated using IQ-TREE tool (default parameters) based on multiple sequence alignment. The ML bootstrap support values are denoted as blue circles as described in the legend.

STATISTICAL ANALYSIS

The Chi-square test and Fisher's-exact test will be utilised to compare two groups using GraphPad Prism, online software. All p-value ≤0.05 will be considered statistically significant.

RESULTS

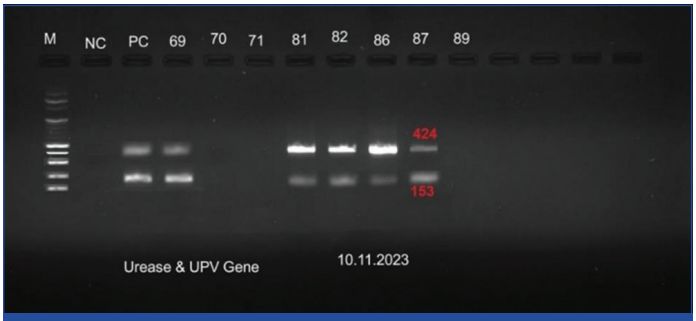
This study was involved with 200 symptomatic women. A greater proportion of the 83 (41.5%) patients were recruited from the age 26-35-year-old. [Table/Fig-2] indicates significance of the patients' ages, which are divided into three groups.

Overall, 200 symptomatic samples, showed 48 (24%) positive for *Ureaplasma* species either one or both targets. In Duplex PCR, both urease and upv gene were showed positive for 24 (12%) samples, followed by 15 (7.5%) and 9 (4.5%) showed only in urease and upv gene, respectively. [Table/Fig-3] shows the PCR amplified products of 424 bp and 153 bp.

[Table/Fig-4] shows the statistical analysis of clinical parameters for positive and negative symptomatic patients. Fibroid uterus (p-value<0.0001), vaginal discharge with odour (p-value<0.0001),

Age (years)	No. of symptomatic patients (n=200) (%)	No. of positive samples (n=48) (%)
18-25	42 (21)	12 (25)
26-35	83 (41.5)	23 (47.9)
36-45	75 (37.5)	13 (27.1)

[Table/Fig-2]: Demographic details of symptomatic patients (n=200).



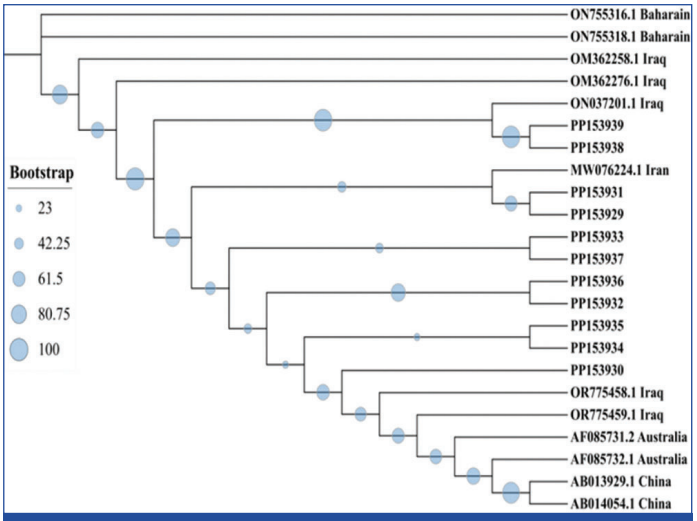
[Table/Fig-3]: Both urease and upv gene showed positive in Duplex PCR.
M: 100 bp DNA Ladder, NC: Negative control; PC: Positive control; Samples 69, 81, 82, 86 and 87 – Showed positive for both gene targets and Samples 70, 71 and 89 were showed negative

Clinical conditions	Number of Patients (n=200) (%)	Urease /Upv gene positive patients (n=48) (%)	Urease /Upv gene negative patients (n=152) (%)	p-value*
Lower abdominal pain	45 (22.5)	11 (22.9)	34 (22.3)	0.9053
Dysuria	11 (5.5)	3 (6.2)	08 (5.2)	0.7273
Irregular menstrual cycle	31 (15.5)	5 (10.4)	26 (17.1)	0.3612
Amenorrhoea	8 (4)	3 (6.2)	05 (3.2)	0.4010
Dysmenorrhea	7 (3.5)	2 (4.2)	05 (3.2)	0.6742
Ovarian cyst	15 (7.5)	3 (6.2)	12 (7.9)	1.0000
Fibroid uterus	6 (3)	0	06 (3.9)	0.0001
Heavy menstrual bleeding	31 (15.5)	7 (14.5)	24 (15.7)	1.0000
Vaginal discharge with odour	18 (9)	18 (37.5)	0	0.0001
Vaginal discharge	13 (6.5)	11 (22.9)	02 (1.3)	0.0001
Vaginal itching	15 (7.5)	10 (20.8)	05 (3.2)	0.0003

[Table/Fig-4]: Clinical conditions of symptomatic patients positive for urease as well as upv gene (n=200).
*p-value ≤0.05 is considered as statistically significant

vaginal discharge (p-value<0.0001) and vaginal itching (p-value=0.0003) were statistically significant. Remaining parameters like lower abdominal pain, irregular menstrual cycle, ovarian cyst, dysuria, amenorrhoea, dysmenorrhoea, heavy menstrual bleeding was not statistically significant.

The specificity of the 11 sequences was determined by using BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi) showed maximum identity of 99-100% with other isolates available in NCBI database. The study sequences were compared with the reference sequences. The aligned 11 sequences PP153929 to PP153939 were available in the GenBank, NCBI database. The phylogenetic tree was constructed based on their genetic sequences obtained by the ML method, which classifying *Ureaplasma* species from both genes as well as the genetic relatedness between various isolates of the same species which is illustrated in [Table/Fig-5]. Study isolates PP153939 and PP153938 forms were closely related with Iraq strain ON037201.1 which gives 100% bootstrap value. Four reference strains ON755316.1 and ON755318.1 from Baharain followed by OM362258.1 and OM362276.1 from Iraq forms a separate ancestral lineages from other isolates. Study sequences pairs PP153933-PP153937, PP153936-PP153932, PP153935-PP153934, and PP153931-PP153929 shared a common ancestor with the cluster formed by Iran isolate MW076224.1, with bootstrap value 80.75%. Other reference



[Table/Fig-5]: Phylogenetic analysis using IQ-TREE tool based on multiple sequence alignment of Ureaplasma species.
The Maximum Likelihood (ML) bootstrap support values are denoted as blue circles as described in the legend

strains from China, Australia and Iraq AB013929.1, AB014054.1, AF085731.2, AF085732.1 and OR775458.1, OR775459.1 are closely grouped with present study strains PP153930. The percentage of sequence similarity was 95.0% covered by reads with the sequences from reference strains.

DISCUSSION

Ureaplasma spp. is a self-replicating organism, appears both in genome size as well as in cellular dimensions. *U. urealyticum* and *U. parvum* are the two important pathogens causing urethritis/cervicitis in infertile patients [1,4,6,12]. In compliance of guidelines for STIs Treatment 2021, urethritis is known to cause by *Ureaplasma* species [18]. Infertility in female patients has notified with few clinical symptoms like MH and Chronic Micturition urethral Pain (CMP) etc., caused by *U. parvum* infections [7]. The investigation was performed by Zhou YH et al., revealed that 19.2% overall prevalence of *Ureaplasma* species was observed in infertile and control groups [12]. In the present study, the prevalence of *Ureaplasma* species was recorded 24.6% in symptomatic women of Puducherry population. *U. parvum* is more predominant among clinical isolates of infertile men when compared to *U. urealyticum* [1,12]. Similarly, present study also found that *U. parvum* was more frequent than *U. urealyticum* in female. Hence, this study deals only with the women reproductive health instead of men. Iranian study was showed the prevalence of *Mycoplasma* and *Ureaplasma* in pregnant women was 5.6% and 11.2%, respectively [8]. A study from South Korea has shown that 16.3% urinary *U. parvum* infected patients were infertile due to tubal factor damages [7]. Culture is the gold standard for determining *Ureaplasma* pathogen identities, yet this method has limitations in terms of contamination and time commitment [1,13].

In Puducherry, a study was conducted on 2019 by Stephen S et al., reported that *U. parvum* is the most significant potential pathogen compared to *U. urealyticum* using Commercial Fast Track Diagnostics (FTD) [15]. Urethritis plus, Real time multiplex PCR kit which targets seven pathogens viz., *Chlamydia trachomatis, Neisseria gonorrhoea, Trichomonas vaginalis, Mycoplasma hominis, M. genitalium, U. urealyticum and U. parvum* in clinical isolates. A few researchers from Puducherry and Korea have performed the efficacy of Real Time multiplex PCR for STI infection and also indicated that *U. parvum* is the most predominant infection in female patients compared to male [15,19]. If a samples were positive for *U. urealyticum* (urease gene), it highly suggests the capable of producing urease enzyme. Other urease producing bacteria includes viz., *Proteus* or some strains of *Staphylococcus* and *Streptococcus* [1]. If specimen is amplified for upv gene, it indicates unique genetic marker for *U. parvum*. Occurrence of

both Urease as well as upv gene it could strongly suggest that may either be a co-infection or the mixed *Ureaplasma* species present in the clinical sample [2].

The diagnostic accuracy of PCR for the detection of *U. urealyticum* and *U. parvum* strains found in Indian adults with symptoms of genital discharge [2,3]. Moreover, 100% of viability was not found in PCR and it may also detect some non viable *Ureaplasma* cells in the clinical isolates [2]. Ideally, in duplex PCR, the amplification of both targets should occur simultaneously. However, there can be instances where one target is amplified more efficiently or preferentially over the other due to various factors. To address preferential amplification, optimisation of PCR conditions, primer design and template concentrations may be necessary to ensure balanced amplification of both targets in duplex PCR [8]. The study from Frølund M et al., were found that the prevalence of *Ureaplasma* species was 31% by both qPCR and culture methods [13].

Another study from Kokkayil P and Dhawan B, suggests that PCR is more sensitive and reliable method to prevent *Ureaplasma* infection and helps to facilitate better clinical management in India [4]. The present study prefers Duplex PCR than culture, due to more time consuming and overgrowth of other bacterial contamination [1,13]. In Indian context, the prevalence of serovar for *U. urealyticum* is 3, 6 and 10 followed by *U. parvum* 1, 3, 6 and 14 with genital tract infections and women with preterm labour as well as recurrent abortions [10,11,13]. About 8.5% of *U. parvum* (biovar 1) was identified in male population diagnosed with Non-Gonococcal Urethritis (NGU) and 13.5% of males are asymptomatic. In case of *U. urealyticum* (biovar 2) was identified in 15.8% of men diagnosed with NGU and 7.8% were diagnosed without urethritis [20].

Clinical conditions were performed between the *Ureaplasma* positive and negative patients showed that the women with symptoms of irregular menstrual cycle, ovarian cyst, vaginal discharge with odour, vaginal discharge and vaginal itching are statistically significant. In future our research will be given to the importance of diagnostic methods for screening of STIs in different populations like pregnant women etc. However, in the present study authors have standardised in-house preparation of Duplex PCR by targeting two different genes of *U. urealyticum and U. parvum*. The amplified PCR products were confirmed by sequencing and these sequences are aligned and published in the NCBI database. The 11 sequences from this study have showed the 99-100% of with other sequences. The phylogenetic analysis of these sequences shows that close genetic relatedness between the various isolates of the same species of *Ureaplasma.* Most of our study sequences have bootstrap value from 61.5 to 100% are shown in [Table/Fig-5] which are closely related with other strains from Iraq, Baharain, Australia and Iran.

Limitation(s)

The study may not account for all potential confounding variables such as socio-economic status, sexual practices, or other underlying health conditions that could affect infertility or *Ureaplasma* infection. These unmeasured factors could influence the study results and the association between *Ureaplasma* species and infertility. Conducting the study at a single hospital might limit the diversity of the sample population and the findings might not be applicable to other regions or settings.

CONCLUSION(S)

This study makes a significant contribution by performing the effectiveness of duplex PCR in identifying the *Ureaplasma* species and providing valuable insights for clinical practice, including improved diagnostic methods and targeted treatment strategies for public health concern. In future, the present research will focus on validating infection in different populations based on these findings and exploring the role of pathogenesis of *Ureaplasma* infections in patients, as well as comparing the reliable diagnostic methods to enhance the management and understanding this type of infections. By addressing these areas, one can improve patient outcomes and existing our knowledge on *Ureaplasma* infections and their impact on human's health.

Acknowledgement

The authors are grateful to the Honourable Chancellor, Vice-Chancellor, Vice-president, Dean-Research, Sri Balaji Vidyapeeth (Deemed-to-be-University), and Dean, Mahatma Gandhi Medical College and Research Institute, Puducherry, India for providing the research facility for this molecular study.

REFERENCES

[1] Hussein MA, Maaroof MN. Isolation and characterization of *Ureaplasma parvum* from the semen of infertile men and detection of virulence factors genes. Int J of Health Sci. 2022;6(S2):8851-63.
[2] Dhawan B, Gupta V, Khanna N, Singh M, Chaudhry R. Evaluation of the diagnostic efficacy of PCR for *Ureaplasma urealyticum* infection in Indian adults with symptoms of genital discharge. Japanese J Infect dis. 2006;59(1):57-58.
[3] Dhawan B, Malhotra N, Sreenivas V, Rawre J, Khanna N, Chaudhry R, et al. *Ureaplasma* serovars & their antimicrobial susceptibility in patients of infertility & genital tract infections. Indian J Med Res. 2012;136(6):991-96.
[4] Kokkayil P, Dhawan B. *Ureaplasma*: Current perspectives. Indian J Medical Microbiol. 2015;33(2):205-14.
[5] Al-khafaji G. Molecular study for diagnosis of *Ureaplasma parvum* in women with recurrent miscarriage. In Obstetrics 2017. IntechOpen.
[6] Ashshi AM, Batwa SA, Kutbi SY, Malibary FA, Batwa M, Refaat B. Prevalence of 7 sexually transmitted organisms by multiplex real-time PCR in Fallopian tube specimens collected from Saudi women with and without ectopic pregnancy. BMC Infect Dis. 2015;15:569.
[7] Kim MS, Lee DH, Kim TJ, Oh JJ, Rhee SR, Park DS, et al. The role of *Ureaplasma parvum* serovar-3 or serovar-14 infection in female patients with chronic micturition urethral pain and recurrent microscopic hematuria. Translational Andrology and Urology. 2021;10(1):96.
[8] Javadinia S, Movahedi Z, Shokrollahi MR, Naghdalipour M, Tabatabaee A, Asgarian R, et al. Prevalence of *Mycoplasma genitalium* and *Ureaplasma urealyticum* in pregnant women of Tehran by duplex PCR. Curr Pediatr Res. 2017;21(4):680-85.
[9] Saigal K, Dhawan B, Rawre J, Khanna N, Chaudhry R. Genital mycoplasma and *Chlamydia trachomatis* infections in patients with genital tract infections attending a tertiary care hospital of North India. Indian J Pathol Microbiol. 2016;59(2):194-96.
[10] Kumar S, Kashyap P, Kumar NB, Kashyap B, Garg IB, Saigal SR. Molecular evidence of *Ureaplasma urealyticum* and *Ureaplasma parvum* colonization in preterm infants with respiratory distress. In Open Forum Infect Dis. 2019;6(Suppl 2):S908.
[11] Kacerovsky M, Kukla R, Bolehovska R, Bostik P, Matulova J, Mls J, et al. Prevalence and load of cervical species with respect to intra-amniotic complications in women with preterm prelabor rupture of membranes before 34 weeks. Front Pharmacol. 2022;13:860498.
[12] Zhou YH, Ma HX, Shi XX, Liu Y. Ureaplasma spp. in male infertility and its relationship with semen quality and seminal plasma components. J Microbiol Immunol Infect. 2018;51(6):778-83.
[13] Frølund M, Björnelius E, Lidbrink P, Ahrens P, Jensen JS. Comparison between culture and a multiplex quantitative real-time polymerase chain reaction assay detecting *Ureaplasma urealyticum* and *U. parvum*. PLoS One. 2014;9(7):e102743.
[14] Mikami Y, Fuwa K, Arima E, Suda Y, Yanagihara I, Ibara S. Validation of the loop-mediated isothermal amplification method for rapid and sensitive detection of *Ureaplasma* species in respiratory tracts of preterm infants. PLoS One. 2021;16(3):e0247618.
[15] Stephen S, Pradeep J, Rajkumar C, Samal R, Vishalakshi A, Ghose S, et al. Molecular diagnosis of Sexually Transmitted Infections (STI) in symptomatic women of puducherry by a commercial real time multiplex PCR, FTD urethritis plus- a preliminary report. J Pure Appl Microbiol. 2019;13(3):1791-96.
[16] Biomedical waste management rules 2016. [Internet]. [cited 2024 Aug 14]. Available from: https://cpcb.nic.in/bio-medical-waste-rules/.
[17] QIAmp DNA Blood Mini Kit Protocol. [Internet]. [cited 2024 Aug 14]. Available from: https://www.qiagen.com/de/resources/download.aspx?id=54278309-afa9-4cb4-af97-e5e46ab5a0bb&lang=en.
[18] Centre for Disease Control and Prevention. Sexually Transmitted Infections Treatment Guidelines, 2021. Recommendations and Reports. Morbidity and Mortality Weekly Report. 2021;70:1-135. Available from: https://www.cdc.gov/std/treatment-guidelines/STI-Guidelines-2021.pdf.

[19] Choe HS, Lee DS, Lee SJ, Hong SH, Park DC, Lee MK, et al. Performance of AnyplexTM II multiplex real-time PCR for the diagnosis of seven sexually transmitted infections: Comparison with currently available methods. Int J Infect Dis. 2013;17:e1134-e1140.

[20] Deguchi T, Yoshida T, Miyazawa T, Yasuda M, Tamaki M, Ishiko H, et al. Association of *Ureaplasma urealyticum* (biovar 2) with nongonococcal urethritis. Sex Transm Dis. 2004;31:192-95.

PARTICULARS OF CONTRIBUTORS:
1. Ph.D. Scholar cum Assistant Professor, Department of Microbiology, School of Allied Health Sciences, Mahatma Gandhi Medical College and Research Institute, Sri Balaji Vidyapeeth (Deemed to be University), Puducherry, India.
2. Undergraduate Student (MLT), Department of Microbiology, School of Allied Health Sciences, Mahatma Gandhi Medical College and Research Institute, Sri Balaji Vidyapeeth (Deemed to be University), Puducherry, India.
3. Assistant Professor, Department of Biotechnology, Mahatma Gandhi Medical Advanced Research Institute, Sri Balaji Vidyapeeth (Deemed to be University), Puducherry, India.
4. Technical Officer, Department of Bioinformatics, Mahatma Gandhi Medical Advanced Research Institute, Sri Balaji Vidyapeeth (Deemed to be University), Puducherry, India.
5. Professor, Department of Obstetrics and Gynaecology, Mahatma Gandhi Medical College and Research Institute, Sri Balaji Vidyapeeth (Deemed to be University), Puducherry, India.
6. Assistant Professor, Department of Bioinformatics, Mahatma Gandhi Medical Advanced Research Institute, Sri Balaji Vidyapeeth (Deemed to be University), Puducherry, India.
7. Professor and Principal, Department of Medical Genetics, School of Allied Health Sciences, Mahatma Gandhi Medical College and Research Institute, Sri Balaji Vidyapeeth (Deemed to be University), Puducherry, India.
8. Assistant Professor, Department of Microbiology, Mahatma Gandhi Medical Advanced Research Institute, Sri Balaji Vidyapeeth (Deemed to be University), Puducherry, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. J Pradeep,
Assistant Professor, Department of Microbiology, Mahatma Gandhi Medical Advanced Research Institute, Sri Balaji Vidyapeeth (Deemed to be University), Puducherry-607402, India.
E-mail: pradeepj@mgmari.sbvu.ac.in

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jun 13, 2024
- Manual Googling: Sep 27, 2024
- iThenticate Software: Sep 30, 2024 (11%)

ETYMOLOGY: Author Origin

EMENDATIONS: 6

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jun 12, 2024**
Date of Peer Review: **Jul 26, 2024**
Date of Acceptance: **Oct 01, 2024**
Date of Publishing: **Dec 01, 2024**