JOURNAL LA LIFESCI

VOL. 06, ISSUE 03 (282-296), 2025
DOI: 10.37899/journallalifesci.v6i3.2566

Molecular Detection of Mycoplasma Hominis in Vaginal Swabs from Women with Urogenital Symptoms

Rajaa Ali Habeeb[1]

[1]*Department of Pathological Analyses, College of Science, University of Al-Qadisiyah, Iraq*

**Corresponding Author: Rajaa Ali Habeeb*
Email: ragaa.ali@qu.edu.iq

Article Info

Article history:
Received 5 August 2025
Received in revised from 20 August 2025
Accepted 10 September 2025

Keywords:
Metamycoplasma Hominis
Genitourinary Syndrome
Polymerase Chain Reaction (PCR)
National Center for Biotechnology Information (NCBI)
Phylogenetic Analysis
Iraq

Abstract

Bacterial infections are a common cause of urogenital symptoms in women of reproductive age, which occurs usually as a result of imbalance in the normal vaginal flora and increase in anaerobic bacteria. Molecular investigation an involvement of M. hominis in urogenital infections of women, sequencing and submission of some positive study M. hominis isolates in the NCBI-GenBank database, and indicating the relationship between infection and some risk factors (age, previous medication, frequent compliance, and marital status). Totally, 191 diseased women with urogenital infections were attended to some private gynecology clinics and subjected to collection of vaginal swabs to be served for DNAs extraction. Targeting 16S rRNA gene, molecular examination was conducted using conventional PCR assay, and the, some positive samples were sequenced, submitted the NCBI-GenBank database, and analysed phylogenetically by the MEGA-11 software to indicate identity between the study and NCB-BLAST M. hominis isolates / strains. Molecular findings of PCR assay showed that 20.42% of study samples were positive to M. hominis. Sequencing data of 10 isolates revealed its identity to the global NCBI-BLAST Indian M. hominis isolate (ID: PP380133.1) at 99.06-99.77% and mutation / changes at 0.0001-0.00025%. Concerning the groups of risk factors, incidence of M. hominis infection was increased significantly in individuals aged 20-40 years than those aged <20 and >40 years, and non-medicated than frequent medication; however, insignificant variations were seen between the groups of frequent compliance and marital status.

Introduction

Mycoplasma hominis, also known *Metamycoplasma hominis*, is a bacterial species belongs to the Mycoplasmataceae family in the Mollicutes class under the Bacillati kingdom (May et al., 2014). This species represents the first *Mycoplasma* species isolated from a Bartholin's gland abscess of human in 1937 by Dienes and Edsall (Singh and Seth, 2016). The bacterium was described as a coccoid in shape and much smaller in size than other bacteria to be passed through the bacteriological filters 0.45µm-pore-size filters that are commonly used to filter-sterilized media (Brown, 2016). *Mycoplasma hominis*, like other *Mycoplasma* species produces adenosine triphosphate by substrate-level phosphorylation of glucose to pyruvate with the help of two enzymes; pyruvate kinase and phosphoglyceric acid kinase of the glycolytic pathway (Shu et al., 2012; Evsyutina et al., 2022). This obligate lifestyle, where the bacterium frequently co-opts host metabolic pathways, significantly contributes to its ability to persist and establish chronic infections (Dessì et al., 2019). The whole genome sequence of *M. hominis* isolated from various pathological conditions, in particular PG21 that considers as a reference strain, shows that this species is the second smallest genome among self-replicating free-living organisms with a gene density a round 89.8%, 537 putative coding sequences (CDSs) and 14

ISSN 2721-1304 (Print), ISSN 2721-1207 (online)

pseudogenes (Ahmed et al., 2021; Vishnyakov, 2021). Thus, the small genome size and limited metabolic capabilities often make it dependent on the host for various essential nutrients, influencing its pathogenic strategies and colonization patterns (Dawood et al., 2022). However, the absence of a rigid cell wall renders it's inherently resistance to antibiotics targeting peptidoglycan synthesis such as penicillins and cephalosporins (Sharratt, 2023).

Under certain circumstances, *M. hominis* can cause a variety of genitourinary tract infections in humans like pyelonephritis, cervicitis, pelvic inflammatory disease, bacterial vaginosis, and urinary tract infections (Qiu et al., 2025). In addition, this bacterium associates with the pregnancy complications and neonatal diseases besides having a pathogenic role in infertility (Cutoiu and Boda, 2024).

In Iraq, although a number of studies have been conducted in women for rapid detection of microbial vaginitis (Al-Dahmoshi, 2017), genital mycoplasmas (Al-Mosawi, 2019), and relationship between Tlr-6 and *Mycoplasma* among urogenital tract infections (Al-Ezzi and Al-Thahab, 2022), as well as in males by studying of *M. hominis* in urethritis (Muhammid et al., 2015), infertility (Al-Jebouri and Mohamed, 2020), and seminal fluid and cervical mucus in infertile Iraqi couples (Shallal et al., 2021). Hence, this might represents the first Iraqi study aims to molecular investigation an involvement of *M. hominis* in urogenital infections of women, sequencing and submission of study *M. hominis* isolates in the NCBI-GenBank database, and indicating the relationship between infection and some risk factors (age, frequent medication, frequent compliance, and marital status).

Methods

Ethical Approval

This study approved by the Department of Pathological Analyses in the College of Science (University of Al-Qadisiyah), and the samples were collected after the oral acceptance of the participants.

Samples and Data

Totally, 191 diseased women who diagnosed clinically with urogenital infections in some private gynecology clinics were subjected to collection of vaginal swabs under aseptic conditions. The obtained swabs were transported to the laboratory using labeled plastic tubes contained 1ml of tryptone (15%) as a transport medium under cooled conditions, and saved frozen (-20°C) until be used. Data related to age, previous medication, frequent compliance, and marital status were recorded and considered as risk factors.

Molecular Testing

After preparation of swab samples, DNAs were extracted following the manufacturer instructions of the Presto^{TM} Mini gDNA Bacteria Kit (Geneaid, Taiwan). Following the manufacturer instructions of the Accupower® PCR PreMix, one set of primers (F: 5′-ACT GAG ATA CGG CCC AGA CT-′3 and R: 5′-GGG TCC CCG TCA ATT CCT TT-′3) was designed according to NCBI-GenBank isolate (ID: OP364020.1) targeting *16S rRNA* gene, and used to preparing the MasterMix tubes at a final volume of 20µL for DNAs amplification in the Thermal Cycler system (BioRad, USA). Electrophoresis of agarose-gel (1.5%) stained with ethidium bromide was done at 100V and 80Am for 90min, and the positive bands were detected at an approximately 604bp using the UV transilluminator system (Clinx Science, China).

The DNAs of some positive isolates were sequenced, and submitted in NCBI-GenBank database. For phylogeny, MEGA-11 software and NCBI-Multiple Sequence Alignment (MSA)

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Viewer were served to analyzing the study *M. hominis* isolates by the ClustralW Alignment, Homology Sequence Identity and Phylogenetic Tree Analysis to indicate identity between the study *M. hominis* isolates and NCB-BLAST *M. hominis* isolates / strains.

Statistical Analysis

Significant variation between the obtained results by the GraphPad Prism Software through applying the *t*-test, One-Way ANOVA in addition to Odds-Ratio (OR) and Relative Risk (RR). The confidence interval (95%CI) was calculated between the obtained data of various age groups (Al-Gharban, 2016; AL-Shaeli et al., 2022).

Results and Discussion

Molecular examination of 191 vaginal swabs by PCR revealed that the positive *M. hominis* isolates were detected in 39 (20.42%) samples (Figure 1).

Sequencing data of 10 *M. hominis* study isolates were documented in the NCBI-GenBank database under the names of *Metamycoplasma hominis* isolate RAH1-*Metamycoplasma hominis* isolate RAH10 with getting specific access numbers that started from PV953408.1 to PV953417.1. Comparative phylogenetic analysis shown the presence of similarity (*) between the study *M. hominis* isolates and the global NCBI-BLAST Indian *M. hominis* isolate (ID: PP380133.1) at 99.06-99.77% and mutation / changes at 0.0001-0.00025% (Figures 2-4, Table 1).

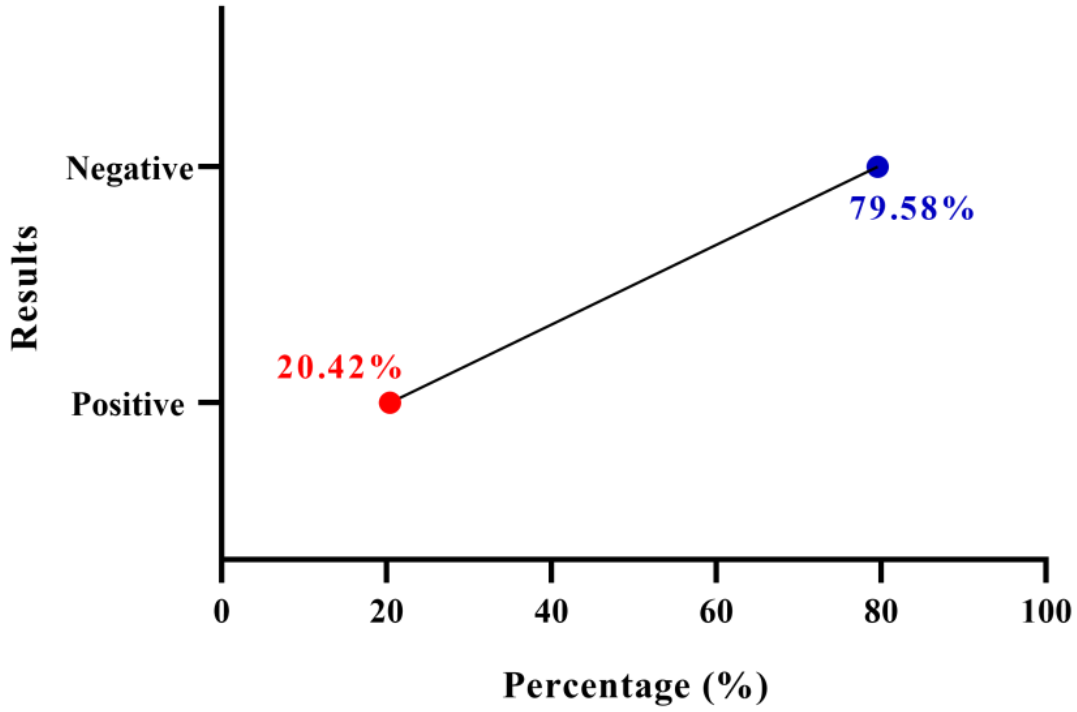

Figure 1. Total molecular results for testing 191 vaginal swab samples by PCR

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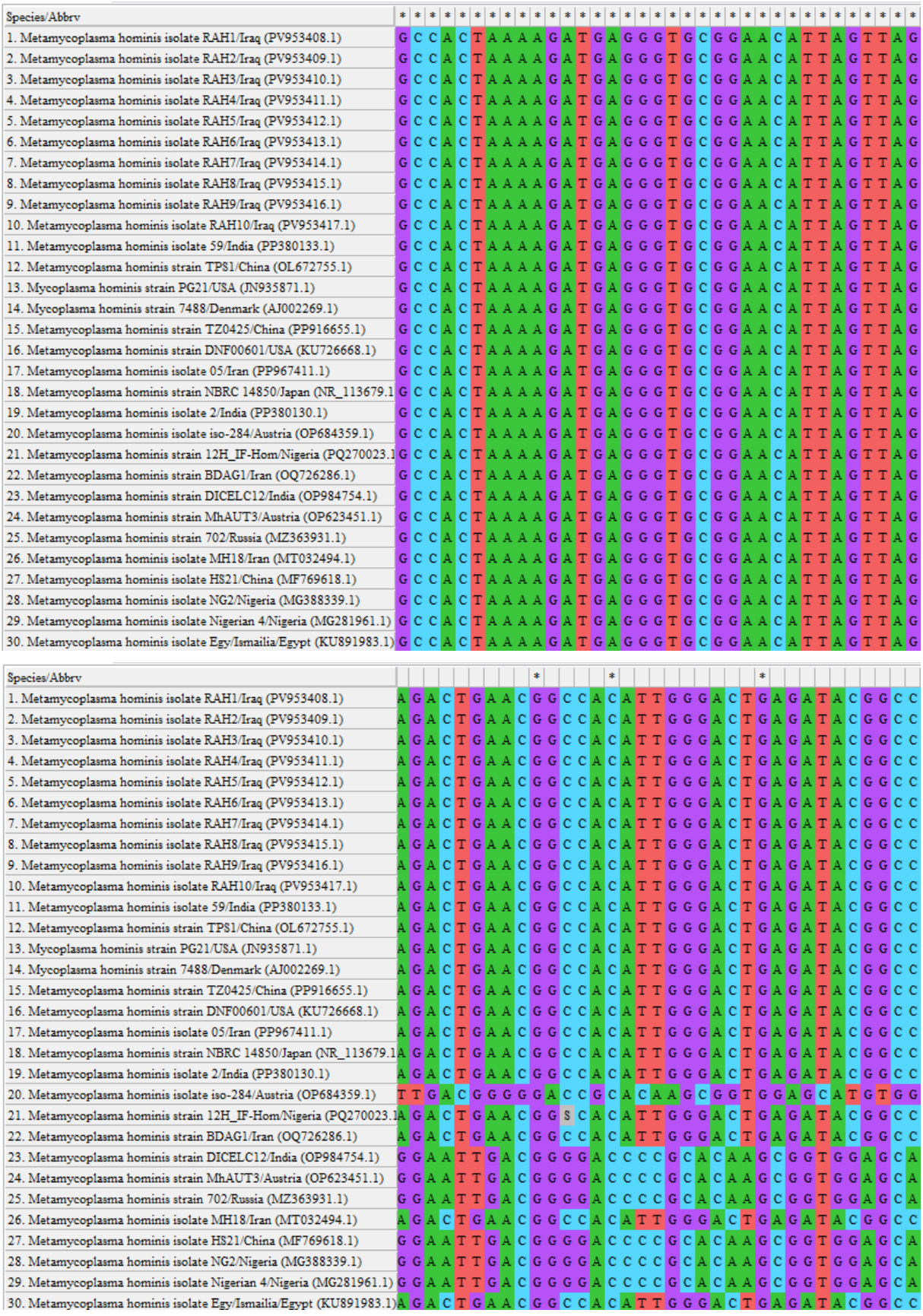

Figure 2. MSA for the local study and global NCBI-GenBank M. hominis isolates / strains by the MEGA-11 Software

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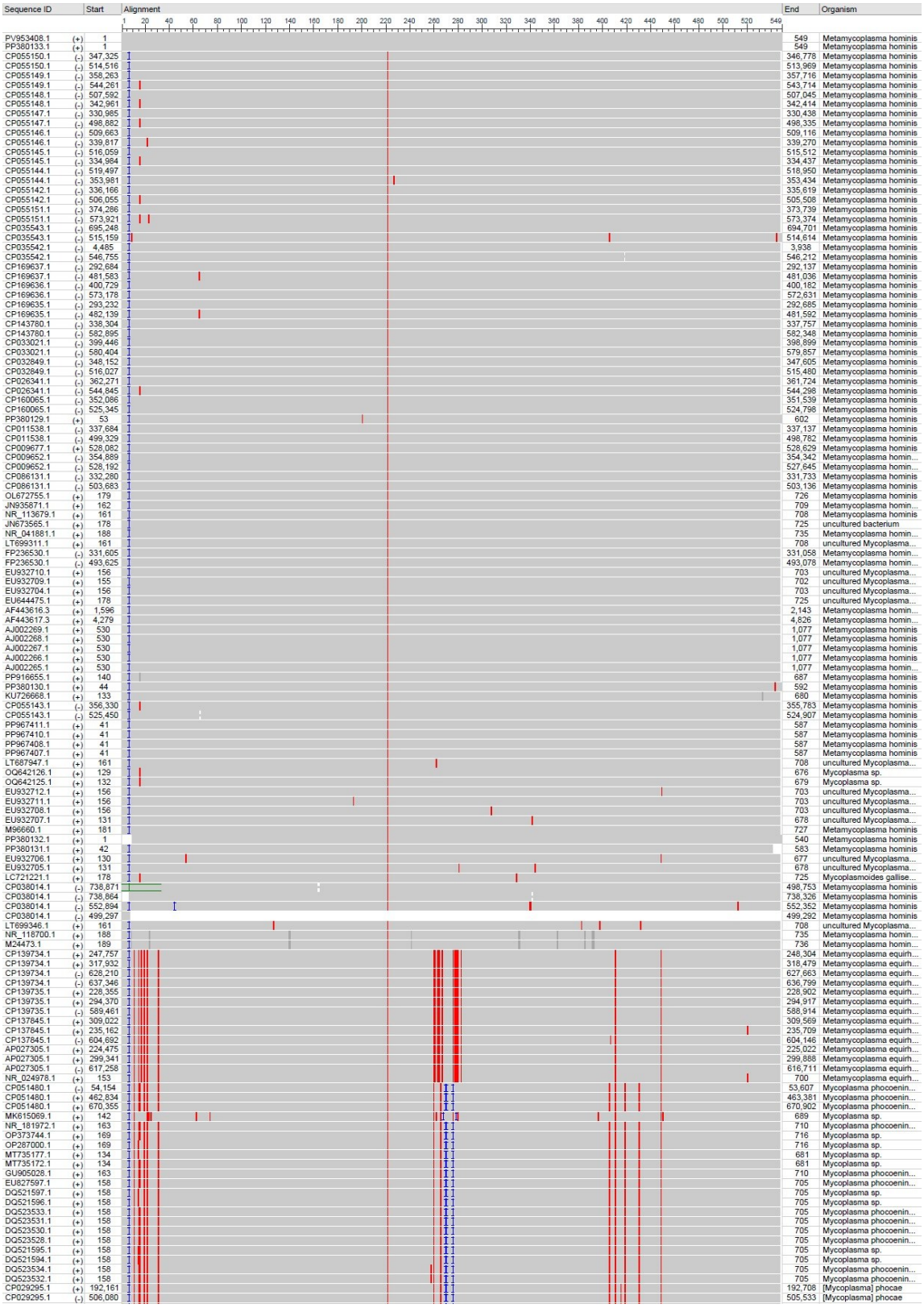

Figure 3. MSA for the local study and global NCBI-GenBank M. hominis isolates / strains by the NCBI-MSA Viewer

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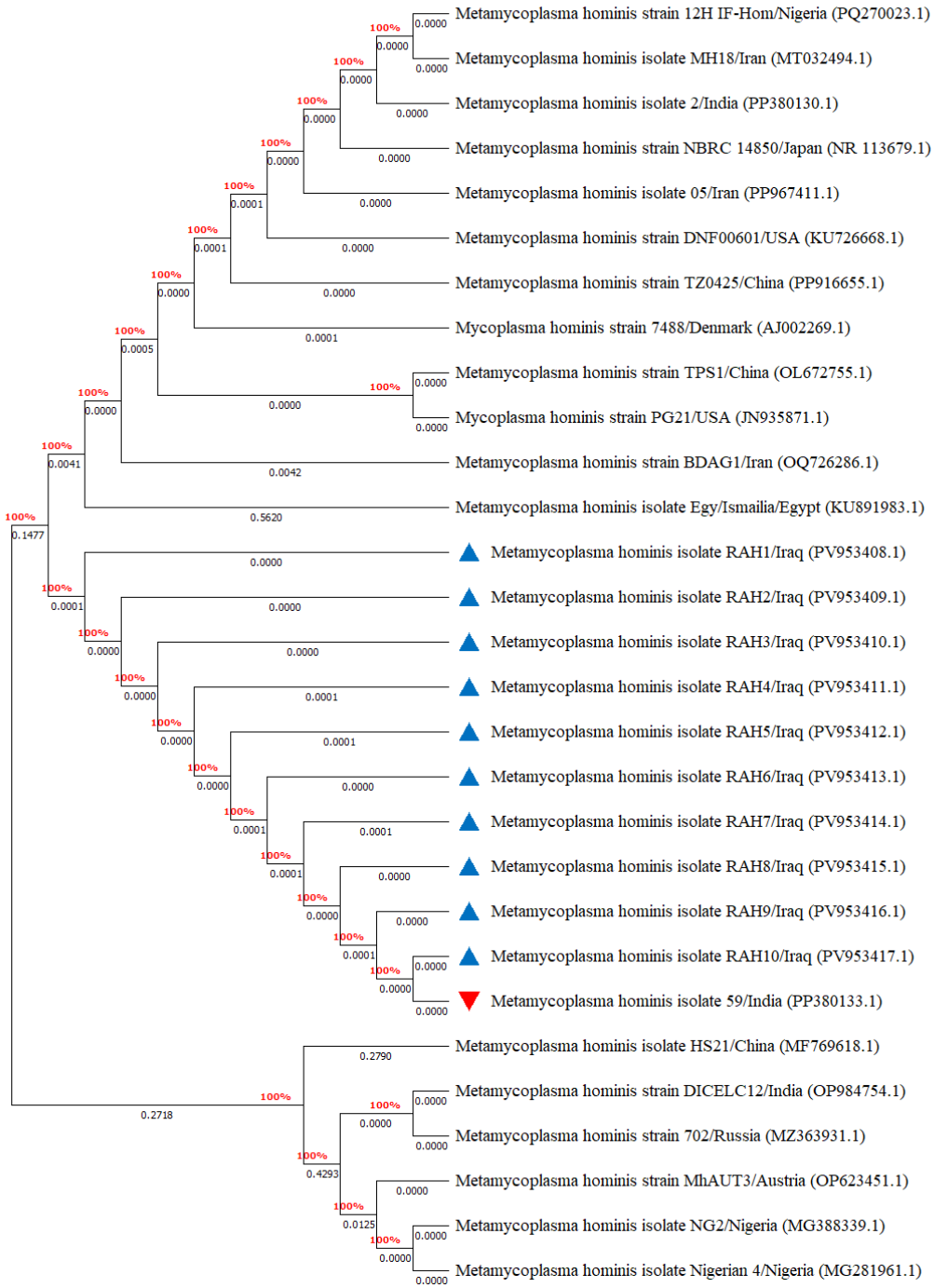

Figure 4. Phylogenetic tree analysis of study local and NCBI-BLAST M. hominis isolates / strains

Table 1. Homology sequence identification for local and NCBI-BLAST *M. hominis* isolates / strains

Local isolate		NCBI-BLAST isolate				Identity (%)
Name	**Access. No.**	**Host**	**Source**	**Country**	**Access. No.**	
RAH1	PV953408.1	Human	Body fluid	India	PP380133.1	99.64
RAH2	PV953409.1	Human	Body fluid	India	PP380133.1	99.58
RAH3	PV953410.1	Human	Body fluid	India	PP380133.1	99.45
RAH4	PV953411.1	Human	Body fluid	India	PP380133.1	99.61

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RAH5	PV953412.1	Human	Body fluid	India	PP380133.1	99.23
RAH6	PV953413.1	Human	Body fluid	India	PP380133.1	99.06
RAH7	PV953414.1	Human	Body fluid	India	PP380133.1	99.71
RAH8	PV953415.1	Human	Body fluid	India	PP380133.1	99.64
RAH9	PV953416.1	Human	Body fluid	India	PP380133.1	99.35
RAH10	PV953417.1	Human	Body fluid	India	PP380133.1	99.77

In current study, the findings of positive infections were distributed significantly ($p<0.05$) among the groups of each risk factors. For age, the incidence of *M. hominis* was increased significantly ($p<0.03734$; 95%CI: 11.30 to 43.77) in women aged 20-40 years [28.16% (29/103)] when compared to those aged >40 years old [14.29% (8/56)] and <20 years old [6.25% (2/32)] (Figure 5).

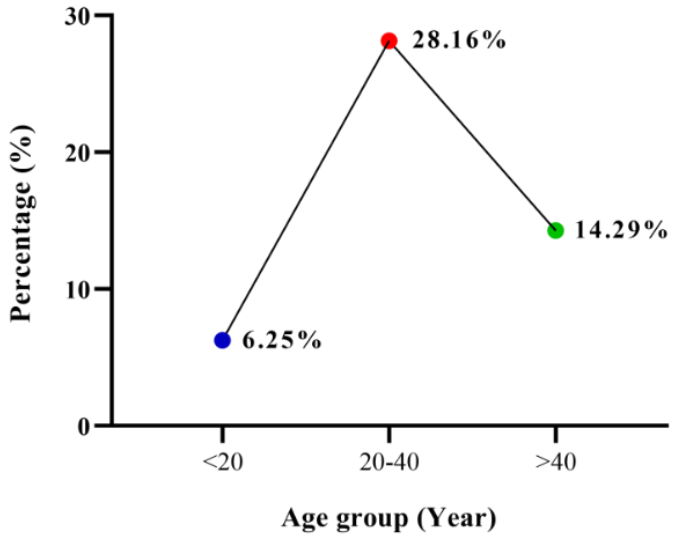

Figure 5. Distribution of positive M. hominis among age groups of study population

Subsequently, the findings of OR and RR recorded significantly ($p<0.0001$; 95%CI: 0.2575 to 51.37 and 0.1822 to 40.64, respectively) that individuals aged 20-40 years were at higher risk of infection (3.063 and 2.474, respectively) when compared to those aged >40 years old (0.56 and 0.622, respectively) and <20 years old (0.221 and 0.268, respectively), (Figure 6).

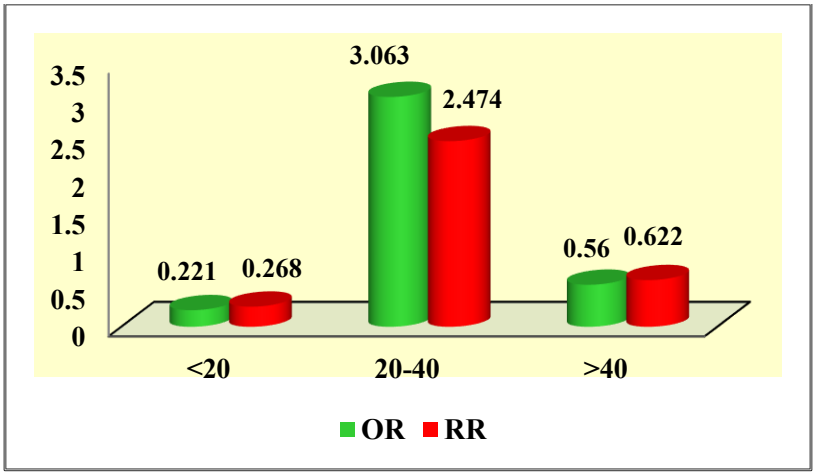

Figure 6. Risk of positive M. hominis infection among age groups of study population

ISSN 2721-1304 (Print), ISSN 2721-1207 (online)

The findings of frequent medication reported a significant higher incidence (p<0.0291; 95%CI: 103.2 to 137.7) of *M. hominis* infection in patients without frequent medication [26.77% (34/127)] than those received it frequently [7.81% (5/64)], (Figure 7).

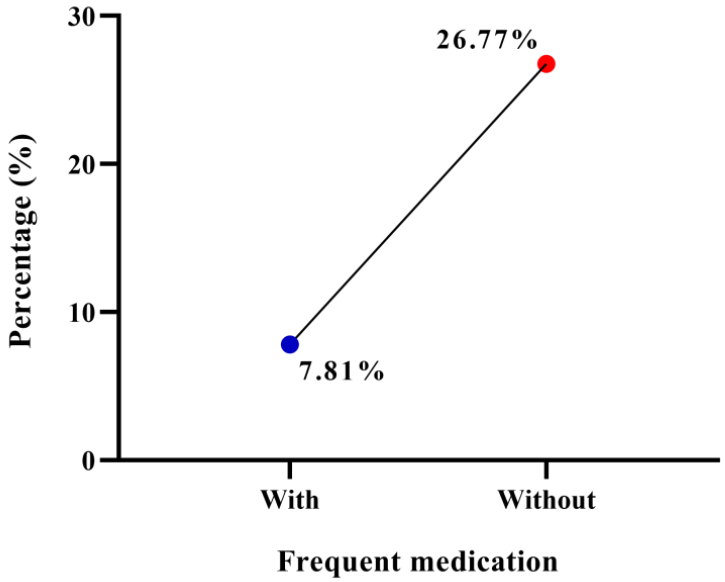

Figure 7. Distribution of positive M. hominis among groups of with and without frequent medication in study population

In addition, the risk of *M. hominis* infection shown a significant higher elevation (p<0.0001; 95%CI: 0.2361 to 28.15 and 95%CI: 0.1812 to 21.84, respectively for OR and RR) in patients without frequent medication (OR: 4.306; RR: 3.436) than those with frequent medication (OR: 0.232; RR: 0.291), (Figure 8).

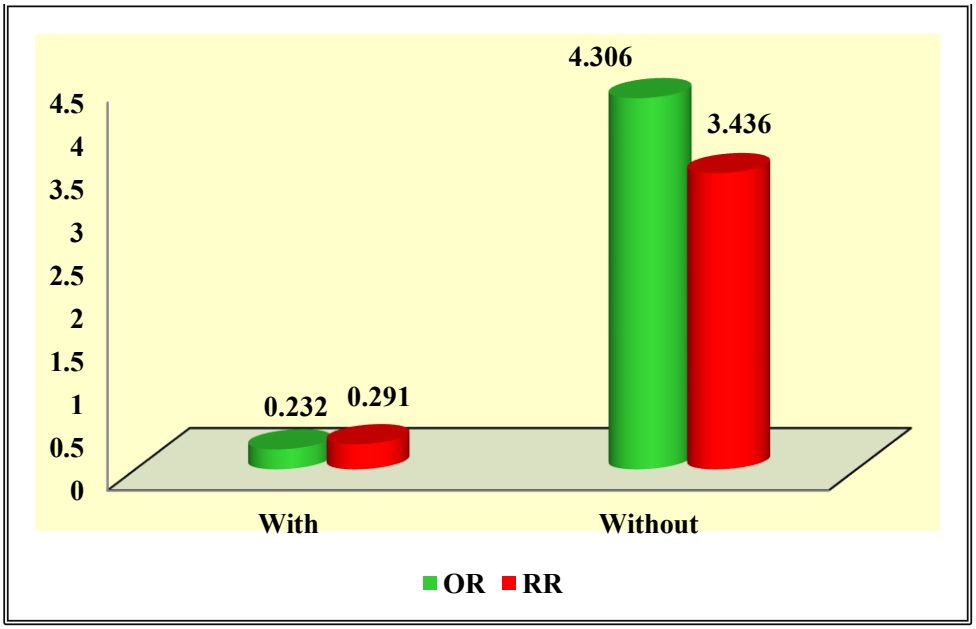

Figure 8. Risk of positive M. hominis infection among groups of with and without frequent medication in study population

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Regarding frequent compliance, no significant variation (p<0.0752; 95%CI: -10.61 to 52.41) was seen in incidence of *M. hominis* infection among those with [23.38% (18/77)] and without [18.42% (21/114)] compliance (Figure 9).

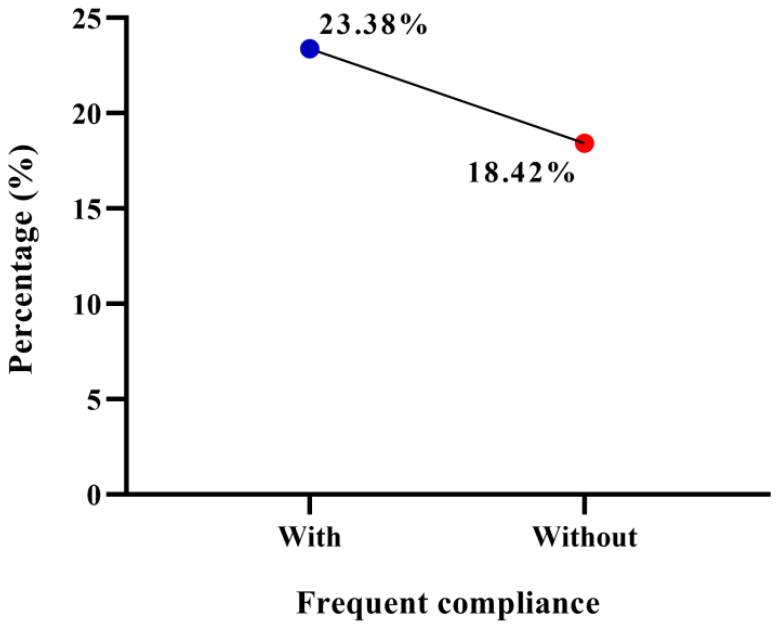

Figure 9. Distribution of positive M. hominis among groups of with and without frequent compliance in study population

However, the risk of *M. hominis* infection was increased significantly (p<0.0001; 95%CI: 0.3035 to 51.36 and 95%CI: 0.2221 to 42.85, respectively for OR and RR) in patients with frequent compliance (OR: 1.372; RR: 1.288) than those without it (OR: 0.729; RR: 0.776), (Figure 10).

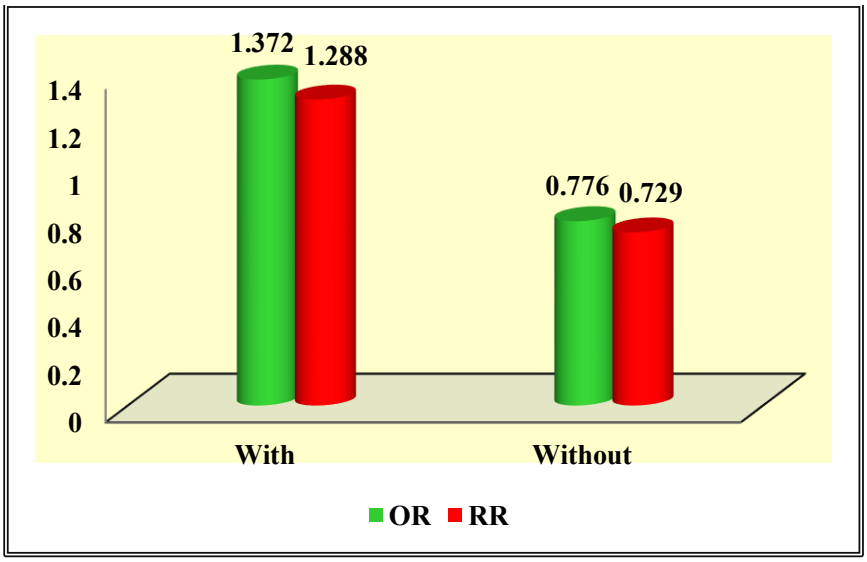

Figure 10. Risk of positive M. hominis infection among groups of with and without frequent compliance in study population

ISSN 2721-1304 (Print), ISSN 2721-1207 (online)

Relation to marital status, incidence rate of *M. hominis* infection was differed insignificantly (p<0.0635; 95%CI: -5.945 to 49.84) between the married [19.75% (32/162)] and not married [24.14% (7/29)] patients (Figure 11).

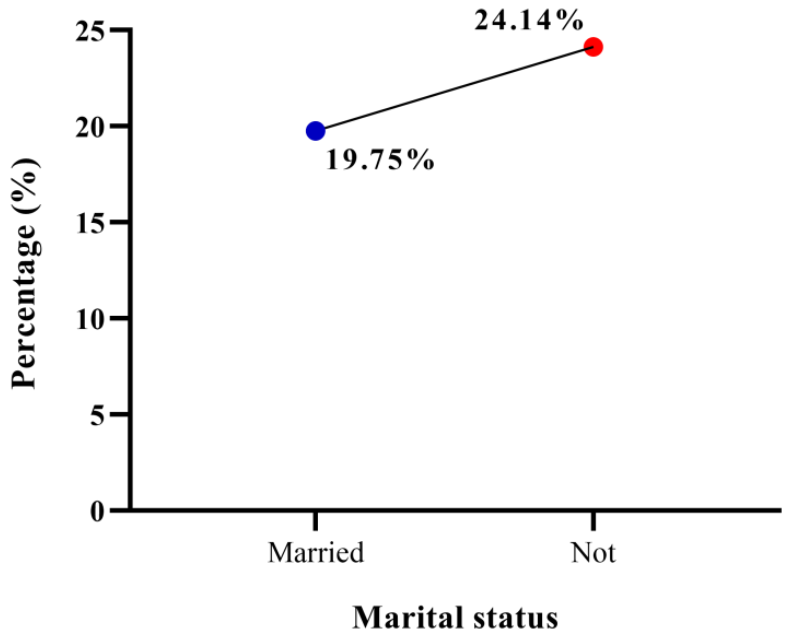

Figure 10. Distribution of positive M. hominis among groups of marital status in study population

However, the risk of *M. hominis* infection was elevated significantly (p<0.0001; 95%CI: 0.2264 to 43.31 and 95%CI: 0.1490 to 35.29, respectively for OR and RR) in not married patients (OR: 1.293; RR: 1.217) in comparison with married (OR: 0.774; RR: 0.822) patients (Figure 12).

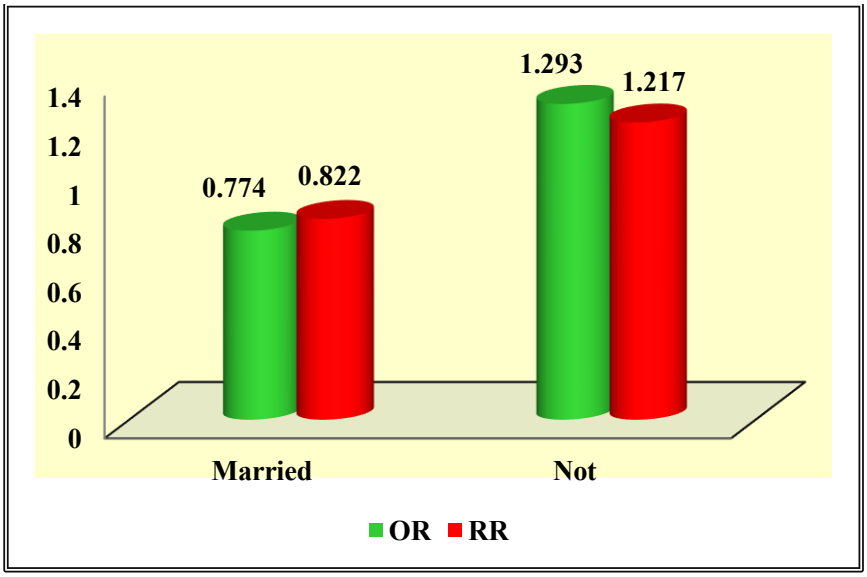

Figure 12. Risk of positive M. hominis infection among groups of marital status in study population

Discussion

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Mycoplasma hominis represents one of the most commonly fastidious bacteria, which having a role in various clinical manifestations, particularly in women presenting with urogenital symptoms. In this study, application of molecular PCR assay detected the incidence of *M. hominis* in 20.42% of study population which comparatively identical with that recorded by Hoxha et al. (2023) who confirm the presence of *M. hominis* in 21.5% of vaginal swabs of female patients using the conventional PCR and targeting the *16S rRNA* gene. However, our findings were lowered than detected by Keane et al. (2000) who observed that *M. hominis* infection was existed in 53% of heterosexual women with bacterial vaginosis, Redelinghuys et al. (2014) who recorded *M. hominis* infection in 30.21% of the vaginal swabs collected from 96 pregnant women; Campos et al. (2015) who found that the prevalence of *M. hominis* in 31.8% of vaginal swabs using of quantitative PCR, and Nundlall et al. (2024) who recorded that the prevalence of *M. hominis* in human immunodeficiency virus infected pregnant women was 81.4% by the real-time PCR assay; but higher than reported by Lee et al. (2016) who identified *M. hominis* in 0.35% of vaginal swabs of pregnant women using the traditional laboratory method (culture). Rumyantseva et al. (2019) tested the prevalence of *M. hominis* in healthy women and patients with altered vaginal microflora, and found that the bacterium was found in 8.9% of normal flora, 26.8% of bacterial vaginosis and 6.4% of aerobic vaginitis. Nevertheless, the association of *M. hominis* infection with the conditions such as bacterial vaginosis, pelvic inflammatory disease, and infertility warrants comprehensive investigation especially given the susceptibility of females to urogenital infections due to anatomical and lifestyle factors. For example, the shorter urethra in women predispose them to ascending infections while factors like sexual activity and spermicide use can alter the vaginal microbiome and increasing susceptibility to various uropathogens (Gholiof et al., 2022; Colella et al.,2023; Elorfaly, 2024). In addition, hormonal changes in particular during the perimenopausal period can affect the vaginal microbiota, and increase the common bacterial infections in women (Muhleisen and Herbst-Kralovetz, 2016; Vieira et al., 2017).

Targeting *16S rRNA* gene, our results of phylogeny indicated that the study *M. hominis* isolates were having a higher identity to the global NCBI-BLAST Indian *M. hominis*. The *16S rRNA* gene serves as a ubiquitous and essential molecular marker for identifying bacterial pathogens including fastidious organisms like *M. hominis* particularly in scenarios where traditional culture methods prove insufficient (Fenollar and Raoult, 2004; Einarsson and Boutin, 2019). Also, its highly conserved and variable regions allow for accurate species-level discrimination, making it a cornerstone in clinical microbiology for bacterial identification (Church et al., 2020). Moreover, advancements in sequencing technologies have revolutionized the characterization of diverse microbial communities including uncultured species (Zhang and Zhang, 2023).

In the present study, incidence of *M. hominis* infection was increased significantly in individuals aged 20-40 years than those aged <20 and >40 years, and non-medicated than frequent medication; however, insignificant variations were seen between the groups of frequent compliance and marital status. Additionally, the risk of *M. hominis* infection was elevated significantly in the 20-40 years old, non-medicated, frequently compliance and non-married patients. These findings were contrast with the results of Lee et al. (2016) who found that the positive rate of genital mycoplasmas and mixed infections was the highest in the pregnant females aged 15-19 years old (88% and 35.3%, respectively), and the results of Nundlall et al. (2024) who found the lack of significant differences ($p<0.081$) between the age groups of study women. Shao et al. (2021) detected significantly that the higher positive rate of infection was observed in females aged 25-29 years (60.5%) and in the 15-19 years (57.7%). As reported by several studies, application of antimicrobial therapy can play an important role

ISSN 2721-1304 (Print), ISSN 2721-1207 (online)

in controlling and prevention of *M. hominis* infection (Waites et al., 2009; Lee et al., 2012; Wang et al., 2016).

Conclusion

This might represent the first molecular phylogeny Iraqi study suggesting the importance of novel diagnostic tools for effectively managing M. hominis infections and preventing severe sequelae. Also, the complex interplay between M. hominis and other microbiota in the female reproductive tract warrants thorough examination to elucidate synergistic or antagonistic effects on disease progression. Hence, it is crucial to understand how shifts in microbial diversity and dominance can create and environment conductive to proliferation of M. hominis and other opportunistic pathogens. Therefore, furthermore studies based on molecular phylogeny appear to be highly important to explain the extent and role of M. hominis in various human infections.

Orcid

Rajaa Ali Habeeb https://orcid.org/0009-0003-6975-9509

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