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Curcumin Antibacterial Effects on *Mycoplasma hominis* Clinical Isolates from Vaginosis

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ABSTRACT

Background: *Mycoplasma hominis* is involved in the development of non-gonococcal urethritis, bacterial vaginosis, postpartum fever, and rarely, bacteremia, peritonitis, arthritis, and meningitis. This study investigated the effect of curcumin against *M. hominis* species isolated from vaginosis.

Materials & Methods: About 550 samples from female patients with vaginosis were examined. The minimum inhibitory concentration (MIC) ranges of tetracycline, doxycycline, clarithromycin, azithromycin, moxifloxacin, ciprofloxacin, and erythromycin were determined. For this purpose, dilutions ranging from 0.25-4 µg/mL were prepared. In addition, curcumin was diluted at 5 and 10 µg/mL concentrations and added to the bacterial suspension in SP-4 broth medium. Quantitative real-time polymerase chain reaction (qRT-PCR) was performed to examine the *Mycoplasma hominis* 16 ribosomal RNA (MH16S rRNA) gene expression upon exposure to 10 µg/mL curcumin.

Findings: Of the 550 samples, 20 were positive for *M. hominis*. The patients' ages ranged from 23- 35 years (mean: 26.5±2). The MIC ranges of antibiotics included 1->4 µg/mL for doxycycline, 2->4 µg/mL for clarithromycin, 2->4 µg/mL for azithromycin, 2->4 µg/mL for moxifloxacin, 2->4 µg/mL for ciprofloxacin, and 1->4 µg/mL for erythromycin. Exposure to 5 µg/mL curcumin resulted in the inhibition of eight isolates. Curcumin at a 10 µg/mL concentration inhibited the growth of 15 out of 20 isolates after 72 hrs of incubation. The mean expression level of the *MH16S rRNA* gene decreased by 0.8-1 fold among the isolates.

Conclusion: Low levels of *M. hominis* were detected in vaginal samples in Samawah, Iraq. Curcumin could inhibit the growth of *M. hominis* causing vaginosis. More in-depth and inclusive studies are required to verify the findings.

Keywords: *Mycoplasma hominis*, Curcumin, Antibiotics, Vaginosis

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Introduction

Mycoplasma spp. are known as opportunistic pathogens that live promiscuously in community settings and form part of the human natural flora. They are highly flexible facultative anaerobes without cell walls, which have various microscopic morphologies such as spherical, coccoid, and budded bacilli [1, 2]. The lack of a cell wall enables *Mycoplasma* spp. to be inherently resistant to some antibiotic classes, such as β-lactams. Also, these species grow slowly on synthetic PPLO (pleuropneumonia-like organisms) agar and broth as well as SP4 agar and broth media containing horse serum and glucose, with 5% CO_2 at 37 °C after 7 days [3]. They also lack flagella, capsules, and spores and tend to adhere to mammalian cell membranes. Pathogenic species for humans include *M. pneumoniae*, *M. genitalium*, *Ureaplasma urealiticum,* and *M. hominis* [4]. Genitourinary tract infections play an important role in male and female infertility, which could be caused by *Mycoplasma* spp. *M. hominis* is involved in the development of non-gonococcal urethritis, bacterial vaginosis, postpartum fever, and rarely bacteremia, peritonitis, arthritis, and meningitis. This bacterium is found in the genitourinary tract of 21 to 54% of women and 4 to 13% of men and therefore could be transmitted sexually, causing multiple infections such as infertility in both sexes [5, 6]. This bacterium is present in the genital tract and semen of fertile and infertile men. *M. hominis* is often associated with *Ureaplasma* species. *M. genitalium* has been isolated from men with urethritis and is a cause of cervicitis and pelvic inflammatory disease [7]. It is less commonly known as a urinary tract infection and is more commonly observed in clinical infections [7]. This species develops its pathogenicity using adhesion factors. Because these two *Mycoplasma* species have a high potential to cause fertility complications during pregnancy and infections during childbirth, their identification and eradication are essential. Identification of *Mycoplasma* spp. using staining, culture, electron microscopy, and biochemical and immunological tests is usually time-consuming (several weeks), has relatively low sensitivity, and requires sufficient experience and facilities. Therefore, rapid identification of *Mycoplasma* infections using rapid approaches and molecular methods with low cost and higher sensitivity, such as PCR (polymerase chain reaction) techniques, is more appropriate [8-11].

The first- and second-line drugs to fight against *Mycoplasma* spp. include the macrolide azithromycin and fluoroquinolone moxifloxacin, respectively. However, the development of resistance has occurred due to several mutations, such as mutations in the *parC* gene. This type of resistance among *M. hominis* and *M. genitalium* species has not been previously reported in our area [1, 12, 13]. Herbal medicinal compounds such as curcumin have been shown to exert antimicrobial effects against both Gram-positive and Gram-negative bacterial species [14, 15]. More importantly, high concentrations of curcumin are not toxic to normal human cells and may not induce antimicrobial resistance [16].

Objectives: In this study, the efficacy of curcumin in inhibiting the growth of *M. hominis* isolates from vaginosis samples was assessed.

Materials and Methods

Samples and bacterial culture: In this study, 550 samples from female patients suffering from vaginosis were examined. Married females aged 20-50 years with clinical signs and no history of antibiotic use were included. For specific culture of *Mycoplasma* spp., PPLO agar and PPLO broth media (BBL

Company, USA) with pH 7.6-8 were used [17]. For this purpose, samples sent to the laboratory in PPLO broth medium were briefly incubated for enrichment at 37 °C for 6 hrs. After enrichment, all PPLO broth media containing the samples were filtered using 0.45 µm filters and gently dissolved and inoculata ed into the second culture medium (PPLO broth, pH 7.6-8). The medium was then placed in a CO_2 incubator and monitored for 3 to 5 days. In case of any change in the color or turbidity of the PPLO broth medium (a sign of bacterial growth), the samples were transferred to a new PPLO broth medium (pH 7.6-8); finally, 200 µL of this passage was cultured on PPLO agar medium and incubated at 37 °C for 72 hrs. PPLO agar media were examined daily by light microscopy for growth and formation of special colonies. PPLO broth medium was used as a negative control, and *M. hominis* ATCC 23114 standard strain was used as a positive control. Arginine hydrolysis (for *M. hominis*) was also performed. Molecular identification of the species was performed by amplification of the specific *16S rRNA* gene.

Amplification of the *16S rRNA* gene: DNA extraction was performed using the protocol of CinnaClon (Iran). DNA concentration was measured using a NanoDrop device (USA) at 260/280 nm. Amplification of the *16S rRNA* gene was performed using a specific primer pair (Table 1). PCR conditions included 94 °C (4 min), followed by 35 cycles of 94 °C (30 s), 80 °C (45 s), and 72 °C (1 min), and a final extension at 72 °C (10 min).

Macrolide and fluoroquinolone exposure: The MIC ranges of antibiotics against *M. hominis* were evaluated by culturing the isolates in SP-4 medium in 96-well plates (in two-fold) and incubating at 37 °C for 4-2 weeks as previously described [19]. Antibiotics used included tetracycline, doxycycline, clarithromycin, azithromycin, moxifloxacin, ciprofloxacin, and erythromycin (Sigma-Aldrich Denmark). There are no CLSI (Clinical and Laboratory Standards Institute) or EUCAST (European Committee on Antimicrobial Susceptibility Testing) breakpoints for these antibiotics against *M. hominis*. Therefore, the results were reported without breakpoints [20, 21].

Curcumin exposure: Curcumin compound (99% purity, Sigma, USA) was diluted at concentrations of 5 and 10 µg/mL and added to the bacterial suspension in SP-4 broth medium and incubated for up to 72 hrs. *M. hominis* ATCC 23114 was used as a positive control. A curcumin-unexposed group was also cultured in SP-4 broth as a control.

Gene expression analysis: Quantitative real-time PCR **(**qRT-PCR) was performed to examine the *MH16S rRNA* gene expression upon exposure to 10 µg/mL curcumin. The average DNA load levels in the sample and control wells were measured and compared to evaluate the effect of the antimicrobial agent. The primer sequences of the *16S rRNA* gene included F: TTTGGTCAAGTCCTGCAACGA and R: CCCCACCTTCCTCCCAGTTA. PCR conditions included 94 °C (4 min), followed by 35 cycles of 94 °C (30 s), 57 °C (45 s), and 72 °C (1 min), and a final extension at 72 °C (10 min) [22].

Data analysis: IBM SPSS Statistics for Windows, Version 20.0 (Armonk, NY: IBM Corp.) was used for all statistical analyses conducted in the current study. Results are presented as mean ± SD (standard deviation) or median (interquartile range).

Table 1) Primer pair used to identify *M. hominis*

Primer	Sequence: 5' to 3'	Annealing (°C)	Product Size (bp)	Reference
MH16S rRNA	F: TGGRGTKAAGTCGTA R: CTGAGATGTTTCAMTTCACM	80	448 bp	[18]

F: forward, R: reverse, bp: base pair

Since the distribution of the dataset was non-parametric, the Mann-Whitney U test was used to compare the two groups. A *p*-value < .05 was considered statistically significant.

Findings

Patients and bacterial isolates: Of the 550 samples, 20 were positive for *M. hominis*. The patients' age ranged from 23- 35 years (mean: 26.5±2). PCR identification of *M. hominis* strains was also performed by detecting 448 bp products (Figure 1).

Antibiotic MIC ranges: Using dilutions of 0.25-4 µg/mL, the MIC ranges of antibiotics were as follows: 1->4 µg/mL for doxycycline, 2->4 µg/mL for clarithromycin, 2->4 µg/mL for azithromycin, 2->4 µg/mL for moxifloxacin, 2->4 µg/mL for ciprofloxacin, and 1->4 µg/mL for erythromycin. Also, 12 *M. hominis* isolates showed MICs of 4 µg/mL or higher for quinolones.

Curcumin exposure: Exposure of isolates to

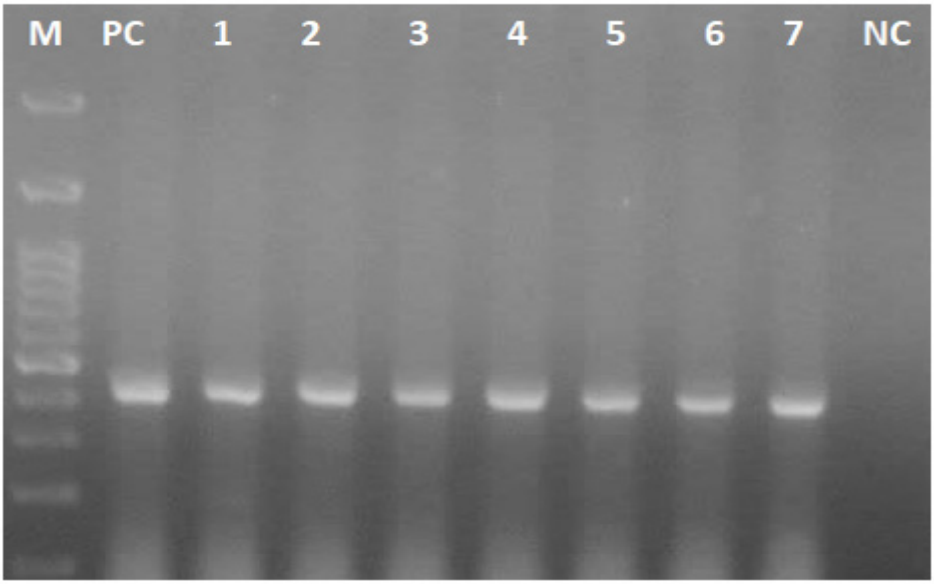


Figure 1) Agarose gel electrophoresis of PCR product of *Mycoplasma hominis* 16 ribosomal RNA gene. Lanes 2-7: positive samples, M: 100 bp DNA marker, PC: positive control, NC: negative control.

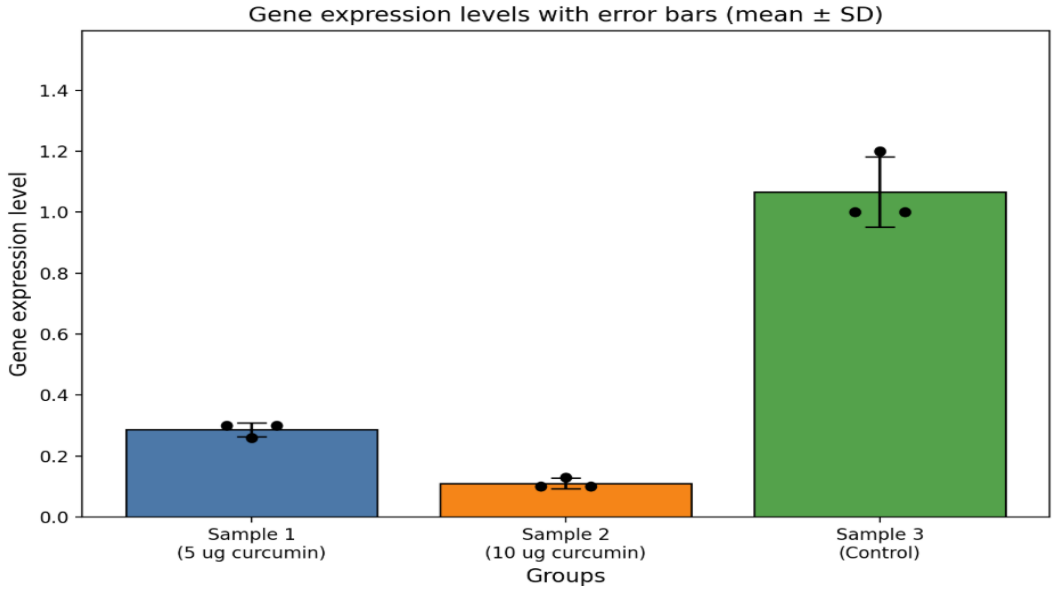


Figure 2) *Mycoplasma hominis* 16 ribosomal RNA (*MH16S rRNA*) gene expression upon exposure to different curcumin concentrations. According to the results, gene expression decreased in a dose-dependent manner.

5 µg/mL curcumin resulted in the inhibition of eight isolates. However, curcumin at a 10 µg/mL concentration inhibited the growth of 15 out of 20 isolates after 72 hrs. Accordingly, the MIC_{50} of curcumin was 10 µg/mL, and its MIC_{90} was >10 µg/mL.

Gene expression analysis: qPCR was performed to examine the expression level of the *MH16S rRNA* gene upon exposure to 10 µg/mL curcumin. The mean expression level of the *MH16S rRNA* gene decreased by 0.8-1 fold among the isolates (Figure 2).

Discussion

In this study, of the 550 vaginosis samples, 20 were positive for *M. hominis*. The patients' age ranged from 23- 35 years (mean: 26.5±2). In previous studies, the mean age of patients with urogenital diseases caused by *M. hominis* has been reported to range from 18-40 years, which is similar to the present study findings [23-25]. Previous antibiotic exposure and hospitalization are significant factors in developing antibiotic resistance, but no significant factor was found in this study. Moxifloxacin is the second-line drug used to eradicate *M. hominis*; therefore, detection of the resistance rate is of great importance [23-25]. Several mutations that may not be occurred by evolutionary pressure confer this resistance [14-17], which were not assessed in this study. Phenotypic evaluation revealed that using dilutions of 0.25-4 µg/mL, the MIC of antibiotics included 1->4 µg/mL for doxycycline, clarithromycin, azithromycin, moxifloxacin, ciprofloxacin, and erythromycin. Also, 12 *M. hominis* isolates showed MIC ≥4 µg/mL for quinolones. There is a need to define cut-off values for *M. hominis* susceptibility to antibiotics.

Due to the failure of treating infections using antibiotics following chemotherapy, it is crucial to search for alternative compounds to prevent the development of resistance and ensure efficient eradication [26, 27]. In this study, the exposure of isolates to 5 µg/mL curcumin resulted in the inhibition of eight isolates. However, curcumin at a 10 µg/mL concentration inhibited the growth of 15 out of 20 isolates after 72 hrs, with MIC_{50} of 10 µg/mL and MIC_{90} of >10 µg/mL. These findings suggest that non-toxic levels of curcumin could be formulated in future studies to eradicate related infections. According to the literature, curcumin at concentrations of 1-10 µg/mL has no cytotoxicity on normal cell lines, and at concentrations of approximately 100 µg/mL decreases cell viability [28-30]. However, the low solubility and rapid mucosal clearance of free curcumin necessitate advanced delivery systems, such as the dendrosome nanocurcumin. Nanoformulations could enhance local residence time and bioavailability, potentially maintaining therapeutic levels within the safety window while overcoming the rapid washout effect of vaginal secretions and mucus [31-33]. There is no previous study regarding the effect of curcumin on *M. hominis* isolates. Moreover, the mean expression level of the *MH16S rRNA* gene decreased by 0.8-1-fold among the isolates upon exposure to 10 µg/mL curcumin. In a study by Shakal et al. (2024), curcumin nanoparticles at a concentration of 1% exerted antibacterial effects against *M. gallisepticum* and *M. synoviae* species isolated from broiler chickens [34]. Accordingly, treated broilers exhibited decreased *Mycoplasma* colonization and improved health conditions. Their study highlights that nanoformulation of curcumin possibly potentiates its effects by lowering the effective concentration.

In a study, *Cinnamomum zeylanicum* at 500 µg/mL was effective against *M. hominis* [35]. In another study, the application of Bioapigyn® herbal ointment compared to doxycycline eradicated all *M. hominis* isolates among female patients

with lower genital tract infections [36]. Curcumin-containing mesoporous silica nanoparticles have also demonstrated acceptable antibacterial effects against *Enterococcus faecalis, Staphylococcus aureus, Acinetobacter baumannii,* and *Escherichia coli* [37]. According to the literature, curcumin likely exerts its antimicrobial effect against *Mycoplasma* spp. through a mechanism that operates on several fronts by destabilizing the membrane and affecting metabolism and biofilm formation [38, 39]. A distinct feature of *Mycoplasma* spp. among bacteria is the absence of a cell wall, which makes them more susceptible to agents targeting plasma membranes compared to Gram-negative bacteria. The lipophilic nature of curcumin permits its penetration into the lipid bilayer and increases fluidity, which in turn causes structural instability of the membrane and the release of intracellular components (ions, proteins), resulting in bacterial cell death [40]. Moreover, curcumin is supposed to interfere with microbiological processes vital for *Mycoplasma* spp. survival, such as the production of membrane sterols [39]. In addition to causing bacterial cell death, curcumin exerts anti-biofilm effects due to its ability to downregulate quorum sensing and influence the EPS (exopolysaccharide) matrix, which prevents the formation of chronic infections [41, 42]. Additionally, curcumin's inhibitory effects on efflux pump activity suggest its potential as a synergistic or alternative agent to antibiotics [43, 44].

Limitations: The main limitation of the present study was the relatively small number of bacterial isolates, affecting the generalizability of the results and statistical robustness. Other limitations were the lack of investigation of the underlying molecular mechanisms (or specific targets) of curcumin and its formulation for antibacterial applications. Moreover, the lack of *in silico* and *in vivo* studies limited the findings related to bioavailability, stability, solubility, and antibacterial efficacy.

Conclusion

In this study, the MIC ranges of quinolones and macrolides were evaluated against *M. hominis* isolates from vaginosis. Curcumin is possibly a potential bioactive compound acting as an alternative agent to inhibit *M. hominis* growth. Moreover, its formulations at lower concentrations could be promising for eliminating *M. hominis* infections. Further in-depth *in silico* and *in vivo* studies are required to confirm its effects.

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Ethical permissions: This study was ethically approved by Al-Muthanna University Review Board (Department of Biology, College of Education for Pure Sciences), Iraq. Consent was also obtained from each patient.

Authors' contributions: D.A.A and F.Q.A. conceptualized the study. A.S.S. performed the work. All authors wrote, edited, and approved the manuscript.

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