Infect Dis Ther (2022) 11:1003–1017
https://doi.org/10.1007/s40121-022-00616-w

ORIGINAL RESEARCH

Clinical and Microbiological Characterization of Bloodstream Infections Caused by *Mycoplasma hominis*: An Overlooked Pathogen

Tong Zeng · Yuan Wu · Zhiyu Yang · Min Luo · Chang Xu · Zhuoran Liu · Jinglin Ouyang · Logen Liu · Xiaotuan Zhang

Received: December 23, 2021 / Accepted: February 23, 2022 / Published online: March 14, 2022

ABSTRACT

Introduction: Bloodstream infection (BSI) is associated with high mortality rates. *Mycoplasma hominis*, which rarely causes extragenital infections, has been shown to induce BSI and presents a clinical diagnostic and therapeutic challenge.

Methods: In this study, we investigated the clinical characteristics, antibiotic resistance, and multilocus sequence typing (MLST) of eight BSI cases caused by *M. hominis* in South China from January 2018 to October 2021.

Results: Underlying immunosuppression and genitourinary tract surgery are important risk factors for *M. hominis* BSI. The most prevalent clinical symptoms and signs were fever. Additional findings included elevated neutrophil count and C-reactive protein level. Furthermore, in this study, all the patients had erythrocytopenia. *M. hominis* harbored the highest rate of resistance to levofloxacin (75.0%), followed by sparfloxacin (50.0%), and gatifloxacin (37.5%). *gyrA* S153L was the most frequent mutation in levofloxacin-resistant strains, followed by *parC* S91I. *parC* K144R may be related to resistance to gatifloxacin and sparfloxacin. Eight strains showed sensitivity to all the other antibiotics analyzed (doxycycline, minocycline, josamycin, and clindamycin). MLST was performed in seven isolates, and seven new sequence types were described. We compared our isolates with all *M. hominis* strains from the PubMLST database, and one major clonal complex and eight singletons were identified.

Conclusions: Our study clarified and expanded the clinical features and antibiotic resistance of *M. hominis* BSI. These findings are useful for the clinical diagnosis and control of *M. hominis* BSI.

Tong Zeng and Yuan Wu contributed equally to this work and share first authorship.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s40121-022-00616-w.

T. Zeng · Y. Wu · Z. Yang · M. Luo · C. Xu · Z. Liu · X. Zhang (✉)
Department of Clinical Laboratory Medicine, The Second Affiliated Hospital, Hengyang Medical School, University of South China, Hengyang 421001, China
e-mail: zxt9289@163.com

X. Zhang
Department of Clinical Laboratory, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, China

J. Ouyang (✉)
Department of Ultrasound Medicine, The Second Affiliated Hospital, Hengyang Medical School, University of South China, Hengyang, China
e-mail: oyjl4879@163.com

L. Liu (✉)
Clinical Research Center, The Second Affiliated Hospital, Hengyang Medical School, University of South China, Hengyang, China
e-mail: llg@fsyy.usc.edu.cn

Keywords: *Mycoplasma hominis*; Bloodstream infection; Sepsis; Antibiotic resistance; Quinolone resistance-determining region; China

Key Summary Points

Why carry out this study?

A comprehensive description of *M. hominis* bloodstream infection (BSI) has rarely been reported. In addition, *M. hominis* lacks a cell wall that complicates both diagnosis and treatment. As a result, understanding the clinical features, antimicrobial resistance, and pathogenic characteristics of *M. hominis BSI* is essential for patient care.

What was learned from the study?

Our results suggest that *M. hominis* can escalate to sepsis, which has life-threatening consequences. There were no specific clinical symptoms of *M. hominis* BSI, therefore blood sample culture is critical.

Antimicrobial-sensitive situations and quinolone-resistant patterns were analyzed, which would guide the empirical selection of antibiotics.

Nine new alleles and seven new sequence types were described. goeBURST analysis revealed that CC-6 was the main clonal complex of *M. hominis* sepsis in South China.

INTRODUCTION

Mycoplasma hominis is an opportunistic human mycoplasma found in regular bacterial cultures. This pathogen typically colonizes the genitourinary tract, and on rare occasions the respiratory system, in a nonvirulent manner [11]. *M. hominis* can cause a variety of genital infections, including bacterial vaginosis [26], nongonococcal urethritis, pelvic inflammatory disease, and prostatitis [23]. In recent years, extragenital *M. hominis* infections have been reported [11, 22, 29]. *M. hominis* BSI is one of the serious invasive infections caused by this pathogen, and treatment is almost certainly required [18]. However, use antibiotics is severely limited because of the pathogen lacks a cell wall [31]. Fluoroquinolones are commonly utilized as empirical therapy for *M. hominis* genitourinary infections, and type II topoisomerases, such as DNA gyrase (encoded by the *gyrA* and *gyrB* genes) and topoisomerase IV (encoded by the *parC* and *parE* genes), interact with fluoroquinolones in *M. hominis* [4, 8, 38]. However, as shown by irrational drug use, fluoroquinolone resistance in *M. hominis* has been increasingly problematic.

Many studies continue to focus on *M. hominis* genitourinary infections; however, the number of reports linked to bloodstream invasiveness is limited. This is, of course, connected to the low frequency or a high prevalence of underdiagnosis of *M. hominis* from blood. In a Public Health England (PHE) reference laboratory in the UK, only five cases of *M. hominis* were isolated from blood over a 10-year period [6]. Clinical, drug resistance, and microbiological features of *M. hominis* BSI are still little known, which might potentially lead to a poor prognosis and even serious clinical crises. A retrospective serial study was conducted, in which the clinical, microbiological, and molecular epidemiological characteristics of eight *M. hominis* BSI cases from a tertiary teaching hospital in South China were investigated and comprehensively assessed from January 2018 to October 2021. We also examined mutations in quinolone resistance-determining regions (QRDRs). The current study may help us better comprehend *M. hominis* from blood, as well as provide treatment recommendations.

METHODS

Ethical Clearance

Ethical clearance to conduct the study was obtained from Medical Research Ethics

Committee at The Second Affiliated Hospital, University of South China (reference number 2021053). The requirement for patient consent to participate in this study was waived because of the retrospective nature of the study. The study followed the latest version of the Helsinki Declaration.

Bacterial Isolates

Between 2018 and 2021, blood samples from all subjects were collected, separated, and cultured in accordance with the Chinese Health Industry Standards. Aerobic and anaerobic bottles (DL Biotech, China) cultures were performed with the use of the BacT/Alert 3D fully automatic blood culture system (BioMerieux, France). The samples from positive blood culture bottles were inoculated onto Columbia blood agar and McConkey plates and incubated at 37 °C in a 5% CO_2 atmosphere until visible colonies appeared. According to the routine method of bacterial identification, Gram staining of suspicious colonies was observed.

Clinical strains of *M. hominis* were screened using the IES kit (Autobio, China) and further identified using real-time fluorescence quantitative PCR (RT-PCR) and 16s rRNA sequencing. For PCR, small-scale preparation of mycoplasmal genomic DNA was performed as previously described [1]. For 16S rRNA sequencing, the following primers were used for PCR amplification: 27f: (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R: (5′-GGTTACCTTGTTACGACTT-3′) [25].

Determination of Antibiotic Resistance

Susceptibility to antibiotics by *M. hominis* isolates was evaluated using a calibrated 10^4–10^5 color-changing units/mL inoculum of each clinical isolate and commercial kits, *Mycoplasma* IES kit (Autobio, China). Antibiotic susceptibility test results were interpreted according to the manufacturer's instructions. For the antibiotics in the *Mycoplasma* kit, which do not include the Clinical and Laboratory Standards Institute (CLSI) breakpoints [33], resistance was determined according to previous studies [13, 19, 23].

Study Subjects

This retrospective study was conducted on patients with *M. hominis* BSI at The Second Affiliated Hospital, University of South China (Hengyang, China) from June 1, 2018 to October 31, 2021. The diagnosis of *M. hominis* BSI was based on the Centers for Disease Control and Prevention and National Healthcare Safety Network (CDC/NHSN) definition [12]: (i) the patient has *M. hominis* cultured from one or more blood cultures and organism cultured from blood is not related to an infection at another site; (ii) the patient has at least one of the following signs or symptoms: fever (> 38 °C), chills, or hypotension. Moreover, signs and symptoms and positive laboratory results were not related to an infection at another site.

Sepsis was defined according to the Sepsis-3.0 criteria [28]: (i) existing evidence of suspected or confirmed infection; (ii) the Sequential Organ Failure Assessment (SOFA) score more than or equal to 2 from baseline or greater than 2 in patients with no baseline score available.

Study Data

Medical records were reviewed to collect the data of patients with *M. hominis* sepsis during the study period. The study data included the following variables: demographic characteristics (age and sex), underlying or concomitant conditions (diabetes mellitus, kidney disease, hepatic disease, history of intra-abdominal trauma or surgery, malignancy, and hypertension), clinical symptoms, *M. hominis* treatment time, admission to intensive care unit (ICU), laboratory values (red blood cell [RBC], platelet, white blood cell [WBC] count, percentage of neutrophils, percentage of lymphocytes, percentage of monocytes, C-reactive protein [CRP], procalcitonin [PCT]), urogenital tract operation as cause of hospitalization, and prognosis (cured or died).

Amplification and Sequencing of QRDRs of *gyrA*, *gyrB*, *parC*, and *parE* Genes

DNA samples were extracted as previously described. Primers for QRDRs of the *gyrA*, *gyrB*, *parC*, and *parE* genes and the tetracycline resistance gene *tetM* were designed as previously described [1, 2, 6]. PCR amplification was performed using the following parameters: 30 s at 98 °C, followed by 28 cycles of 20 s at 98 °C, 30 s at 58 °C, and 30 s at 72 °C, and a final extension of 7 min at 72 °C. Positive PCR products were visualized on agarose gel, excised, and sequenced using the Sanger method. Gene mutations and amino acid substitutions of QRDRs were analyzed using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the SeqMan software and compared to the reference strain, *M. hominis* PG21 (ATCC23114 GenBank accession number FP236530.1).

Multiple Sequence Alignment Analysis

BLASTp was performed on the *parC* and *parE* protein sequences of *M. hominis*, and the comparison species were limited to *Mycoplasma* (taxid: 31969). Nonredundant matched sequences were downloaded, with 1357 sequences (containing *gyrA*) for *parC* and 1319 sequences (containing *gyrB*) for *parE*. MAFFT software was used to perform a multiple sequence alignment analysis. The amino acid sequences of *parC* from residues 72 to 151 and of *parE* from residues 408 to 495 were extracted using *M. hominis* coordinates to build the sequence logos in WebLogo Version 2.8.2 (http://weblogo.berkeley.edu) [7].

Multilocus Sequence Typing

MLST was performed by sequencing five housekeeping loci (*uvr*A, *GyrB*, *fts*Y, *tuf*, and *gap*) [5] according to a previously described method. The *M. hominis* MLST database (https://pubmlst.org/organisms/mycoplasma-hominis) was used to assign allele numbers and sequence types (STs). The MLST sequence data for the other 59 strains were obtained from Boujemaa et al. [5]. Sequences were aligned, and a phylogenetic tree was constructed using MEGA software version X [16]. The phylogenetic tree was based on concatenated sequences using the neighbor-joining method. Support for internal nodes was estimated using the nonparametric bootstrap method with 1000 replications. Global optimal goeBURST diagrams were drawn up using PHYLOViZ software version 2 [21] to analyze the relatedness between STs.

RESULTS

Clinical Characteristics

The current study examined instances of *M. hominis* BSI that met the inclusion criteria. Eight strains of *M. hominis* BSI were collected among the 1148 patients diagnosed with BSI from January 2018 to October 2021 in The Second Affiliated Hospital, University of South China. Table 1 summarizes the clinical features of patients with *M. hominis* BSI. There were no differences in these instances between men and women, and the median age was 40 (23–-81) years. Five patients who suffered severe vehicle accident, cerebral hemorrhage or liver failure developed *M. hominis* sepsis and were admitted to the ICU, two of whom died. The other three patients, all of whom received urogenital tract surgery, had stable disease, and their body temperature had fallen before *M. hominis* was identified. Fever was the most frequent clinical symptom (Fig. 1). Before therapy, four patients had WBC levels that were above normal and recovered to normal levels in two patients after treatment. Neutrophils were above the normal range in all patients, whereas lymphocytes and RBCs were below the normal range. The platelets of five patients showed large fluctuations. The mononuclear cells were relatively stable before and after treatment. CPR and PCT in Fig. 1 are the values of the day when a positive blood culture bottle was sent. On the day of diagnosis, all patients had CRP levels above the normal range (13.4–296 mg/L), with only two patients having PCT levels within the normal range. In summary, decreased lymphocyte and RBC counts, increased neutrophil counts, and CRP levels were the most frequently observed.

Table 1 Clinical details of eight patients with *M. hominis* bloodstream infection

No.	Department	Admission cause	Underlying disease	Urogenital tract operation	Prior antibiotics therapy	Final treatment	SOFA	Treatment cycle[a] (days)	Outcome
MH-BL01	ICU	Multiple injuries in car accident	No	UC	TZP	LEV + DOX	4	20	Cured
MH-BL02	ICU	Chronic liver failure	Cirrhosis	UC	NA	NA	10	NA	NA
MH-BL03	Obstetrics	Premature rupture of membrane	No	Caesarean section; UC	CMZ	CLI	0	3	Cured
MH-BL04	Gynecology	Uterine fibroids	No	Hysteromyomectomy; UC	TZP + MTR	TZP + MTR + CLI	0	3	Cured
MH-BL05	Urology	Hemonephrosis	Hyperthyroidism; hypertension	Percutaneous nephrostomy; UC	TZP	TZP + PN	1	0[b]	Cured
MH-BL06	ICU	Multiple injuries in car accident	Diabetes; hypertension; coronary heart disease	UC	MEM + PMB	PMB + DOX + FCZ + LNZ	5	Died (12 days)[c]	Death
MH-BL07	ICU	Multiple injuries in car accident	No	UC	TZP	TZP + MIN	4	8	Cured
MH-BL08	ICU	Brainstem hemorrhage	Hypertension; appendectomy; gastrorrhagia	UC	TZP	CMZ + LEV + MIN	7	Abandoned therapy (27 days)[d]	Abandoned therapy

CMZ cefmetazole, *TZP* piperacillin–tazobactam, *MTR* metronidazole, *MEM* meropenem, *CLI* clindamycin, *LEV* levofloxacin, *MIN* minocycline, *DOX* doxycycline, *PMB* polymyxin B, *FCZ* fluconazole, *LNZ* linezolid, *ICU* intensive care unit, *UC* urethral catheterization, *NA* not available, *SOFA* Sequential Organ Failure Assessment

[a]Treatment cycle refers to the diagnosis of *M. hominis* bloodstream infection from the beginning of the use of sensitive antibiotics to the stoppage of *M. hominis*-sensitive antibiotics after infection control

[b]*M. hominis*-sensitive antibiotics were not used because the infection was controlled before the diagnosis was confirmed

[c]Died after 12 days of *M. hominis*-sensitive antibiotic treatment

[d]Treatment was abandoned after 27 days of *M. hominis*-sensitive antibiotic treatment

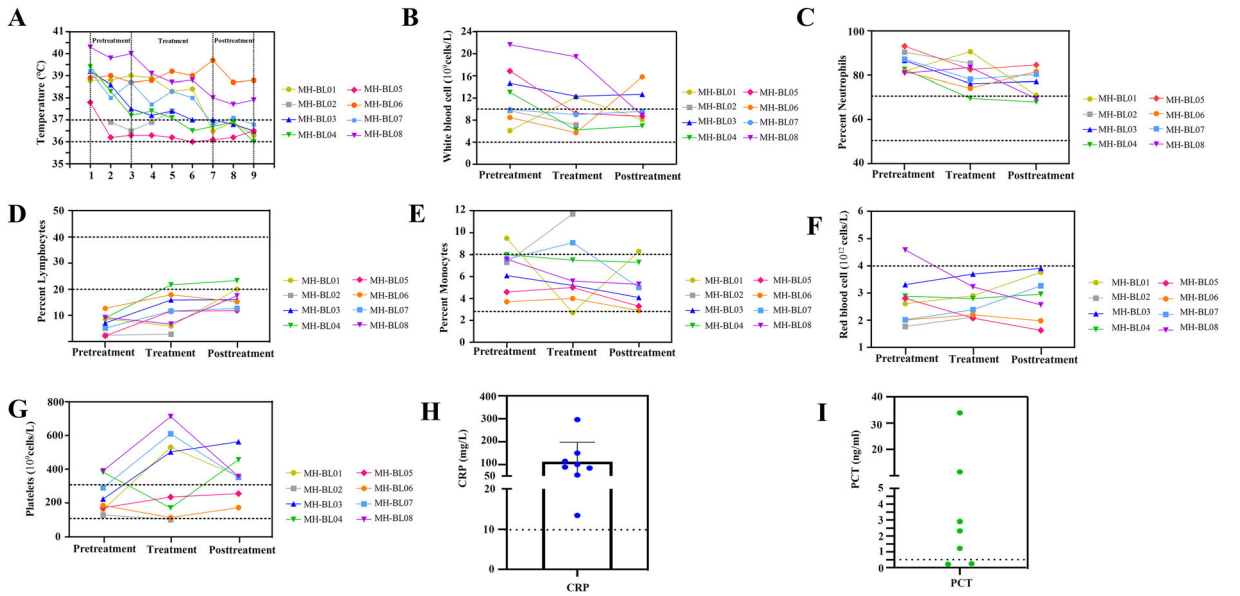


Fig. 1 Body temperature and hematology parameters of patients with *M. hominis* BSI. **a** Body temperature; **b** white blood cells; **c** neutrophils; **d** lymphocytes; **e** monocytes; **f** red blood cells; **g** platelets; **h** C-reactive protein (CRP); **i** procalcitonin (PCT)

In terms of clinical care, all patients received empiric antibiotic therapy with β-lactam antibiotics before the infection was identified. However, this effect was not significant. When the pathogen was discovered, tetracyclines or fluoroquinolones were the most frequently used antibiotic regimens. Combination antibiotic therapy was used in two cases (MH-BL01, MH-BL08), with one patient receiving levofloxacin (LEV) combined with doxycycline (DOX) and the other receiving LEV combined with minocycline (MIN). Three patients (MH-BL03, MH-BL04, and MH-BL05) who had not progressed to sepsis showed decreasing trends in temperature and inflammatory parameters before the use of sensitive antibiotics. One patient (MH-BL02) was asked to be transferred to another hospital after being discharged from the ICU, and his follow-up clinical data was not available.

Mutations in Quinolone Resistance-Determining Regions

All isolates were sensitive to DOX, MIN, josamycin (JOS), and clindamycin (CLI). The eight *M. hominis* strains exhibited resistance to LEV (75.0%), sparfloxacin (SPA, 50.0%), and GAT (37.5%) (Table 2). We performed molecular analysis on seven *M. hominis* strains, except for one strain that could not be preserved. The seven *M. hominis* strains, as verified via molecular identification using 16S rRNA sequencing and qPCR (Supplementary Fig. S1), were further amplified for the QRDRs of the *gyrA*, *gyrB*, *parC*, and *parE* genes. Six types of amino acid substitutions in the QRDRs of the seven strains, based on DNA sequencing, were identified (Table 3): *gyrA* S153A (one sample) and S153L (five samples), *parC* S91I (seven samples), K144R (four samples), and *parE* V417I (seven samples). The double substitution *parC* S91I/*parE* V417I was identified in an LEV-sensitive and six LEV-resistant isolates. A mutation in *gyrA* S153 was detected in six resistant LEV-resistant strains. Four *M. hominis* strains harbored four substitutions (*gyrA* S153L or *gyrA* S153A, *parC* S91I, *parC* K144R, and *parE* V417I), with MICs greater than fourfold increase against SPA and GAT compared with other strains without mutation at *parC* K144R. Three nucleotide alterations (G1323A, A1347T, and G1428A) (Supplementary Table S2) were identified in the *gyrB* genes

Table 2 Antimicrobial susceptibilities of *M. hominis* from blood

Strain	DOX		MIN		JOS		LEV		SPA		GAT		CLI	
	MIC	[13]	MIC	[23]	MIC	[23]	MIC	CLSI	MIC	[19]	MIC	[19]	MIC	CLSI
MH-BL1	≤ 2	S	≤ 1	S	≤ 1	S	≤ 0.5	S	≤ 0.5	S	≤ 0.5	S	≤ 0.25	S
MH-BL2	≤ 2	S	≤ 1	S	≤ 1	S	≥ 8	R	≥ 8	R	4	I	≤ 0.25	S
MH-BL3	≤ 2	S	≤ 1	S	≤ 1	S	≥ 8	R	≥ 8	R	≥ 8	R	≤ 0.25	S
MH-BL4	≤ 2	S	≤ 1	S	≤ 1	S	≥ 8	R	2	I	≤ 0.5	S	≤ 0.25	S
MH-BL5	≤ 2	S	≤ 1	S	≤ 1	S	≥ 8	R	≥ 8	R	≥ 8	R	≤ 0.25	S
MH-BL6	≤ 2	S	≤ 1	S	≤ 1	S	≥ 8	R	≥ 8	R	≥ 8	R	≤ 0.25	S
MH-BL7	≤ 2	S	≤ 1	S	≤ 1	S	≥ 8	R	≤ 0.5	S	2	I	≤ 0.25	S
MH-BL8	≤ 2	S	≤ 1	S	≤ 1	S	≤ 0.5	S	≤ 0.5	S	≤ 0.5	S	≤ 0.25	S

The breakpoints (mg/L) according to the Clinical and Laboratory Standards Institute (CLSI) or other studies were as follows: doxycycline S ≤ 4, R ≥ 8; minocycline S ≤ 2, R ≥ 8; josamycin S ≤ 2, R ≥ 8; levofloxacin S ≤ 1, R ≥ 2; sparfloxacin S ≤ 1, R ≥ 4; gatifloxacin S ≤ 1, R ≥ 4; clindamycin S ≤ 0.25, R ≥ 0.5

S sensitive, *I* intermediate, *R* resistance, *DOX* doxycycline, *MIN* minocycline, *JOS* josamycin, *LEV* levofloxacin, *SPA* sparfloxacin, *GAT* gatifloxaxin, *CLI* clindamycin

Table 3 Antibiotic phenotype and molecular characterization of fluoroquinolone-resistant *M. hominis* isolates from blood

Strain	MIC (μg/mL)			QRDRs			
	LEV	SPA	GAT	*gyrA*	*gyrB*	*parC*	*parE*
MH-BL01	≤ 0.5	≤ 0.5	≤ 0.5	ND	ND	G272T(S91I)	G1249A(V417I)
MH-BL02	≥ 8	≥ 8	4	C458T(S153L)	ND	G272T(S91I) A431G(K144R)	G1249A(V417I)
MH-BL03	≥ 8	≥ 8	≥ 8	C458T(S153L)	ND	G272T(S91I) A431G(K144R)	G1249A(V417I)
MH-BL04	≥ 8	2	≤ 0.5	C458T(S153L)	ND	G272T(S91I)	G1249A(V417I)
MH-BL05	≥ 8	≥ 8	≥ 8	C458T(S153L)	ND	G272T(S91I) A431G(K144R)	G1249A(V417I)
MH-BL06	≥ 8	≥ 8	≥ 8	C458T(S153L)	ND	G272T(S91I) A431G(K144R)	G1249A(V417I)
MH-BL07	≥ 8	≤ 0.5	2	T457G(S153A)	ND	G272T(S91I)	G1249A(V417I)

M. hominis positions gyrA 153, *parC* 91,104, and 144, and *parE* 417 correspond to *Escherichia coli* coordinates *gyrA* 83, *parC* 80, 93, and 133, and *parE* 410, respectively

LEV levofloxacin, *SPA* sparfloxacin, *GAT* gatifloxacin, *ND* no significant amino acid mutations detected

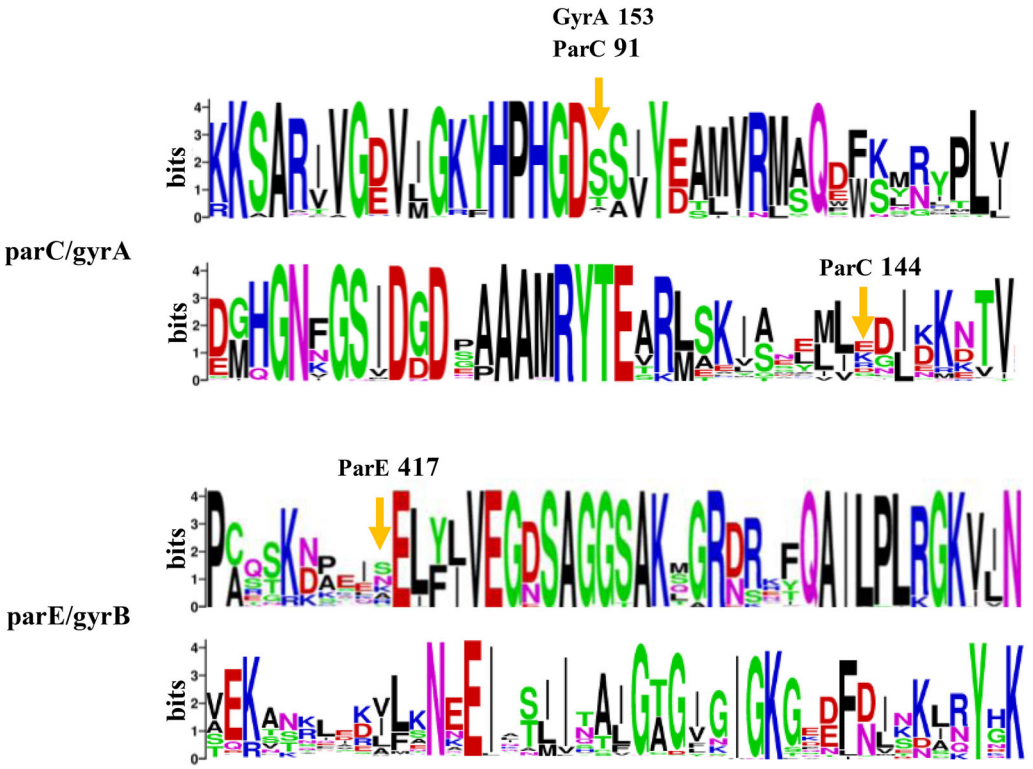
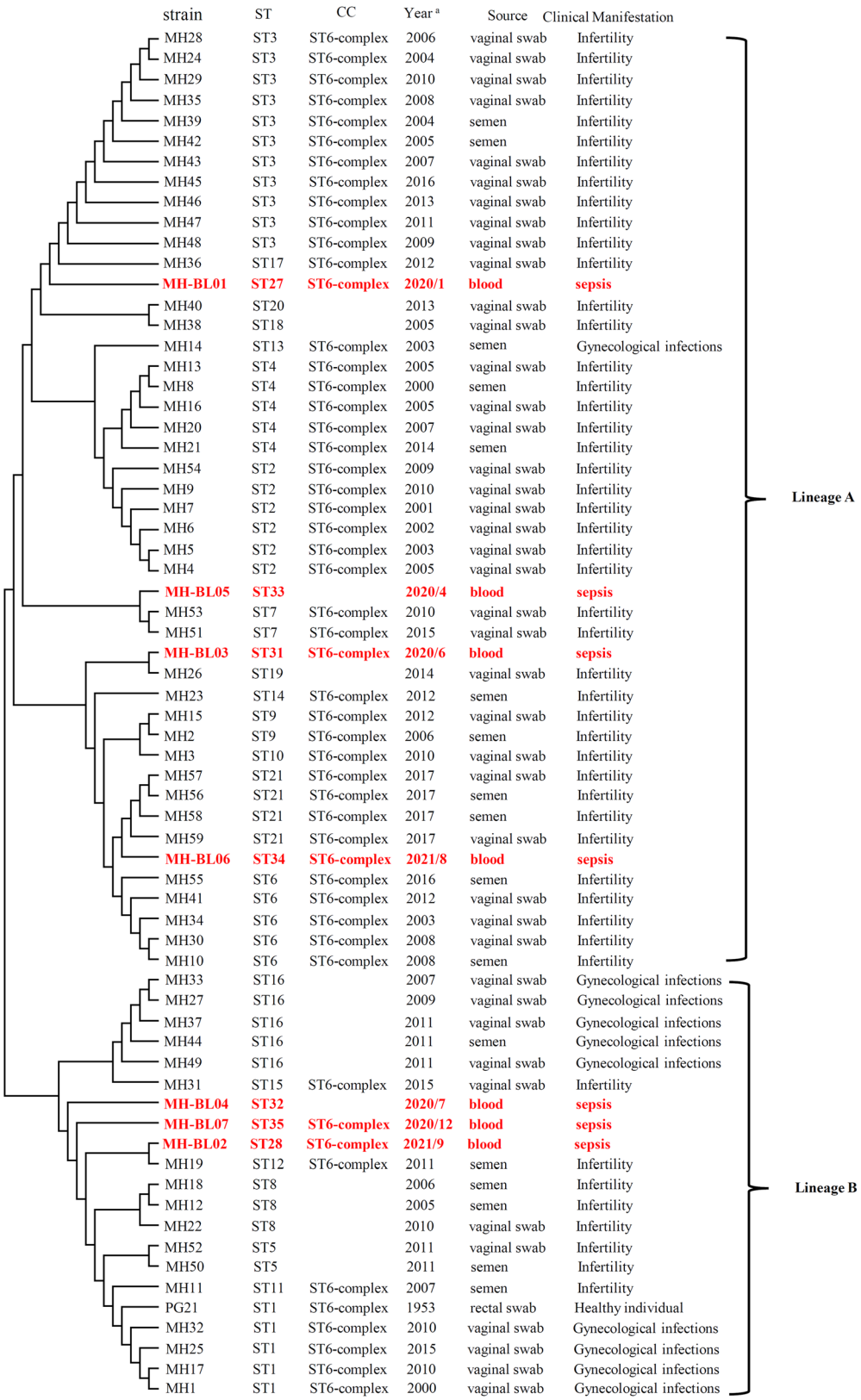


Fig. 2 Sequence conservation of the quinolone resistance-determining region (QRDR). The logos for *parC* and *gyrA* and *parE* and *gyrB* were computed for 1357 and 1319 nonredundant sequences, respectively. A logo represents the height of each letter proportional to the observed frequency of the corresponding amino acid. The mutation sites that were based on the *M. hominis* coordinates are indicated above the symbols

of seven clinical isolates. However, these nucleotide mutations did not cause amino acid changes during translation. Figure 2 provides a graphical representation of amino acid multiple sequence alignment of *parC* and *gyrA* and *parE* and *gyrB* among *Mycoplasma*. The overall height of the stack indicates the sequence conservation at this position. The QRDR conservativeness of *parC* and *gyrA* was higher than that of *parE* and *gyrB*. In the present study, *parC* and *gyrA* mutations occurred in a relatively conserved region, which is a hot region of current mutation studies. In comparison, the *parC* 144 and *parE* 417 positions were relatively less conserved.

Multilocus Sequencing Type

MLST revealed seven sequence types among the seven *M. hominis* strains, including nine new alleles and seven new STs. All STs were first discovered and are shown in Supplementary Table S3. We compared our isolates with clinical *M. hominis* isolates from other countries by selecting 59 *M. hominis* isolates from the PubMLST database. Almost all isolates were from the genitourinary tract in Africa (Fig. 3). The 66 human *M. hominis* isolates were divided into 28 STs. In addition, goeBURST (PHYLOViZ) analysis revealed that one clonal complex (CC) was CC-6 and eight singletons (Fig. 4). CC-6 included 77.3% (51/66) isolates covering 20 STs, and ST6 was defined as the founder of the homologous complex. In this study, five ST types (71.4%, 5/7) belonged to the CC-6 complex group. According to a previous study [5], two major lineages, A and B, were differentiated. The dendrogram generated from the MLST data showed that the majority of STs were grouped into lineage A (18 STs), and it also


strain ST CC Year [a] Source Clinical Manifestation
MH28 ST3 ST6-complex 2006 vaginal swab Infertility
MH24 ST3 ST6-complex 2004 vaginal swab Infertility
MH29 ST3 ST6-complex 2010 vaginal swab Infertility
MH35 ST3 ST6-complex 2008 vaginal swab Infertility
MH39 ST3 ST6-complex 2004 semen Infertility
MH42 ST3 ST6-complex 2005 semen Infertility
MH43 ST3 ST6-complex 2007 vaginal swab Infertility
MH45 ST3 ST6-complex 2016 vaginal swab Infertility
MH46 ST3 ST6-complex 2013 vaginal swab Infertility
MH47 ST3 ST6-complex 2011 vaginal swab Infertility
MH48 ST3 ST6-complex 2009 vaginal swab Infertility
MH36 ST17 ST6-complex 2012 vaginal swab Infertility
MH-BL01 ST27 ST6-complex 2020/1 blood sepsis
MH40 ST20 2013 vaginal swab Infertility
MH38 ST18 2005 vaginal swab Infertility
MH14 ST13 ST6-complex 2003 semen Gynecological infections
MH13 ST4 ST6-complex 2005 vaginal swab Infertility
MH8 ST4 ST6-complex 2000 semen Infertility
MH16 ST4 ST6-complex 2005 vaginal swab Infertility
MH20 ST4 ST6-complex 2007 vaginal swab Infertility
MH21 ST4 ST6-complex 2014 semen Infertility
MH54 ST2 ST6-complex 2009 vaginal swab Infertility
MH9 ST2 ST6-complex 2010 vaginal swab Infertility
MH7 ST2 ST6-complex 2001 vaginal swab Infertility
MH6 ST2 ST6-complex 2002 vaginal swab Infertility
MH5 ST2 ST6-complex 2003 vaginal swab Infertility
MH4 ST2 ST6-complex 2005 vaginal swab Infertility
MH-BL05 ST33 2020/4 blood sepsis
MH53 ST7 ST6-complex 2010 vaginal swab Infertility
MH51 ST7 ST6-complex 2015 vaginal swab Infertility
MH-BL03 ST31 ST6-complex 2020/6 blood sepsis
MH26 ST19 2014 vaginal swab Infertility
MH23 ST14 ST6-complex 2012 semen Infertility
MH15 ST9 ST6-complex 2012 vaginal swab Infertility
MH2 ST9 ST6-complex 2006 semen Infertility
MH3 ST10 ST6-complex 2010 vaginal swab Infertility
MH57 ST21 ST6-complex 2017 vaginal swab Infertility
MH56 ST21 ST6-complex 2017 semen Infertility
MH58 ST21 ST6-complex 2017 semen Infertility
MH59 ST21 ST6-complex 2017 vaginal swab Infertility
MH-BL06 ST34 ST6-complex 2021/8 blood sepsis
MH55 ST6 ST6-complex 2016 semen Infertility
MH41 ST6 ST6-complex 2012 vaginal swab Infertility
MH34 ST6 ST6-complex 2003 vaginal swab Infertility
MH30 ST6 ST6-complex 2008 vaginal swab Infertility
MH10 ST6 ST6-complex 2008 semen Infertility
MH33 ST16 2007 vaginal swab Gynecological infections
MH27 ST16 2009 vaginal swab Gynecological infections
MH37 ST16 2011 vaginal swab Gynecological infections
MH44 ST16 2011 semen Gynecological infections
MH49 ST16 2011 vaginal swab Gynecological infections
MH31 ST15 ST6-complex 2015 vaginal swab Infertility
MH-BL04 ST32 2020/7 blood sepsis
MH-BL07 ST35 ST6-complex 2020/12 blood sepsis
MH-BL02 ST28 ST6-complex 2021/9 blood sepsis
MH19 ST12 ST6-complex 2011 semen Infertility
MH18 ST8 2006 semen Infertility
MH12 ST8 2005 semen Infertility
MH22 ST8 2010 vaginal swab Infertility
MH52 ST5 2011 vaginal swab Infertility
MH50 ST5 2011 semen Infertility
MH11 ST11 ST6-complex 2007 semen Infertility
PG21 ST1 ST6-complex 1953 rectal swab Healthy individual
MH32 ST1 ST6-complex 2010 vaginal swab Gynecological infections
MH25 ST1 ST6-complex 2015 vaginal swab Gynecological infections
MH17 ST1 ST6-complex 2010 vaginal swab Gynecological infections
MH1 ST1 ST6-complex 2000 vaginal swab Gynecological infections
Lineage A
Lineage B

◀**Fig. 3** Neighbor-joining trees of 66 *M. hominis* isolates based on the concatenated sequences of MLST. The isolates originating from this study are marked in red. [a]Year of bacteria acquisition

revealed that sepsis-associated STs were distributed in both lineages.

DISCUSSION

In previous reports, *M. hominis* often caused maternal postpartum or postabortal fever and often settles without treatment [32], and immune functions play an essential role in regulating *M. hominis* sepsis [9]. In the immunocompetent host, *M. hominis* can be efficiently cleared from the circulation by phagocytes and macrophages in the blood and the reticuloendothelial system [9]. In our study, those patients who had a clear urogenital source presented with a transient BSI and both of them required irrelevant antibiotics and survived, whereas patients admitted with major trauma to ICU progressed to sepsis and needed effective therapy for a longer time but nevertheless in some cases died. Although most *M. hominis* BSI have been transitory in prior research [34], the possibility of progression should not be discounted. This could be related to the patient's immunological function, although it was not investigated in this study.

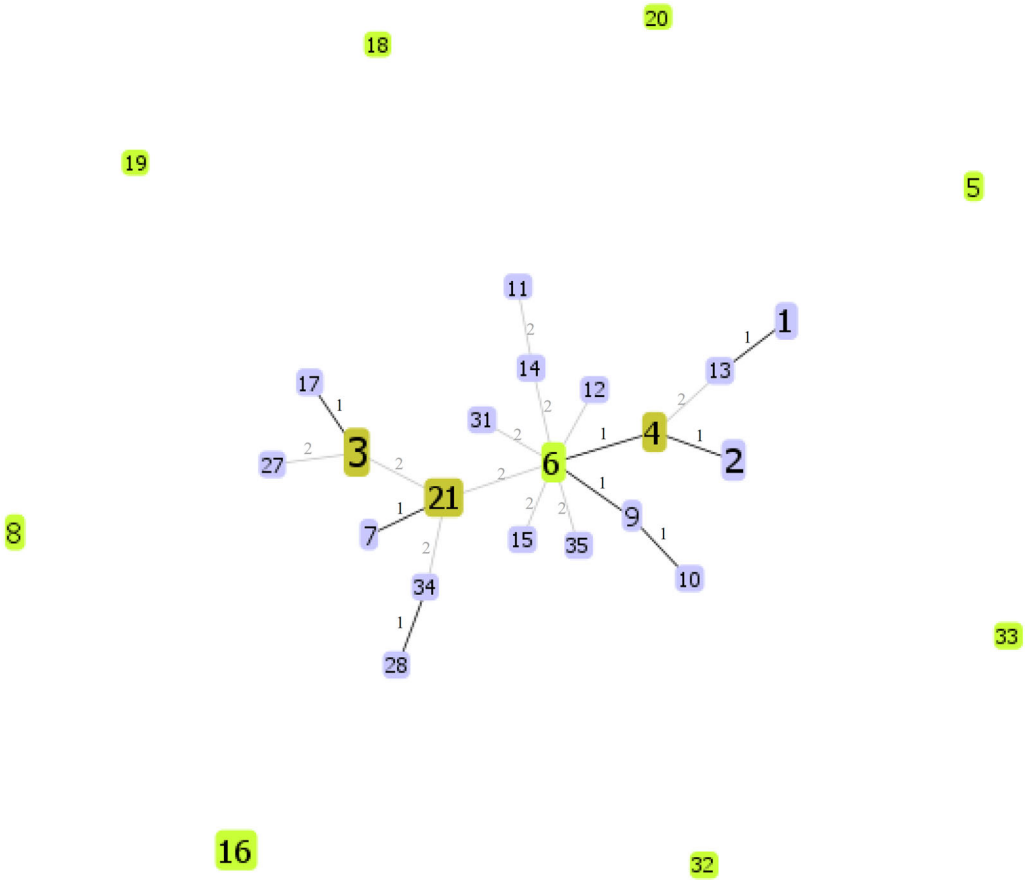


Fig. 4 Phylogenetic analysis. Minimum spanning tree analysis of the 66 *M. hominis* isolates based on MLST. Isolates are represented by circles, and the size of the circle is proportional to the number of isolates. Branches and numbers represent allelic differences between isolates. Each blue dot represents one ST, and green dots indicate individual founders, while yellow spots denote sub-founders

Several elements of the clinical presentation of *M. hominis* sepsis were validated via our investigation (Fig. 1). The most common clinical features were fever. Additional findings included lymphopenia, increased neutrophil count and CPR, and only two patients having PCT levels within the normal range. Furthermore, although all our patients had erythropenia, there is no evidence to support a link between this clinical presentation and *M. hominis* BSI. When compared with patients who had BSI caused by other bacteria of the same period, no significant differences in temperature, CRP, PCT, WBC, neutrophil count, platelet and RBC were observed ($P > 0.05$) (Supplementary Table S1), which suggested that there were no characteristic changes in inflammatory indicators of *M. hominis* BSI. Therefore, it is extremely important to analyze blood cultures from patients with severe infections, and those who have failed to respond to β-lactams should be warned about the risk of *M. hominis* BSI. (The inclusion criteria and results of statistical analysis for the control group are described in the Supplementary Material.)

M. hominis is inherently resistant to all β-lactams because of the lack of a cell wall. To further limit treatment choices, two antibiotic families, fluoroquinolones and tetracyclines, showed strong action against these bacteria [3]. In antibiotic susceptibility studies of *M. hominis* in the urinary tract, a German study [15] showed that ciprofloxacin (CIP) may be used in empirical treatment; CLI and JOS could be used as alternatives. Italian [17] and Hungary studies [24] also showed low resistance to fluoroquinolones. A lower sensitivity to JOS (79.2%) for *M. hominis* in Athens has been reported [14]. In a study in Beijing, the resistance rates of *M. hominis* to CIP, LEV, and SPA were 82.2%, 62.2%, and 80.0%, respectively, and few strains showed resistance to MIN, DOX, and JOS [35]. These results were similar to those of some other studies in China [37–39] and current research. As a result, the antibiotic sensitivity profile of *M. hominis* isolated in various regions of the genitourinary tract can be utilized as a reference for the treatment of *M. hominis* BSI. In China, MIN, DOX, or JOS could be used as empirical therapy for *M. hominis* BSI.

The predominance of reduced susceptibility to fluoroquinolones among *M. hominis* isolates is an alarming sign. Sequence analysis of QRDRs in LEV-resistant *M. hominis* strains indicated that *parC* S91 and *gyrA* S153 were mutation hotspots (Table 3). Previous research has found that these two mutations are the most common genetic changes in fluoroquinolone-resistant *M. hominis* [8, 20, 38]. Furthermore, these two positions exhibited considerably higher conservation (Fig. 2). *parC* S91 (corresponding to mutations in *Ureaplasma* spp. *parC* S83) substitution was the most frequent mutation in *Ureaplasma* spp. [27, 30]. However, a mutation in *parC* S91I was observed in one of the bacterial strains susceptible to LEV. This might be because mutations in *gyrA* S153 constitute the major mechanism of LEV resistance in clinical isolates of *M. hominis*, whereas changes in *parC* S91 allow resistance to increase to a greater degree. Similar findings have been previously published in the literature. According to Zhang et al. [38], a single *parC* S91I mutation increased the MIC of LEV from 0.5 to 4–8 g/mL. The MIC of LEV was enhanced to 128 g/mL when ParC S91I was coupled with *gyrA* S153L. According to Yang et al. [36], strains carrying the two mutations had a two- to fourfold higher MIC for LEV than those carrying *parC* S91 alone. Structural analysis of the wild-type topoisomerase IV (ParC–ParE) complex coupled to LEV in *Ureaplasma* spp. revealed that *parC* Ser83 or its analogous mutations in *M. hominis* (*gyrA* S153A, *gyrA* S153L, and *parC* S91I) precluded interactions with LEV. Controversially, some studies have indicated that the *ParC* K144R mutation does not contribute to fluoroquinolone resistance [8, 36, 38], while Meng et al. [19] indicated that this mutation point substitution in *parC* of *M. hominis* might be related to its resistance to ofloxacin and LEV. This mutation site was also discovered in our clinical isolates, but it appeared to increase the MIC values of SPA and GAT. Because a single mutation was found in sensitive strains in prior research, it is possible that K144R with *parC* S91 and/or *gyrA* S153 might promote resistance only when present concurrently, although the biological relevance of these modifications remains unknown. We discovered that all isolates had identical ParE

aberrations that resulted in an amino acid exchange (Val 417 Ile) compared to the type strain PG21. This might be related to genetic variation. Several nucleotide changes occurred in the *gyrB* gene, consistent with previous results [10, 20] and our findings; however, these were nonsense mutations with no amino acid change.

MLST analysis revealed the molecular epidemiological characteristics of *M. hominis*. We also highlighted the higher genetic diversity of sepsis-associated *M. hominis* in South China. We identified a total of seven STs, including seven new STs, which means all of the STs were the new types. Because the data we jointly evaluated were only from genitourinary tract isolates from one hospital in North Africa, it is unknown if this genetic diversity is related to the area or the causal location, and it is worth additional investigation when the database is richer. CC-6 was not only the main CC of *M. hominis* isolated from the genitourinary tract in North Africa but was also isolated from the blood. CC-6 may be more closely associated with BSI. In addition, the dendrogram generated from the MLST data in this study showed that the majority of STs were grouped into lineage A (57.1%, 4/7), and our strains were not concentrated in a particular lineage or formed a new lineage. However, none of the MLST-derived data could be correlated with the year and source of isolation of the clinical strains.

Our study had a few limitations. First, the extremely limited instances and strains are a potential drawback of our investigation. At the same time, however, few similar cases of this kind have been reported, so we hope that our report will provide some help in clinical management. Second, because this study was retrospective, the inconsistency of the clinical data prevented further utilization and analysis of more significant indicators.

CONCLUSIONS

A serial study of *M. hominis* bloodstream infection was conducted. Substantial levels of fluoroquinolone resistance in *M. hominis* were found in South China. It is critical to promptly identify the features of *M. hominis* sepsis, followed by effective therapeutic strategies. Furthermore, more research is necessary to confirm the connections between the genotypes, resistant spectrum, and clones of *M. hominis* isolates from blood. Further studies are required to improve the awareness of *M. hominis* and to develop effective therapies for patients with *M. hominis* sepsis.

ACKNOWLEDGEMENTS

Funding. The work and Rapid Service Fee were supported by the Natural Science Foundation of Hunan Province (2021JJ40483), Health Commission Program of Hunan province (202211004530 and 202203034469), and the Key Laboratory for Molecular Diagnosis and Precision Medicine in Hengyang (202010041574).

Medical Writing and Editorial Assistance. The authors acknowledge CaiHong Yan, a specialist at the intensive care unit, who played an important role in collecting and discussing clinical data. The authors would also like to thank Bei Li and Lu Wang for providing samples and isolates for analysis. The authors thank Editage for English language editing.

Authorship. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Author Contributions. Tong Zeng and Xiaotuan Zhang conceived and designed the study and wrote the manuscript. Zhuoran Liu collected the clinical data and gave advice for the experiments. Zhiyu Yang, Chang Xu, and Min Luo performed the determination of antibiotic resistance and qPCR. Yuan Wu, Logen Liu, and Jinglin Ouyang acquired and analyzed the data. All authors read and approved the final manuscript.

Disclosures. Tong Zeng, Yuan Wu, Zhiyu Yang, Min Luo, Chang Xu, Zhuoran Liu, Jinglin Ouyang, Logen Liu, and Xiaotuan Zhang have nothing to disclose. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Compliance with Ethics Guidelines. Ethical clearance to conduct the study was obtained from Medical Research Ethics Committee at The Second Affiliated Hospital, University of South China (reference number 2021053). The requirement for patient consent to participate in this study was waived because of the study was retrospective.

Data Availability. All data generated or analysed during this study are included in this manuscript/ as supplementary information files.

REFERENCES

1. The Bébéar CM, Renaudin H, Charron A, Bové JM, Bébéar C, Renaudin J. Alterations in topoisomerase IV and DNA gyrase in quinolone-resistant mutants of *Mycoplasma hominis* obtained in vitro. Antimicrob Agents Chemother. 1998;42:2304–11.

2. Bebear CM, Bové JM, Bebear C, Renaudin J. Characterization of *Mycoplasma hominis* mutations involved in resistance to fluoroquinolones. Antimicrob Agents Chemother. 1997;41:269–73.

3. Bebear CM, Renaudin H, Bryskier A, Bebear C. Comparative activities of telithromycin (HMR 3647), levofloxacin, and other antimicrobial agents against human mycoplasmas. Antimicrob Agents Chemother. 2000;44:1980–2.

4. Bebear CM, Renaudin J, Charron A, et al. Mutations in the gyrA, parC, and parE genes associated with fluoroquinolone resistance in clinical isolates of *Mycoplasma hominis*. Antimicrob Agents Chemother. 1999;43:954–6.

5. Boujemaa S, Ben Allaya A, Mlik B, Mardassi H, Ben Abdelmoumen Mardassi B. Phylogenetics of *Mycoplasma hominis* clinical strains associated with gynecological infections or infertility as disclosed by an expanded multilocus sequence typing scheme. Sci Rep. 2018;8:14854.

6. Chalker VJ, Sharratt MG, Rees CL, et al. Tetracycline resistance mediated by tet(M) has variable integrative conjugative element composition in *Mycoplasma hominis* strains isolated in the United Kingdom from 2005 to 2015. Antimicrob Agents Chemother. 2021;65(4):e02513–20.

7. Crooks GE, Hon G, Chandonia JM, Brenner SE. WebLogo: a sequence logo generator. Genome Res. 2004;14:1188–90.

8. Fürnkranz U, Walochnik J, Henrich B. *Mycoplasma hominis* shows strain-dependent increase in resistance to selected antibiotics after symbiosis with *Trichomonas vaginalis*. J Glob Antimicrob Resist. 2018;14:169–75.

9. Fernández Guerrero ML, Manuel Ramos J, Soriano F. *Mycoplasma hominis* bacteraemia not associated with genital infections. J Infect. 1999;39:91–4.

10. Gruson D, Pereyre S, Renaudin H, Charron A, Bébéar C, Bébéar CM. In vitro development of resistance to six and four fluoroquinolones in *Mycoplasma pneumoniae* and *Mycoplasma hominis*, respectively. Antimicrob Agents Chemother. 2005;49:1190–3.

11. Henao-Martínez AF, Young H, Nardi-Korver JJ, Burman W. *Mycoplasma hominis* brain abscess presenting after a head trauma: a case report. J Med Case Rep. 2012;6:253.

12. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control. 2008;36:309–32.

13. Jang YS, Min JW, Kim YS. Positive culture rate and antimicrobial susceptibilities of *Mycoplasma hominis* and *Ureaplasma urealyticum*. Obstet Gynecol Sci. 2019;62:127–33.

14. Kechagia N, Bersimis S, Chatzipanagiotou S. Incidence and antimicrobial susceptibilities of genital mycoplasmas in outpatient women with clinical vaginitis in Athens, Greece. J Antimicrob Chemother. 2008;62:122–5.

15. Krausse R, Schubert S. In-vitro activities of tetracyclines, macrolides, fluoroquinolones and clindamycin against *Mycoplasma hominis* and *Ureaplasma* ssp. isolated in Germany over 20 years. Clin Microbiol Infect. 2010;16:1649–55.

16. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33:1870–4.

17. Leli C, Mencacci A, Bombaci JC, et al. Prevalence and antimicrobial susceptibility of *Ureaplasma urealyticum* and *Mycoplasma hominis* in a population of Italian and immigrant outpatients. Infez Med. 2012;20:82–7.

18. Madoff S, Hooper DC. Nongenitourinary infections caused by *Mycoplasma hominis* in adults. Rev Infect Dis. 1988;10:602–13.

19. Meng DY, Sun CJ, Yu JB, Ma J, Xue WC. Molecular mechanism of fluoroquinolones resistance in *Mycoplasma hominis* clinical isolates. Braz J Microbiol. 2014;45:239–42.

20. Meygret A, Le Roy C, Renaudin H, Bébéar C, Pereyre S. Tetracycline and fluoroquinolone resistance in clinical *Ureaplasma* spp. and *Mycoplasma hominis* isolates in France between 2010 and 2015. J Antimicrob Chemother. 2018;73:2696–703.

21. Nascimento M, Sousa A, Ramirez M, Francisco AP, Carriço JA, Vaz C. PHYLOViZ 2.0: providing scalable data integration and visualization for multiple phylogenetic inference methods. Bioinformatics. 2017;33:128–9.

22. Noska A, Nasr R, Williams DN. Closed trauma, *Mycoplasma hominis* osteomyelitis, and the elusive diagnosis of Good's syndrome. BMJ Case Rep. 2012;2012:bcr2012007056.

23. Ozturk S, Yildiz S, Dursun P, Yener Ilce B, Kaymaz O. Mycoplasma hominis profile in women: culture, kit, molecular diagnosis, antimicrobial resistance, and treatment. Microb Pathog. 2019;135:103635.

24. Pónyai K, Mihalik N, Ostorházi E, et al. Incidence and antibiotic susceptibility of genital mycoplasmas in sexually active individuals in Hungary. Eur J Clin Microbiol Infect Dis. 2013;32:1423–6.

25. Petti CA, Bosshard PP, Brandt ME, Clarridge JE, Procop G. Interpretive criteria for identification of bacteria and fungi by DNA target sequencing; approved guideline. Clinical and Laboratory Standards Institute (CLSI) document MM18-A. Wayne: CLSI; 2008.

26. Plummer EL, Vodstrcil LA, Bodiyabadu K, et al. Are *Mycoplasma hominis*, *Ureaplasma urealyticum* and *Ureaplasma parvum* associated with specific genital symptoms and clinical signs in nonpregnant women? Clin Infect Dis. 2021;73:659–68.

27. Schneider SC, Tinguely R, Droz S, et al. Antibiotic susceptibility and sequence type distribution of *Ureaplasma* species isolated from genital samples in Switzerland. Antimicrob Agents Chemother. 2015;59:6026–31.

28. Singer M, Deutschman CS, Seymour CW, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). JAMA. 2016;315:801–10.

29. Smith BJ, Crowe A, Comahig J, Sheorey H, Stanley P. A 27-year-old woman with an unusual cause of periprosthetic joint infection. Clin Infect Dis. 2016;62:1463.

30. Song J, Qiao Y, Kong Y, et al. Frequent topoisomerase IV mutations associated with fluoroquinolone resistance in *Ureaplasma* species. J Med Microbiol. 2015;64:1315–20.

31. Taylor-Robinson D. The role of mycoplasmas in pregnancy outcome. Best Pract Res Clin Obstet Gynaecol. 2007;21:425–38.

32. Taylor-Robinson D, Lamont RF. Mycoplasmas in pregnancy. BJOG. 2011;118:164–74.

33. Waites KB, Bade DJ, Bébéar C, et al. Methods for antimicrobial susceptibility testing for human mycoplasmas; approved guideline. CLSI standards: guidelines for health care excellence. Report No. M43-A. Wayne: Clinical and Laboratory Standards Institute; 2011.

34. Waites KB, Katz B, Schelonka RL. Mycoplasmas and ureaplasmas as neonatal pathogens. Clin Microbiol Rev. 2005;18:757–89.

35. Wang QY, Li RH, Zheng LQ, Shang XH. Prevalence and antimicrobial susceptibility of *Ureaplasma*

urealyticum and *Mycoplasma hominis* in female outpatients, 2009–2013. J Microbiol Immunol Infect. 2016;49:359–62.

36. Yang T, Pan L, Wu N, et al. Antimicrobial resistance in clinical *Ureaplasma* spp. and *Mycoplasma hominis* and structural mechanisms underlying quinolone resistance. Antimicrob Agents Chemother. 2020;64(6):e02560–19.

37. Zeng XY, Xin N, Tong XN, Wang JY, Liu ZW. Prevalence and antibiotic susceptibility of *Ureaplasma urealyticum* and *Mycoplasma hominis* in Xi'an, China. Eur J Clin Microbiol Infect Dis. 2016;35:1941–7.

38. Zhang H, Zheng L, Zhao J, Ding S, Xia Y. Investigation of fluoroquinolone resistance mechanism in *Mycoplasma hominis* isolated from urogenital samples in a Chinese hospital. J Med Microbiol. 2019;68:206–10.

39. Zhang W, Li L, Zhang X, Fang H, Chen H, Rong C. Infection prevalence and antibiotic resistance levels in *Ureaplasma urealyticum* and *Mycoplasma hominis* in gynecological outpatients of a tertiary hospital in China from 2015 to 2018. Can J Infect Dis Med Microbiol. 2021;2021:8842267.

Publisher's Note