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Clinical and Microbiological Features of Postoperative Extragenitourinary Mycoplasma hominis Infections: A Retrospective Analysis of Three Cases

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Case Report

Keywords: Mycoplasma hominis, extragenital infection, surgical operation, MALDI-TOF MS, 16S rRNA gene sequencing

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Clinical and Microbiological Features of Postoperative Extragenitourinary Mycoplasma hominis Infections: A Retrospective Analysis of Three Cases

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Abstract

Objective: To characterize the clinical and microbiological features of postoperative extragenitourinary Mycoplasma hominis (M. hominis) infections and to provide a reference for optimizing diagnostic and therapeutic strategies.

Methods: A retrospective analysis was performed on three cases of postoperative M. hominis infection diagnosed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) at our hospital between December 2023 and May 2025. Clinical characteristics, microbiological findings, and diagnostic and therapeutic data were collected and analyzed. The isolates were further confirmed by 16S rRNA gene sequencing.

Results: All three cases of M. hominis infection occurred at surgical incision sites. The

predominant clinical manifestations were delayed wound healing accompanied by persistent purulent discharge. No characteristic bacterial morphology was observed by Gram staining. After 48 h of incubation on Columbia blood agar, small, pinpoint, translucent colonies were observed. All three isolates were identified as M. hominis by MALDI-TOF MS and confirmed by 16S rRNA gene sequencing.

Conclusion: Postoperative M. hominis infection lacks specific clinical manifestations and is easily missed by conventional bacteriological examination. The combination of MALDI-TOF MS and 16S rRNA gene sequencing enables rapid and accurate identification of M. hominis. M. hominis infection should be considered in patients with persistently non-healing wounds, negative results on conventional bacterial culture, and poor response to empirical antibiotic therapy.

Keywords: Mycoplasma hominis; extragenital infection; surgical operation; MALDI-TOF MS; 16S rRNA gene sequencing

Introduction

Mycoplasma hominis (M. hominis) belongs to the class Mollicutes and is a species of the genus Mycoplasma. M. hominis is surrounded by a cell membrane and lacks a cell wall. In humans, M. hominis primarily colonizes the mucosal surfaces of the respiratory and urogenital tracts and rarely penetrates the submucosal layer. However, following immunesuppression or invasive procedures, M. hominis colonizing the urogenital tract may proliferate extensively and breach local barriers, resulting in ectopic dissemination and subsequent invasion of tissues or organs outside the urogenital tract, thereby causing extragenital infection. In recent years, with increasing clinical recognition of the pathogenic potential of M. hominis and its associated infections, extragenital M. hominis infections have gradually attracted greater attention. In this

study, we systematically analyzed the clinical manifestations, laboratory findings, and microbiological diagnostic characteristics of three cases of extragenital M. hominis infection, with the aim of improving clinicians' awareness of this uncommon infection and providing a reference for its early and accurate diagnosis and appropriate therapeutic management.

1 Materials and Methods

1.1 General Data

Three patients with M. hominis-caused surgical site infections (SSIs) after surgical procedures were included from our hospital between December 2023 and May 2025.

1.2 Methods

1.2.1 Medical-record data collection

(1) General clinical data: sex, age, surgical procedure, infection characteristics, antimicrobial usage, infection site, and related information.

(2) Laboratory examinations: bacterial isolation and culture, Gram-stained smear microscopy, and species identification.

1.2.2 Reagents and Instruments

Bacterial culture was performed using Columbia blood agar and rose-bengal China-blue lactose agar plates (Thermo Fisher Scientific, USA). Gram staining and Wright–Giemsa staining were performed using reagents from Zhuhai Baso Biotechnology Co., Ltd. The automated Gram-staining instrument used was the PREVI Color Gram automated slide stainer (bioMérieux, France). Species identification was performed using the VITEK MS fully automated rapid microbial mass-spectrometry system (bioMérieux, France).

1.2.3 Isolation and Culture of Clinical Strains

Clinical specimens were routinely inoculated onto Columbia blood agar and China-blue rose-bengal agar plates and incubated at 35 °C in 5% CO_2. Plates were inspected daily for bacterial growth. After 48 h, pinpoint-sized colonies were Gram-stained and Wright–Giemsa-stained; colonial morphology was examined microscopically, followed by purification and subculturing.

1.2.4 Species Identification

(1) MALDI-TOF MS: VITEK MS (bioMérieux, France) was used. A single colony from the Columbia blood-agar plate was applied onto the target slide, overlaid with 1 μL CHCA matrix, air-dried, and analysed. The acquired spectrum was matched to the built-in library using statistical clustering to generate the identification result.

(2) 16S rRNA Gene Sequencing: Universal primers 27F-sgF (5'-AGAGTTTGATCATGGCTC-3') and 1492R-sgR (5'-TAGGGTTACCTTGTTACGACTT-3') were used for Polymerase Chain Reaction (PCR) amplification of the 16S rRNA gene. Amplicons were sequenced using the Sanger method by Beijing Ruibo Xingke Biotechnology Co., Ltd.

(3) Phylogenetic analysis: After obtaining the 16S rRNA gene sequences of the three strains to be tested, phylogenetic trees were constructed using MEGA 12 software based on the Neighbor-Joining method, incorporating relevant reference sequences of closely related species downloaded from the National Center for Biotechnology Information (NCBI) database. A bootstrap test with 1000 replicates was performed to evaluate the reliability of each branch node.

2 Results

2.1 Clinical Characteristics

Three patients (2 males, 1 female; age range 34~74 years) were identified. Two patients developed

89 localized SSIs, and one patient presented with intracranial infection following extensive burns
90 complicated by craniocerebral trauma. Detailed clinical features and therapeutic courses are
91 summarized in Table 1. White blood cell (WBC), neutrophil percentage (NEUT%), C-reactive
92 protein (CRP), and procalcitonin (PCT) values were measured on the day of positive specimen
93 collection.

94 Table 1 Clinical characteristics and management of infected patients

Item	Case 1	Case 2	Case 3
Gender	Male	Male	Female
Age (years)	74	34	44
Surgical Procedure	Laparoscopic radical prostatectomy, pelvic lymph node dissection	Intracranial hematoma evacuation + decompressive craniectomy, external ventricular drainage	Lumbosacral posterior spinal fracture reduction with pedicle screw fixation + pelvic closed reduction with external fixator
Infection Features	Abdominopelvic effusion, right anterior lower abdominal wall subcutaneous exudation	Head surgical site wound exudate	Wound edema, pain, swelling, tenderness, minimal needle tract bleeding
Maximum Temperature (℃)	38.9	37.6	36.8
WBC Count ($\times 10^9$/L）	11.86	16.88	10.17
NEUT%	79.8	88.6	70.8

CRP (mg/L)		-	190.6	23.0
PCT (ng/L)		0.415	2.360	0.048
Antimicrobial Use	Pre-adjustment	Cefuroxime sodium	Teicoplanin + ceftazidime-avibactam	Ceftazidime + vancomycin
	Post-adjustment	Cefuroxime sodium	Linezolid + moxifloxacin	Linezolid + levofloxacin
Wound Management		Drainage	Drainage tube	Surgical debridement, vacuum-assisted drainage
Infection Site		Surgical incision	Head surgical site	Pelvic traumatic incision
Identification Method		VITEK MS	VITEK MS	VITEK MS
Prognosis		Discharged without cure	Discharged without cure	Cured

-: not tested

(2) In Case 3, the postoperative wound was erythematous, non-healing, and purulent before antimicrobial therapy (Fig.1A). After levofloxacin treatment, the wound healed completely, with no residual erythema or swelling (Fig. 1B).

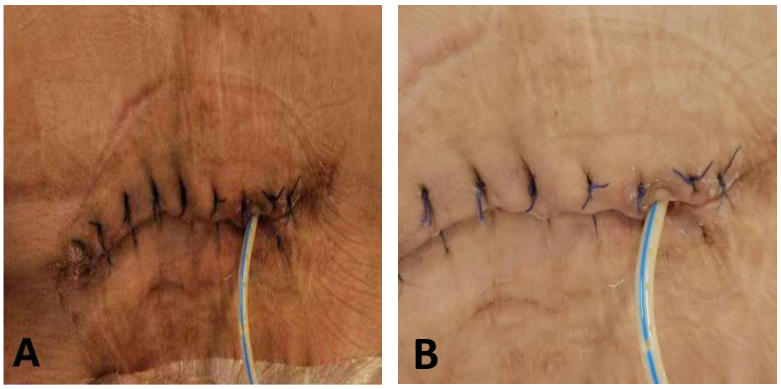


Fig. 1A and B: Wound status before and after levofloxacin treatment

2.2 Bacterial Isolation, Culture, and Microscopic Examination

Two specimens consisted of postoperative wound exudates, and one specimen was cerebrospinal fluid (CSF). All samples appeared pale-yellow or blood-tinged. Direct wet mounts revealed abundant red blood cells and WBC, but Gram staining did not detect bacteria in any of the three samples (Table 2). After incubation for 48 h at 35 °C in 5% CO_2, pinpoint, transparent, or semi-transparent colonies were observed on Columbia blood-agar plates. Wright–Giemsa staining of these colonies did not reveal typical bacterial morphology; only granular aggregates appeared. Gram staining again failed to detect individual bacterial cells (Fig. 2).

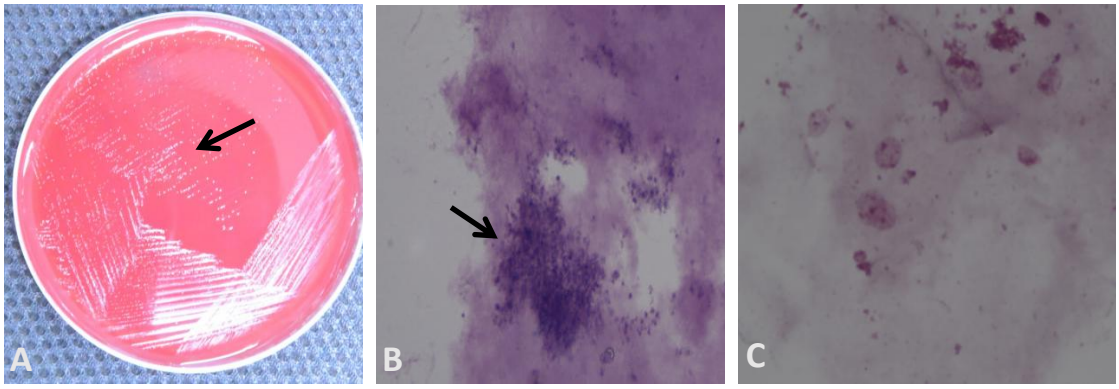


Fig. 2 **(A)** Colony morphology of M. hominis on Columbia blood-agar plate; **(B)** Wright–Giemsa stain of M. hominis colonies (×1000); **(C)** Gram stain of M. hominis colonies (×1000)

Table 2 Microbiological findings of infected patients

Case	Specimen Type	Specimen Characteristics	Cytological Examination	Gram Stain	Culture Result
1	Drainage fluid	Pale yellow	Leukocytes +++、Erythrocytes +	No bacteria detected	M. hominis
2	Cerebrospinal fluid	Red	Leukocytes +++、Erythrocytes +++	No bacteria detected	M. hominis
3	Secretions	Light red	Leukocytes ++、	No bacteria	M. hominis

		Erythrocytes +	detected

2.3 Identification of M. hominis

(1) MALDI-TOF MS results: Target colonies from Columbia blood-agar plates were identified as M. hominis by VITEK MS. Protein mass peaks and confidence values are listed in Table 3. Representative mass spectra of the three isolates are shown in Fig. 3.

Table 3 Major protein peaks and confidence scores of M. hominis

Case	1	2	3
Peak 1 (Da)	3478.31	3478.40	3478.31
Peak 2 (Da)	4341.14	4369.29	4369.29
Peak 3 (Da)	5878.83	5878.95	5879.06
Peak 4 (Da)	6534.22	6534.10	6663.63
Peak 5 (Da)	6960.07	6960.20	6960.32
Peak 6 (Da)	7588.28	7587.89	7588.15
Peak 7 (Da)	9263.08	-	9263.23
Confidence (%)	99.9	99.9	99.9

-: no protein peak signal obtained

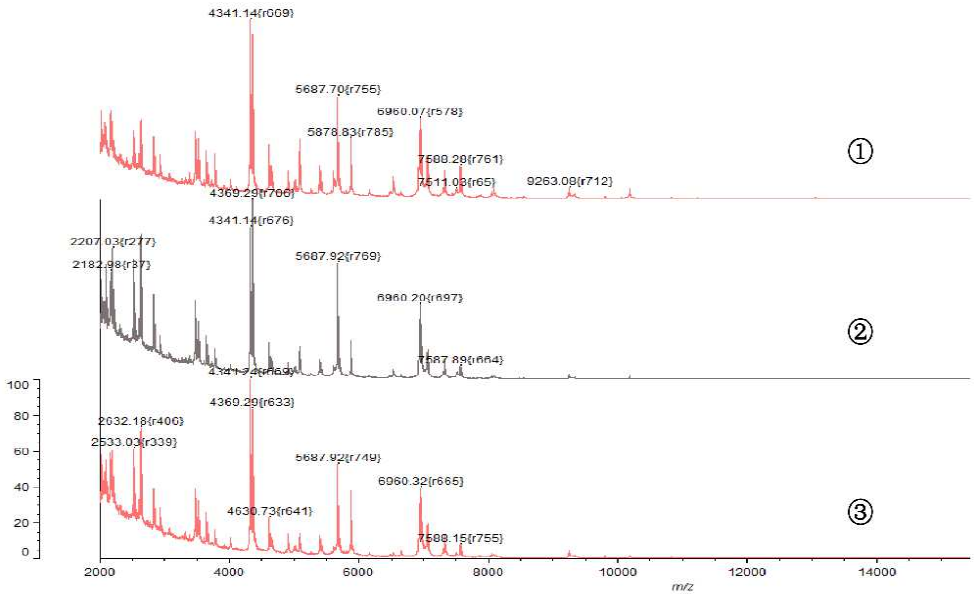


Fig.3 Relative molecular mass spectra from VITEK MS Identification of 3 M. hominis Strains

Note: ①②③ represent the mass spectrometry identification spectra of Cases 1, 2, and 3, respectively.

(2) Sequencing results: The target strains showed a single clear target band upon gene amplification.The target strain amplified a single clear target band after gene amplification. The sequencing results compared by Basic Local Alignment Search Tool showed that the sequence similarity between cases 1, 2, and 3 and M. hominis was 100%, 100%, and 99.856%, respectively.

(3) The phylogenetic tree shows that Sample1, Sample2, and Sample3 are phylogenetically closely related and cluster in the same branch as Metamycoplasma hominis strain NBRC 14850, with a bootstrap value of 100%. Among them, Sample2 and Sample3 exhibit the closest genetic relationship, while Sample1 forms a highly supported monophyletic group with both. These results suggest that the three samples under testing have a close phylogenetic relationship with M. hominis, providing phylogenetic evidence for their taxonomic identification. A phylogenetic tree based on 16S rRNA sequences was constructed (Fig. 4).

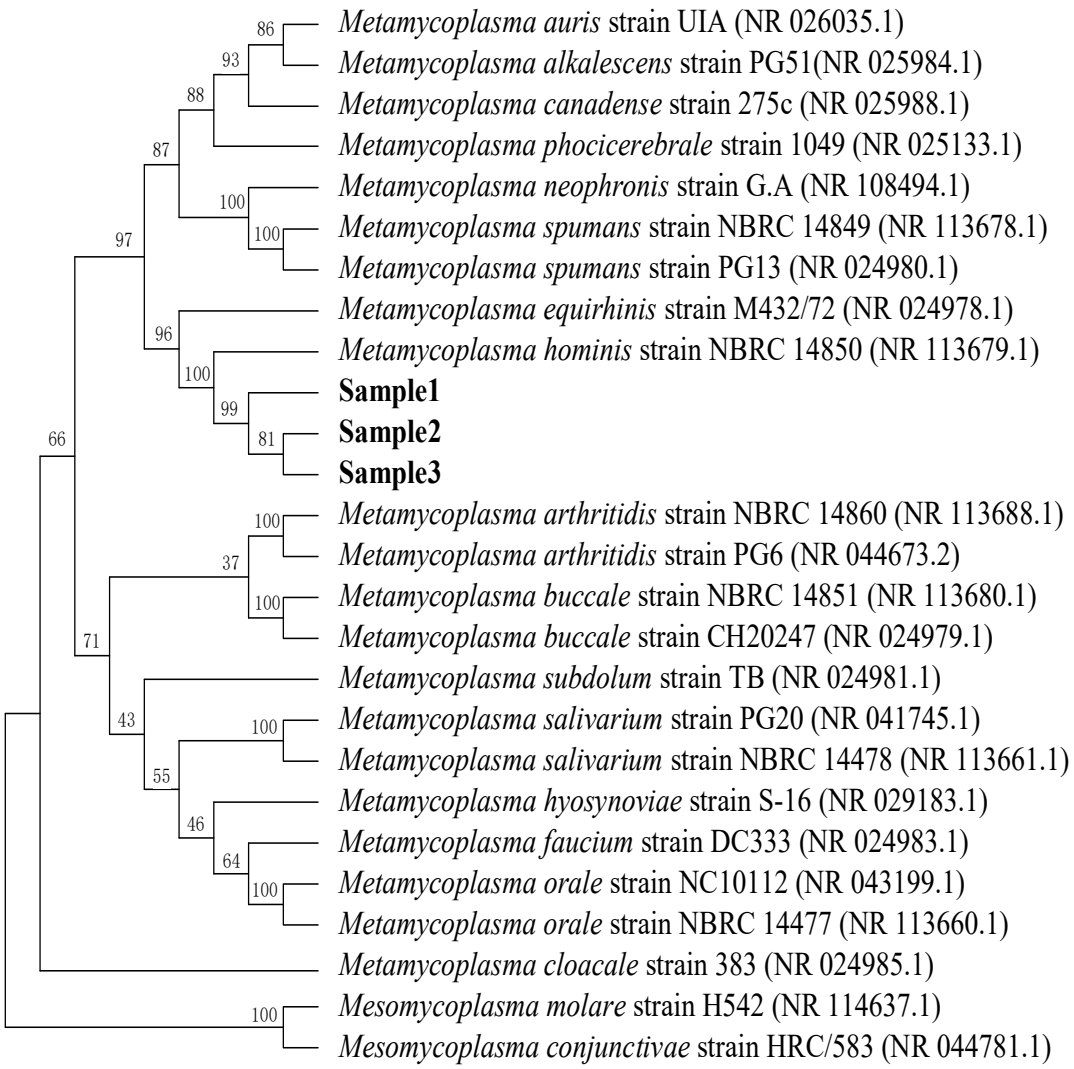


Fig. 4 Phylogenetic tree based on 16S rRNA gene sequences.

Note: Sample1, Sample2, and Sample3 represent Cases 1, 2, and 3, respectively.

3 Discussion

M. hominis primarily colonizes the urinary and respiratory tracts. The colonization rate in the female urogenital tract is 21%–54%, whereas it is relatively lower in males, at 4%–13%. Among healthy adults, the colonization rate of M. hominis in the upper respiratory tract is approximately 1%–3%; it is about 8% in patients with chronic respiratory diseases and can be as high as 30% in patients with chronic tonsillitis[1]. Extragenital M. hominis infections are commonly seen in suppurative arthritis, trauma, prosthetic joint infections after joint replacement surgery, and

139 immunosuppressive therapy. In addition, M. hominis has been reported in central nervous system
140 (CNS) infections, including brain abscesses and meningitis following head trauma or neurosurgery,
141 infective endocarditis after prosthetic valve replacement, and abscess formation after
142 transplantation or surgical procedures[2-5] . Madoff S et al. [6] classified these extra-genitourinary
143 infections into five major categories: septicemia, CNS infections, respiratory tract infections, joint
144 infections, and wound infections, and noted that most patients with M. hominis infection have
145 impaired immune function or severe disruption of anatomical barriers.

146 M. hominis is the only Mycoplasma species that grows on conventional blood agar media.
147 After approximately 48 hours of culture, smooth, pinhead-sized colonies can usually be observed,
148 displaying a characteristic “fried-egg” appearance under low-power microscopy. M. hominis has
149 stringent nutritional requirements, grows slowly, and lacks a cell wall, making it difficult to
150 identify by Gram staining and easily missed. Laboratory testing commonly uses the arginine
151 hydrolysis test to identify Mycoplasma. During growth, M. hominis breaks down arginine to
152 produce ammonia, increasing the pH of the culture medium. Whether M. hominis is growing is
153 determined by observing if the phenol red indicator changes from yellow to red. However, liquid
154 culture methods have methodological limitations in practical application and may yield
155 false-negative or false-positive results. Therefore, combining solid culture methods for cultivation
156 and identification can help improve the accuracy of M. hominis detection and identification[7-9].

157 MALDI-TOF MS is currently a routine method for species identification in clinical
158 microbiology, with the advantages of simple operation, rapid detection, and low cost. In addition,
159 it can also achieve good results in the identification of mycoplasma[10-12]. All three M. hominis
160 isolates in this study were rapidly identified using MALDI-TOF MS and f0.211urther validated by

16S rRNA gene sequencing. The two methods yielded concordant identification results, indicating that MALDI-TOF MS provides high accuracy for the identification of Mycoplasma species.

However, the MALDI-TOF MS technology relies on the completeness of its database to a certain extent. If the mass spectrometry database does not have the pre-stored target strain spectrum, the identification may fail. Therefore, the laboratory should update the database and introduce standard spectra in time. Mass spectrometry has certain advantages in rapid detection and high-throughput analysis, but its sensitivity and specificity need to be further optimized and improved. Traditional nucleic acid detection of M. hominis mainly uses 16S rRNA as the gene target. At present, studies have developed other PCR detection methods using different targets such as gap, fstY and yidC[9,13]. Combining molecular biological methods such as PCR with MALDI-TOF MS technology can significantly improve the detection rate of Mycoplasma infection.

M. hominis mainly colonizes the genitourinary tract, and the pathogen is mostly infected backward through the genitourinary tract. Especially when surgery or other invasive procedures are performed in adjacent areas of the genitourinary tract, the local tissue barrier and normal defense mechanisms may be destroyed, thereby increasing the risk of colonized bacteria translocating to adjacent tissues or other parts and causing infection. Based on the clinical data of the three patients with M. hominis infection in this study and an analysis of relevant literature, the possible routes of infection mainly include the following two: (1) Surgery-associated infection: M. hominis colonized in the genitourinary tract translocates and causes adjacent tissue infection when the body is subject to trauma or invasive procedures[6]; (2) Post-head trauma infection: Head trauma, especially open craniocerebral injury, can destroy the body's normal tissue barrier, making

it possible for M. hominis colonized in the upper respiratory tract to enter the cranium through the trauma site, thereby causing CNS infection[14]. In the M. hominis infection cases reported by Li et al. [15], the pathogenic bacteria were all isolated from the surgical site, suggesting that the destruction of the surgery-related tissue barrier may be an important trigger for extragenitourinary M. hominis infection. Therefore, combined with the above cases and literature reports, the possibility of M. hominis invading through the surgical incision and causing infection cannot be ruled out in all 3 patients in this study.

M. hominis infection can cause fever and elevated inflammatory markers, including WBC, NEUT%, CRP, and PCT, with CRP elevation being particularly pronounced. In addition, the site of infection often presents with localized redness and swelling, accompanied by inflammatory reactions such as exudation. Qiu et al. [16] demonstrated that CRP levels are closely associated with disease progression and suggested that CRP levels should be continuously monitored throughout the course of post-traumatic infection. In the report by Wang et al. [17], physicians performed only debridement and drainage of the wounds in two patients with M. hominis infection after sternotomy, without administering targeted antimicrobial therapy; consequently, the duration of infection was relatively prolonged. Therefore, when patients develop clinical manifestations such as persistent fever, continuously elevated inflammatory markers, and long-term nonhealing wounds after trauma surgery, and Gram staining and routine bacterial cultures fail to identify the pathogen, clinicians should consider the possibility of M. hominis infection, promptly adjust the treatment regimen, and perform additional targeted tests to identify the causative organism.

M. hominis lacks a cell wall and is intrinsically resistant to β-lactam and glycopeptide antibiotics, which target bacterial cell wall synthesis. However, it retains varying degrees of

susceptibility to antimicrobial agents with other mechanisms of action, including fluoroquinolones and macrolides. Previous studies[18,19] have reported that M. hominis exhibits relatively high susceptibility to doxycycline, josamycin, and minocycline, whereas resistance rates to azithromycin, clarithromycin, and ciprofloxacin are relatively high. In recent years, with the increasing inappropriate and excessive use of antimicrobial agents, the resistance rate of M. hominis to fluoroquinolones has shown an upward trend[20-22]. Zeng et al. [23] reported that the increasing fluoroquinolone resistance in M. hominis may be associated with alterations at S91 of the parC gene and S153 of the gyrA gene in Mycoplasma strains.At present, no standardized and universally accepted treatment regimen has been established for M. hominis infection. In clinical practice, treatment strategies for Mycoplasma genitalium infection may serve as a reference, with regimens such as doxycycline followed by sitafloxacin or doxycycline followed by moxifloxacin selected according to the patient's clinical condition and antimicrobial susceptibility profile[7].

In conclusion, M. hominis can cause postoperative SSIs and is easily missed by conventional laboratory diagnostic methods owing to its distinctive growth characteristics and lack of a cell wall. MALDI-TOF MS can serve as a reliable method for the rapid identification of M. hominis in clinical practice and, when necessary, can be combined with 16S rRNA gene-targeted sequencing for further confirmation. In patients with SSIs, M. hominis infection should be suspected when conventional bacterial cultures yield negative results and empirical antimicrobial therapy is ineffective. In such cases, efforts should be made to obtain microbiological evidence promptly and to adjust the antimicrobial treatment regimen in a timely manner.

Declarations

Abbreviations

M. hominis	Mycoplasma hominis
MALDI-TOF MS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
SSIs	Surgical site infections
PCR	Polymerase Chain Reaction
NCBI	National Center for Biotechnology Information
WBC	White blood cell
NEUT%	neutrophil percentage
CRP	C-reactive protein
PCT	Procalcitonin
CSF	Cerebrospinal fluid
CNS	Central nervous system

Ethics approval and consent to participate

This retrospective study was performed in line with the principles of the Declaration of Helsinki. The study was approved by the Ethics Committee of the Fourth Medical Center of Chinese PLA General Hospital (approval number: 2025KY198-KS001). The requirement for patient consent to participate in this study was waived because of the study was retrospective.

Consent for publication

Patients have written provided informed consent for the case details and images to be published.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare no conflicts of interest in this work.

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Authors' contributions

XYL conceived and designed the study, acquired funding, provided supervision, and critically revised the manuscript for important intellectual content. HXW curated the data and drafted the manuscript. SY, HLJ, and WY performed the microbiological identification and phylogenetic analysis. LSZ, LX, and ZLH performed the laboratory experiments and collected clinical data. ZK and WHB contributed to the interpretation of results. All authors read and approved the final manuscript and agreed to be accountable for all aspects of the work.

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