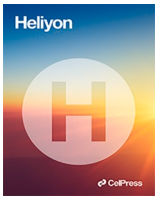
Heliyon 10 (2024) e28730

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Case report

# Osteomyelitis of the femur caused by Metamycoplasma orale in an immunocompromised patient using metagenomic next-generation sequencing: A case report

Hanxiao Zhu [a,1], Jingzhi Zhu [b,c,1], YiFei Wang [d,1], Xiaotong Xi [b,c], Keyi Wang [a], Yongkang Wang [a], Ran Ding [b,c,*], Hang Li [a,**]

[a] The Second Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou, China
[b] The State Key Laboratory of Neurology and Oncology Drug Development, Nanjing, China
[c] Nanjing Simcere Medical Laboratory Science Co., Ltd, Nanjing, China
[d] The First Hospital of Hunan University of Chinese Medicine, Changsha, China

ARTICLE INFO

*Keywords:*
*Metamycoplasma orale*
Metagenomic next generation sequencing
Osteomyelitis
Immunocompromise
Case report

ABSTRACT

*Background: Metamycoplasma orale (M.orale),* a symbiotic bacterium observed in the human oral cavity, is generally regarded as non-pathogenic to humans. Although infrequent, symptomatic infections caused by *M.orale* may occur in individuals with compromised humoral immunity. Accurate identification and early diagnosis of *M.orale* still present significant challenges due the limitations associated with conventional detection methods. Although metagenomic next-generation sequencing (mNGS) is currently widely utilized in clinical practices and exhibits a remarkable specificity and sensitivity for detecting various pathogens, its application in the diagnosis of *M.orale*-induced osteomyelitis remains largely unexplored.

*Case description:* In this report, we present a case study of osteonecrosis caused by *M.orale* in a 20-year-old female patient with nephrotic syndrome and other comorbidities. She was administered long-term hormone therapy and immunosuppressants, leading to her admission to the hospital due to recurrent fever, hip abscess and left thigh pain. Imaging examination revealed bilateral mid-femoral lesions, with the extensive nature of the left femoral lesion suggesting a potential secondary infection. Although no pathogen was detected in pus culture, mNGS analysis identified *M.orale* in the sample. Following treatment with doxycycline and levofloxacin, the patient's symotoms improved and she was discharged with favorable outcomes.

*Conclusion:* mNGS enables rapid identification of etiology in patients with osteomyelitis caused by the rare pathogen *M.orale*. This case accentuate the strength of mNGS for early detection and targeted clinical treatment of infectious diseases caused by uncommon pathogens.

## 1. Introduction

*Metamycoplasma orale (M.orale)* is an infrequent etiology of invasive infection in immunocompromised hosts, which characterized

* Corresponding author. The State Key Laboratory of Neurology and Oncology Drug Development, Nanjing, China.
** Corresponding author.
*E-mail addresses:* ran.ding@simceredx.com (R. Ding), hanglihz@zju.edu.cn (H. Li).
[1] Equal contributions.

https://doi.org/10.1016/j.heliyon.2024.e28730
Received 24 November 2023; Received in revised form 13 March 2024; Accepted 22 March 2024
Available online 27 March 2024

by clinical manifestations such as pyrexia, cachexia, arthralgia, or abscess formation. The diagnosis of *M.orale* is impeded by the specific requirements of specialized culture media and prolonged incubation, which are not commonly found in conventional microbiology laboratories (incubations lasting longer than 5 days) [1]. Consequently, the conventional approaches fall short in meeting the clinical diagnostic requirements for pathogenic infections, both in terms of accuracy and promptness.

In the past few years, metagenomic next-generation sequencing (mNGS) has gained increasing application in the field of clinical microbiology [2,3]. This culture-independent and unbiased pathogen detection tool offers distinct advantages for identifying pathogenic microbes that cannot be isolated using conventional microbial culture methods. Herein, we present a case study of osteomyelitis caused by *M.orale* in a young and immunocompromised woman, which was promptly diagnosed the utilization of through metagenomic nextgeneration sequencing (mNGS).

## 2. Case presentation

The patient, a 20-year-old woman, presented with symptoms of edema and oliguria since May 2005 following vaccination. Subsequently, she was diagnosed with nephrotic syndrome and treated with hormones until 2013. She administered biologics and cyclophosphamide until achieving stabilization of her nephrotic condition in September 2015. By the end of 2016, she voluntarily discontinued hormone usage. In July 2020, she was diagnosed with hemophagocytic syndrome, agranulocytosis, sepsis, duodenal perforation, severe anemia, and hypogammaglobulinemia. The patient presented with recurrent hyperpyrexia persisting for 3 years with a maximum temperature of 40 °C, and bilateral thigh pain lasting over 1 year. There was no history of trauma, and despite undergoing anti-infective therapy at other medical facilities, there was no significant alleviation of symptoms. On February 8, 2023, the patient sought further treatment at the outpatient department of the Second Affiliated Hospital Zhejiang University School of Medicine. The examination revealed localized swelling in the left thigh, while magnetic resonance imaging (MRI) confirmed bilateral femoral infarction with infection. Surgical treatment was recommended and the patient was admitted for management of acquired

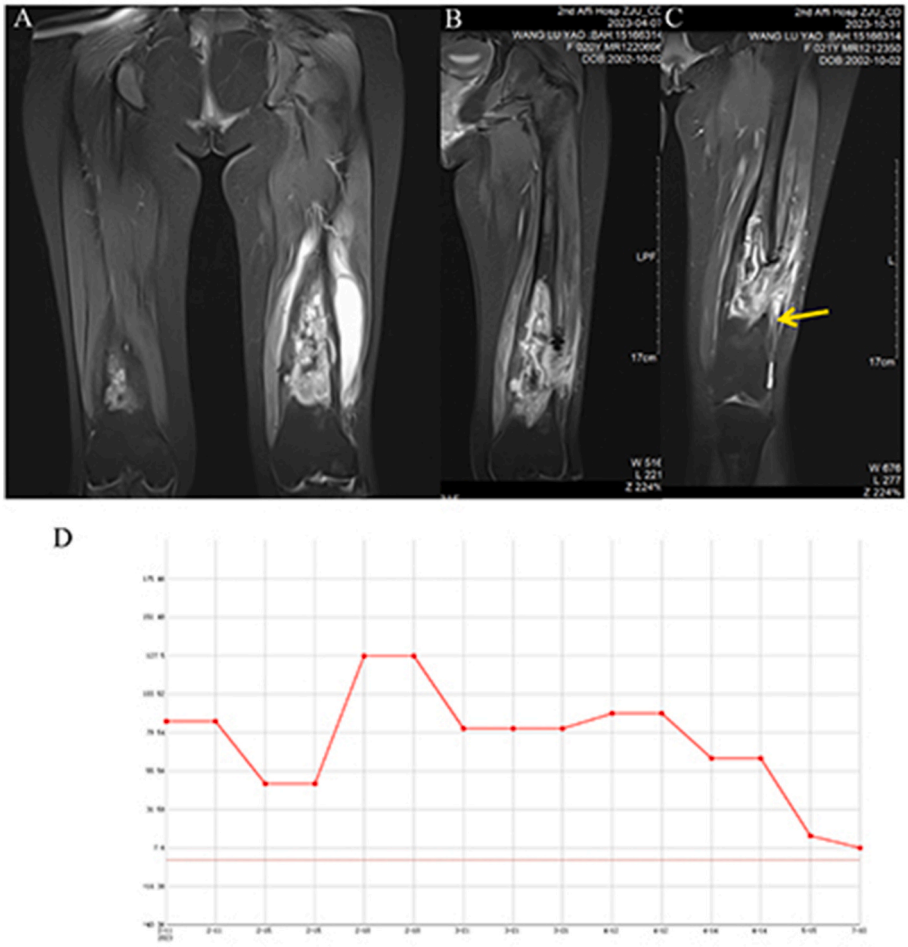



**Fig. 1.** The findings from the in-hospital examination. (A) Magnetic resonance imaging (MRI) of the patient before surgery. (B) MRI examination at 2 months post surgery. (C) MRI examination at 8 months post surgery. The arrow points to the lateral thigh muscle flap that fills the medullary cavity. (D) CRP data of the patient during follow-up in hospital.

femoral deformity.

After admission, a comprehensive physical examination was conducted, revealing a body temperature of 37.2 °C and pulse rate of 100 beats/min. Upon palpation of her thighs, she experienced tenderness. Her left thigh exhibited swelling, while the other limbs appeared normal. The routine blood test indicated that the levels of erythrocyte sedimentation rate, C-reactive protein, white blood cell count, and neutrophil percentage of the patient were high, while the eosinophil percentage remained within normal range. The MRI demonstrated bilateral mid-femur lesions, with a prominent lesion observed in the left femur (Fig. 1A). Considering the possibility of secondary infection, a diagnosis of infectious osteomyelitis was established based on clinical symptoms and imaging findings. Expirical anti-infection treatment with clindamycin was initiated.

To identify the causative pathogen, the pus sample was punctured and subjected for mNGS examination for unbiased pathogen detection on February 10th. DNA from the sample was isolated using Tiangen extraction kit and generated libraries with the Hieff NGS®OnePotTM II DNA kit, following instructions for end repair, adapter ligation, and PCR amplification. The libraries were sequenced on a NextSeq 550 platform with single-end reads of 75bp. After filtering low-quality, low-complexity, and short sequences as well as removing human reference genome sequences, high-quality sequencing data was compared to microbial genome databases and performed advanced data analysis. The identified *Aspergillus flavus* (17 specific reads) and *Metamycoplasma orale* (24,788 specific reads) in mNGS hold significant importance on February 11th [Fig. 2(A-B)]. Considering the patient's symptoms and other laboratory tests, clinical exclusion of *A. flavus* infection occurred due to its wide environmental presence which could have been introduced from external sources. Simultaneously, the pus culture results remained inconclusive. We employed a specific PCR analysis to further verify the accuracy of *M. orale* using the same pus sample, wherein primers were designed to target the specific region of *M. orale*: forward primer-5′-GACATACTTGCCGCTTTCTAT-3′ and reverse primer-5′-GTCTCTGTTGCACTAGTCGT-3'. Positive verification results were obtained (Fig. 3).

Based on the mNGS results and symptoms, the patient was diagnosed with *M. orale*-induced osteomyelitis, leading to a change in antibiotic therapy to levofloxacin. Following thorough examination and exclusion of surgical contraindications, the patient underwent left femoral lesion resection on February 17, 2023. After 6 days of anti-infection treatment with doxycycline and levofloxacin, the patient's body temperature returned to normal and the symptoms of the patient improved significantly, resulting in discharge from the hospital. Subsequent follow-up indicated a decrease in CRP levels (Fig. 1D) and improved condition of the left femur (Fig. 1B–C) following treatment with levofloxacin (1#qd) and doxycycline (100mg bid). The entire treatment process is summarized in Fig. 4. No

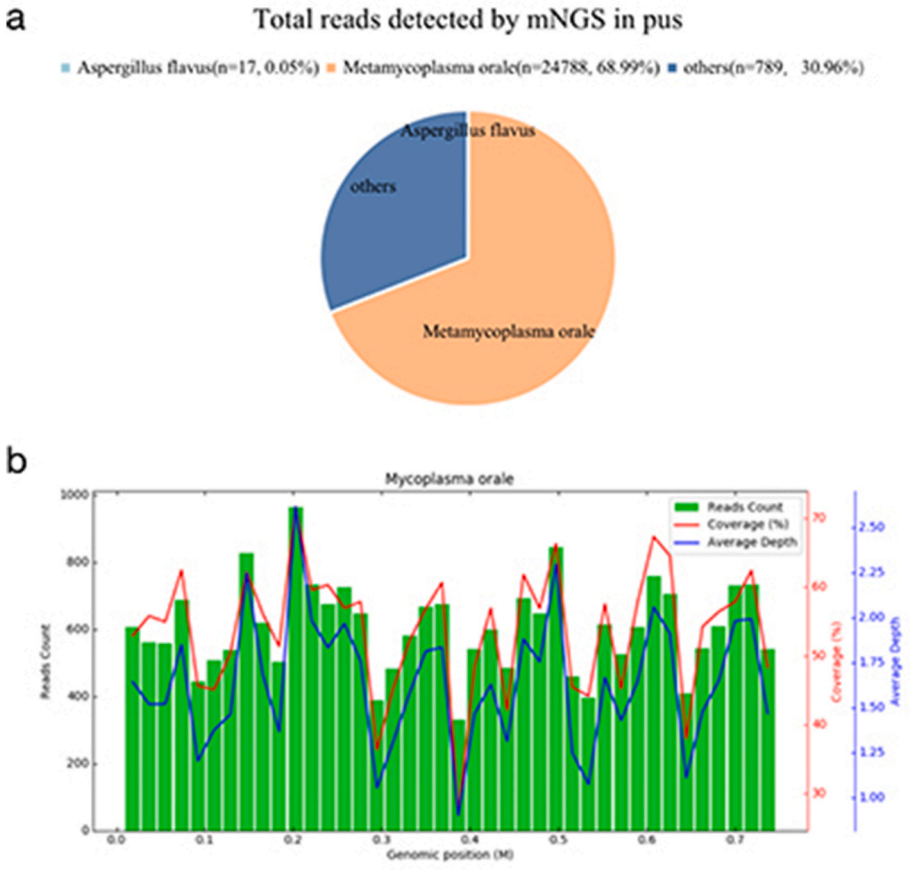



**Fig. 2.** MNGS results. (A) *M.orale* detceted in pus by mNGS. (B) Coverage picture of *M.orale.* The genome coverage of *M.orale* is 53.835%.

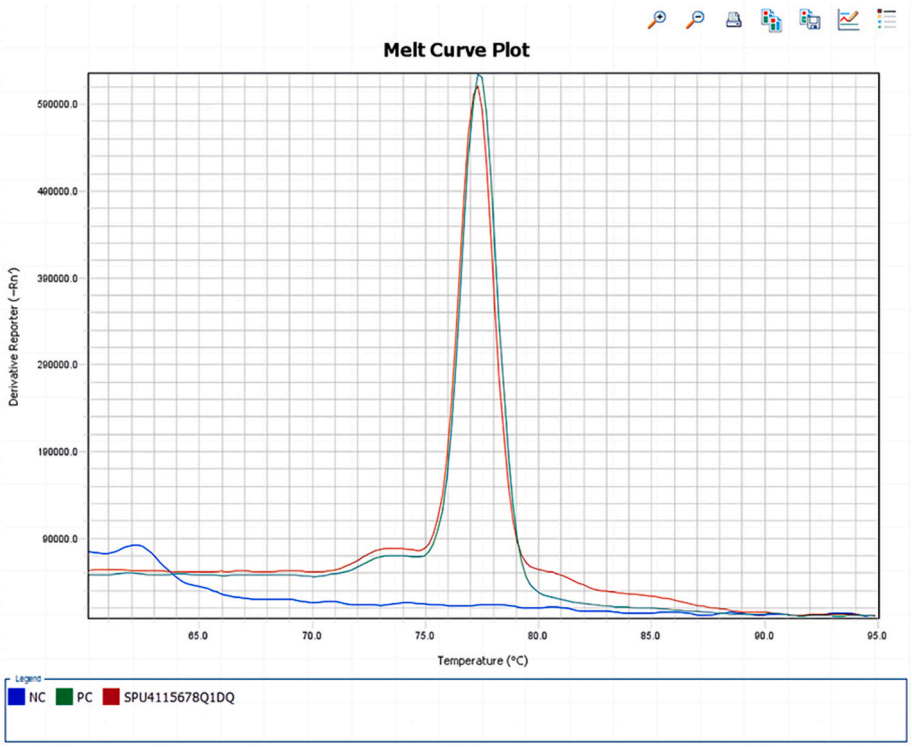



**Fig. 3.** QPCR verification results of *M.orale.* blue line: negative control; green line: positive control; red line: the pus sample of the patient.

adverse and unanticipated events occurred.

## 3. Discussion

*Metamycoplasma* is a class of minimal prokaryotic organisms lacking cell wall structure, occupying an intermediate position between independent life and intracellular parasitic life [4]. Over 20 species of *Metamycoplasma* have been identified in humans, with *M. pneumoniae*, *M. genitalium*, *M. hominis* and *M. urealyticum* being the primary species conventionally associated with the process of infection [5]. They can induce diseases in humans and animals, primarily through droplet transmission, sexual contact transmission, and mother-to-child transmission, resulting in respiratory tract infections, genitourinary tract infections, perinatal infections and neonatal suppurative infections [6–8], such as pneumonia and encephalitis. *M.orale* is typically a commensal bacterium residing in the human oral cavity and is generally not regarded as pathogenic. However, in 1974, L.G. Tallgren successfully isolated *M.orale* from the bone marrow of a patient with eosinophilic leukemia and postulated its potential etiological role in the disease [9]. In 2002, Michelle Paessle cultured an anaerobic left joint abscess obtained from a male patient with chronic variable immunodeficiency and conducted comparative analysis using 16S rRNA PCR, to highlight the pivotal role of molecular technology in diagnosing challenging infectious disease [10]. In 2020, Jeffrey Ketchersid reported that *M.orale* was implicated in the development of multiple abscesses among patients with secondary hypogammaglobulinemia, thereby emphasizing the significance of *Metamycoplasma* infection as a potential etiology in

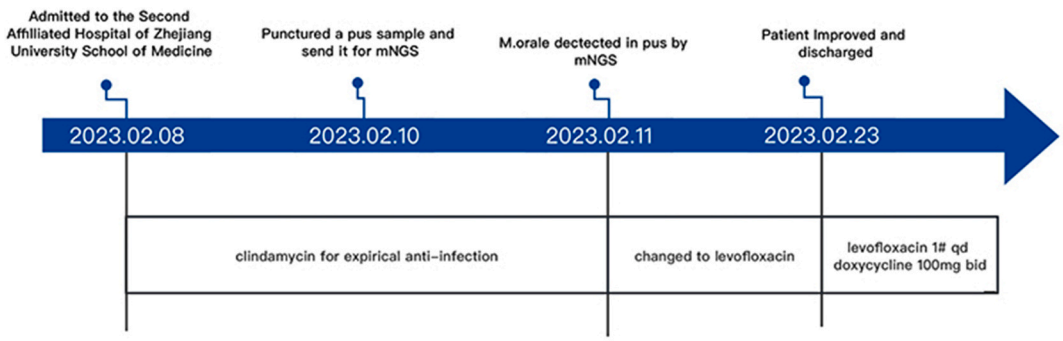



**Fig. 4.** Patient progression timeline.

cases presenting with culture-negative abscesses and septic arthritis [11]. In addition, Aaron C Liu MSc documented a case wherein 16S RNA was employed to identify *M. orale* from the synovial fluid of an immunocompromised boy presenting with septic arthritis, splenic lesions, and agammaglobulinemia. These instances exclusively manifested in individuals with underlying medical conditions, thereby signifying a significantly heightened susceptibility to Metamycoplasma infection among immunocompromised patients.

The detection methods of *Metamycoplasma* primarily encompass culture, serological examination, and molecular diagnostic techniques. Clinically, the identification and diagnosis of *M. orale* infection pose challenges due to limitations in traditional culture strategies. One major obstacle lies in absence of a cell wall structure, rendering its observation under a light microscope. Futhermore, conventional gram staining methods have proven ineffective for testing clinical specimens [12]. Serological examinations also encounter difficulties with low positivity rates and limited specificity. The constraints imposed by current detection techniques hinder many patients from fully benefiting from optimal treatment opportunities. Consequently, non-conventional techniques such as 16S rRNA sequencing, PCR, and mNGS play a pivotal role in facilitating prompt identification of *M.orale*. In the treatment of *Metamycoplasma*, empirical antibiotics face challenges due to its lack of a cell wall, ability to change shape easily, sensitivity to osmotic pressure, and resistance to drugs that inhibit cell wall synthesis such as penicillins and cephalosporins [13]. Currently, the commonly employed drugs in clinical practice primarily encompass tetracyclines, macrolides and quinolones [14]. Furthermore, the emergence of *Metamycoplasma* mutations and the escalation of antimicrobial resistance have rendered the prompt diagnosis and treatment of *Metamycoplasma* infection intricate and targeted [15].

In this report, the patient has a history of nephrotic syndrome since childhood, accompanied by underlying conditions such as hemophagocytic syndrome and hypogammaglobulinemia. Prolonged use of corticosteroids has resulted in compromised systemic resistance. The aforementioned case serves to further underscore the significantly heightened susceptibility to *Metamycoplasma* infections among immunocompromised individuals. *Metamycoplasma* colonization in the femoral head leads to non-responsive osteomyelitis due to impaired antibacterial activity. However, due to the lack of continuous imaging data, it is difficult to determine whether there is bone necrosis before the occurrence of osteomyelitis. Our patient exhibited a favorable response to levofloxacin and doxycycline treatment, highlighting the importance of considering *Metamycoplasma* infection in individuals with osteomyelitis when conventional diagnostic tests yield negative results. Furthermore, it is imperative to consider the patient's immune status during treatment, as alterations in their immune response may lead to potential reinfection upon cessation of therapy [11]. Notably, mNGS results were promptly reported within a day following sample delivery, underscoring the significant reduction in detection time achieved by mNGS and its valuable contribution towards early diagnosis. However, mNGS solely indicates the presence of pathogenic nucleic acid fragments in clinical specimens and does not provide information regarding the relationship between the pathogen and infection. Therefore, under appropriate circumstances, it is essential to employ additional methods or infection-related markers for cross-validation.

In conclusion, *M.orale* is an exceptionally rare opportunistic pathogen with limited documented cases of osteomyelitis in immunocompromised patients. The identification of *M.orale* was achieved through mNGS analysis of the pus sample. The aforementioned case underscores the potential of mNGS as a rapid approach for antibiotic treatment, thereby improving patient prognosis.

## Consent for publication

We have obtained written informed consent from the patient for the publication of this case report, any accompanying data and images.

## Ethics declarations

The patient provided informed consent for the publication of his anonymised case details and images.

## Data availability statement

The datasets presented in this study can be found in online repository at https://ngdc.cncb.ac.cn/omix: accession no.OMIX005008.

## CRediT authorship contribution statement

**Hanxiao Zhu:** Writing – review & editing. **Jingzhi Zhu:** Writing – original draft. **YiFei Wang:** Writing – original draft. **Xiaotong Xi:** Writing – original draft. **Keyi Wang:** Formal analysis, Data curation. **Yongkang Wang:** Formal analysis, Data curation. **Ran Ding:** Writing – review & editing. **Hang Li:** Writing – review & editing, Project administration.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## References


[1] A.H. Totten, G. Uzel, P.P. Khil, J.H. Youn, J. Treat, C.D. Soutar, S.M. Holland, J.P. Dekker, C.S. Zerbe, Disseminated mycoplasma orale infection in patients with phosphoinositide-3-kinase regulatory subunit 1 mutations, Open Forum Infect. Dis. 9 (9) (2022) ofac472, https://doi.org/10.1093/ofid/ofac472.

[2] Y. Zheng, X. Qiu, T. Wang, J. Zhang, The diagnostic value of metagenomic next-generation sequencing in lower respiratory tract infection, Front. Cell. Infect. Microbiol. 11 (2021) 694756, https://doi.org/10.3389/fcimb.2021.694756.

[3] Y.Y. Qian, H.Y. Wang, Y. Zhou, H.C. Zhang, Y.M. Zhu, X. Zhou, Y. Ying, P. Cui, H.L. Wu, W.H. Zhang, J.L. Jin, J.W. Ai, Improving pulmonary infection diagnosis with metagenomic next generation sequencing, Front. Cell. Infect. Microbiol. 10 (2021) 567615, https://doi.org/10.3389/fcimb.2020.567615.

[4] A. Uusküla, P.K. Kohl, Genital mycoplasmas, including Mycoplasma genitalium, as sexually transmitted agents, Int. J. STD AIDS 13 (2) (2002) 79–85, https://doi.org/10.1258/0956462021924695.

[5] D. Gómez Rufo, E. García Sánchez, J.E. García Sánchez, M. García Moro, Implicaciones clínicas de las especies del género Mycoplasma [Clinical implications of the genus Mycoplasma], Rev. Española Quimioter. : publicacion oficial de la Sociedad Espanola de Quimioterapia 34 (3) (2021) 169–184, https://doi.org/10.37201/req/014.2021.

[6] P.K. Kutty, S. Jain, T.H. Taylor, A.M. Bramley, M.H. Diaz, K. Ampofo, S.R. Arnold, D.J. Williams, K.M. Edwards, J.A. McCullers, A.T. Pavia, J.M. Winchell, S. J. Schrag, L.A. Hicks, Mycoplasma pneumoniae among children hospitalized with community-acquired pneumonia, Clin. Infect. Dis. : an official publication of the Infectious Diseases Society of America 68 (1) (2019) 5–12, https://doi.org/10.1093/cid/ciy419.

[7] R. Gnanadurai, H. Fifer, Mycoplasma genitalium: a review, Microbiology (Reading, England) 166 (1) (2020) 21–29, https://doi.org/10.1099/mic.0.000830.

[8] P.A. Quinn, Mycoplasma infection of the fetus and newborn, Progress in clinical and biological research 281 (1988) 107–151.

[9] L.G. Tallgren, R. Wegelius, L.C. Andersson, E. Jansson, Eosinophilic leukaemia–recovery of mycoplasma orale from the bone marrow, Acta Med. Scand. 195 (1–2) (1974) 87–92, https://doi.org/10.1111/j.0954-6820.1974.tb08102.x.

[10] M. Paessler, A. Levinson, J.B. Patel, M. Schuster, M. Minda, I. Nachamkin, Disseminated Mycoplasma orale infection in a patient with common variable immunodeficiency syndrome, Diagn. Microbiol. Infect. Dis. 44 (2) (2002) 201–204, https://doi.org/10.1016/s0732-8893(02)00429-7.

[11] J. Ketchersid, J. Scott, T. Lew, N. Banaei, S. Kappagoda, Recurrent Multifocal Mycoplasma Orale Infection in an Immunocompromised Patient: A Case Report and Review, Case reports in infectious diseases, 2020 8852115, https://doi.org/10.1155/2020/8852115, 2020.

[12] A.H. Totten, G. Uzel, P.P. Khil, J.H. Youn, J. Treat, C.D. Soutar, S.M. Holland, J.P. Dekker, C.S. Zerbe, Disseminated mycoplasma orale infection in patients with phosphoinositide-3-kinase regulatory subunit 1 mutations, Open Forum Infect. Dis. 9 (9) (2022) ofac472, https://doi.org/10.1093/ofid/ofac472.

[13] Y. Dong, Y. He, X. Zhou, X. Lv, J. Huang, Y. Li, X. Qian, F. Hu, J. Zhu, Diagnosis of mycoplasma hominis meningitis with metagenomic next-generation sequencing: a case report, Infect. Drug Resist. 15 (2022) 4479–4486, https://doi.org/10.2147/IDR.S371771.

[14] Z. Samra, S. Rosenberg, Y. Soffer, In vitro susceptibility of Mycoplasma hominis clinical isolates to tetracyclines, quinolones and macrolides, Diagn. Microbiol. Infect. Dis. 44 (4) (2002) 359–361, https://doi.org/10.1016/s0732-8893(02)00459-5.

[15] R. Krausse, S. Schubert, In-vitro activities of tetracyclines, macrolides, fluoroquinolones and clindamycin against Mycoplasma hominis and Ureaplasma ssp. isolated in Germany over 20 years, Clin. Microbiol. Infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 16 (11) (2010) 1649–1655, https://doi.org/10.1111/j.1469-0691.2009.03155.x.