Journal of Microbiology, Immunology and Infection 56 (2023) 1284—1287

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

Short Communication

Metagenomics assists in the diagnosis of a refractory, culture-negative pyoderma gangrenosum-like ulcer caused by *Helicobacter cinaedi* in a patient with primary agammaglobulinemia

Li Zhang [a,1], Menglan Zhou [b,c,1], Wei Lv [a], Taisheng Li [a], Yingchun Xu [b,c,*], Zhengyin Liu [a,**]

[a] *Department of Infectious Disease, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China*
[b] *Department of Clinical Laboratory, State Key Laboratory of Complex Severe and Rare Diseases, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China*
[c] *Beijing Key Laboratory for Mechanisms Research and Precision Diagnosis of Invasive Fungal Diseases, Beijing, China*

Received 26 November 2022; received in revised form 31 May 2023; accepted 9 July 2023
Available online 18 July 2023

KEYWORDS
Helicobacter cinaedi;
Pyoderma gangrenosum;
Metagenomic next-generation sequencing;
Agammaglobulinemia

Abstract *Helicobacter cinaedi* is known to cause various infections in immunocompromised hosts ranging from skin lesions to disseminated septicemia. Identification of *H. cinaedi* is difficult through conventional identification methods due to its fastidious nature. We reported a refractory and culture-negative pyoderma gangrenosum-like ulcer caused by *H. cinaedi* in a patient with primary agammaglobulinemia. Metagenomic next-generation sequencing (mNGS) was applied for the identification of *H. cinaedi* and prolonged minocycline and amoxicillin-clavulanate potassium was used to eradicate the infection. Given the difficulties in culturing this organism, it's highly possible that *H cinaedi* infections have been overlooked. We suggest that early consideration of *H. cinaedi* infection should be suspected in immunocompromised patients presenting with unexplained skin lesions as the appropriate antibiotic choice plus a

* Corresponding author. Department of Clinical Laboratory, State Key Laboratory of Complex Severe and Rare Diseases, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China.
** Corresponding author.
E-mail addresses: xycpumch@139.com (Y. Xu), zhengyinl@hotmail.com (Z. Liu).
[1] Contributed equally.

https://doi.org/10.1016/j.jmii.2023.07.001

prolonged treatment course is essential for the prognosis. Application of mNGS could contribute to the early identification of rare and cryptogenic pathogens.

A 26-year-old man developed edema and redness, first on his right pretibial area then on his left pretibial area after trauma in the right ankle. The edema gradually increased in size and progressed with repeated attacks of fever (T_{max}39.5 °C) for more than two months despite self-medication with moxifloxacin, cefoperazone/sulbactam and cefaclor. He was admitted to Peking Union Medical College hospital on August 21st, 2019. Lymphocyte subsets analysis revealed zero B cells and all immunoglobulins (Ig) were extremely low (IgG 0.09 g/L, IgA 0.01 g/L, IgM 0.05 g/L). Since the patient didn't have any history possibly leading to the immune deficiency status, primary agammaglobulinemia was considered and the patient started intravenous immunoglobulin (IVIg) replacement therapy (600 mg/kg). Peripheral blood culture and skin secretion/biopsy was negative of all microbiological staining and culture. Empirical treatment with cefmetazole and quinolones were switched to vancomycin and ceftazidime due to the persistent fever. Intravenous vancomycin and ceftazidime were lasted for two weeks and degraded to oral sulfamethoxazole-trimethoprim (Fig. 1-A). The patient maintained afebrile and discharged with oral sulfamethoxazole-trimethoprim on September 20th, 2019. IVIG and oral sulfamethoxazole-trimethoprim were regularly applied after discharge.

Repeated redness, swelling and tenderness of right pretibial area were gradually noticed around four months later followed by recurred fever since April, 2020. The patient was re-admitted again on April 26th, 2020. On physical examination, an 11 × 17 cm skin lesion with ulceration and effusion was observed in the right lower limb (Fig. 1-B). A similar size skin lesion manifesting as hyperpigmentation without swollen or ulceration was noticed in the left lower limb. Similarly, no microbiological evidence was found in blood or skin culture. Skin biopsy pathology showed inflammatory and necrotic exudates accompanied with epidermal hyperplasia, pseudoepitheliomatous hyperplasia and hemosiderosis. A possible diagnosis of infection or pyoderma gangraenosum was suspected. His fever persisted despite successive administration of cefmetazole, levofloxacin, vancomycin and ceftazidime. Antibiotic regimen was changed to linezolid and meropenem then and his body temperature returned to normal immediately the next day. Intravenous linezolid and meropenem were administered for two weeks and the patient's right lower limb was much improved (Fig. 1-B). The patient was discharged on June 30th.

Long-term antibiotic treatment was not continued after the last discharge due to the possible diagnosis of pyoderma gangraenosum but IVIG (20 g) was regularly infused every month. On September 27th, the patient felt the skin lesions of the right lower limb became red and swollen with increased warmth. At the time, he was still afebrile and self-medicated with oral moxifloxacin and levofloxacin. The skin lesion progressed despite the antibiotic treatment and he started to fever intermittently since October 7th (T_{max}39.6 °C). The patient was admitted once again on October 13th. Physical examination revealed a 15 × 20 cm skin lesion with redness, swelling and warmth in the right lower limb and a 6 × 10 cm old skin lesion with pigmentation in the left lower limb (Fig. 1-C). Skin biopsy was negative of all microbiological staining and culture. A total of four sets of peripheral blood culture were collected during the pyrogenic stage and only one of the aerobic bottles flagged positive after 235 h. However, neither staining nor extension of the incubation revealed visible growth. Pus was aspirated from the skin lesion in the right lower limb and sent for microbiological analysis and mNGS. All the staining and culture were negative but mNGS reported *Helicobacter* spp. Due to the lack of microbiological evidence at first, empirical treatment of intravenous vancomycin and cefepime were initiated on Oct 14th and lasted for two weeks. Considering the possibility of *Helicobacter* spp infection, antibiotics were tentatively degraded to minocycline and cefepime for five days and then minocycline and amoxicillin-clavulanate potassium for a week. The skin ulcer on the right lower limb gradually healed after treatment (Fig. 1-C). The patient remained afebrile for more than two weeks and was discharged on November 16th, 2020 with oral minocycline and amoxicillin-clavulanate potassium for one year. We have kept a regular follow-up on him after discharge. The skin lesions on the right leg have completely healed after six months and no recurrence has been observed after one year (Fig. 1-D). The residual hyperpigmentation was possibly due to the side effects of minocycline after consultation with the dermatologist.

Helicobacter cinaedi, originally known as *Campylobacter cinaedi*, is a Gram-negative spiral bacillus that colonized in the normal intestinal of humans, hamsters, dogs, cats, foxes, monkeys and many other animals.[1,2] *H. cinaedi* was firstly isolated from the rectal swabs in homosexual men with proctitis, proctocolitis, and enteritis in 1984.[3] *H. cinaedi* can cause various infections such as enteritis, bacteremia, cellulitis, arthritis and in rase cases, meningitis and infective endocarditis.[4] Recent studies also revealed that *H. cinaedi* was involved in the development of atherosclerosis.[5,6] It is known that *H. cinaedi* is a notoriously fastidious organism thus making the detection and cultivation very challenging. *H. cinaedi* is mainly isolated from blood samples, and, to a lesser extent, stools, skin and pus.[7,8] Due to its slow-growing nature, prolonged incubation is often required to obtain positive results. Comparison between different automated blood culture systems

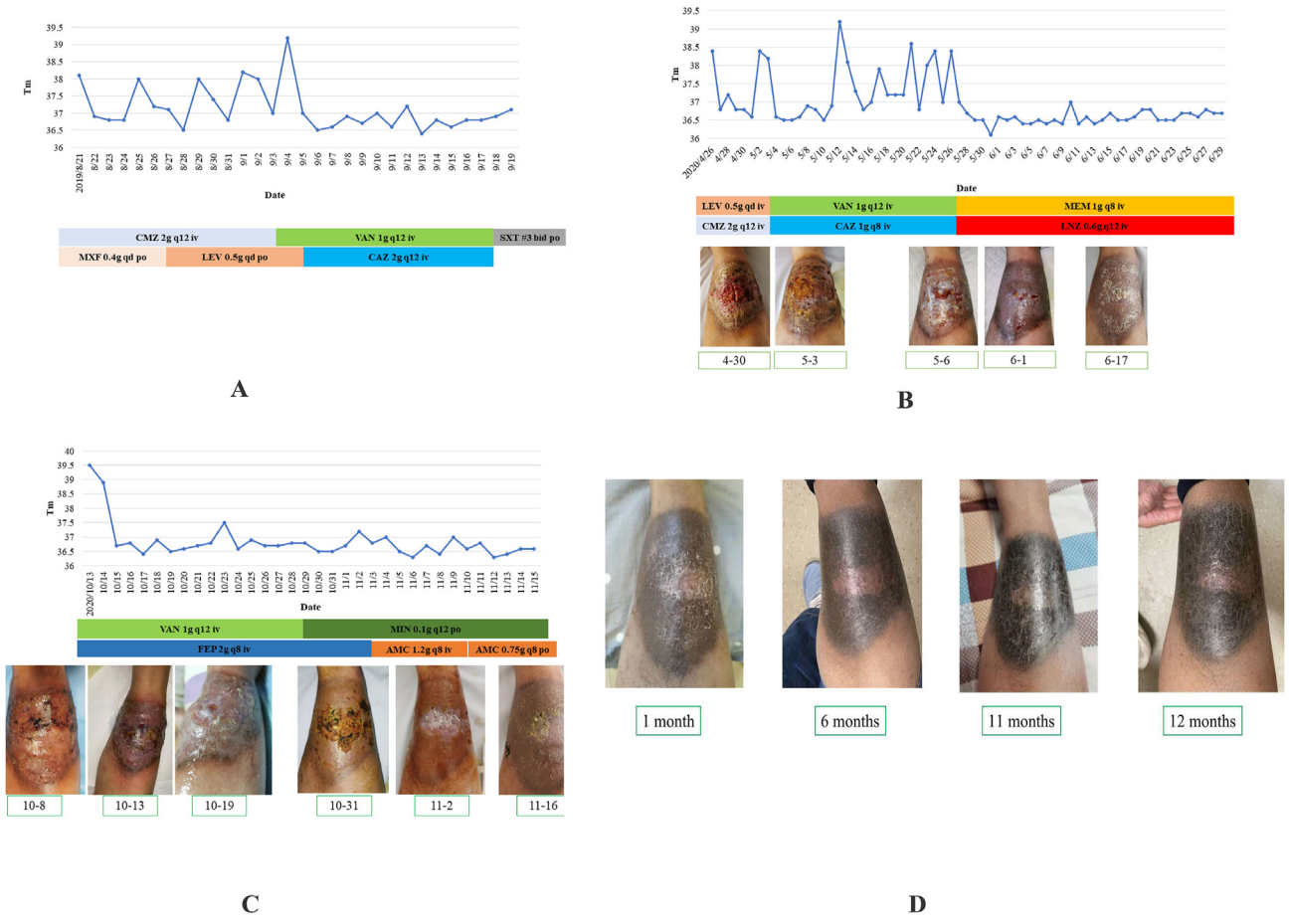

Figure 1. Clinical characteristics and treatment process of the refractory *Helicobacter cinaedi* infection. A. Temperature fluctuations during the treatment course in the first admission. B. Variations of the skin lesions in the right lower limb and temperature fluctuations during the treatment course in the second admission. C. Variations of the skin lesions in the right lower limb and temperature fluctuations during the treatment course in the third admission. D. Follow-up monitoring of the skin lesions in the right lower limb after the last discharge. Tm: temperature, iv: intravenous, po: oral administration, qd: ocne a day, bid: twice a day, q8: once every 8 h, q12: once every 12 h, CMZ: cefmetazole, MXF: moxifloxacin, LEV: levofloxacin, VAN: vancomycin, CAZ: ceftazidime, SXT: sulfamethoxazole-trimethoprim, #3: 3 tablets, MEM: meropenem, LNZ: linezolid, FEP: cefepime, MIN: minocycline, AMX: amoxicillin-clavulanate.

revealed that the VersaTREK system had an advantage over the BacT/ALERT system in detecting *H. cinaedi* with a higher positivity (3 days with both aerobic and anaerobic bottles versus 4—10 days with only an aerobic bottle).[4,9] Despite being flagged positive, subculture on solid media was not necessarily successful because of its special growth requirements. *H. cinaedi* is a microaerophilic bacterium which requires 5—10% O_2 and high humidity. Supplementation of 5—10% H_2 to the microaerobic conditions can possibly accelerate the growth of *H. cinaedi*. Gas conditions containing 6% O_2, 7% H_2, 7% CO_2, and 80% N_2 was suggested in the initial culture of clinical specimen.[4] Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) and gene-sequencing based methods have facilitated the fast and accurate identification of *H. cinaedi*.[2,10]

Concerning the antimicrobial susceptibility testing and treatment of *H. cinaedi* infection, no established guideline has been published so far. According to published studies, carbapenems, aminoglycosides, and tetracyclines exhibit great *in vitro* activity against *H. cinaedi* isolates with low minimum inhibitory concentrations (MIC) values.[4,11] Penicillins and cephalosporins demonstrate moderate MIC values while macrolides have particularly high MIC values.[4,11] A decrease in susceptibility in quinolones such as ciprofloxacin and levofloxacin has been observed in recent years due to point mutations of DNA gyrase genes.[12,13] Long-term therapy of 2—6 weeks or even longer was suggested for the treatment the prevention of *H. cinaedi* infections.[14,15] In this case, the patient was re-admitted twice due to refractory infection in the right limb. Despite that *H. cinaedi* was only detected during the third hospitalization, it is highly possible *H. cinaedi* had been the persistent causative agent throughout the whole infection course. This is strongly supported by that the addition and prolonged therapy of minocycline and amoxicillin-clavulanate potassium finally cured the infection and no recurrence has been observed for more than one year. Failure in the early detection of this organism may be attributed to the fastidious nature itself and the limitation

of traditional identification methods. Applying mNGS in the early stage could have prevented the recurrence and protraction of the infection.

In conclusion, we reported the first *H. cinaedi* infection case in a patient with primary agammaglobulinemia in mainland China. *H. cinaedi* was negative of all traditional microbiological tests and finally identified using mNGS and eliminated through prolonged antibiotic treatment. Given the difficulties in culturing the organism, it's highly possible that *H cinaedi* infections have been overlooked. Patients with hypogammaglobulinemia are susceptible to *H. cinaedi* infection and recurrence. Thus, *H cinaedi* infection should be suspected in immunocompromised patients presenting with unexplained refractory skin lesions. Application of mNGS contributed to the early identification of *H. cinaedi* and the initiation of appropriate treatment. There is a need to establish a standard guideline for the diagnosis and treatment of *H cinaedi* infection.

Funding

This work was supported by National Science Foundation for Young Scientists of China (82202541) and the Non-profit Central Research Institute Fund of Chinese Academy of Medical Sciences (3332022012).

Ethics statement

This study was conducted in accordance with the recommendations of Peking Union Medical College Hospital Ethics Committee, and written informed consent was obtained from the patient.

Authors contribution

LZ and **MZ** conceived and designed the work. **MZ** did the literature search and figures preparation work. **WL**, **TL**, **YX** and **ZL** revised and approved the manuscript.

Main point

We reported here a patient with primary agammaglobulinemia suffering from refractory, pyoderma gangrenosum-like ulcer in his lower limbs despite advanced antibiotic treatment. No definitive pathogen was found until the last admission when metagenomics was applied and identified *H. cinaedi*.

Acknowledgments

None.

References

1. Shimizu S, Shimizu H. Cutaneous manifestations of Helicobacter cinaedi: a review. *Br J Dermatol* 2016;**175**:62—8.
2. Minauchi K, Takahashi S, Sakai T, Kondo M, Shibayama K, Arakawa Y, et al. The nosocomial transmission of Helicobacter cinaedi infections in immunocompromised patients. *Intern Med* 2010;**49**:1733—9.
3. Fennell CL, Totten PA, Quinn TC, Patton DL, Holmes KK, Stamm WE. Characterization of Campylobacter-like organisms isolated from homosexual men. *J Infect Dis* 1984;**149**:58—66.
4. Kawamura Y, Tomida J, Morita Y, Fujii S, Okamoto T, Akaike T. Clinical and bacteriological characteristics of Helicobacter cinaedi infection. *J Infect Chemother : official journal of the Japan Society of Chemotherapy* 2014;**20**:517—26.
5. Khan S, Okamoto T, Enomoto K, Sakashita N, Oyama K, Fujii S, et al. Potential association of Helicobacter cinaedi with atrial arrhythmias and atherosclerosis. *Microbiol Immunol* 2012;**56**:145—54.
6. Khan S, Rahman HN, Okamoto T, Matsunaga T, Fujiwara Y, Sawa T, et al. Promotion of atherosclerosis by Helicobacter cinaedi infection that involves macrophage-driven proinflammatory responses. *Sci Rep* 2014;**4**:4680.
7. Harada K, Nakamura I, Gotoh M, Tsuboi R. Pyoderma gangrenosum-like ulcer caused by Helicobacter cinaedi in a patient with hypogammaglobulinemia. *J Dermatol* 2017;**44**:e334—5.
8. Hill A, Byrne A, Bouffard D, Luong ML, Saber M, Chapdelaine H. Helicobacter cinaedi bacteremia mimicking eosinophilic fasciitis in a patient with X-linked agammaglobulinemia. *JAAD Case Rep* 2018;**4**:327—9.
9. Tomida J, Tsurunaga M, Hosoda T, Hayakawa S, Suematsu H, Sawamura H. Evaluation of automated blood culture systems about the detection capability for Helicobacter cinaedi. *J Jpn Soc Clin Microbiol* 2012:233.
10. Sugimoto M, Takeichi T, Muramatsu H, Kojima D, Osada Y, Kono M, et al. Recurrent cellulitis caused by Helicobacter cinaedi in a patient with X-linked agammaglobulinaemia. *Acta Derm Venereol* 2017;**97**:277—8.
11. Inoue K, Sasaki S, Yasumi T, Imai K, Kusunoki T, Morio T, et al. Helicobacter cinaedi-associated refractory cellulitis in patients with X-linked agammaglobulinemia. *J Clin Immunol* 2020;**40**:1132—7.
12. Kiehlbauch JA, Brenner DJ, Cameron DN, Steigerwalt AG, Makowski JM, Baker CN, et al. Genotypic and phenotypic characterization of Helicobacter cinaedi and Helicobacter fennelliae strains isolated from humans and animals. *J Clin Microbiol* 1995;**33**:2940—7.
13. Kuijper EJ, Stevens S, Imamura T, De Wever B, Claas EC. Genotypic identification of erythromycin-resistant campylobacter isolates as helicobacter species and analysis of resistance mechanism. *J Clin Microbiol* 2003;**41**:3732—6.
14. Kiehlbauch JA, Tauxe RV, Baker CN, Wachsmuth IK. Helicobacter cinaedi-associated bacteremia and cellulitis in immunocompromised patients. *Ann Intern Med* 1994;**121**:90—3.
15. Lasry S, Simon J, Marais A, Pouchot J, Vinceneux P, Boussougant Y. Helicobacter cinaedi septic arthritis and bacteremia in an immunocompetent patient. *Clin Infect Dis* 2000;**31**:201—2.