

Case Report

***Herbaspirillum huttiense* with Multi-Drug Resistance Genes Isolated from Patient with Pneumonia-like Symptoms in Vietnam**

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Abstract

Whole-genome sequencing of a bacterial isolate from a throat swab of a 20-year-old woman in Vietnam with pneumonia-like symptoms identified *Herbaspirillum huttiense*, a bacterium rarely associated with human infections. The isolated strain harbored genes resistant to various antibiotic classes. This case highlights the potential for *H. huttiense* to cause human illness and the emergence of multi-drug resistance in this organism. Further research is needed to determine the role of *H. huttiense* in respiratory infections.

Keywords: *Herbaspirillum huttiense*, Pneumonia, Vietnam, *bla*NDM-1, *bla*OXA-58

Background

Herbaspirillum huttiense (*H. huttiense*) is a gram-negative, aerobic bacterium typically found in soil and water environments. While not a common human pathogen, a limited number of cases have been documented where *H. huttiense* has been associated with human infections [1]. This case

report describes the isolation of *H. huttienne* from a patient presenting with pneumonia-like symptoms in Vietnam.

Case presentation

On August 23rd, 2023, a previously healthy 20-year-old woman from Dien Bien province, presented to Dien Bien Provincial General Hospital with fatigue, high fever, and a sore throat. Initially diagnosed with myocarditis, she received treatment for five days with no improvement. Transferred to the Department of Tropical Diseases on August 27th, she was discharged at her caregiver's request and tragically passed away at home. The caregiver authorized the use of her clinical data for further investigation. Coinciding with the last reported diphtheria case in the province, a throat swab sample was sent to the National Institute of Hygiene and Epidemiology on August 27th for analysis, suspecting diphtheria due to the ongoing outbreak and the patient's symptoms. Laboratory findings hinted at initial recovery with rising RBC and Hb, but persistent inflammation marked by elevated WBC, NEU%, and CRP suggested further investigation. Myocarditis, potential kidney injury, and rough liver parenchyma added complexity. While the cause of the lower respiratory symptoms remained unclear, a chest X-ray showed a normal lung appearance. Antibiotic therapy failure raised concerns about resistance

Because the patient was living in the region of the diphtheria outbreak, a throat swab was collected and sent to the Department of Bacteriology, National of Hygiene and Epidemiology (Vietnam) for diagnosis. Following WHO guidelines [2], the swab was inoculated on Columbia blood agar and Tinsdale agar plates. *H. huttienne* was detected using the MALDI-TOF MS with MALDI-biotyper 3.1 software (Bruker Daltonik, GmbH, Bremen, Germany) based on predominated colonies on agar plates, and the isolated strain was designed as NIHE-VKDB-102023. Susceptibility test with MIC on the Vitek 2 system (BioMérieux, France) and disc diffusion methods according to the Clinical and Laboratory Standards Institute, M100, 32nd Edition (M100 Performance Standards for Antimicrobial Susceptibility Testing). Colistin, Piperacillin/ Tazobactam, Ceftazidime/Avibactam, Ceftolozane/Tazobactam, and Meropenem were included for MIC, and Ceftazidime and Ceftriaxone were included for disc diffusion method. Quality control was performed using *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. Results of Antimicrobial Susceptibility Testing are shown in Table 1.

Table 1. Susceptibility test results of <i>Herbaspirillum huttiense</i> isolated in a pneumonia-like symptoms case in Vietnam		
Antimicrobial agents	MIC (mg/L)	Inhibitory zone diameter (mm)
Ceftazidime	-	27
Ceftriaxone	-	27
Colistin	8	-
Piperacillin-tazobactam	1	-
Ceftazidime-avibactam	1	-
Ceftolozone-tazobactam	0.25	-
Meropenem	0.25	-
- Not performed		

As a precaution for suspected diphtheria, real-time PCR was performed according to WHO guidelines [2], yielding a negative result for the diphtheria pathogen during the culture methods. NIHE-VKDB-102023 strain was then extracted with QIAamp® DNA Mini Kit (Qiagen, Germany). Whole-genome sequencing method was performed using NEBNext Ultra II DNA Library Prep kit for Illumina (NEB) (New England Biolabs, Massachusetts, United States) and then sequenced using Miseq platform (Illumina, CA, United States). Pair-end short-read sequencing data in this study was deposited into NCBI Sequence Read Archive (SRA, accession number: PRJNA1056759). *H. huttiense* identification was performed by Kraken2 software using a standard database [3]. Genome assembly and annotation were performed using previously described methods [4]. Antimicrobial resistance (AMR) genes, plasmids, and virulence genes were searched using Staramr and VFDB [5-8]. As a result, no virulence genes or plasmids were found. However, AMR genes *aac(3)-IId*, *aph(3')-VI*, *bla_{NDM-1}*, *bla_{OXA-58}*, *catB3*, *flor*, *mph(E)*, *msr(E)*, *sul2*, *tet(39)* were detected. Of which, *aac(3)-IId*, *sul2*, *aph(3')-VI*, and *bla_{NDM-1}* were determined as mobile elements using MGEfinder tool [9]. In addition, a phylogenetic tree construction with 100 replicates was performed similarly to methods described previously [10]. Finally, the phylogenetic tree was visualized using Figtree version 1.4.4 [11], and the phylogenetic tree is shown in Figure 1

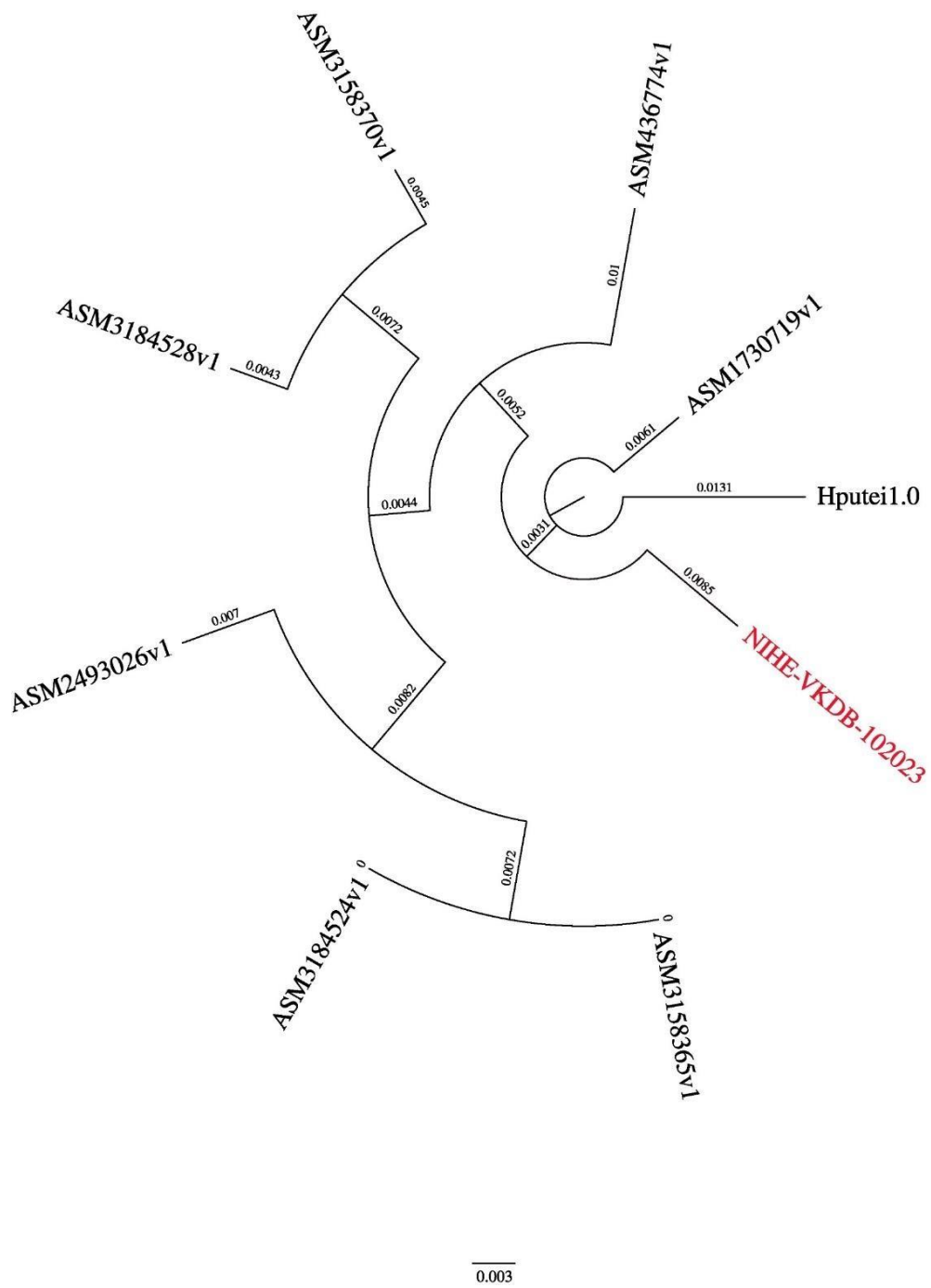


Figure 1. The phylogenetic tree of a *Herbaspirillum huttiense* strain isolated in a pneumonia-like symptoms case in Vietnam

Discussion and conclusion

While sporadic *H. huttienne* infections have been reported globally [12], the negative chest X-ray in this case makes definitively linking it to pneumonia challenging. However, isolating this organism from a patient with respiratory symptoms warrants further investigation of its potential role. Currently, both CLSI and EUCAST lack cutoff breakpoints for susceptibility testing, making sensitivity interpretation challenging. However, our isolated strain carries multi-drug resistance genes, indicating potential resistance to a broad range of antibiotics. While chromosomal genes suggest a lower risk of immediate spread, mobile elements could facilitate horizontal gene transfer among bacteria, posing a significant challenge for treatment.

Phylogenetic analysis revealed that the isolated *H. huttienne* strain (NIHE-VKDB-102023) clustered closely with a strain previously isolated from a blood sample in China (strain ASM436774v1). This suggests a potential link between *H. huttienne* and human infections. The absence of blood cultures in this case is a limitation. Blood cultures could have helped differentiate between pneumonia and potential bloodstream infection caused by *H. huttienne*. Without blood cultures, definitively attributing the patient's symptoms to *H. huttienne* pneumonia remains challenging.

This case highlights the potential pathogenicity of *H. huttienne* infections, especially in individuals exposed to water, soil, or domestic animals. Identifying multi-drug resistant genes in *H. huttienne* underlines the growing threat of antimicrobial resistance (AMR) in opportunistic pathogens. Enhanced surveillance, responsible antibiotic use, and continued research are necessary to combat this challenge and protect vulnerable individuals from potentially life-threatening infections.

Data availability

WGS data can be accessed using accession number PRJNA1056759.

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Declaration of competing interest

None to declare.

Ethical approval statement

The patient's caregiver provided written informed consent for the use of clinical details.

Authors contribution

Sample analysis: LMH, PTH, DBN, HTBN, LTM, and HTTH; genomic analysis: LHH; epidemiology information: PQT, TND, PTL, PDC; manuscript writing: LHH, PQT, and HTTH; revision and supervision: LHH, PQT, and HTTH.

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