Infection and Drug Resistance

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ORIGINAL RESEARCH

Genomic Characterization of a Fluoroquinolone Intermediate Resistance *Herbaspirillum* sp. Strain HhutSZ1 Rarely Causing Bacteremia

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Purpose: This study aims to conduct a whole-genome analysis of the isolated strain HhutSZ1, providing more reliable clinical experience for the treatment of patients infected with such bacteria.

Patients and Methods: A patient with IgA nephropathy failed to respond to treatment with ciprofloxacin for an infection. A rare strain of *Herbaspirillum* was isolated from the patient's blood. The VITEK MS Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) System and VITEK 2 Automated Microbial Identification System were used to conduct a preliminary identification and antibiotic susceptibility tests. Then we performed whole-genome sequencing of this strain along with comparative genomic analysis.

Results: VITEK 2 System identified HhutSZ1 as *Burkholderia cepacia*, while the VITEK MS System identified it as *Herbaspirillum huttiense* with a high confidence coefficient. The phylogenetic tree based on the 16S rRNA gene showed that HhutSZ1 belonged to the genus *Herbaspirillum*. Average Nucleotide Identity (ANI) analysis showed that the scores of this strain compared with other *Herbaspirillum huttiense* strains were all lower than 95, confirming that this strain did not belong to *Herbaspirillum huttiense*. The patient's infection failed to resolve despite ciprofloxacin treatment. Subsequent antimicrobial susceptibility testing revealed that HhutSZ1 exhibited intermediate resistance to ciprofloxacin, which is consistent with the clinical treatment failure. Six genes were detected in the Comprehensive Antibiotic Resistance Database (CARD). And among them, five RND family efflux pump genes resistant to fluoroquinolone were all located on chromosomes.

Conclusion: For the genus *Herbaspirillum*, mass spectrometry identification cannot accurately identify the species. The analysis based on 16s rRNA combined with ANI can be more accurate. Some *Herbaspirillum spp* may have inherent resistance to fluoroquinolone antibiotics. In conclusion, our findings suggest that the low detection rate and low drug resistance of this strain cannot be overlooked, and the study provides valuable clinical insight for managing infections in immunocompromised patients.

Keywords: *Herbaspirillum hutitense*, bloodstream infection, whole genome sequence, antibiotic resistance

Introduction

Herbaspirillum species are non-fermenting, microaerophilic, motile, Gram-negative bacilli belonging to the class *Betaproteobacteria*.[1–3] While commonly found in environmental reservoirs such as soil and water, these organisms are increasingly recognized as opportunistic human pathogens, particularly in immunocompromised hosts or individuals with significant comorbidities.[4–8] The clinical management of *Herbaspirillum* infections is complicated by significant diagnostic challenges. In clinical microbiology laboratories, automated biochemical identification systems (eg, the VITEK® 2 system) frequently misidentify *Herbaspirillum* spp. as *Burkholderia cepacia* complex due to phenotypic similarities.[9] This misidentification carries critical therapeutic implications, as *Herbaspirillum* spp. are frequently pan-susceptible, in stark contrast to the inherent multidrug resistance often associated with *B. cepacia*.[10] Consequently, a biochemical identification of a pan-susceptible "B. cepacia" should raise suspicion of a possible *Herbaspirillum* species.

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To achieve accurate species-level identification, methods beyond biochemical profiling are essential. It is generally established that MALDI-TOF MS and molecular methods (eg, 16S rRNA gene sequencing) offer superior accuracy compared to biochemical identification systems alone.[6,11,12]

Furthermore, the antibiotic resistance profile of *Herbaspirillum* remains poorly defined. Existing literature suggests that overt antibiotic resistance in *Herbaspirillum* infections is rare, with most isolates demonstrating susceptibility to a broad range of antibiotics, although intermediate resistance has been occasionally observed.[8] However, robust clinical evidence linking this intermediate resistance to actual treatment failure has been lacking.

Therefore, the objective of this study is to report the first case of ciprofloxacin treatment failure associated with a blood culture isolate of *Herbaspirillum* sp. that showed intermediate resistance to fluoroquinolone antibiotics. Through comprehensive genomic and phenotypic characterization of the isolate, designated HhutSZ1, we aim to elucidate the mechanisms underlying this resistance and highlight the potential for mobile genetic elements to facilitate horizontal gene transfer. This case provides valuable new insights into the evolving pathogenicity and treatment challenges posed by this emerging pathogen.

Materials and Methods

Bacterial Identification and Antimicrobial Susceptibility

The clinical isolate studied in this work was obtained from a patient at Shenzhen Second People's Hospital (Shenzhen, China). Per standard clinical procedures, blood samples were collected aseptically and inoculated into aerobic and anaerobic blood culture bottles (BACT/ALERT® FA Plus/BACT/ALERT®FN Plus) and incubated in the BACTALERT3D system (bioMérieux, Marcy-l'Étoile, France). A positive signal triggered Gram staining and subculture of the blood onto Columbia blood agar with 5% sheep blood (bioMérieux). The agar plates were incubated at 37°C under 5% CO_2 for 24–48 hours This study was approved by the Institutional Review Board/Ethics Committee of Shenzhen Second People's Hospital, and the requirement for informed consent was waived due to the anonymized nature of the microbiological data.

Pure colonies from the subculture were used for identification via MALDI-TOF MS on a VITEK® MS (bioMérieux, Marcy-l'Étoile, France) platform. The isolate was designated as "HhutSZ1" in this study for internal reference, where "SZ" refers to Shenzhen, and this designation has been used consistently throughout our subsequent investigations.

Antimicrobial susceptibility testing (AST) was performed using the VITEK®2 COMPACT system (bioMérieux, Marcy-l'Étoile, France) with the AST-N335 susceptibility card for Gram-negative bacteria. Results were interpreted according to the breakpoints defined by the Clinical and Laboratory Standards Institute (CLSI) M100-Ed34 document. As there are no species-specific breakpoints for *Herbaspirillum* sp., the breakpoints for Non-*Enterobacteriaceae* were applied. Given that *Herbaspirillum* spp. are rare and frequently misidentified by routine biochemical systems, 16S rRNA gene sequencing was employed for definitive species confirmation.

Whole-Genome Sequencing and Bioinformatics Analysis

Genomic DNA was extracted from an overnight pure culture of HhutSZ1. For PacBio long-read sequencing, high-molecular-weight DNA was mechanically sheared using g-TUBE (Covaris) to generate fragments of >10 kb. A SMRTbell library was constructed through end-repair, A-tailing, and adapter ligation, followed by sequencing on the PacBio Revio platform. For short-read sequencing, the DNA was fragmented by ultrasonication to an insert size of ~350 bp. A paired-end library was prepared using the NEBNext Ultra DNA Library Prep Kit and sequenced on the DNBSEQ platform.

De novo assembly was performed using a hybrid approach with Canu, and the assembly was polished with short reads. The final complete genome consists of one circular chromosome (5.47 Mb) and one plasmid (0.47 Mb). Gene prediction and functional annotation were performed using Glimmer and multiple databases, respectively.

The Functional annotation of the predicted genes was performed by querying against multiple public databases. The specific database versions used were as follows: NR (2024–04-16), Swiss-Prot (release-2024_01), COG (2020–11-25), KEGG (Release 109.0), and Gene Ontology (GO, release 2019–07-01). Pathogenicity and resistance traits were analyzed

against the Virulence Factor Database (VFDB, 2024–03-26), the Comprehensive Antibiotic Resistance Database (CARD, v3.0.9), and the Antibiotic Resistance Genes Database (ARDB, v1.1). Genomic islands and integrative/conjugative elements were predicted using ICEfinder (https://bioinfo-mml.sjtu.edu.cn/ICEfinder/ICEfinder.html). The accuracy of species identification was validated by comparing the 16S rRNA gene sequence with known bacterial sequences in the GenBank database via BLAST. The phylogenetic tree was established using the MEGA11.0 software. A circular representation of the genome was created by Circos software (circos-0.69–9.tgz). ANI heatmap was created using fastANI software (Version 1.32). All databases were accessed during the period of February to March 2025.

Results

The Patient and Bacteria Identification

The patient is a 26-year-old male diagnosed with IgA nephropathy 2 years ago and was treated with prednisone acetate, which was gradually tapered until it was discontinued a year ago. He was admitted to the hospital 5 days ago with symptoms of cough and sputum, bilateral lower extremity pitting edema, and decreased urine output after a cold. After admission, he was given methylprednisolone sodium succinate 40 mg qd, chest CT suggesting lung infection and pleural effusion, and was treated with ciprofloxacin for anti-infection. After 5 days of treatment, the patient had a sudden onset of high fever, with a maximum temperature of 38.5 degrees Celsius.

We isolated a Gram-negative bacterium named HhutSZ1 from the patient's blood, and the colony looked translucent white and lacked a visible hemolytic zone (Figure 1). Although it was recognized as *B. cepacia* by the Vitek® 2 system, its antimicrobial susceptibility did not correspond to that of multidrug-resistant *B. cepacia*. Then, the strain was identified by Vitek® MS and obtained a 99.9% score of homology with *H. huttiense* (Figure 2).

To further identify its species, we compared the 16S rRNA gene with the Genbank database after performing whole-genome sequencing of HhutSZ1. *H. huttiense* ATCC14670 produced the highest match score. Sequences with the top 15 matches were collected to build a phylogenetic tree with HhutSZ1, and the phylogenetic tree showed that HhutSZ1 was in a separate branch (Figure 3A). Then, we conducted a comparison based on the whole genome between HhutSZ1 and four *H. huttiense* strains from the NCBI database. The Average Nucleotide Identity (ANI) between our clinical blood isolate HhutSZ1 and other clinical isolates of *H. huttiense* (including H. hutt-CLJ01, H. hutt-CLJ02, and H. hutt-NFYY) were 94.07%, 94.07%, and 94.12%, respectively. The ANI value with the plant-derived strain H. hutt-ZXN111 was 93.80% (Figure 3B). All pairwise values are distinctly below the 95% species threshold, confirming that HhutSZ1 represents a novel species within the genus, even when compared to closely related clinical isolates.

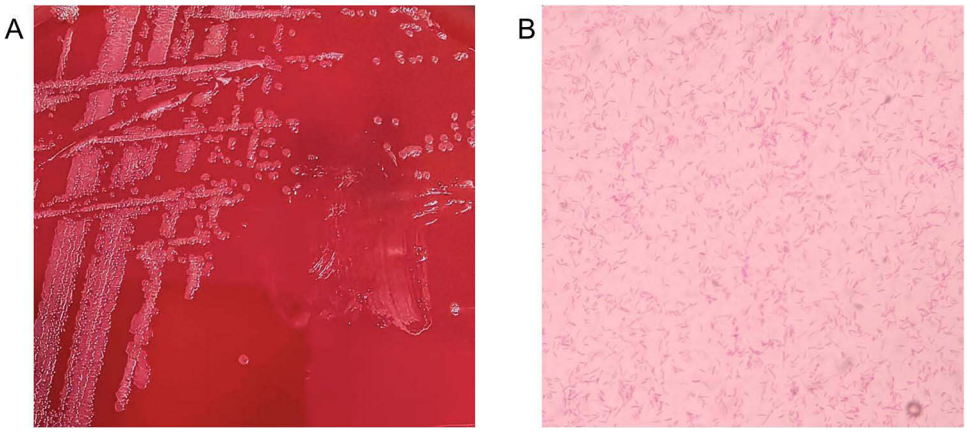

Figure 1 HhutSZ1 was conducted subsequent to the detection of a positive blood culture after 24 hours of incubation; (**A**) Colony morphology on the blood plate; (**B**) Gram staining of HhutSZ1.

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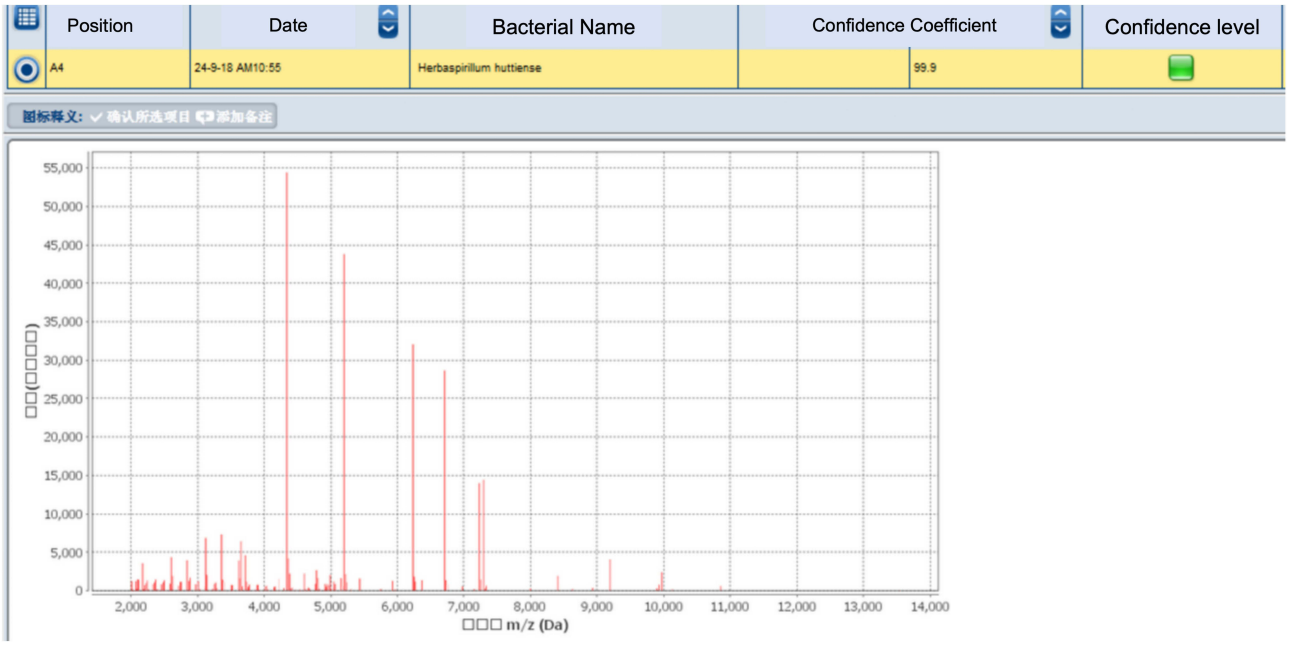

Figure 2 The mass spectra of *H. huttiense* revealed high accuracy.

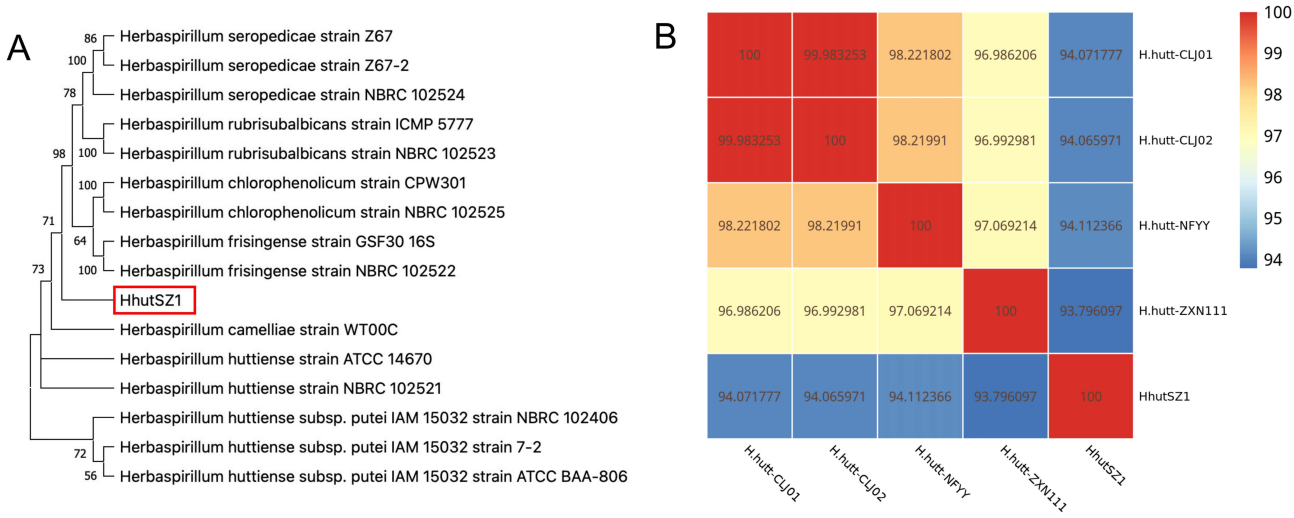

Figure 3 Phylogenetic and genomic relatedness analysis of HhutSZ1. (**A**) Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, showing the phylogenetic position of HhutSZ1 among closely related *Herbaspirillum* species. (**B**) Average Nucleotide Identity (ANI) heatmap between HhutSZ1 and other *H. huttiense* strains. Values represent percentage ANI. This indicates that HhutSZ1 represents a distinct species from the compared *H. huttiense* strains.

Whole Gene Sequencing and Analysis

WGS results showed that HhutSZ1 carries a chromosome with a GC content of 62.86% and a length of 5,474,608 bp and a plasmid with a GC content of 53.97% and a length of 468,427 bp (Figure 4). Detailed information is available in Table 1. A total of 5464 genes were predicted, including 36 drug resistance-related genes and 404 virulence-related genes, and three virulence genes were located on the plasmid (Supplementary Tables 1 and 2). Sixty-seven genes related to exotoxins, biofilms, immune regulation, flagella, adhesion, invasion and other aspects were identified in the VFDB database. Among them, the virulence genes related to flagella were the most numerous, with 21. Numerous genes with unknown functions were present on the sole plasmid that HhutSZ1 possesses. Furthermore, we identified a 91,060 bp

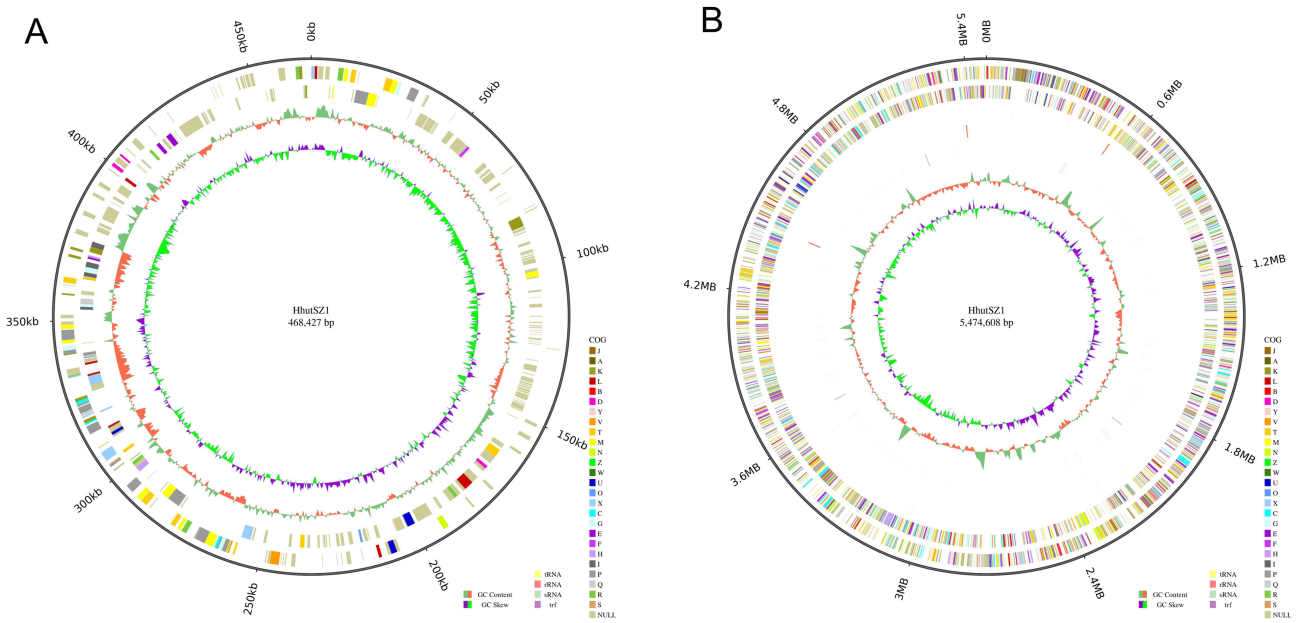

Figure 4 Circular representation of HhutSZ1; (**A**) Genome; (**B**) Plasmid.

ICE element that demonstrates the characteristics of a Type IV secretion system. A collection of Type II toxin-antitoxin system genes was identified within this fragment.

Antibiotic Susceptibility Tests and Resistance Genes Analysis

Antibiotic susceptibility test of HhutSZ1 showed that it was sensitive to most commonly used clinical antibiotics but presented a low level of resistance to fluoroquinolone antibiotics. The MICs results are shown in Table 2. Six antibiotic resistance-related genes were detected in the CARD database, all located on chromosomes, among which five were RND family efflux pump genes resistant to fluoroquinolone antibiotics and tetracycline family antibiotics (Table 3).

Table 1 Genomic Characteristics of HhutSZ1

Sample Name	Genome Type	Total Length (bp)	GC Content (%)	Gene Number	Gene Average Length (bp)	Genome Length (%)
HhutSZ1	Chromosome	5,474,608	62.86	5156	—	—
	Plasmid1	468,427	53.97	308	—	—
	All	5,943,035	62.16	5464	932.62	85.74

Notes: The gene average length for the complete genome ("All") is provided. This value was calculated directly from the aggregated annotation data. The average gene length for individual replicons (chromosome and plasmid) was not separately calculated by our pipeline and is thus indicated as "—".

Table 2 Antibiotic Susceptibility Tests Results of HhutSZ1

Antibiotics	MIC (mg/L)	Antibiotics	MIC (mg/L)	Antibiotics	MIC (mg/L)
Ticacillin - Clavulanic acid	≤8	Imipenem	≤0.25	Doxycycline	1
Piperacillin - tazobactam	≤4	Meropenem	≤0.25	Minocycline	≤1
Ceftazidime	1	Tobramycin	≤1	Tigecycline	1
Cefoperazone/Sulbactam	≤8	Ciprofloxacin	2	Amikacin	8
Cefepime	0.25	Levofloxacin	4	Colistin	2

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Table 3 Identification of Resistance Genes from CARD Database

Gene	Identities (%)	Drug Class	Resistance Mechanism	AMR Gene Family
adeF	84.11	Fluoroquinolone antibiotic; tetracycline antibiotic	Antibiotic efflux	Resistance-nodulation-cell division (RND) antibiotic efflux pump
adeF	42.28	Fluoroquinolone antibiotic; tetracycline antibiotic	Antibiotic efflux	Resistance-nodulation-cell division (RND) antibiotic efflux pump
adeF	42.7	Fluoroquinolone antibiotic; tetracycline antibiotic	Antibiotic efflux	Resistance-nodulation-cell division (RND) antibiotic efflux pump
adeF	61.3	Fluoroquinolone antibiotic; tetracycline antibiotic	Antibiotic efflux	Resistance-nodulation-cell division (RND) antibiotic efflux pump
adeF	42.47	Fluoroquinolone antibiotic; tetracycline antibiotic	Antibiotic efflux	Resistance-nodulation-cell division (RND) antibiotic efflux pump
SPG-1	100	Carbapenem	Antibiotic inactivation	SPG beta-lactamase

Discussion

Herbaspirillum species are uncommon but emerging opportunistic pathogens, with infections primarily occurring in immunocompromised individuals or those with underlying malignancies, and most frequently presenting as bacteremia.[6,8,13] However, the true clinical burden of *Herbaspirillum* is likely underestimated due to its frequent misidentification as closely related genera, such as *B. cepacia* complex, using conventional diagnostic methods.[9–11] This diagnostic challenge has direct implications for clinical laboratory practice. Our findings suggest that when conventional systems identify a pan-susceptible "B. cepacia" from a sterile site in an immunocompromised patient, confirmation by 16S rRNA sequencing or ANI analysis should be pursued to rule out *Herbaspirillum*.

Our study, focusing on the clinical isolate HhutSZ1 identified as *H. huttiense*—the most prevalent species in human infections. We demonstrated that even a high-confidence score from MALDI-TOF MS could not reliably differentiate *H. huttiense* from other *Herbaspirillum* species. This finding highlights a significant limitation of mass spectrometry for the precise taxonomy of this genus and corroborates the necessity of a polyphasic approach.[14,15] Our use of 16S rRNA sequencing and Average Nucleotide Identity (ANI) analysis was therefore critical for achieving accurate species-level classification, a step that is essential for reliable epidemiological tracking and association of specific species with clinical outcomes. The antibiotic resistance profile of HhutSZ1 presents an intriguing clinical puzzle. While previous reports suggest that *Herbaspirillum*, particularly *H. huttiense*, generally exhibits no notable drug resistance,[5,8,16] our isolate displayed low-level resistance to fluoroquinolones. This discrepancy may partly stem from the historical misidentification of clinical isolates, which could have led to unreliable phenotypic data in the past. The case of our patient, who acquired the infection despite ciprofloxacin prophylaxis, aligns with this phenotypic finding. Importantly, our genomic analysis did not detect acquired fluoroquinolone resistance genes. This suggests that the observed resistance may be intrinsic, potentially mediated by chromosomal mutations in genes, or efflux pump activity, as has been hinted at in other studies.[8] Therefore, for immunocompromised patients receiving fluoroquinolone prophylaxis or empiric therapy, our case sounds a note of caution. It underscores that antimicrobial susceptibility testing is indispensable, and reliance on historical susceptibility profiles may be misleading.

Beyond the intrinsic resistance, the most significant finding of our study is the identification of a conjugative plasmid in HhutSZ1. Although the functions of most plasmid-borne genes remain unknown, its demonstrated ability to transfer and be stably maintained in a recipient strain represents a potential evolutionary leap for this pathogen. Conjugative plasmids are primary vehicles for the horizontal transfer of acquired antibiotic resistance genes and virulence factors.[17] The current absence of known high-level resistance genes on this plasmid is little consolation; its very existence provides a ready platform for the future acquisition and dissemination of such traits. Should this plasmid capture resistance genes —for instance, to carbapenems or other last-resort antibiotics—it could rapidly transform *Herbaspirillum* from a typically susceptible organism into a formidable multidrug-resistant pathogen.

In conclusion, our characterization of HhutSZ1 reveals a multifaceted threat. It underscores the diagnostic challenges that have likely obscured the true prevalence of *Herbaspirillum* infections. It demonstrates that low-level intrinsic resistance to clinically relevant antibiotics like fluoroquinolones exists and may have clinical consequences. Future work must focus on sequencing a larger collection of clinical isolates to determine the prevalence and diversity of such mobile genetic elements, and on functionally characterizing plasmid pSZ1 to identify its full genetic cargo. Enhanced vigilance through accurate molecular identification and antimicrobial surveillance is crucial to mitigate the potential future crisis posed by this emerging pathogen.

Despite the significant findings, this study has several limitations. Firstly, it is a single-case report, which inherently limits the generalizability of our conclusions. The association between the intermediate resistance of HhutSZ1 to fluoroquinolones and the clinical treatment failure, while compelling in this instance, requires validation through larger surveillance studies or cohort analyses. Secondly, the precise molecular mechanisms underlying the observed resistance and the full functional repertoire of the conjugative plasmid warrant further investigation.

Conclusion

In our case, biochemical identification systems misidentified HhutSZ1 as *B. cepacia*, whereas MALDI-TOF MS provided a result consistent with the *Herbaspirillum* genus, though it could not achieve reliable species-level resolution. Ultimately, definitive identification required molecular methods. This experience in our study suggests that for accurate characterization of *Herbaspirillum*, molecular techniques are indispensable. Therefore, our findings suggest that fluoroquinolones may not be a reliable empiric therapeutic option for *Herbaspirillum* infections. Clinical laboratories should prioritize accurate species identification and AST for guiding definitive therapy in such cases. Our study highlights the importance of not overlooking its low-level drug resistance and provides clinical experience for prophylactic drug use in immunosuppression or immune deficiency patient populations.

Data Sharing Statement

The genome assembly number of HhutSZ1 in GenBank is ASM4730081v1. The authors confirm that the data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical Statement and Informed Consent

The Ethics Committee of the Shenzhen Second People's Hospital approved the study. The patient provided written informed consent for publication of this study.

Acknowledgments

Our study complies with the Declaration of Helsinki.

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Disclosure

The authors declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

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