Preplanned Studies

Genomic Insights into Genetic Characteristics of *Chromobacterium haemolyticum* — China, 2023

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Summary

What is already known about this topic?

Chromobacterium haemolyticum (*C. haemolyticum*) is an emerging multidrug-resistant and potentially extensively drug-resistant pathogen capable of causing invasive, lethal infections in humans. Conventional biochemical and mass spectrometry identification methods used in clinical laboratories cannot reliably distinguish it from *C. violaceum*.

What is added by this report?

This study provides the first report characterizing the genomic features of *C. haemolyticum* isolated from a young patient in China and reveals the evolutionary patterns of global *C. haemolyticum* isolates.

What are the implications for public health practice?

This research highlights the advantages of whole-genome sequencing for accurate differentiation of *Chromobacterium* species, raises public awareness about this uncommon pathogen, and provides scientific foundations for improved detection and prevention strategies.

ABSTRACT

Introduction: *Chromobacterium haemolyticum* (*C. haemolyticum*) can cause invasive infections in humans. This study aims to reveal the genomic characteristics of *C. haemolyticum* and provide guidance for clinical diagnosis, treatment, prevention, and control.

Methods: Species identification was performed through isolation culture and matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Antibiotic susceptibility testing determined resistance phenotypes. High-throughput sequencing and bioinformatics methods were used to predict antibiotic resistance genes and virulence genes and to analyze the evolutionary characteristics of global *C. hemolyticus* genomes.

Results: In this study, a *C. haemolyticum* strain was isolated from the bronchoalveolar lavage fluid of a patient in Guangxi Zhuang Autonomous Region, China. The isolate was sensitive to chloramphenicol, macrolides, and trimethoprim, while resistant to beta-lactams. Comparative genomics analysis revealed that most global strains carry carbapenemase-encoding genes. Phylogenetic analysis showed that the strain from this patient was closely related to a pond-derived *C. haemolyticum* isolate from Yangzhou, China.

Conclusions: This study uncovered the genetic characteristics of *C. haemolyticum* from various sources worldwide, including antibiotic resistance and virulence factors, providing an important reference for clinical treatment.

The genus *Chromobacterium* belongs to the family *Neisseriaceae* and comprises 19 species (*1*). *Chromobacterium violaceum* (*C. violaceum*) is a zoonotic pathogen found in tropical and subtropical regions that can cause severe sepsis with high mortality rates in humans (*2*). Since the first report of *C. haemolyticum* in 2008 (*3*), most invasive infection cases (e.g., pneumonia and bacteremia) have been associated with exposure to water bodies (*4*). However, genomic data on *C. haemolyticum* remains insufficient worldwide.

Here, we report the first case of pulmonary infection caused by *Chromobacterium* spp. in Guigang City, Guangxi Zhuang Autonomous Region, China. On the evening of November 4, 2023, an 18-year-old patient was admitted to the Qintang District People's Hospital following a traffic accident in Guigang City. The patient subsequently developed pneumonia and a *Chromobacterium* spp. strain was isolated from bronchoalveolar lavage fluid. After combined treatment with cefoperazone sodium/sulbactam sodium, meropenem, and levofloxacin, the patient recovered.

Antibiotic susceptibility testing (AST) was conducted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines for non-*Enterobacteriaceae* bacteria to determine the minimum

inhibitory concentration (MIC) of the *C. haemolyticum* strain (*5*). The AST results are presented in Table 1. Overall, the strain demonstrated sensitivity to most antibiotics tested while exhibiting resistance to several beta-lactam and aminoglycoside antibiotics (Table 1 and Supplementary Table S1, available at https://weekly.chinacdc.cn/).

The isolate was initially identified as *C. violaceum* using blood agar culture, biochemical experiments, and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) (model: VITEK MS, version: VITEK MS V3.0, BioMérieux, France). Due to similar cultural characteristics between the two species, *C. haemolyticum* is frequently misidentified as *C. violaceum* (*6*). Definitive identification as *C. haemolyticum* was subsequently achieved (Supplementary Table S2, available at https://weekly.chinacdc.cn/) using MetaphlAn4 with database version mpa_vOct22_CHOCOPhlAnSGB_202212 (*7*).

We downloaded 19 publicly available genomes of *C. haemolyticum* from the National Center for Biotechnology Information (NCBI) to characterize genomic features. After excluding two low-quality genomes (GCF_000285415.1 and GCF_003332145.1), the remaining 17 genomic datasets were used for subsequent analysis (Supplementary Table S3, available at https://weekly.chinacdc.cn/). We detected 12 antibiotic resistance genes (ARGs) classified into 7 categories (Supplementary Tables S1 and Supplementary Table S4, available at https://weekly.chinacdc.cn/). The predominant ARGs were *rpsJ* and four mutated genes: *gidB*, *MurA*, *folP*, and *gyrA*. Additionally, carbapenemase-encoding genes, including $bla_{\text{CRH-1}}$, $bla_{\text{CRH-2}}$, and $bla_{\text{CRH-3}}$ were detected in 72.22% (13/18), 11.11% (2/18), and 16.67% (3/18) isolates, respectively. Moreover, we identified five types of multiple efflux pump systems-encoding genes (EmrAB-OMF, EmrAB-TolC, MdfA/CMr, MdtABC-TolC, and MacAB-TolC) that can reduce drug susceptibility.

We detected 9 virulence factors (VFs), with 33.33% (3/9) belonging to the type 3 secretion system (Supplementary Table S5, available at https://weekly.chinacdc.cn/). Among these VFs, *sicA*, *spaQ*, *spaT*, *fba*, *hfq*, and *recA* were present in all strains. To investigate potential drivers mediating ARGs and VFs transfer, we identified 59 intact prophages belonging to 19 types (Supplementary Table S6, available at https://weekly.chinacdc.cn/). The most prevalent prophage was *Mannhe_vB_MhM_3927AP2* (83.33%), followed by *Ralsto_RSA1* (33.33%), *Haemop_SuMu* (27.78%), and *Burkho_phiE125* (27.78%).

To explore the population evolution of *C. haemolyticum*, we analyzed 18 public *C. haemolyticum* genomes and 2 *C. violaceum* genomes (*8*). The phylogenetic tree revealed two distinct lineages corresponding to the two different species, spanning

TABLE 1. Results of antibiotic susceptibility testing.

Antibiotic	MIC (μg/mL)	Interpretation
Imipenem	4	S
Meropenem	≤1	S
Piperacillin	≥128	R
Piperacillin/tazobactam	≤4/4	S
Cefepime	≤2	S
Ceftazidime	≤2	S
Minocycline	≤4	S
Cefoperazone/Sulbactam	≤8/4	S
Sulfamethoxazole/Trimethoprim	≤1/19	S
Gentamicin	≤2	S
Amikacin	32	I
Levofloxacin	≤1	S
Ciprofloxacin	≤0.5	S
Chloramphenicol	≤8	S
Aztreonam	≤2	S

Note: S indicates sensitivity, R indicates resistant, and I indicates intermediate.

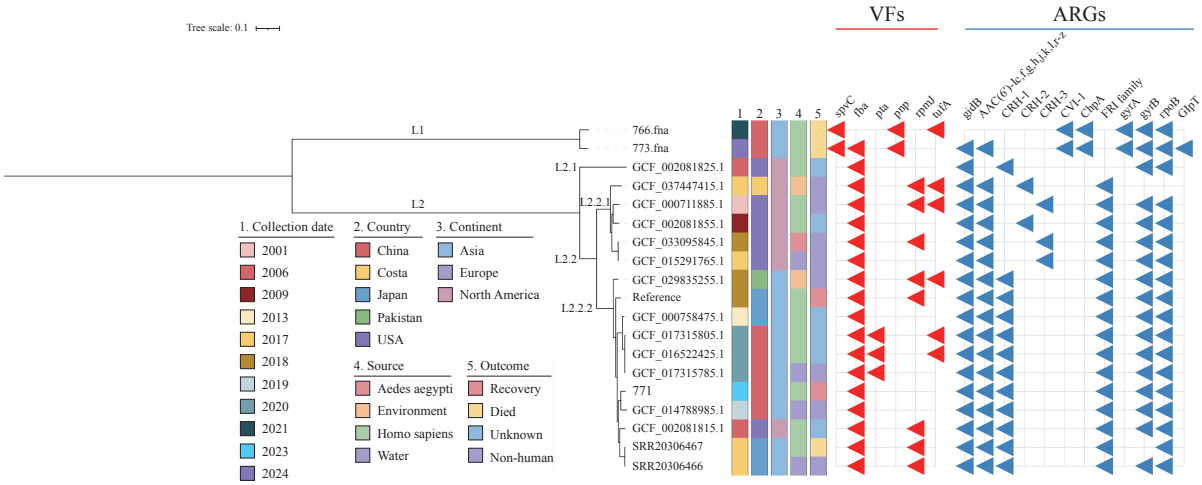

FIGURE 1. Phylogenetic analysis of 19 *C. haemolyticum* genomes.
Note: Phylogenetic tree-built details and analysis are described in the text. Collection date, country, source, continent, and outcome are labeled with different colors. The red and blue triangles represent the existence of VFs and ARGs, respectively.
Abbreviation: VFs=virulence factors; ARGs=antibiotic resistance genes.

five countries and four diverse sources (human, water, environment, and *Aedes aegypti*) (Figure 1). Lineage one (L1) consisted of 2 *C. violaceum* strains from China, while lineage two (L2) comprised global *C. haemolyticum* strains. The isolate from the patient in this study showed a close genetic relationship with a pond-source *C. haemolyticum* strain from Yangzhou, China. Both isolates exhibited fewer virulence factors, with 6 VFs each.

DISCUSSION

Advances in whole-genome sequencing (WGS) enable rapid and accurate species identification and tracking of potential factors and evolutionary patterns of existing and emerging antibiotic resistance (AMR) and virulence factors in bacteria that inhabit organisms and the environment (*8*–*12*) Using WGS, we successfully distinguished the genetic differences between the *C. haemolyticum* strain in our study and *C. violaceum*, confirming the identify of our isolate as *C. haemolyticum* (Supplementary Table S6, available at https://weekly.chinacdc.cn/).

Carbapenem-resistant gram-negative bacteria pose a significant health burden (*13*–*15*). Carbapenemase-encoding genes naturally exist in the chromosomes of *Chromobacterium* species (*16*). We detected $bla_{\text{CRH-1}}$ in 13 strains of *C. haemolyticum*, while $bla_{\text{CRH-2}}$ and $bla_{\text{CRH-3}}$ were detected in 2 and 3 strains, respectively. Additionally, we identified mutated fluoroquinolone resistance genes (*gyrA* and *gyrB*) and the tetracycline resistance gene *rpsJ*. However, the *C. haemolyticum* isolate from our patient remained sensitive to ciprofloxacin, nalidixic acid, meropenem, tetracycline, and tigecycline, suggesting these ARGs may not be expressed (*12*).

In summary, this is the first report characterizing the genome of *C. haemolyticum* causing human pulmonary infection in China. We found that the strain isolated from the recovered patient carried fewer virulence factors and shared a close genetic relationship with a water isolate from Yangzhou, China. Due to the limited number of available genomes, which may underestimate the global transmission risk of carbapenem-resistant *C. haemolyticum*, increased genomic surveillance is needed to better understand its spread and evolutionary trajectory. Our findings enhance awareness of this uncommon species and provide a scientific basis for the prevention and treatment of infections caused by *C. haemolyticum* in humans, food animals, and ornamental animals in the future.

Conflicts of interest: No conflicts of interest.

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