

# *Clostridium ramosum* Bacteremia in an Immunocompetent Patient with SARS-CoV-2 Infection: A Case Report

Danni Bao<sup>1</sup>, Xiaohong Xu<sup>1</sup>, Yizhang Wang<sup>1</sup>, Fengjiao Zhu<sup>1</sup>, Yanhong Wu<sup>1</sup>, Hongzhang Li<sup>2</sup>

<sup>1</sup>Department of Clinical Laboratory, Sanmen People's Hospital, Taizhou, Zhejiang, People's Republic of China; <sup>2</sup>Department of Gastroenterology, Sanmen People's Hospital, Taizhou, Zhejiang, People's Republic of China

Correspondence: Hongzhang Li, Department of Gastroenterology, Sanmen People's Hospital, Taizhou, Zhejiang, People's Republic of China, Email lihongzhang@zjsmyy.com

**Abstract:** We report a case of *Clostridium ramosum* bacteremia in a 73-year-old patient with SARS-CoV-2 infection and right lower abdominal tenderness in China. The microbiological features and genomic epidemiological characteristics of *C. ramosum* worldwide were investigated to identify the possible sources of infection. Whole-genome sequencing of *C. ramosum* WD-I2 was performed using an Illumina NovaSeq 6000 platform. Phylogenetic analysis of *C. ramosum* WD-I2 and other publicly available *C. ramosum* isolates was performed and visualized using the interactive Tree of Life (iTOL) web server. The resistome of *C. ramosum* WD-I2 consists of two antimicrobial resistance genes (*tetM* and *ermB*), which explains the antimicrobial resistance trait to tetracycline and macrolides. Phylogenetic analysis showed that the strain closest to our isolated strain WD-I2 was SUG1069, recovered from a pig feces sample from Canada, which differed by 589 SNPs. To our knowledge, this is the first report of *C. ramosum* bacteremia in China. Our findings highlight the potential risk of invasive *C. ramosum* infections during the COVID-19 pandemic.

**Keywords:** *Clostridium ramosum*, bacteremia, antimicrobial resistance, SARS-CoV-2, whole genome sequencing

## Introduction

*Clostridium ramosum* was first identified and characterized in 1898 following its isolation from a patient with pulmonary gangrene and appendicitis.<sup>1</sup> This species, initially identified as *Bacillus ramosum* and later referred to as *C. ramosum*, was renamed *Erysipelatoclostridium ramosum* in 2013.<sup>2</sup> However, its taxonomic name has not been legally published according to the International Code of Nomenclature of Bacteria.<sup>3</sup> *C. ramosum* is a gram-positive, anaerobic, non-motile Bacillus species capable of forming spores. It is commonly found as part of the normal intestinal flora in humans, with over 83% of adult patients having it present in their feces. This high prevalence makes it one of the most widespread commensal clostridial species in humans.<sup>4</sup> Although *C. ramosum* can be isolated from clinical specimens, it is rarely identified as the primary cause of infection; therefore, its pathogenicity has been largely underestimated.<sup>5</sup> Infections caused by *C. ramosum* have been predominantly reported in young children (under 5 years old) with inner ear infections and immunocompromised elderly individuals.<sup>6</sup> However, in certain cases, *C. ramosum* has been reported to cause infections in healthy individuals. These infections are usually opportunistic and occur under certain circumstances such as trauma, surgery, or in the presence of other underlying medical conditions.<sup>7–9</sup>

In certain instances, *C. ramosum* causes bacteremia, which has been associated with a higher mortality rate, particularly in immunocompromised hospitalized patients.<sup>6</sup> The predominant source of bloodstream infection is the translocation of strains from the digestive system to cutaneous decubitus.<sup>5</sup> Although rifampin resistance was discovered in 1971, tetracycline, aminoglycosides, and fluoroquinolone resistance were also noted; *C. ramosum* did not show multidrug resistance to most antimicrobial agents.<sup>6,10</sup>

Increasing evidence suggests that SARS-CoV-2 infection can compromise the integrity of the intestinal barrier by adversely affecting all layers of defense, including biological, mechanical, and immune barriers.<sup>11</sup> This broken gut barrier allows luminal bacteria, fungi, and endotoxins to spread to typically sterile extraintestinal locations and the bloodstream, thereby promoting systemic inflammation and immune activation.<sup>11</sup> Patients with COVID-19 have been found to have microbiota that are more enriched in virulent bacteria such as enterococci, as well as opportunistic pathogens such as *Clostridium hathewayi*, *Clostridium ramosum*, *Actinomyces viscosus*, *Streptococcus* spp., and *Rothia* spp., when compared to healthy individuals.<sup>12–14</sup> Here, we report a case of septic shock associated with *C. ramosum* bacteremia in a patient with SARS-CoV-2 infection and its genomic characterization.

## Case Report

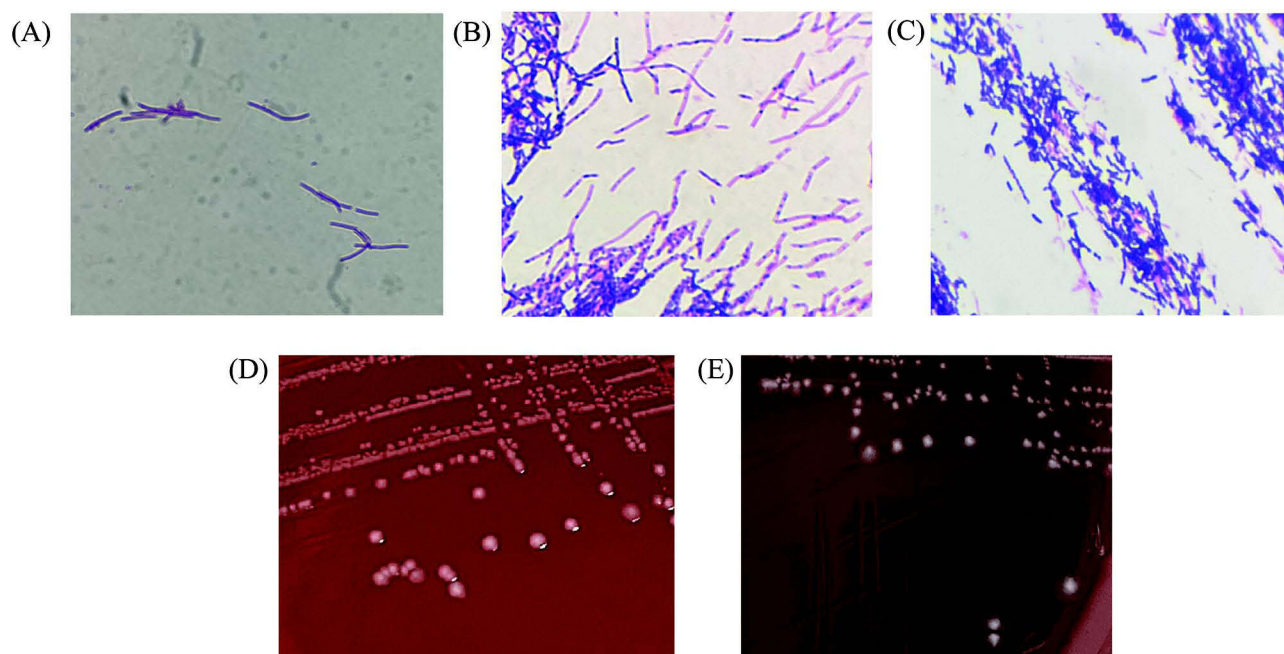
A 73-year-old female patient sought medical attention during the COVID-19 pandemic in China (December 2022) due to persistent right lower limb pain resulting from a fall four days earlier. Upon admission, the patient was in a frail state, exhibited tenderness in the right lower abdominal area, and tested positive for COVID-19. Upon admission, the patient had a normal body temperature. However, the patient presented with symptoms such as chills, a mild cough with phlegm, frequent urination, and two episodes of diarrhea prior to admission.

Laboratory testing showed a white blood cell count of  $3.9 \times 10^9/L$  (94.7% neutrophils), platelets  $131 \times 10^9/L$ , hemoglobin 110g/L, pH value (T) 7.54, carbon dioxide partial pressure (T) 22.4 mmHg, partial pressure of oxygen (T) 186 mmHg, lactic acid 4.7 mmol/L, glutamic pyruvic transaminase 49 U/L, glutamic oxaloacetic transaminase 159 U/L, albumin 22.3 g/L, potassium 3.08mmol/L, urea 14.52 mmol/L, creatine kinase 2885 U/L, creatine kinase isoenzyme 58 U/L, lactate dehydrogenase 519 U/L, and high sensitivity C-reactive protein 319.9 mg/L. The patient was treated with imipenem 0.50g q6h and vancomycin 0.5 q6h for two days. Unfortunately, the patient was discharged on the third day after admission and died. Her laboratory test results were notable for a white blood cell count of  $11.9 \times 10^9/L$  (89.2% neutrophils), platelets  $11 \times 10^9/L$ , high sensitivity C-reactive protein >370mg/L, IL-6 956.65 pg/mL, and lactic acid concentration 6.5 mmol/L. Abdominal computed tomography (CT) revealed free air around the right retroperitoneal area (Figure 1), which was diagnosed as intestinal perforation.

Blood specimen from the patient was injected into conventional aerobic and anaerobic bioMérieux BacT/Alert bottles. One of the anaerobic bottles (bottle 1) became positive after 57.4 hours of incubation, and the other anaerobic bottle (bottle 2) became positive after 68.5 hours of incubation. Gram staining revealed a weakly gram-positive rod (Figure 2A). After 24 h of anaerobic incubation, the organism produced a pure culture on blood agar but did not grow aerobically in subculture. A pure culture of tiny, grayish, smooth, spherical, and non-hemolytic colonies with intact edges



**Figure 1** Images of abdomen computed tomography (CT). Free air was observed around the right retroperitoneal area (indicated by red arrows).



**Figure 2** (A) Weakly gram-positive bacilli were stained by *C. ramosum* anaerobic bottle culture for 24 hours. (B) The staining of *C. ramosum* in blood agar for 24 hours was gran-variable/negative. (C) The staining of *C. ramosum* in blood agar for 48 hours was gram-positive. (D) A pure culture of small, grayish, smooth, round, non-haemolytic colonies with whole edges after 24 hours in blood agar. (E) The colonies of *C. ramosum* in blood agar for 48 hours were turns to fried egg-shaped, and the color turns to light brown.

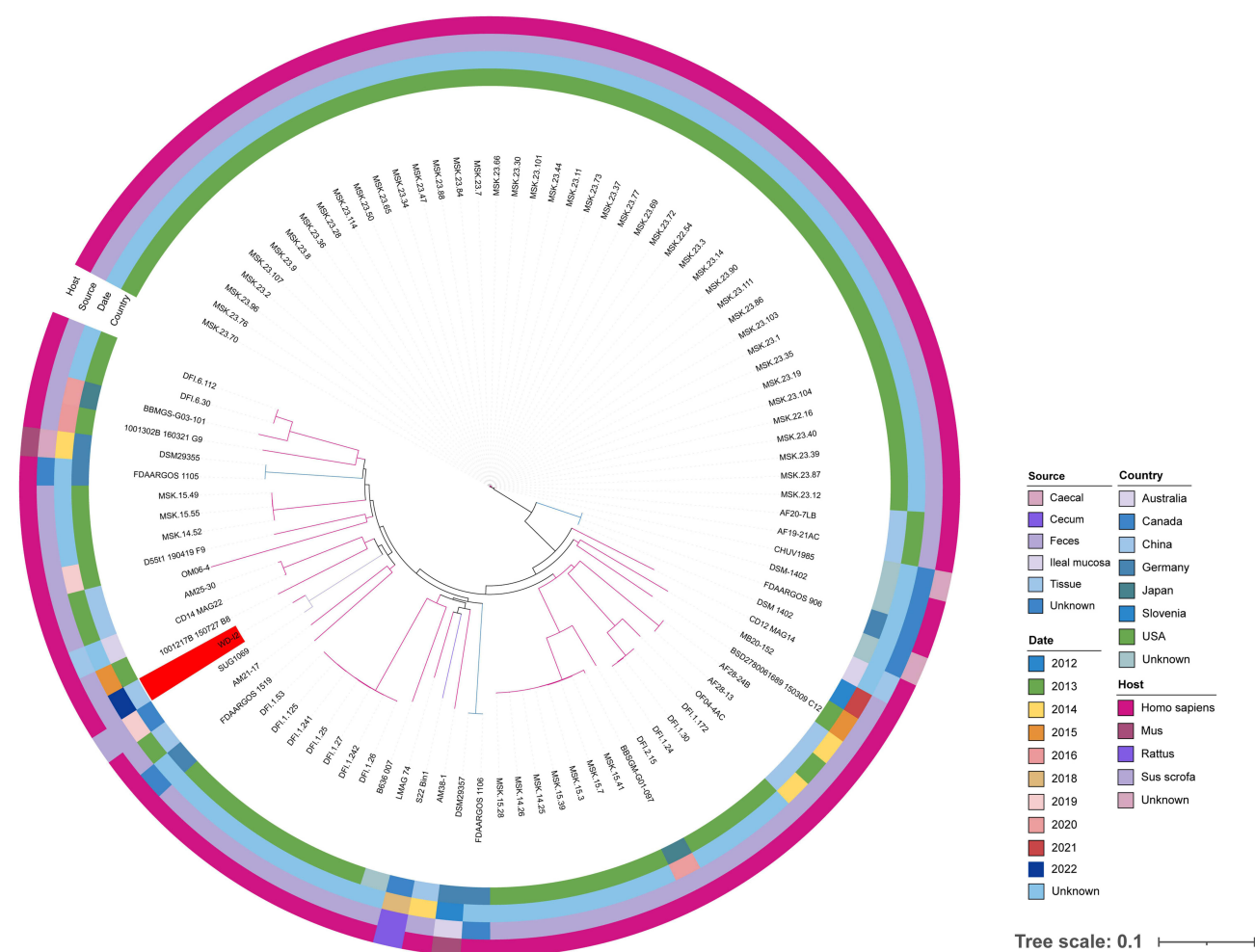
was obtained after 24 h (Figure 2D). The colonies of *C. ramosum* grown in blood agar for 48 h turned egg-shaped, and the color turned light brown (Figure 2E). Gram staining showed gram-variable/negative rods after 24 h (Figure 2B), which became more strongly gram-positive later in culture (Figure 2C). *C. ramosum* isolate WD-I2 was first identified using matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS VITEK, bioMérieux) and then verified using 16S rRNA gene sequencing. Antimicrobial susceptibility testing for the following antimicrobial agents: penicillin, ampicillin, ceftriaxone, tetracycline, meropenem, imipenem, clindamycin, chloramphenicol, sulfamethoxazole and metronidazole was performed by broth microdilution method and interpreted using the Clinical and Laboratory Standards Institute (CLSI) guidelines (M100-S32 and M11-A8), except for vancomycin, erythromycin, gentamicin, and ciprofloxacin, which does not have a valid breakpoint.

Whole-genome sequencing of *C. ramosum* WD-I2 was performed using an Illumina NovaSeq 6000 platform (Illumina Inc., San Diego, CA, USA). A total of 98 *C. ramosum* strains were retrieved from the NCBI GenBank database to obtain all publicly available *C. ramosum* genomes on January 8th, 2023. Phylogenetic analysis of *C. ramosum* WD-I2 and other publicly available *C. ramosum* isolates was performed using the Snippy software (<https://github.com/tseemann/snippy>). The phylogenetic tree was visualized using the interactive Tree of Life (iTOL) V5 web server.<sup>15</sup> The genome sequence of *C. ramosum* WD-I2 has been deposited in NCBI GenBank under the accession number JAJQMP000000000.

The genome of *C. ramosum* strain WD-I2 comprises 3,580,417 bp and exhibits a G +C content of 31.5%. The strain was predicted to have 77 contigs, 3536 coding sequences (CDSs), and 68 RNAs (62 tRNA, 3 rRNA, and 3 ncRNA) genes. The resistome of *C. ramosum* strain WD-I2 consists of two antimicrobial resistance genes (*tetM* and *ermB*). The *tetM* gene encodes an efflux pump that actively expels tetracycline and related antibiotics from the bacterial cell, thereby conferring resistance. The *ermB* gene modifies the 23S rRNA component of the bacterial ribosome through methylation, reducing the binding affinity of clindamycin and leading to resistance. Antimicrobial susceptibility testing showed that the isolate was resistant to clindamycin (MIC > 16 µg/mL) and tetracycline (MIC = 16 µg/mL), but still susceptible to a varieties of antimicrobial agents, including penicillin (MIC = 0.25 µg/mL), ampicillin (MIC < 0.25 µg/mL), ceftriaxone (MIC < 0.5 µg/mL), meropenem (MIC < 0.125 µg/mL), imipenem (MIC < 0.125 µg/mL), chloramphenicol (MIC = 1 µg/mL), sulfamethoxazole (MIC < 0.125 µg/mL) and metronidazole (MIC = 2 µg/mL). The MIC of vancomycin, erythromycin,

gentamicin, and ciprofloxacin were 1 µg/mL, >16 µg/mL, 16 µg/mL and 4 µg/mL, respectively. These findings explain the antimicrobial resistance of WD-I2 to tetracycline and macrolides. Notably, the *C. ramosum* strain WD-I2 carries multiple virulence genes, including adherence (*fbpA/fbp68*, *groEL*, *lap*, *flmH*, and *plr/gapA*), regulation (*LisR/LisK*), toxin (*nagH*, *nagK*, and *cy/R2*), antiphagocytosis (*m1B* and *cpsA*), enzyme (*eno*), immune evasion (*cps2K*, *cpsY*, *galE*, *acpXL*, *gtaB*), iron uptake (*hemL*), and serum resistance (*rmlA*), which may be related to the poor prognosis of infection. Among these, *groEL* is a heat shock protein that serves an adhesive function in *Clostridium* spp.<sup>16</sup> and MU-toxins (*nagH* and *nagK*) are modular hydrolytic enzymes that contribute to their pathogenicity.<sup>17</sup> The virulence gene *cy/R2* encodes an exotoxin, which is lethal for a wide range of gram-positive bacteria and plays key roles in the host-pathogen dialog.<sup>18</sup>

The phylogenetic relationship between *C. ramosum* WD-I2 and 97 *C. ramosum* strains currently deposited in the NCBI GenBank database was investigated to evaluate the genomic epidemiological features of *C. ramosum* strains in a global context (Figure 3). These isolates were mainly distributed in USA (71, 72.45%), and the host of isolation mainly distributed in humans (92, 93.88%), which were recovered from the fecal and mucosal tissues with Crohn's disease, except for the isolate collected from a blood sample in this study. To our knowledge, this is the first report of *C. ramosum* bacteremia in China. Phylogenetic analysis showed that the strain closest to our isolated strain WD-I2 was SUG1069, recovered from a pig feces sample from Canada, which differed by 589 SNPs.



**Figure 3** Recombination-filtered core genome phylogeny for a total of 98 *C. ramosum* isolates worldwide deposited in the NCBI GenBank database. The isolation date, host, source, and country are represented by squares of different colors. The isolate recovered by this study was highlighted in the red color.



## Discussion

*C. ramosum* is a gram-positive anaerobic enteric bacterium that typically exists in the human gut microbiota. It is a member of the RIC group, which also includes *Clostridium innocuum* and *Clostridium clostridioforme*. *C. ramosum* rarely affects children (under 5 years old) with inner ear infections or elderly individuals with impaired immune systems.<sup>6,9</sup> The production of IgA proteases by *C. ramosum* may facilitate its penetration of intestinal mucosal defenses, especially in patients who are particularly vulnerable to infection.<sup>19,20</sup> Previous studies have demonstrated that *C. ramosum* can cause a variety of life-threatening conditions, including but not limited to bacteremia, osteomyelitis, septic arthritis, mastoiditis, spondylodiscitis, otitis media, pyelonephritis, septic arterial emboli, endocarditis, gas gangrene, septic pseudoarthrosis, peritoneal dialysis-related peritonitis, and liver abscess.<sup>10,21</sup> The majority of *C. ramosum* isolates have been identified in blood cultures,<sup>21,22</sup> which is one of the three species with the highest case fatality rates in *Clostridium* bacteremia.<sup>23</sup> Adults with underlying cancer or immunosuppression and patients with bowel perforation and abscess formation are particularly vulnerable to *C. ramosum* infections.<sup>8</sup> The source of the bacteremia was probably translocation from the gastrointestinal tract and soft tissue infections.<sup>5</sup> Moreover, the incidence of *C. ramosum* infections is likely underestimated, as *Clostridium* spp. may be unrecognized in cases of polymicrobial bacteremia due to its long incubation periods. Therefore, accurate processing of blood cultures and careful analysis of gram stains are crucial for bacteriological diagnosis of invasive *C. ramosum* infections.

Currently, there are limited studies on bacteremia during the COVID-19 pandemic in China. However, previous reports have indicated that bacteremia is more common in COVID-19 patients than in non-COVID-19 patients.<sup>24–27</sup> The compromised integrity of the intestinal barrier due to SARS-CoV-2 infection and the higher prevalence of *C. ramosum* in the microbiota of COVID-19 patients may contribute to the increased incidence of bacteremia. Additionally, the production of IgA protease by *C. ramosum* may aid in the breach of intestinal mucosal barriers and cause bacteremia.<sup>19,20</sup> It is worth noting that glucocorticoids, which have potent anti-inflammatory actions, can have negative effects on gut barrier function.<sup>28</sup>

## Conclusions

This study presents the first report on the clinical manifestations, antimicrobial resistance mechanisms and genomic features of *C. ramosum* bacteremia recovered from an immunocompetent patient in China with the aim of exploring the potential relationship between *C. ramosum* and COVID-19 infection. Continuous monitoring of *C. ramosum* infections using conventional and whole-genome sequencing techniques is essential for a better understanding of the mechanisms of resistance and transmission dynamics of this emerging bacterial pathogen.

## Ethical Approval

The study was approved by the Ethics Committee of Sanmen People's Hospital, Taizhou, China.

## Patient Consent for Publication

The patient provided written informed consent for the publication of case details and accompanying images.

## Author Contributions

All authors made a significant contribution to the work reported, whether in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas, took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

We declare that we have no conflicts of interest in connection with this paper, and that we received no payment or services from a third party in relation to this study.

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