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Case report

Identification of *Terrisporobacter muris* isolated from human blood using whole-genome sequencing: A case report

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ABSTRACT

We describe a case of *Terrisporobacter muris* bacteremia in a critically ill elderly man with a sigmoid colonic perforation. Initially, MALDI-TOF MS identified the bacterium as *T. glycolicus*; however, 16S rRNA gene sequencing suggested the presence of *T. glycolicus*, *T. mayombei*, or *T. petrolearius*. Owing to the limitations of these methods in distinguishing among the *Terrisporobacter* species, we employed whole-genome sequencing, which revealed the involvement of *T. muris* in the infection. This case represents the second report of *T. muris* isolated from human blood culture and is the first to describe the clinical course of this infection. Our findings underscore the diagnostic utility of whole-genome sequencing in accurately identifying novel infectious agents.

1. Introduction

The genus *Terrisporobacter*, reclassified from the species *Clostridium glycolicum* and *Clostridium mayombei*, comprises anaerobic, spore-forming, gram-positive, or gram-variable, rod-shaped bacteria [1]. Only a small number of human infections involving *Terrisporobacter* species have been reported [2–9]. In 2022, Afrizal et al. described *Terrisporobacter muris* sp. nov from mouse cecal matter [10]. In their study, the average nucleotide identity (ANI) and genome-to-genome distance calculator (GGDC) values of *T. muris* to the two closest *Terrisporobacter* species were below species delineation thresholds, leading them to propose the creation of a new species within the genus *Terrisporobacter*. Notably, the isolate was found to represent the same species as *T. othiniensis*, a proposed species isolated from human blood culture for the first time in 2015 [6,10].

Here, we report the second known case of *T. muris* bloodstream infection identified using whole-genome sequencing (WGS). Our approach included a database search and the construction of a phylogenetic tree for *T. muris* using WGS data. Additionally, we described the phenotypic characteristics and antimicrobial susceptibility profile of the bacterium.

2. Case report

An 86-year-old Asian male with a history of chronic obstructive pulmonary disease (COPD), chronic kidney disease, hypertension,

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and sarcopenia presented to the emergency department with worsening shortness of breath and a feeling of tightness in his chest. The patient reported a sudden worsening of his chronic shortness of breath over the past 24 hours, escalating from modified Medical Research Council (mMRC) dyspnea grade 2 to grade 4 (too breathless to leave the house). On initial presentation, he appeared moderately ill and the patient's vital signs were within normal. Auscultation revealed bilateral wheezes and crackles at the lung bases, with reduced air entry in the lower lobes. Physical examination identified a left-sided pleural effusion. A non-contrast-enhanced chest computed tomography (CT) scan revealed a 7.0 × 7.2 cm consolidation in the left upper lobe lingular segment with obstruction of the segmental bronchus, borderline-sized lymph nodes in the lower paratracheal, subcarinal, and subaortic areas, a massive left pleural effusion, and several nodular infiltrations in the posterior costophrenic angle and pleura of the left lower hemithorax. These findings suggest the possibility of lung cancer with multiple metastases. A pleural fluid study also supported the likelihood of cancer. A CT-guided core needle biopsy confirmed the diagnosis of adenocarcinoma, and a positron emission tomography (PET) CT scan revealed multiple metastases. Subsequently, the patient received three cycles of monthly chemotherapy consisting of Pemetrexed (483 mg, 489 mg, 477 mg), Pembrolizumab (200 mg each cycle), and Carboplatin (153.9 mg, 167.4 mg, 170.1 mg), administered intravenously. When he visited our hospital for regular checkups after his third chemotherapy sessions, he appeared chronically ill but without signs of acute distress. Laboratory tests indicated elevated creatinine and blood urea nitrogen levels, rising from 1.36 to 2.65 mg/dL and from 25.8 to 50.2 mg/dL, respectively, suggesting acute kidney injury superimposed on chronic kidney disease. The following day, the patient was admitted for hydration and nutritional support. Upon admission, the patient developed a fever of 38.3 °C and pneumonia in both lungs, with an increasing demand for oxygen. Empirical antibiotic therapy was initiated with intravenous ceftriaxone (2 g per day) and metronidazole (500 mg three times per day). As his condition worsened, the antibiotic therapy was switched to piperacillin/tazobactam (3.375 g four times per day). He was subsequently transferred to the intensive care unit (ICU) and intubated due to the progression to acute respiratory distress syndrome. The patient developed hematochezia in the following days, and sigmoidoscopy revealed gross perforation of the distal sigmoid colon, which required emergency hemoclipping. Contrast-enhanced abdominal CT scan also showed evidence of colonic perforation. For definite bleeding control, the patient underwent segmental resection of the colon the following day. Postoperative bronchoscopy revealed purulent discharge in the right lower bronchus and severe mucosal flares in both the bronchi. When pneumonia worsened, trimethoprim and sulfamethoxazole (360mg and 1800mg, three times per day) were added to the antibiotic regimen. After 3 weeks of treatment in the ICU, the patient died from the exacerbation of pneumonia. During the hospitalization, a total of 33 sets of bacterial, fungal, and mycobacterial cultures from sputum, peripheral blood, central vein blood, oral cavity swab, urine, IV catheter, rectal swab, Hemovac drainage, Hemovac tip, and bronchial washing were performed. Among them, seven cultures tested positive, whose results are summarized in Table 1. Notably, two sets of central venous blood cultures obtained from the right jugular catheter following a bronchoscopy examination tested positive. Following 24 hours of incubation, the central venous blood cultures were observed as small bacterial colonies on Blood agar and Brucella agar plates. Each culture yielded a single organism. The colonies from the blood cultures appeared tiny and grayish on Brucella agar plates. Gram staining revealed gram-variable rod-shaped bacteria (Fig. 1).

We employed matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to identify the organism. Following the manufacturer's instructions, we isolated the colonies and transferred them to a target plate, overlaid them with a matrix (alpha-cyano-4-hydroxycinnamic acid), and analyzed them using the MALDI Biotyper Sirius (Bruker Daltonics, Bremen, Germany). The resulting spectra were compared to a reference library (MBT IVD Library v12.0.0.0) for identification. MALDI-TOF MS initially identified the bacterium from the blood cultures as *C. glycolicum*, which was the former designation of *T. glycolicus*. However, this identification was considered inconclusive because the database only included *C. glycolicum* and *C. mayombei* (former designations of *T. glycolicus* and *T. mayombei*, respectively) among *Terrisporobacter* species.

The 16S ribosomal RNA gene sequencing revealed percentage identities of 98.76 %, 98.72 %, and 97.56 % with *T. mayombei*, *T. glycolicus*, and *T. petrolearius*, respectively, for the isolated bacterium. Bacterial DNA was extracted using the boiling method from a 0.5 McFarland suspension of bacterial cells, and sequencing was performed using the Sanger method with BigDye® terminator chemistry (Applied Biosystems, Waltham, MA, USA) on an ABI 3730xl DNA Analyzer (Applied Biosystems, Waltham, MA, USA). The primer sets used for 16S rRNA gene sequencing were as follows: 4F-TTGGAGAGTTTGATCCTGGCTC, 534R-TACCGCGGCTGCTGGCAC, 27F-AGAGTTTGATCMTGGCTCAG, 801R- GGCGTGGACTTCCAGGGTATCT.

According to the Clinical and Laboratory Standards Institute (CLSI) guideline MM18, 2nd edition, "Interpretive Criteria for Identification of Bacteria and Fungi by Targeted DNA Sequencing," species-level identification requires a % identity of 99 % or higher,

Table 1
Positive culture results obtained during hospitalization.

Date (day/month)	Material	Culture result	Remarks
19/06	Rectal swab	*Klebsiella pneumoniae*	CPE
20/06	Rectal swab	*Klebsiella pneumoniae*	CPE
26/06	Hemovac drainage	*Klebsiella pneumoniae*	CPE
		Enterococcus faecium	
28/06	Bronchial washing	*Acinetobacter baumannii* complex	
30/06	Urine	*Enterococcus faecium*	
	Blood	*Terrisporobacter* spp.	
03/07	Hemovac tip	*Klebsiella pneumoniae*	ESBL(+) CPE

Abbreviations: CPE, Carbapenemase-producing*Enterobactarales*; spp., Species pluralis; ESBL, Extended-spectrum beta-lactamases.

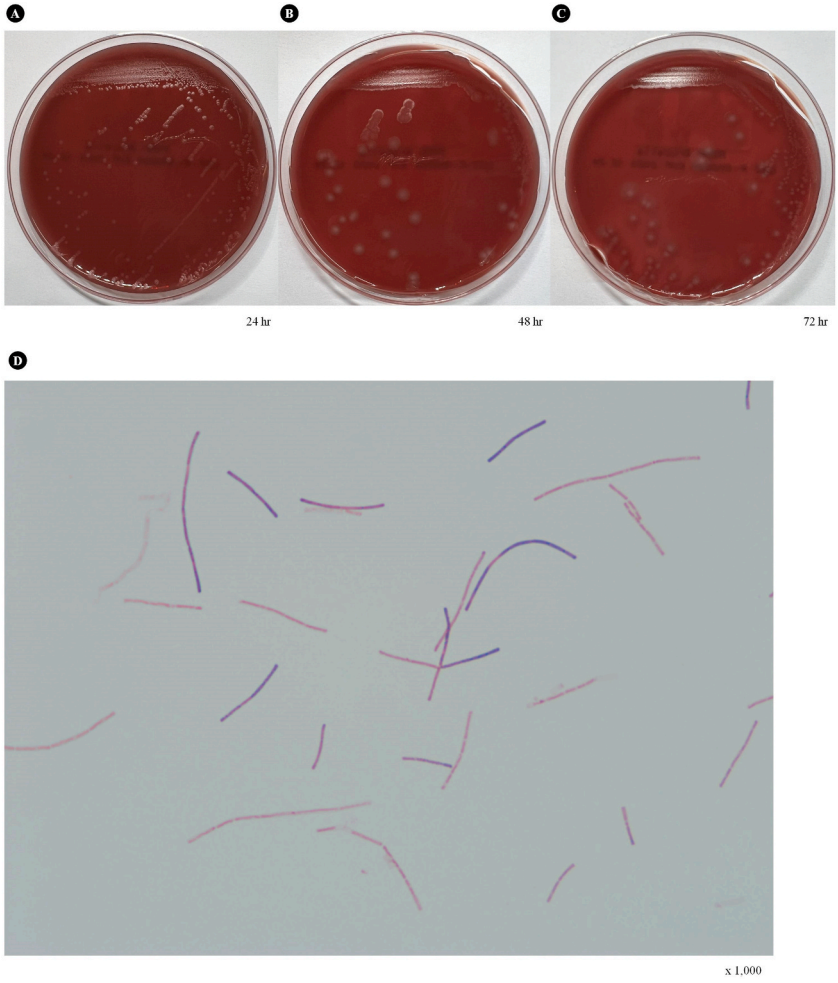

Fig. 1. Subculture of the isolate on Brucella agar plates (A, B, C). D. Gram staining of the isolate.

while a range of 97 %–99 % indicates genus-level identification [11]. In our case, the % identity values of 98.76 %, 98.72 %, and 97.56 % with *T. mayombei*, *T. glycolicus*, and *T. petrolearius*, respectively, indicated genus-level identification as *Terrisporobacter* species. Due to the inability to achieve species-level identification, the isolate was reported as "Most closely related to *Terrisporobacter* spp." This ambiguity of the 16S rRNA gene sequencing result, together with the limitations of the MALDI Biotyper Sirius database, presented the challenges in the precise identification of the bacterium.

To identify the bacterium at the species level, we performed WGS on a NextSeq2000 system (Illumina, San Diego, CA, USA) using NextSeq 2000 P2 Reagents (300 Cycles) v3, following library preparation with the Illumina DNA Prep Library Preparation Kit (#20060060, Illumina). For 16S rRNA analysis, we extracted 16S rRNA sequences from the assembly and compared them with those from five reference genomes of the genus *Terrisporobacter*. All reference genomes were downloaded from the National Center for Biotechnology Information (NCBI) database. The accession numbers are as follows: *T. muris*: GCF_020809405.1, *T. othiniensis*: GCA_020809405.1, *T. mayombei*: GCF_032598785.1, *T. glycolicus*: GCA_036812735.1, *T. hibernicus*: GCA_032598785.1, and *T. petrolearius*: GCF_036812735.1.

Subsequently, we used CLUSTALW to perform multiple sequence alignment [12]. A maximum likelihood tree for the 16S rRNA data was constructed using IQ-TREE (v2.2.03) [13], applying ModelFinder3 [14], tree reconstruction, and ultrafast bootstrapping (1000 replicates) [15]. The best-fit model based on the Bayesian Information Criterion (BIC) was HKY (unequal transition/transversion rates and unequal base frequencies) + F (empirical base frequencies). For WGS analysis, we performed pairwise comparisons between each genome of *Terrisporobacter* species and the assembled sequences using pyani (v0.2.12) with the MUMmer3 algorithm [16] to determine ANI value and coverage. Phylogenetic trees based on 16S rRNA and ANI were constructed using the Interactive Tree Of Life (ITOL) [17] (Fig. 2).

The WGS results indicated that the bacterium was *T. muris*, exhibiting an ANI of 99.72 % and an ANI coverage of 90.44 %. When we used the 16S rRNA sequence of *T. muris* as the reference, our isolate demonstrated a complete identity match of 100.00 % (Table 2).

In addition, we uploaded the WGS data to the Type (Strain) Genome Server (TYGS) for whole genome-based taxonomic analysis

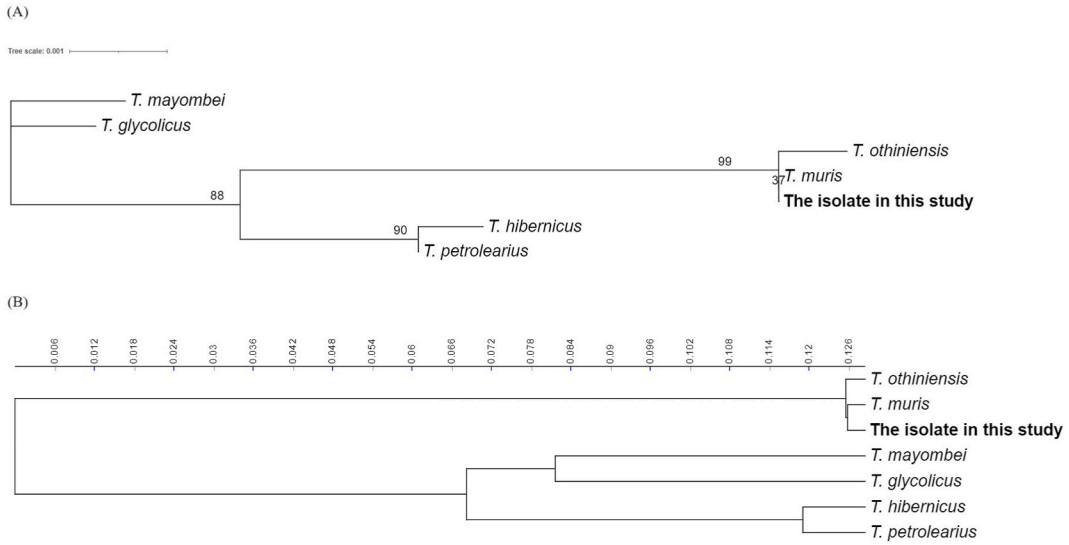

Fig. 2. Phylogenetic analysis based on (A) 16S rRNA sequences using IQ-TREE (v2.2.03), applying ModelFinder3, tree reconstruction, and ultrafast bootstrap (1000 replicates) options, or (B) a whole genome-based dendrogram built with distance matrices based on overall sequence similarity from pairwise comparisons obtained using ANIm (average nucleotide identity MUMmer3) algorithms.

[18]. According to the TYGS results, the bacterium is equally likely to belong to *T. muris* or *T. othiniensis*, based on the pairwise digital DNA-DNA hybridization (dDDH) values calculated using formula d4, which is the sum of all identities found in high-scoring segment pairs (HSPs) divided by overall HSP length [19]. The dDDH values between our strain and *T. muris* and *T. othiniensis* were 97.3 % and 96.4 %, respectively.

Using this clinical isolate, we determined the minimal inhibitory concentration (MIC) of a range of antibiotics against *T. muris* using an epsilometer test (Table 3). The epsilometer test was replicated three times, with identical results across all replicates.

3. Discussion

In 1963, Gaston and Stadtman first identified *C. glycolicum* (later reclassified as *T. glycolicus*) in mud [20]. However, the clinical relevance of *Terrisporobacter* species has only recently been acknowledged. Blood cultures from a bone marrow transplant patient diagnosed with Hodgkin's lymphoma yielded *T. glycolicus* in 2007 [2]. It has also been isolated from an otogenic brain abscess [3], a scalp wound containing a foreign body [4], a wrist wound sustained in a motor vehicle accident [4], blood from a patient with acalculous cholecystitis [5], a deep surgical site with prosthetic material [7], blood from a patient with urinary tract infection [8], and blood from a patient with cirrhosis [9]. In addition, Lund et al. isolated a possible novel species belonging to the *Terrisporobacter* genus from a sepsis patient's blood culture and named it '*Terrisporobacter othiniensis*' [6]. More recently, a mouse gut microbiome study proposed the name '*Terrisporobacter muris*' for bacteria isolated from the cecal content of a specific pathogen-free (SPF) mouse, as a heterotypic synonym for '*Terrisporobacter othiniensis*' [10].

In our study, the initial 16S rRNA gene sequencing was inconclusive, suggesting that the organism could be any of three species: *T. mayombei*, *T. glycolicus*, or *T. petrolearius*. In a separate case report of *T. glycolicus*, in which a surgical site infection with prosthetic material was described, the phenotypic identification method misidentified the organism as *Clostridium difficile* [7]. Consistent with our report, 16S rRNA gene sequencing was unable to achieve species-level identification within the genus *Terrisporobacter*, whereas MALDI-TOF MS identified the isolate as *C. glycolicum* (the former designation of *T. glycolicus*). The authors of this report attributed the misidentification of the phenotypic analysis to an unresolved taxonomic dispute between *C. difficile* and *T. glycolicus* [21]. Notably,

Table 2
Whole-genome sequence assembly results used to identify *Terrisporobacter muris*.

Species (Hit taxon)	ANI (%)	ANI coverage (%)	16S rRNA gene identity (%)[a]
Terrisporobacter muris	99.72	90.44	100.00
Terrisporobacter othiniensis	99.61	93.34	99.93
Terrisporobacter mayombei	88.30	62.10	99.23
Terrisporobacter glycolicus	88.11	64.82	99.65
Terrisporobacter hibernicus	87.89	60.00	99.20
Terrisporobacter petrolearius	87.86	62.98	99.27

Abbreviations: ANI, Average nucleotide identity; rRNA, Ribosomal ribonucleic acid.
[a] Sequence data obtained from whole-genome sequences.

Table 3
Antimicrobial susceptibility of *Terrisporobacter muris* clinical isolate determined using an epsilometer test.

Antimicrobial	MIC (μg/mL)
Piperacillin/Tazobactam	16
Penicillin	1
Metronidazole	0.064
Meropenem	6
Vancomycin	0.25
Cefoxitin	24
Ceftriaxone	>32
Imipenem	1
Trimethoprim/sulfamethoxazole	>32

Abbreviations: MIC, Minimum inhibitory concentration.

they did not perform further studies, such as analysis of genomic DNA G + C content, predominant cellular fatty acids, and products from peptone yeast extract, to pinpoint the exact identity of the organism. This case and our study illustrate the challenges in reliably identifying *Terrisporobacter* species using conventional methods.

T. othiniensis and *T. muris*, both names not yet validated, were originally described as different strains [6,10]. The ambiguous TYGS results in our study, with dDDH values of 97.3 % and 96.4 % for *T. muris* and *T. othiniensis*, respectively, indicate challenges in definitively identifying our bacterium. Recent genome-based metabolic and phylogenomic analyses suggest that both strains belong to the same species [22]. In our WGS study, the organism isolated from the patient showed the highest ANI and 16S rRNA gene identity with the *T. muris* strain, exceeding the species-level identification threshold. According to the study describing the taxonomy of *T. muris*, *T. muris* has close 16S rRNA gene sequence similarities of 99.23 % with *T. mayombei* and 99.16 % with *T. glycolicus*, indicating significant genetic resemblance among these organisms as well [10].

In cases where rare bacteria cannot be definitively identified to the species level using routine tests, previous studies have demonstrated that species-level identification can be achieved using WGS methods [23–25]. Our results highlight the significant role of WGS in the precise identification of novel pathogens, particularly in situations where routine testing yields inconclusive results.

Several reported cases of *T. glycolicus* infections suggest that hematogenous seeding from the gastrointestinal (GI) tract is a likely origin because of the bacteria's known residence in the GI tract. For instance, in the case of a bone marrow transplant recipient [2], the authors speculated that chemotherapy regimens for Hodgkin lymphoma might damage the gut mucosa. This damage may facilitate the translocation of *T. glycolicus* from the gut to the bloodstream. Similarly, in a patient with acalculous cholecystitis [5], the presence of the bacterium in the blood was hypothesized to have arisen from the gallbladder, given its anatomical proximity to the GI tract. In line with these interpretations, perforation in our patient's sigmoid colon provided a plausible route for *T. muris*, which is likely a part of the gut microbiota, to enter the bloodstream.

4. Conclusion

In summary, we report a case of bacteremia caused by *T. muris* in a critically ill patient with a sigmoid colon perforation. We also present the results of antibiotic susceptibility tests. Because this is the second reported case of *T. muris* infection, the clinical significance of *T. muris* merits further investigation. Notably, the successful use of WGS for the precise identification of bacteria highlights the importance of a whole-genome approach for identifying emerging infectious agents.

Ethical declaration

The Institutional Review Board of Korea University Guro Hospital, Seoul, Korea approved this study (IRB no. 2023GR0387) and waived the need for informed consent. This manuscript follows the ethical principles included in the declaration of Helsinki.

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Consent to publish

Not applicable due to insufficient data to recognize the patient.

Data availability

The sequenced genomes in the current study have been deposited at the NCBI Sequence Read Archive (SRA): PRJNA1133157 and

are publicly available as of the date of publication.

CRediT authorship contribution statement

Imseok Kang: Writing – original draft, Methodology, Data curation. **Dong-Jae Youm:** Formal analysis. **Inhee Kim:** Software. **Jongmun Choi:** Methodology. **Jung Yoon:** Conceptualization. **Soo Young Yoon:** Supervision, Funding acquisition. **Chae Seung Lim:** Funding acquisition, Conceptualization. **Min-Chul Cho:** Writing – review & editing, Investigation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Soo Young Yoon reports article publishing charges was provided by the Korea Medical Device Development Fund grant. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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