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Case Report

Can *Bacillus paranthracis* cause bacteremia in a T-ALL patient? WGS-based diagnosis

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Abstract

Introduction: Most of *Bacillus* spp. generally avirulent species in healthy patients, but if there is a fragile in the patient, especially their immune system, *Bacillus* spp. can be an agent for infections.
Case Report: In this case, we report that *Bacillus paranthracis*, diagnosed by whole genome sequencing, is responsible for bacteremia in a T-ALL patient. A 26-year-old male patient was diagnosed with T-cell acute lymphoblastic leukemia. *Bacillus paranthracis* was isolated from two sets of blood cultures obtained from a patient with febrile neutropenia.
Results: The bacteria was identified as *Bacillus cereus* group in a routine microbiology laboratory by MALDI TOF MS. Then whole genome sequencing (WGS) confirmed its name as *Bacillus paranthracis*. The pathogenicity of the bacterium, especially in immunocompromised patients, has also been demonstrated by WGS.
Conclusions: In a microbiology laboratory, the use of Whole Genome Sequencing (WGS) is important for diagnosing diseases, especially in immunocompromised patients. It will serve the management of these patients for infection control.

Key words: *Bacillus paranthracis*; bacteremia; hematologic malignancy; whole genome sequencing.

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Introduction

Although medical advancements have enhanced the efficacy of treatment, chemotherapy and interventional procedures increase the chances of contracting life-threatening infections, especially sepsis [1]. The low immunity of immunocompromised patients makes them vulnerable to many microorganisms. Bacterial growths that are considered to be contaminants for patients who are not immunocompromised may act as causative agents of infections in immunocompromised patients. Therefore, contaminants need to be correctly identified in immunosuppressive patients for their effective clinical management [2]. With an increase in the diagnostic capacity of microbiology laboratories, the diversity of microorganisms isolated in bacteremia and sepsis has also increased. In these laboratories, the diagnosis from a blood culture bottle with a positive signal starts with the gram stain, and then laboratories can make many tests according to their capacity. MALDI TOF MS can be used to quickly identify culture samples from a vial with a positive signal. However, in some cases, identification by MALDI TOF MS may also be insufficient [3].

Bacillus cereus group 21 is a member of the family Firmicutes. *Bacillus paranthracis*, another member bacteria in this group, which is isolated as environmental isolates and rarely as causative agents in humans [4,5]. In this article, we described the process of the microbiological diagnosis of *B. paranthracis* isolated from four blood culture bottles of the two sets of blood cultures obtained from an immunocompromised patient with hematologic malignancy.

Case Report

A 26-year-old male patient, a lesion with a size of 30 mm was observed in anterior mediasten on computed tomography (CT), diagnosed with T-cell

acute lymphoblastic leukemia (T-ALL) was hospitalized on the 20th of October 2023. Hyper-CVAD regimen (the combination of hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone alternating with high-dose methotrexate and cytarabine), was started as induction treatment. His body temperature did not increase for 10 days. On the 10th day following hospitalization, febrile neutropenia started and his body temperature increased to 38.9 °C, and two sets of blood cultures were collected. Moreover, he was taking piperacillin tazobactam.

Microbiology Laboratory Examinations

Blood culture

We detected a positive signal from four blood culture bottles among two sets of blood culture bottles loaded into the Autobio 120BC (Autobio, China) blood culture system at the sixth hour. Following gram staining of the blood culture bottles with positive signals, large gram-positive bacilli were detected. The blood cultures were inoculated with 5% sheep blood agar and incubated overnight in an incubator at 35 °C under normal atmospheric conditions. Beta-hemolyzed, dull, granular colonies were detected.

MALDI TOF Mass spectrometry

Based on the culture results obtained after incubation, the bacterial species were identified as *Bacillus cereus* (score 2.32) by matrix-assisted laser desorption ionization-time of flight mass spectrometry analysis (MALDI Biotyper® Sirius System) (Bruker, Germany). As MALDI-TOF MS cannot reliably differentiate between strains in the *B. cereus* group, whole-genome analysis was performed.

Antibiotic susceptibility

Based on a discussion among experts in the clinic, the bacteria grown were considered to be the causative agent and an antibiotic susceptibility test was performed by the disc diffusion method, according to EUCAST; briefly, discs containing imipenem (10 µg), meropenem (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), and vancomycin (5 µg) were used to conduct the test. The results of the antibiotic susceptibility test showed that the bacteria were susceptible to imipenem, meropenem, and vancomycin, and they were also susceptible to ciprofloxacin and levofloxacin, but in a dose-dependent manner [6].

Whole-genome sequencing analysis

The DNA was extracted from the samples collected from different sites, including oropharyngeal, skin lesion, and nasopharyngeal regions, using the EZ1&2™ Virus Mini Kit (QIAGEN, Germany), following the manufacturer's instructions. The extracted DNA was quantified using a Qubit 4.0 Fluorometer (Invitrogen, Singapore) using a dsDNA High-Sensitivity Assay Kit (Life Technologies, USA), following the manufacturer's guidelines. The concentration of DNA was found to be 8.4 ng/µL. A barcoded sequencing library was prepared, and whole-genome sequencing was performed with 700 ng of the extracted DNA using the Native Barcoding Genomic DNA Kit (SQK-NBD114.24) - Oxford Nanopore Technologies (ONT, UK). The library was loaded onto FLO-MIN114, R10 version flow cells on the GridION device (ONT, UK).

We performed de novo assembly using the Flye (v.2.9.3) [7] assembler to analyze the sequencing dataset. The contigs generated were aligned to the reference genomes downloaded from the "nucleotide" database in NCBI using BLAST (v.2.15) [8]. Species with more than 90% pairwise identity and 80% query coverage were identified, and sequencing reads were mapped to the respective genomes using minimap2 (v.2.26) [9]. The best alignment was determined by performing a downstream analysis (evaluating mapping statistics), according to the reference sequence percentage and mean coverage statistics. Polymorphisms were identified, and a consensus genome was generated using the Geneious Prime® 2022.2.2 analysis program. Genome annotation was performed using Geneious Prime. Antibiotic resistance genes were identified using the Resistance Gene Identifier (RGI) tool in the Comprehensive Antibiotic Resistance Database (CARD) [10]. Secondary metabolites were identified using antiSMASH (bacterial version) [11]. Moreover, virulence factors and their related genes within representatives of the Bacillus cereus group were identified through analysis using the Virulence Factor Database (VFDB) [12].

The isolated causative agent was identified as *Bacillus paranthracis* strain PR1 with 94.3% equivalence by whole-genome sequencing (Oxford Nanopore) (Supplementary Table 1). The *van*Y gene of the strain was positive in the whole-genome analysis; however, this is not the gene primarily responsible for glycopeptide resistance; gene positivity is generally not dependent on glycopeptide resistance. Bacteria might develop resistance using the efflux system with the *qac*J gene, especially quaternary ammonium resistance and antiseptic resistance. Pathogenicity genes related to

enzymes, toxins, the secretion system, capsule, and iron acquisition were also identified from the whole-genome analysis: phosphatidylinositol-specific phospholipase C (*pip*lc), sphingomyelinase (*sph*), phosphatidylcholine-preferring phospholipase C (PC-PLC) (*plc*A), immune inhibitor A metalloproteinase (*inh*A), Bacillibactin responsible for iron acquisition (*dhb*C and *dhb*A), Type VII secretion system (*esx*B and *ess*C), Cereolysin O (*alo*), Hemolysin III (*hem*III), non-hemolytic enterotoxin (*nhe*A, B, and C), and polysaccharide capsule Capsular polysaccharide (*wec*C) (Supplementary Table 2).

Discussion

The *Bacillus paranthracis* strain, which was isolated from a patient with hematologic malignancy and evaluated as the causative agent, was identified as a part of the *Bacillus cereus* group by the MALDI system and identified as *B. paranthracis* by whole-genome analysis. Due to an improvement in the diagnostic capability of microbiology laboratories, the likelihood of accurate identification of causative agents at the species level has also increased. Accurate identification is required for the effective management of patients. Whole-genome analysis provides more information about the characteristics of the bacteria and facilitates a more accurate determination of the virulence properties of bacteria. The bacterial strain we isolated as the causative agent had efflux genes that provided resistance to quaternary ammonium compounds and antiseptics. Characteristics that promote pathogenicity, such as phospholipase, sphingomyelinase, metalloproteinase, cerolysin O, hemolysin, non-hemolytic enterotoxin, and capsule, may also contribute to the infectivity of *B. paranthracis*, especially in immunocompromised patients. These characteristics helped us determine that the bacteria were responsible for causing infection in the immunocompromised patient.

Opportunistic pathogens are frequently found to be the causative agents of infection in immunocompromised patients. Although *Bacillus* species are less likely to cause sepsis, especially in patients with hematological malignancy, they should not be ignored because they may cause serious complications in immunocompromised patients. *Bacillus* sp. should also be kept in mind in prophylactic treatment. In a study conducted in Japan, 12 patients were diagnosed with *Bacillus cereus* septicemia. Thus, *Bacillus cereus* should be monitored in patients with hematological malignancy, especially those who are administered intensive chemotherapy. *Bacillus* growth in even a single blood culture bottle in this group of patients should not be considered as contamination and new blood culture samples should be collected for evaluation as the causative agent. In the same study, the probability of sepsis cases caused by *Bacillus* species was found to increase, especially if the patient had a central venous catheter. The researchers recommended that healthcare-associated infections should be monitored in these clinics [13].

The ability of *B. paranthracis* to form endospores and biofilms, metabolize polyhydroxybutyrate, and the presence of genes that impart resistance to carbon starvation help this species survive under adverse conditions in different types of environments. These characteristics allow them to thrive on the surface of book pages in the library, in the depths of the Pacific Ocean, and even in human intestinal flora [4].

In another study, *B. paranthracis* was found to be the causative agent through WGS in an outbreak caused by a foodborne *Bacillus cereus* group. The emetic toxin produced by these bacteria may be responsible for this outbreak. That study also provided insights into the use of WGS in foodborne outbreaks [14]. Identifying the main source of outbreaks can also help predict possible causative agents, especially in immunocompromised patients.

These properties that make *Bacillus* species pathogenic may help them colonize the transient microbiota and the intestinal epithelium, and may also give rise to bacteremia with such properties. However, further comprehensive studies are needed to confirm this speculation. A study reported that *B. paranthracis*, which was also isolated from the blood of a patient infected with Ebola, may act synergistically with the Ebola virus [15].

Although MALDI TOF MS has made routine microbiology laboratory tests fast and reliable, it fails to identify the *Bacillus cereus* group at the species level. Therefore, species-level identification of these bacteria requires molecular and sequencing methods, such as PCR and WGS [5].

Conclusions

Whole-genome analysis also reveals the pathogenicity of bacteria and can help determine whether they are causative agents. The data obtained by whole-genome analysis can be used to develop new algorithms for evaluating blood culture contaminants, especially in immunocompromised patients. Although the routine use of WGS is in an early stage, we speculate that its application may become a part of normal clinical protocol within a few years, especially

in laboratories that deal with immunocompromised patients.

Nucleotide sequence accession number

The draft genome sequence of strain *B. ovenparanthracis* PR1 was deposited in the GenBank database under accession number CP040515.1.

Authors' contributions

SSY, AP, EMO, TD, IM, and TD wrote this paper. SSY, AP, EMO, SY, TD, KO, IM, and TD contributed to the diagnosis and clinical decision for diagnosis. All authors reviewed the manuscript. All authors read and approved the final version of this manuscript.

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Conflict of interests

No conflict of interests is declared.

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Annex – Supplementary Items

Supplementary Table 1. The whole genome sequencing properties of of *Bacillus paranthracis* PR1.

RefSeq ID	Average Coverage	# Contigs	Largest Contig	Total Length	N50	N90	L50	L90	GC (%)
NZ_CP040515.1	9X	77	1114862	5461998	770821	82289	3	13	35,93

Supplementary Table 2. The virulence properties of *Bacillus paranthracis* PR1 by identified by WGS.

VF class	Virulence factors	Related genes	***B. paranthracis* PR1 (Prediction)**
			Bacillus paranthracis strain PR1 consensus sequence
Adherence	BslA	bslA	-
Enzyme	Phosphatidylinositol-specific phospholipase C (PI-PLC)	piplc	orf02420
	Immune inhibitor A metalloproteinase	-	orf03346; orf05415
	Sphingomyelinase (SMase)	sph	orf06186
	Phosphatidylcholine-preferring phospholipase C (PC-PLC)	plcA	orf06187
	Immune inhibitor A metalloproteinase	inhA	orf06192
Immune evasion	B. cereus exo-polysaccharide (BPS)	bpsA	-
		bpsB	-
		bpsC	-
		bpsD	-
		bpsE	-
		bpsF	-
		bpsG	-
		bpsH	-
		bpsX	-
	Hyaluronic acid (HA) capsule	hasA	-
		hasB	-
		hasC	-
	Polyglutamic acid capsule	capA	-
		capB	-
		capC	-
		capD	-
		capE	-
	Polysaccharide capsule	-	orf00310; orf00312; orf00313; orf00314; orf00326; orf00327; orf00328
Iron acquisition	Bacillibactin	dhbB	-
		dhbE	-
		dhbF	-
	Hal	hal	-
	Petrobactin	asbA	-
		asbB	-
		asbC	-
		asbD	-
		asbE	-
		asbF	-
	Bacillibactin	dhbC	orf04092
		dhbA	orf04093
	IlsA	ilsA	orf05346
Regulation	AcpAB	acpA	-
		acpB	-
	AtxA	atxA	-
	PagR-XO1	pagR-XO1	-
	PagR-XO2	pagR-XO2	-
	PlcR-PapR quorum sensing	plcR	orf00199
		papR	orf00200
Secretion system	Type VII secretion system	esxL	-
		-	orf04277
		-	orf04280
		esxB	orf04281
		essC	orf04282
Toxin	Anthrax toxin	cya	-
		lef	-
		pagA	-
	Cereulide	cesA	-
		cesB	-
		cesC	-
		cesD	-

		cesH	-
		cesP	-
		cesT	-
	Certhrax	cer	-
	Cytotoxin K (Hemolysin IV)	cytK	-
	Insecticidal crystalline toxins	cry	-
		cyt	-
		vip	-
	Hemolysin III homolog	-	orf00043
	Hemolysin II	hlyII	orf02754
	Anthrolysin O/Cereolysin O/Hemolysin I	alo	orf03010
	Hemolytic enterotoxin HBL	hblC	orf03198
		hblD	orf03199
		hblA	orf03200; orf03201
	Hemolysin III	hlyIII	orf04236
	Non-hemolytic enterotoxin (Nhe)	nheC	orf04680
		nheB	orf04684
		nheA	orf04687
Antiphagocytosis	Capsular polysaccharide (Vibrio)	wecC	orf00316