Supplementary Methods

1. NTS

A 2 mL blood sample was centrifuged at 200 ×g for 2 minutes to remove blood cells, and the was analyzed using Oxford Nanopore MinION or GridION platform. The detailed protocol of NTS has been published as a methodological study in reference 3 of this letter.

2. Blood Culture

Blood samples were collected during a period of high fever (body temperature over 38.5 ℃) with chills. For each blood culture, a total of 40 mL of blood was collected from both a peripheral vein (20 mL) and a venous catheter (20 mL), and subjected to cultivation of aerobic and anaerobic bacteria, and fungi. Blood culturing was performed on an Automated Blood Culture System (Thermo Fisher Scientific, Waltham, MA, USA), according to the manufacturer's instructions.

3. Selection of Antibiotics

The institution-specific clinical guidelines for hematopoietic stem cell transplantation patients with febrile neutropenia are as follows: (1) neutropenia without fever, the prophylactic antibiotics are moxifloxacin or gentamicin + oral fluconazole or voriconazole; (2) after pathogen identification, the therapeutic antibiotics are prescribed according to *The Sanford Guide to Antimicrobial Therapy*; and (3) if no pathogen is identified, empirical antibiotics are prescribed according to the personal experience of the attending physician.

Since no antibiotic resistance mechanisms were detected by the NTS, we chose the antibiotics with the lowest likelihood of drug resistance according to *The Sanford guide to antimicrobial therapy* as well as drug-resistance data from the past 2 years in our local laboratory.

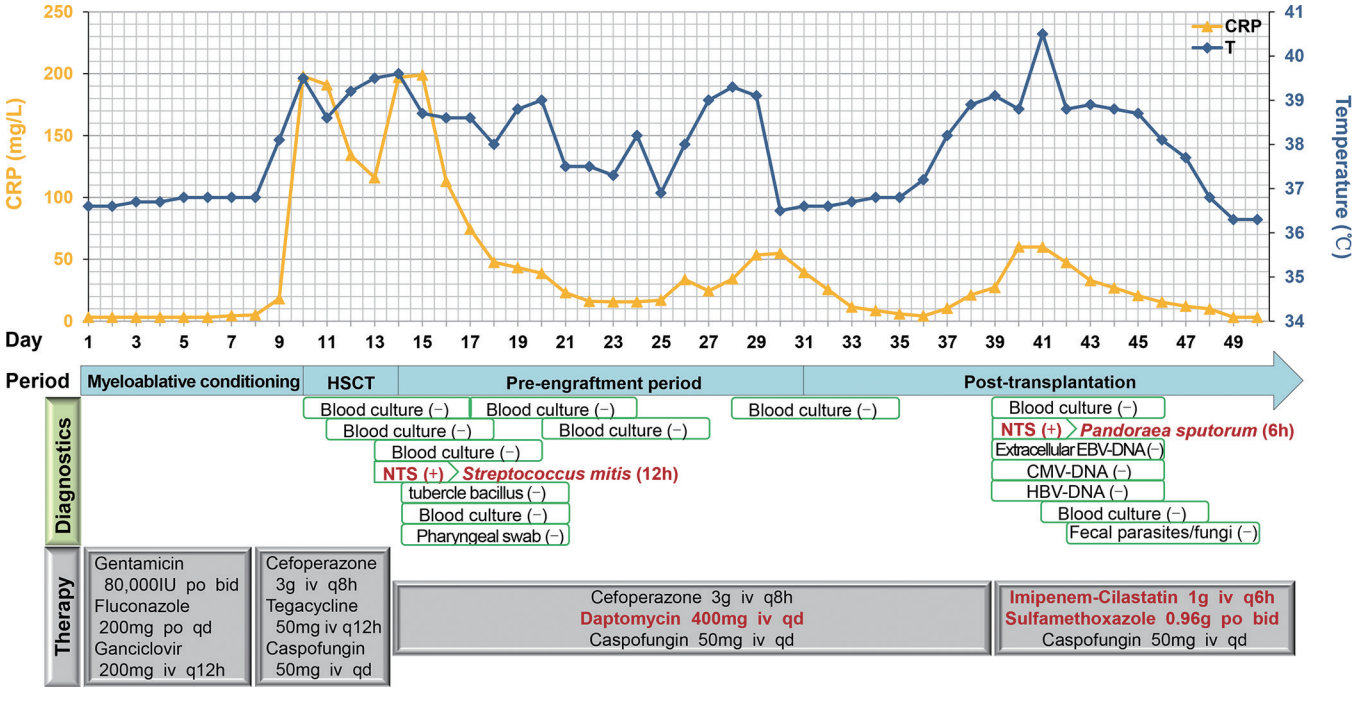

Figure S1 Timeline of clinical management progression of case 1 who underwent allo-HSCT. Timing of laboratory diagnostic tests, including conventional culture and nanopore targeted sequencing, and early antimicrobial therapies are depicted. CRP: C-reactive protein; temperature: top body temperature of the day; myeloablative conditioning regimen: etoposide + busulfan + cyclophosphamide + antithymocyte globulin; allo-HSCT: allogeneic hematopoietic stem cell transplantation; NTS: nanopore targeted sequencing; CMV: cytomegalovirus; EBV: Epstein-Barr virus; HBV: hepatitis B virus.

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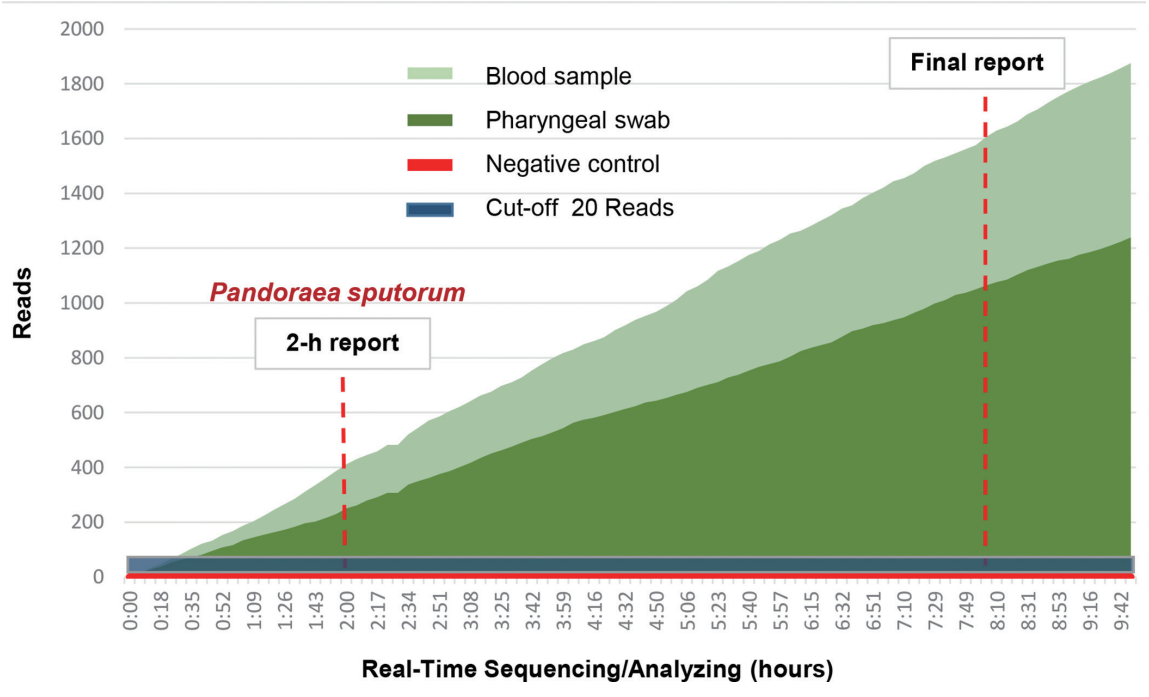

Figure S2 The second real-time nanopore-targeted sequencing of case 1. Highly abundant, multidrug-resistant *Pandoraea sputorum* was detected in blood after 25 min of sequencing, as well as in pharyngeal swab after 40 min of sequencing. The pathogen was report within 2 h after NTS. The clinical turnaround time was less than 6 hours.

Table S1 Clinical characteristics of the 11 patients with severe infections

Case	Gender	Age (years)	Primary disease	Treatment for primary disease	Immunosuppressive agents	Symptoms	WBC (10^9/L) at infection
1	Female	15	ALL	Allo-HSCT	FK506+MTX+MMF+anti-CD25+ATG	High fever, oral ulcer, diarrhea	0.02
2	Female	41	AML	Allo-HSCT	FK506+MTX+MMF+anti-CD25+ATG	High fever	0.26
3	Male	25	MAL	Allo-HSCT	FK506+MTX+MMF+anti-CD25+ATG	High fever, oral ulcer	0.04
4	Male	52	MDS	Allo-HSCT	FK506+MTX+MMF+anti-CD25+ATG	High fever, cough, diarrhea	0.3
5	Male	50	AML	Allo-HSCT	FK506+MTX+MMF+anti-CD25+ATG	High fever	0.02
6	Male	17	ALL	Allo-HSCT	FK506+MTX+MMF+anti-CD25+ATG	High fever	0.01
7	Female	31	AML	Allo-HSCT	FK506+MTX+MMF+anti-CD25+ATG	Low fever, vomiting, diarrhea	3.77
8	Male	61	AML	Chemotherapy	None	High fever, cough, diarrhea, septic shock	0.92
9	Male	37	ALL	Allo-HSCT	CSA+MTX+ anti-CD25	High fever	0.01
10	Male	31	CML-AP	Punatinib + allo-HSCT	FK506+MTX+MMF+anti-CD25+ATG	High fever	0.82
11	Male	49	ALL	allo-HSCT	FK506+MTX+MMF+anti-CD25+ATG	High fever, oral ulcer	0.05

AML: acute myeloid leukemia; ALL: acute lymphoblastic leukemia; MAL: mixed-phenotype acute leukemia: MDS: myelodysplastic syndrome; CML-AP: accelerated phase of chronic myeloid leukemia; allo-HSCT: allogeneic hematopoietic stem cell transplantation; WBC: white blood cell; FK506: tacrolimus; MTX: methotrexate; MMF: mycophenolate mofetil; ATG: antithymocyte globulin; CSA: cyclosporine A.

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