Metagenomic next-generation sequencing contributes to the diagnosis of *Gardnerella vaginalis* bacteremia associated with a rare extragenital infection and diagnostic challenges in a 13-year-old female patient: A case report

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Abstract. *Gardnerella vaginalis* (*G. vaginalis*) is a well-recognized cause of bacterial vaginosis but is rarely associated with extragenital infections, particularly in individuals without a history of sexual activity. Metagenomic next-generation sequencing (mNGS) enables unbiased detection of pathogens and has emerged as a valuable diagnostic approach for challenging infectious diseases. The current report outlines a case of *G. vaginalis* bacteremia in a 13-year-old female patient presenting with fever and acute bronchitis. Peripheral blood mNGS, combined with conventional microbiological investigations, was performed to identify the causative pathogen. Initial empirical treatment with piperacillin-tazobactam and azithromycin failed to control the recurrent fever. Traditional cultures of blood, cerebrospinal fluid and bone marrow aspirate all returned negative results, whereas peripheral blood mNGS identified *G. vaginalis* as the potential pathogen. Following targeted therapy with metronidazole for 3 days, the patient's body temperature returned to normal, and follow-up blood mNGS at discharge was negative for the pathogen. Due to its fastidious growth requirements, *G. vaginalis* is difficult to detect using conventional culture methods, which may lead to delayed diagnosis and false-negative results. The present case highlights the utility of mNGS for timely and accurate pathogen identification in diagnostically challenging infections. The current case also broadens the recognized clinical spectrum of *G. vaginalis* infections by demonstrating its potential to cause bloodstream infection with persistent fever in an adolescent without a reported history of sexual activity.

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Introduction

Gardnerella vaginalis (*G. vaginalis*) is a common cause of bacterial vaginosis (BV), especially in sexually active women. Although it has been isolated from vaginal samples of asymptomatic healthy children (1), symptomatic vulvovaginal infection caused by *G. vaginalis* is uncommon during childhood and adolescence. In addition to BV, *G. vaginalis* has been detected in intrauterine infections, intra-amniotic infections, chorioamnionitis, postabortal pelvic inflammatory disease and postpartum endometritis following cesarean section (2). Extragenital infections caused by *G. vaginalis*, especially bacteremia in individuals without sexual activity, are rare and have not been frequently reported. As a difficult-to-culture, gram-negative or variable bacillus (2), the culture process for *G. vaginalis* usually lasts a few days and may return a negative result (3). Consequently, the true incidence of invasive *G. vaginalis* infection may be underestimated. The limited number of reported cases may be due to the lack of proper diagnostic tools, particularly molecular methods such as 16S rRNA gene sequencing and metagenomics next-generation sequencing (mNGS) (4). These techniques enable culture-independent pathogen detection and may improve diagnosis of fastidious organism that are difficult to identify using conventional microbiological methods. The present report outlines a clinical case of *G. vaginalis* bacteremia in an adolescent girl, diagnosed with the help of mNGS. This case expands the limited literature on invasive *G. vaginalis* infection in non-sexually active adolescents and highlights the importance

of considering this organism in the differential diagnosis of unexplained culture-negative bloodstream infections.

Case report

A 13-year-old girl was admitted to the Department of Pediatrics, The Affiliated Hospital of Yunnan University (Kunming, China), in November 2023, with fever and symptoms suggestive of acute bronchitis. A total of 3 weeks earlier, the girl started coughing repeatedly with sputum and then developed fever >10 days before admission. A chest computed tomography (CT) scan performed at another hospital was reported to show signs of bronchitis, while craniocerebral CT showed no significant abnormalities. However, repeat chest CT examinations performed on hospital days 2 and 7 showed no evidence of bronchitis (data not shown).

On admission (before the first hospitalization day), the initial vital signs of the patient were as follows: Body temperature, 38.3˚C; respiratory rate, 25 breaths/min; pulse, 111 beats/min, indicating mild tachycardia; general condition, stable. Two palpable cervical lymph nodes, ~1x1 cm in size, were observed on the girl's neck; they were mobile, non-tender and not adhering to surrounding tissues, and the neck was supple, with no nuchal rigidity. Physical examination revealed pharyngeal congestion without nasal obstruction, and the bilateral tonsils were normal. Breath sounds were coarse in both lungs, without rale or pleural friction. The abdomen was flat and soft, with no tenderness or rebound tenderness. Neurological examination showed intact physiological reflexes, with no pathological reflexes elicited. Peripheral blood smear showed no heterotypic lymphocytes. Biochemical analysis revealed normal liver and kidney function, electrolyte levels, myocardial enzyme levels, C-reactive protein (CRP) and procalcitonin (PCT). Urine and stool tests were normal. Tests for influenza A virus, influenza B virus and coronavirus disease-19 were negative. Preoperative serological markers, including hepatitis B virus markers (surface antigen/antibody, e antigen/antibody, core antibody), hepatitis C virus, human immunodeficiency virus and *Treponema pallidum* antibodies, were all negative. Tests for cytomegalovirus, rubella, herpes and toxoplasma were also negative. Widal test, Weil-Felix test and fungal (1,3)-β-D-glucan test (G-test) yielded normal results. The antinuclear antibody and antineutrophil cytoplasmic antibody profiles were negative. However, plasma levels of multiple cytokines, measured using a 12-Cytokine Detection Kit fluorescence assay (cat. no. 281601HN; Hunan Wellgrow Biotech Co., Ltd.), were above the normal ranges.

Lumbar puncture and bone marrow aspiration were conducted. The cerebrospinal fluid (CSF) was clear, but the red blood cell count was $4x10^6$/l (normal range: 0), the white blood cell count was $252x10^6$/l (normal range: 0-$15x10^6$/l) [3% neutrophils (normal range: 0-6%), 92% lymphocytes (normal range: 40-80%), 5% monocytes (normal range: 15-45%)], and the protein level was 561 mg/l (normal range: 150-450 mg/l).

Mycoplasma pneumoniae IgG and IgM were positive, with IgG at 113.0 arbitrary units/ml (AU/ml) (normal range: 0-36.0 AU/ml) and IgM near the threshold at 1.13 cut-off index (COI) (normal range: <1.00 COI). After anti-infective treatment with piperacillin-tazobactam (4.5 g every 8 h for 4 days) and azithromycin (0.5 g/day for 4 days), infection markers, including CRP and PCT, did not increase, as indicated by normal blood test results. Piperacillin-tazobactam was then discontinued, while azithromycin (0.5 g/day for another 2 days) was continued for anti-infective treatment, but the patient still had recurrent fever (maximum body temperature, 39.5˚C) until targeted treatment was initiated.

To investigate potential pathogenic infections, conventional cultures of blood, CSF and bone marrow aspirate were performed. No organism was detected in any of the three specimens after incubation under both aerobic and anaerobic conditions at 37˚C for 7 days.

Concurrently, peripheral blood was subjected to mNGS for unbiased pathogen detection. No typical skin flora was detected during microbiological investigations. Briefly, plasma was separated, and 300 μl was used for DNA extraction using a Nucleic Acid Extraction Kit (cat. no. Z000100194; BGI Genomics). The extracted DNA was then processed to construct libraries through fragmentation, end-repair, adapter ligation and PCR amplification using the PMseq™ Infectious Pathogen High-throughput Gene Detection Kit (cat. no. Z000100051; BGI Genomics). DNA and library concentrations were measured using Qubit 3 fluorometer (Thermo Fisher Scientific, Inc.). Pooled libraries were sequenced on the MGISEQ-200 platform (MGI Tech Co., Ltd.) after generation of DNA nanoballs. Stringent quality controls, including no-template negative control, positive control containing known microbial nucleic acids and internal reference standards, were run in parallel to monitor contamination and assay performance. Bioinformatics analysis consisted of quality filtering using the in-house get_umhost_IC_qc pipeline (BGI Genomics) (5), host read removal by alignment to the GRCh38.p14 reference genome and taxonomic classification against multiple databases, including the NCBI nucleotide database (https://www.ncbi.nlm.nih.gov/nuccore), Food and Drug Administration-ARGOS (https://data.argosdb.org/) and the Genome Taxonomy Database (https://gtdb.ecogenomic.org/). Microbial identification was based on multiple parameters, such as specific read counts, genome coverage and relative abundance compared with negative controls and samples processed in the same batch. The stringently mapped read number (SMRN) was used as the primary criterion for interpretation. A microorganism was considered a suspected pathogen when its SMRN exceeded that of the negative control and met the following thresholds: SMRN ≥3 for bacteria, viruses, fungi, mycoplasma and chlamydia; SMRN ≥100 for parasites; and SMRN ≥1 for *Mycobacterium tuberculosis* (6).

In contrast to conventional cultures, mNGS identified *G. vaginalis* as the predominant pathogen in the patient's blood. A total of 664 specific reads of *G. vaginalis* were identified, which covered 1.86% of its genome (Fig. 1A). To validate this detection, microbial DNA was extracted from the patient's plasma using a Nucleic Acid Extraction Kit (cat. no. Z000100194; BGI Genomics). PCR targeting a fragment of *G. vaginalis* 16S rRNA was conducted using a pair of previously reported primers (forward, 5'-CTCTTGGAA ACGGGTGGTAA-3'; reverse, 5'-TTGCTCCCAATCAAA AGCGGT-3') (7) and the GoldenStar T6 Super PCR Mix (Beijing Tsingke Biotech Co., Ltd.). The PCR conditions were as follows: Initial denaturation at 98˚C for 2 min; 35 cycles of denaturation at 98˚C for 10 sec, annealing at 55˚C for 10 sec

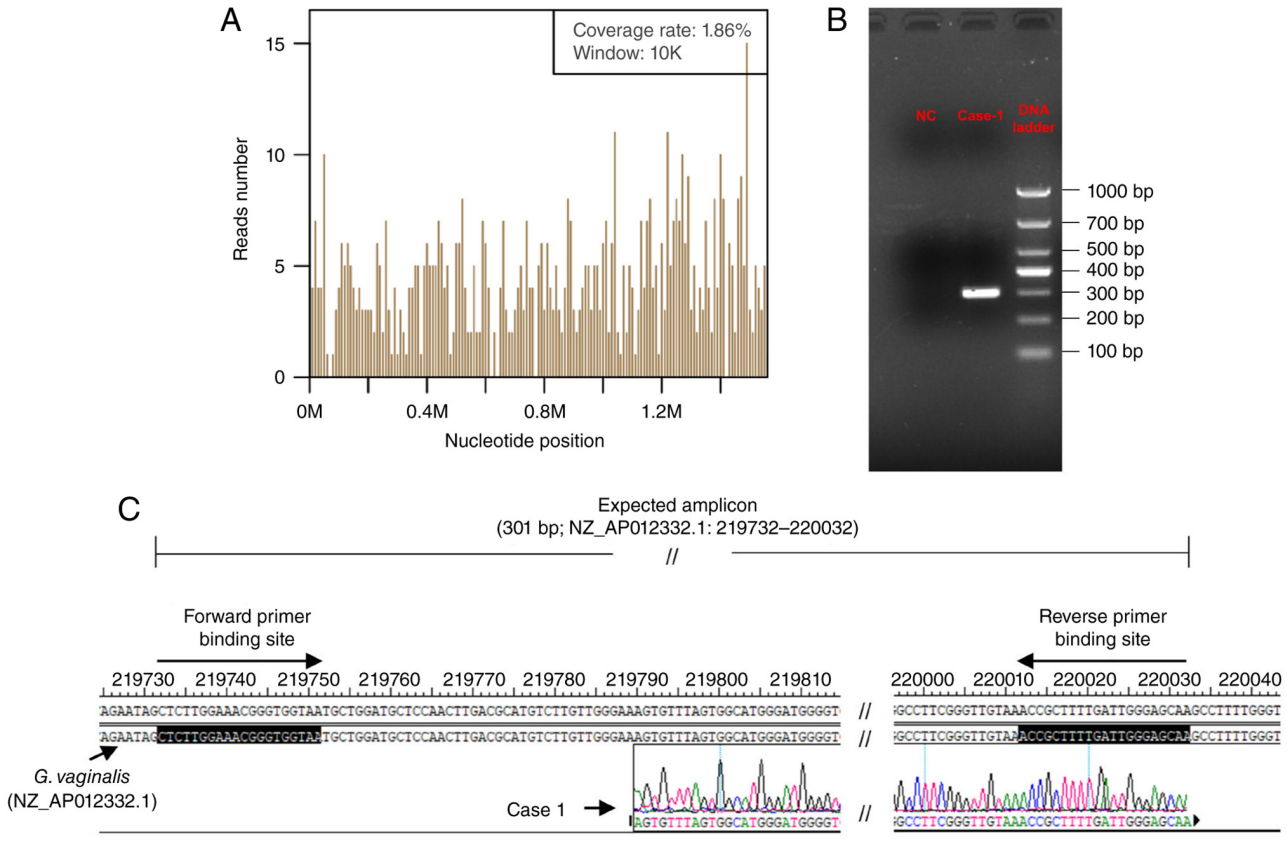

Figure 1. Identification of *G. vaginalis* from peripheral blood of the patient. (A) Sequencing reads distribution of *G. vaginalis* detected by metagenomic next-generation sequencing from the patient's plasma. (B) A fragment of the 16S rRNA of *G. vaginalis* from plasma was amplified by PCR. (C) Alignment of the PCR product with the *G. vaginalis* 16S rRNA gene based on Sanger sequencing. The forward primer binds directly to the reference sequence, whereas the reverse primer binding site is shown as the reverse complementary sequence corresponding to the opposite DNA strand. The expected amplicon is 301 bp. Primer binding sites are highlighted in black, and the intervening sequence is omitted for clarity. *G. vaginalis*, *Gardnerella vaginalis*; NC, negative control derived from plasma obtained from a healthy donor; M, million; K, kilobases.

and extension at 72˚C for 15 sec; followed by a final extension at 72˚C for 1 min. PCR products were analyzed by electrophoresis on a 1.5% agarose gel and visualized using ethidium bromide (Fig. 1B). The amplified products were further confirmed by Sanger sequencing (Fig. 1C).

Given the patient's recurrent fever, a multidisciplinary consultation was held, and *G. vaginalis* was considered the most likely causative pathogen. A gynecologic examination was performed to further investigate a possible source of infection. The external genitalia showed an intact hymen, but were heavily congested, with a large amount of yellowish, curd-like discharge without obvious malodor. Vaginal secretion was examined using an Aerobic Vaginitis/Bacterial Vaginosis Detection Kit (JY-Po-Color AV/BV set; Beijing Zhongsheng Jinyu Diagnostic Technology Co., Ltd.) in the clinical laboratory. The results showed a cleanliness grade of II according to the manufacturer's interpretation criteria and was negative for *Gardnerella* but positive for mycetes and peroxidase. The positive mycetes result indicated the presence of molds, whereas the positive peroxidase result suggested vaginal inflammation.

Based on the mNGS findings and clinical presentation, targeted therapy with metronidazole (0.5 g every 8 h for 8 days before discharge) was initiated. Concurrently, empirical acyclovir therapy (0.5 g every 8 h for 8 days before discharge) was administered for suspected viral meningitis. After 3 days of treatment, the patient's body temperature and resting pulse rate gradually returned to stable and normal (Fig. 2A). At that time, blood, CSF and bone marrow cultures were all negative. After 8 days of treatment, the patient's family requested discharge because of their stable clinical condition, although the recommended course of anti-infective treatment had not yet been completed. On the day of discharge, peripheral blood was sampled for follow-up mNGS, and a negative result was found for *G. vaginalis*. In addition, the plasma levels of all 12 cytokines decreased after treatment (Fig. 2B), indicating partial recovery of the inflammatory response. The diagnostic and therapeutic timeline from admission to discharge is summarized in Fig. 3. At a telephone follow-up conducted in August 2026, the patient's family reported that they remained in good clinical condition, with no recurrence of similar symptoms.

Discussion

The present report describes a case of bloodstream infection of *G. vaginalis* diagnosed by mNGS in an adolescent girl with no reported sexual history. Previous studies have shown that *G. vaginalis* is frequently detected in the vaginal microbiota of women with BV, and can also be isolated from healthy women at lower abundances (8,9). Vaginal *Gardnerella* biofilm has been suggested to be associated with sexual

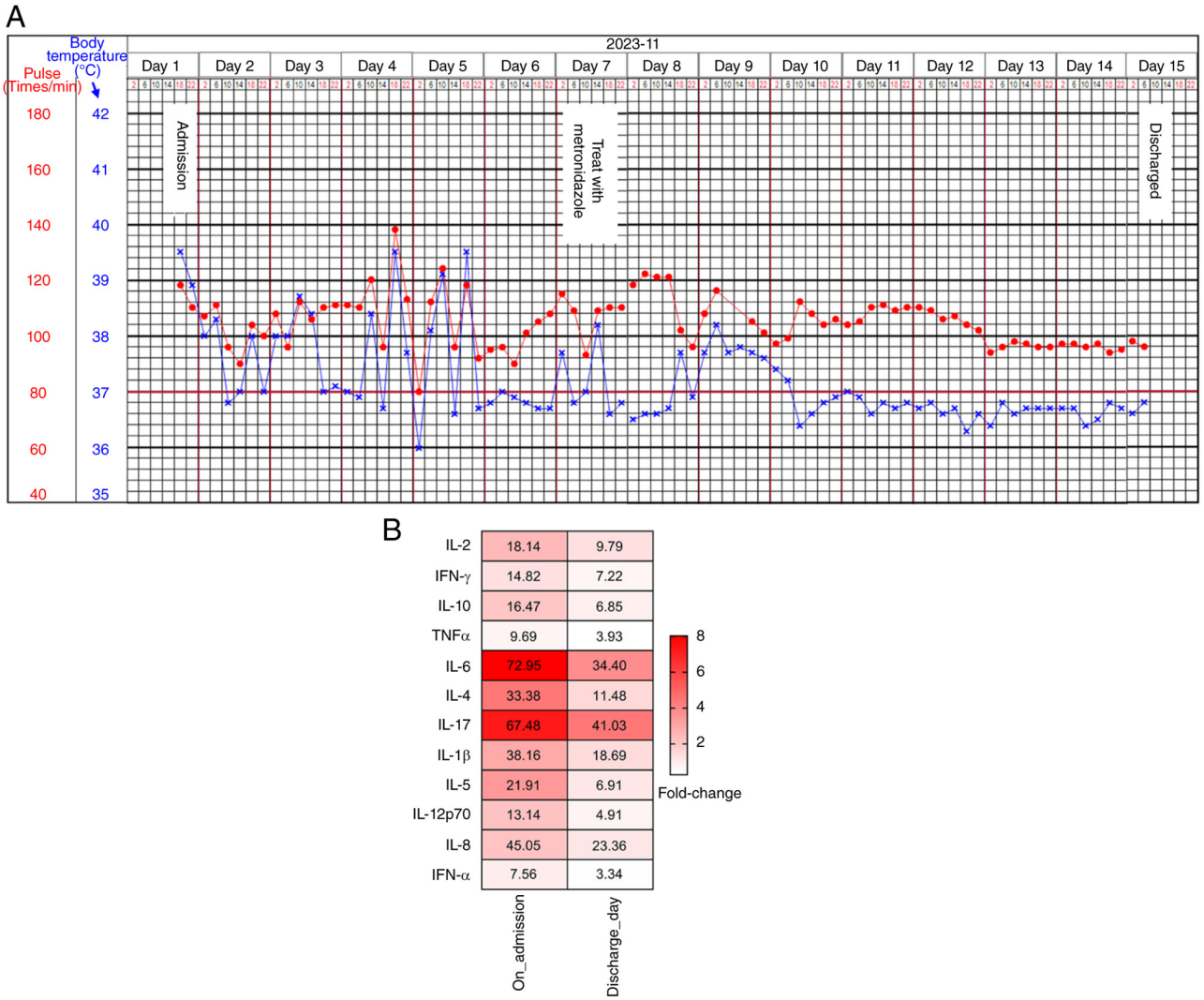

Figure 2. Symptoms of the patient before and after treatment targeting *Gardnerella vaginalis* infection. (A) Pulse and body temperature monitoring of the patient during hospitalization. The values shown represent serial measurements obtained during hospitalization and '2, 6, 10, 14, 18, 22' correspond to time of measurement. (B) Plasma cytokine concentrations before and after treatment of the patient. The fold change is calculated relative to the upper limit of the reference range for each cytokine, so the fold change from 0 to 1 represents the normal level of each cytokine. The reference ranges (pg/ml) were as follows: IL-2, 0-7.394; IFN-γ, 0-11.25; IL-10, 0-8.051; TNFα, 0-14.192; IL-6, 0-9.10; IL-4, 0-7.541; IL-17, 0-9.31; IL-1β, 0-13.04; IL-5, 0-6.398; IL-12p70, 0-6.024; IL-8, 0-20.98; IFN-α, 0-8.50. The numerical values shown in each cell indicate the measured cytokine concentrations (pg/ml).

transmission, with evidence that some heterosexual couples share identical strains (10). *G. vaginalis* has been implicated in a series of genitalia-associated infections, including BV, intrauterine infections, intra-amniotic infections, chorioamnionitis, postabortal pelvic inflammatory disease and postpartum endometritis following cesarean section (2). Notably, extragenital and male infections have also been reported, such as pulmonary infections (11), bronchial infections and ventilator-associated pneumonia (12), prosthetic joint infections (13,14), blood infections with bacteremia (3), CSF infections and purulent meningitis (4).

G. vaginalis infections have been reported across various age groups. Most extragenital infections occur in adults or adolescents with sexual partners (4). In adult men, reported infections are predominantly originated in the genitourinary tract (3), while in adult women, infections are mainly observed in gynecologic and obstetric patients (15). In pediatric patients, *G. vaginalis* infection is limited to neonates (16,17). By contrast, there was an unusual case of urinary tract infection in a 2-month-old infant, without maternal chorionic amniotic inflammation or BV (17). Reports of *G. vaginalis* infection in juveniles remain uncommon. To date, only two previously published cases involving adolescents/young adults have been identified (Table I). One was a 14-year-old boy with purulent meningitis, who had a girlfriend with a history of vaginitis (4). Another was a 19-year-old woman with bacteremia associated with severe acute encephalopathy, preceded by abnormal vaginal discharge (18).

In the current report, the patient reported no history of sexual contact, and gynecological examination confirmed an intact hymen. The patient had their first menstruation at the age of 12 years. Their vaginal discharge was yellow and curd-like, and was positive for mycetes and peroxidase, suggesting local vaginal inflammation. Their vaginitis may have been due to poor perineal hygiene management. As microbiome profiling or quantitative culture data from genital samples were not

Table I. Cases of *Gardnerella vaginalis* infection in adolescents and young adults.

First author/s, year	Sex	Age	Identification method	Case presentation	Treatment	Outcome	(Refs.)
Tankovic *et al*, 2017	Female	19	Blood culture (positive)	Bacteremia, acute encephalopathy	Amoxicillin-clavulanate	Recovered	(18)
Lu *et al*, 2022	Male	14	CSF culture (negative), mNGS (positive)	Purulent meningitis	Meropenem, linezolid and amoxicillin	Recovered	(4)
Current report	Female	13	Blood/CSF/puncture-fluid culture (negative), mNGS (positive)	External vaginitis, bacteremia	Metronidazole	Recovered	-

CSF, cerebrospinal fluid; mNGS, metagenomic next-generation sequencing.

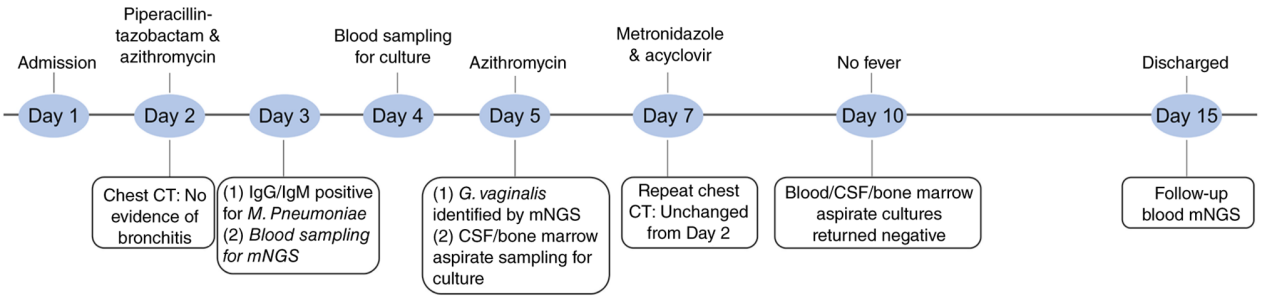

Figure 3. Timeline of diagnosis and treatment of the patient. *G. vaginalis*, *Gardnerella vaginalis*; *M. pneumoniae*, *Mycoplasma pneumoniae*; CSF, cerebrospinal fluid; CT, computed tomography; mNGS, metagenomic next-generation sequencing.

available, the source of the infection cannot be definitively determined.

G. vaginalis secretes vaginolysin (VLY), a pore-forming toxin that generally recognizes and binds to membrane cholesterol to create pores and lyse target cells (19). High amounts of VLY may damage the brain endothelial cells and pass the blood-brain barrier (4,18). The patient in this case was diagnosed with bacteremia with a probable presentation of central nervous system infection, since the CSF protein level was outside the normal range. Given the clinical suspicion of viral meningitis, empirical treatment with azithromycin and acyclovir was initiated. However, no direct microbiological evidence supporting viral meningitis was obtained. Since CSF was not subjected to mNGS, central nervous system involvement cannot be ruled out definitively.

Although quantitative cultures and serial pre-treatment samples were unavailable, several lines of evidence support the clinical significance of *G. vaginalis* detection in the current case. Firstly, the patient's persistent fever failed to respond to empirical antibiotics but resolved rapidly after initiation of metronidazole, accompanied by negative follow-up blood mNGS at discharge. This temporal association is inconsistent with a transient or incidental detection event. Secondly, mNGS identified 664 specific reads covering 1.86% of the *G. vaginalis* genome, a level far exceeding contamination or typical background observed in negative controls, and the result was independently validated by 16S rRNA PCR and Sanger sequencing, supporting the authenticity of microbial identification. Thirdly, blood samples were obtained via peripheral venipuncture, and no typical skin flora was detected, reducing the likelihood of exogenous contamination. Although transient colonization cannot be completely excluded, the available evidence collectively supports *G. vaginalis* as the most likely cause of bacteremia in this patient.

G. vaginalis is fastidious and requires complex and harsh growth conditions, including enriched media, a CO_2-enriched atmosphere and prolonged incubation. Conventional identification relies on culturing infected specimens, such as urine (3), blood (3,20) or pus from the pathological site (21). However, this approach has notable limitations. Firstly, conventional cultures take a relatively long time to obtain results, and it is usually necessary to extend the incubation time to as long as 5-7 days (3,22). In the current report, the blood, CSF and bone marrow aspirate cultures were prolonged to 7 days. These time-consuming methods may lead to a delayed diagnosis and treatment. By contrast, the turn-around-time of mNGS is much shorter at approximately 24-48 h (23). Secondly, usual aerobic conditions also return negative results (3,11), because *G. vaginalis* grows poorly under routine aerobic culture conditions, potentially leading to missed identification of the organism. Conventional cultures only detect viable microorganisms with an overall positivity rate of only 30-40% (24). Given the consistently negative results in previous reports (11,24) and the current case, we hypothesized that missed detection may occur more frequently than generally recognized. Thirdly, conventional cultures cannot always directly pinpoint the specific species involved, and additional methods such as biochemical tests or 16S rRNA gene sequencing are often

required for adjunctive identification (18). Taken together, mNGS is superior to traditional methods in both detection speed and accuracy. Because of these advantages, mNGS has been recommended to be considered as a front-line diagnostic tool in chronic and recurrent infections (23).

Vaginal flora such as *G. vaginalis* is rarely considered pathogenic in juveniles without a sexual partner. This diagnostic blind spot is further compounded by the fact that traditional blood, CSF and bone marrow aspirate cultures all returned negative results. The application of mNGS successfully identified this unexpected pathogen, thereby broadening the understanding of *G. vaginalis* infection in sexual naïve adolescents. Future reductions in the cost and improved accessibility of mNGS may facilitate larger-scale studies and clinical diagnosis in undiagnosed patients.

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Availability of data and materials

The raw sequencing data generated in the present study may be found in the Genome Sequence Archive database under accession number CRA022671 or at the following URL: https://ngdc.cncb.ac.cn/gsa/browse/CRA022671. The other data generated in the present study may be requested from the corresponding author.

Authors' contributions

TCZ, YHJ, JiW and BL were involved in the conceptualization of the study. XL, TCZ and JiW wrote the main manuscript text. JuW, YHJ and TTY collected the diagnosis and treatment information. XL, FWL, CM and QL conducted mNGS, PCR and Sanger sequencing analyses. XL, TCZ, FWL and TTY conducted molecular analysis and data interpretation. TCZ, XL and YHJ confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Written informed consent was obtained from the patient's parents for publication of this case report after omitting any identifying information of the patient.

Competing interests

The authors declare that they have no competing interests.

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