AKADÉMIAI KIADÓ

Acta Microbiologica et Immunologica Hungarica

68 (2021) 4, 286–290

DOI:
10.1556/030.2021.01358

ORIGINAL RESEARCH PAPER

A case of bacteremia caused by *Leptotrichia trevisanii* in pediatric patient with febrile neutropenia and review of literature

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Received: December 4, 2020 • Accepted: April 28, 2021
Published online: May 27, 2021

ABSTRACT

Leptotrichia species are fastidious anaerobic, fusiform, pencil-shaped Gram-negative bacilli that reside in microbiota of humans. *Leptotrichia* species have increasingly been recognized as an opportunistic pathogen in humans, mainly in the immunocompromised patient. Anaerobic organisms have rarely been isolated from blood cultures of pediatric patients. In our study, we isolated *Leptotrichia trevisanii* from central venous catheter culture of a five-year-old male patient. It was identified with both matrix-assisted laser desorption ionization time-of-flight mass spectrometry and confirmed via 16S rRNA gene sequencing. The early recognition of anaerobic bacteremia and administration of appropriate antimicrobial and play an important role preventing mortality and morbidity in children. In our study we report a rarely diagnosed case of *L. trevisanii* bacteremia in a pediatric patient.

KEYWORDS

Leptotrichia trevisanii, anaerobic infections

INTRODUCTION

Leptotrichia species are fastidious anaerobic, fusiform, pencil-shaped Gram-negative bacilli that reside in the mouth, intestines and female genital tract of humans. *Leptotrichia* is one of six genera in family Fusobacteriaceae in the phylum Fusobacteria [1]. The genus *Leptotrichia* contains a wide variety of species, such as *L. buccalis, L. trevisanii, L. amnionii, L. goodfellowi, L. hofstadii, L. shahii, L. wadei,* and *L. hongkongensis* [2]. *Leptotrichia* species have increasingly been recognized as an opportunistic pathogen in humans, mainly in the immunocompromised patient. *Leptotrichia* spp. have been implicated in periodontal disease, endocarditis, chorioamnionitis and arthritis, especially in immunocompromised patients [3]. *Leptotrichia trevisanii* bacteremia has been reported with acute myeloid leukaemia [4, 5], myelodysplastic syndrome [2] and febrile neutropenia [6]. Recent studies have demonstrated that the level of anaerobic bacteremia in children is much lower than that in adults [7]. Herein we present the case of bacteremia caused by *L. trevisanii* in a child with febrile neutropenia with the diagnosis of non-Hodgkin's lymphoma.

CASE REPORT

A five-year-old male patient was diagnosed with non-Hodgkin's lymphoma in April 2019. He was followed up in Hacettepe University Faculty of Medicine Pediatric Oncology clinic. The patient was admitted to the Pediatric Oncology Clinic with the symptoms of fever and diarrhoea after the 4th cycle of chemotherapy. Laboratory blood analysis was examined. A blood test demonstrated the following findings: a white blood cell count of 1,0/μL (neutrophils 28.3 %: lymphocytes: 70.9%); red blood cell count, 4,74/μL; haemoglobin level, 12.3 g/dL;

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hematocrit, 36,1; platelet count, 108/μL and C-reactive protein, 13,5 mg/dL Empirical antimicrobial therapy was initiated with ciprofloxacin and metronidazole. Blood samples from a peripheral vein and central venous catheter were obtained during the initial febrile episode and were submitted to the Clinical Microbiology Laboratory. Bactec Peds Plus (BD; USA) bottles were incubated in the Bactec FX (BD, USA) automated blood culture system. A positive signal was received from the central venous catheter blood culture bottle after 14 hours and 43 minutes. Long, filamentous, Gram-negative bacilli were observed in routine Gram-staining made from central venous catheter blood culture bottle giving a positive signal. Gram-staining result has been reported in the patient's clinic. To isolate and identify this microorganism, subcultures of the positive central venous catheter blood culture bottle were performed. They were inoculated into Schaedler agar supplemented with vitamin K1 and hemin (Oxoid) and Columbia 5% sheep blood agar (BD, USA), MacConkey (BD, USA), chocolate agar plates (BD, USA). Schaedler agar was incubated at 37 °C in anaerobic conditions for 48 hours. AneroGen gas-generating sachets (Thermo Scientific, Dreieich, Germany) were used to create anaerobic conditions. A chocolate blood agar plate was streaked and incubated in 5% CO_2 atmosphere at 37 °C to assess aerotolerance. Colonies formed after 48 hours incubation in Schaedler agar. It performed grey and smooth colonies in Schaedler agar (Fig. 1). Unsporulated, elongated, fusiform Gram-negative bacilli were showed with Gram-staining preparation of the isolated colony in anaerobic condition (Fig. 2). There was no growth in the chocolate plate. Gram-staining, catalase and oxidase tests were performed on the growing microorganism. Catalase and oxidase tests were found to be negative.

The isolate was identified as *L. trevisanii* with matrix-assisted laser desorption ionization time-of-flight mass spectrometry [MALDI-TOF/MS (Bruker, Germany)] that yielded high identification score value of 2.1. Anaerobic Schaedler plate was directly referred for DNA sequencing. Finally, the result was confirmed with polymerase chain reaction-based amplification of 16S rRNA *L. trevisanii* strain (NR 029805.1) was used as a positive control.

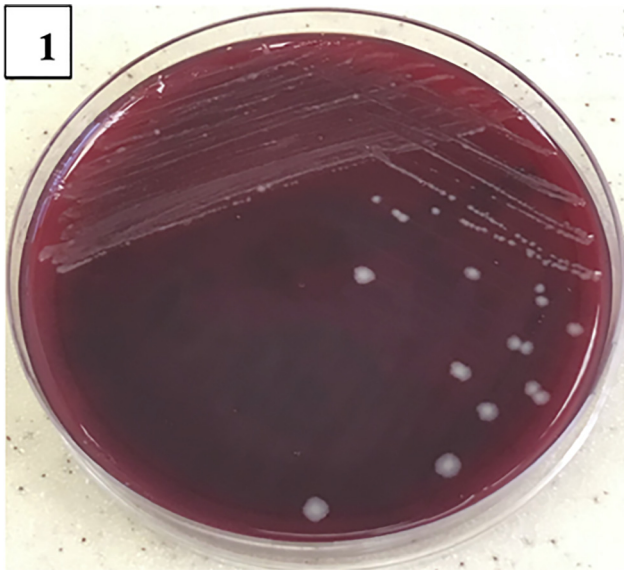

Fig. 1. The form of colonies on a Schaedler agar plate

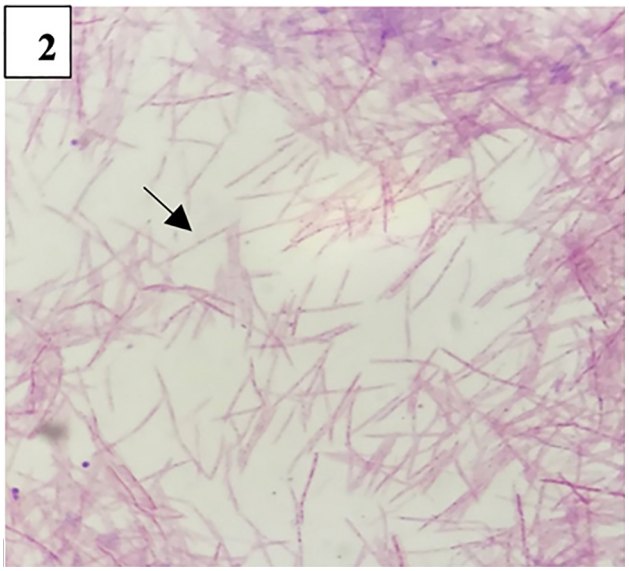

Fig. 2. Gram-staining preparation of the isolated colony in anaerobic condition (grown on a Schaedler) Gram stain × 1,000

DNA was isolated from 0.5 to 1 g of cell paste using MasterPure DNA purification kit Qiagen, Hilden, Germany) following the manufacturer's recommendations, with a modification of st/DL for cell lysis as described by Wu et al. [8]. The primers used for amplification were 5F (5′-TTGGAGAGTTTGATCCTGGCTC-3′) and 1194R (5′-ACGTCATCCCCACCTTCCTC-3′), which bind to conserved regions near bp 5 and 1194 of the 16S rRNA gene. The cycles are initial denaturation at 95 °C for 3 min, 35 cycles of 95 °C for 20 s, annealing at 51 °C for 30 s, extension 72 °C for 1 min, and final extension 72 °C for 5 min. The amplified products were run and viewed in a 1.5% agarose gel (Sigma, St. Louis, MO, USA). Sequencing was performed using BigDye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems, MA, USA). DNA sequences of the purified products were identified using ABI Prism 3700 Genetic Analyzer (Applied Biosystems) [9]. The isolates were identified comparing the DNA reference isolates with data stored in the GenBank using the Basic Local Alignment Search Tool (BLAST version 2.0 http://www.ncbi.nlm.nih.gov/BLAST) program. A phylogenetic tree analysis was created using ClustalW MegAlign. The molecular 16S rRNA gene sequence was found to be a similarity >99 % compared to the reference sequence of the type strain *L. trevisanii.*

To determine the susceptibility to antibiotics, we measured the minimum inhibitory concentration (MIC) of antibiotics against the strain of *L. trevisanii* isolated, using the Etest (bioMerieux, Mercy. L'Etoile, France) on a 1 McFarland suspension of the bacteria incubated for 48 hours in anaerobic conditions on Brucella agar with 5% defibrinated horse blood supplemented with 1 mg/l vitamin K1. The

bacteria were susceptible to all antibiotics tested: penicillin (MIC ≤0.02 mg/mL), ampicillin (MIC 0.032 mg/mL), meropenem (MIC 0.012 mg/mL), ceftriaxone (MIC 0.08 mg/mL), metronidazole (MIC 0.75 mg/mL), clindamycin (MIC <0.016 mg/mL), and piperacillin/tazobactam (MIC 0.064 mg/mL), cefoxitin (MIC 0.094 mg/mL), tetracycline MIC (<0.125 mg/mL), tigecycline MIC (≤0.016 mg/mL).

Our patient's therapy was arranged as piperacillin/tazobactam plus amikacin. Central venous catheter was removed from the patient. There was no reproduction in control of blood cultures. He was discharged after two weeks of inpatient management. Good clinical outcome was obtained for the patient.

DISCUSSION

Leptotrichia species are opportunistic pathogens, anaerobic, Gram-negative rods that inhabit the micrflora of humans. The factors predisposing to bacteremia caused of anaerobes include neoplasms, hematologic disorders, organ transplant, recent gastrointestinal, obstetric or gynecologic surgery, intestinal obstruction, decubitus ulcers, dental extraction, the newborn age, sickle cell disease, diabetes mellitus, postsplenectomy, and the use of cytotoxic agents or corticosteroids [10, 11]. Infections caused by *L. trevisanii* are not prevalent, but these infections can be severe in immunosuppressed patients. In literature, 17 cases have been published in which *L. trevisanii* was identified as the cause of infection. Summary of *L. trevisanii* cases reported in Table 1. The first bacteraemia due to *L. trevisanii* was reported in a man with acute myeloid leukaemia by Tee et al. [5]. Hematologic malignancy is the most common underlying disease in patients with *Leptotrichia* bacteremia and the second most common underlying disease is solid cancer. Other characteristics of patients with *Leptotrichia* bacteremia include neutropenia, a history of chemotherapy, and

Table 1. Characteristics of patients with *Leptotrichia trevisanii* bacteremia

Patient	Age/ sex	Underlying disease	Sample	Treatment	İdentification	References
Case 1	46/ male	Acute myeloid leukaemia	Blood culture	Ceftriaxone, gentamicin and metronidazole.	16S rRNA	[5]
Case 2	55/ male	Myelodysplastic syndrome	Blood culture	Ceftazidime and teicoplanin, Based on the results of the antibiogram: amoxicillin	16S rRNA	[2]
Case 3	12/ female	Acute myeloid leukaemia	Blood culture	Tazobactam/piperacillin plus amikacin treatment	16S rRNA	[6]
Case 4	66/ male	Oesophagal cancer	Blood culture	Cefepime and cefcapene pivoxil	16S rRNA	[6]
Case 5	74/ female	Acute myeloid leukaemia	Blood culture	Meropenem and clindamycin	16S rRNA	[4]
Case 6	69/ female	Diffuse large B-cell lymphoma	Blood culture	Piperacillin-tazobactam plus amikacin; meropenem	16S rRNA	[19]
Case 7	33/ female	Systemic lupus erythematosus	Blood culture	Empirical therapy with metronidazole and cefoperazone/tazobactam	16S rRNA	[20]
Case 8	53/ male	Multiple myeloma	Blood culture	Vancomycin	16S rRNA	[16]
Case 9	56/ male	Multiple myeloma	Blood culture	Cephalosporin	16S rRNA	[16]
Case 10	63/ female	Myelodysplastic syndrome	Blood culture	Carbapenem	16S rRNA	[16]
Case 11	62/ female	Multiple myeloma	Blood culture	Piperacillin-tazobactam	16S rRNA	[21]
Case 12	7/male	Burkitt's lymphoma	Blood culture	Not reported	16S rRNA	[12]
Case 13	37/ male	Diffuse large B-cell lymphoma	Blood culture	Not reported	16S rRNA	[12]
Case 14	34/ female	Double primary cancer (colon & gastric cancer)	Blood culture	Not reported	16S rRNA	[12]
Case 15	19/ male	Ewing sarcoma	Blood culture	Not reported	16S rRNA	[12]
Case 16	45/ female	No medical or surgical history	Cervical canal exudate	Clindamycin and ceftriaxone	16S rRNA	[22]
Case 17	36/ female	Pregnancy/Chorioamnionitis	Amniotic fluid	Ampicillin and gentamicin	16S rRNA	[23]
Present report	5/male	Non-hodgkin lymphoma	Blood culture	Piperacillin-tazobactam plus amikacin	16S rRNA	

oropharyngeal mucosal damage [12]. In this report patient's underlying disease is non-Hodgkin lymphoma.

Bloodstream infection is life-threatening for hospitalized, elderly and pediatric patients who are particularly susceptible to bloodstream infection caused by anaerobic bacteria [13]. The use of anaerobic bottles has contributed to the diagnosis of a significant number of bacteremia [16]. Recent studies have demonstrated that the level of anaerobic bacteremia in children is much lower than that in adults. The low prevalence of pediatric anaerobic bacteremia has also been reported in other studies [7, 14]. In bloodstream infection caused by anaerobic bacteria the 96.6% of the patients were adult and 3.4% were pediatric patients and this data is consistent with the information that anaerobic bacteremia is rare in the pediatric population in the literature [15]. Therefore, we have described the case of rare bacteremia by *L. trevisanii* in a pediatric patient who was followed up with the diagnosis of non-Hodgkin lymphoma.

L. trevisanii antibiotic susceptibility profile reviewed from literature. The optimal therapy for *L. trevisanii* infections has not been established. Most strains are susceptible to penicillin, tetracycline, rifampicin, metronidazole, chloramphenicol, clindamycin and carbapenems [5]. Although *Leptotrichia* spp. show *in vitro* resistance to gentamicin, kanamycin, erythromycin, vancomycin and fluoroquinolones [16].

Identification of *Leptotrichia* species can be difficult to distinguish from *Fusobacteria and Lactobacillus* species with conventional laboratory assays [17]. MALDI-TOF/MS appears to be more accurate and faster than routine biochemical identification methods when 16S rRNA sequencing is used as the gold standard for verifying the identity of the organism. MALDI-TOF/MS can be used to identify *Leptotrichia* species in the rutin microbiology laboratory. In this report *L. trevisanii* was identified by MALDI-TOF/MS and confirmed by 16S rRNA sequence analysis as in other studies [18].

CONCLUSION

Anaerobic bloodstream infection has a low prevalence in the pediatric patient so anaerobic inoculation may be most helpful when bacteremia is clinically suspected in a child who is severely immunocompromised, or after a recent abdominal surgery, or source of bacteremia is not identified by clinical evaluation [7]. Therefore this report, where the case of rare bacteremia caused by *L. trevisanii* in a child with febrile neutropenia with the diagnosis of non-Hodgkin's lymphoma, is important for literature.

MALDI-TOF/MS enables precise, rapid identification of many bacterial isolates that can not be effectively identified by conventional methods [24]. The time period for diagnosis was shortened by MALDI-TOF/MS and a successful clinical result was obtained for the patient. MALDI-TOF/MS is to be used to identify anaerobic bacteriae in the rutin microbiology laboratory.

Funding: No funding sources.

Conflict of interest: The authors declare that there is no conflict of interest regarding the publication of this paper.

ACKNOWLEDGMENT

None.

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