Rapid identification of a *Leptotrichia trevisanii* catheter-related bloodstream infection using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

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**Introduction:** *Leptotrichia trevisanii* is a large, fusiform, non-sporulating, motile, Gram-negative rod, and is a member of the family *Fusobacteriaceae* in the phylum *Fusobacteria*. Although *L. trevisanii* bacteraemia is extremely rare, severe infections have been reported in immunocompromised patients.

**Case presentation:** We present a case of a 69-year-old woman diagnosed with diffuse large B-cell lymphoma, who suffered a catheter-related bloodstream infection due to *L. trevisanii* during the post-autologous peripheral blood progenitor-cell transplantation aplasia phase. In this case, rapid identification of the opportunistic pathogen was achieved using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry directly from a blood culture, with correct identification of the micro-organism within 2 h after the blood culture became positive.

**Conclusion:** Rapid identification of this opportunistic pathogen allowed initiation of appropriate antimicrobial therapy, which contributed to a successful clinical outcome for the patient.

**Keywords:** catheter-related infection; *Leptotrichia trevisanii*; MALDI-TOF.

**Introduction**

*Leptotrichia* spp. are fastidious, anaerobic, fusiform, Gram-negative rods that reside in the oral cavity, intestines and female genital tract of humans (Eribe & Olsen, 2008; Liu, 2011). This genus, included in the family *Fusobacteriaceae*, phylum *Fusobacteria*, has increasingly been recognized as an opportunistic pathogen in humans, mainly in immunocompromised patients (Couturier et al., 2012). Although *Leptotrichia buccalis* is the most commonly isolated causative bacterium, six other species have been found to cause infection in humans: *Leptotrichia wadei* and *Leptotrichia hongkongensis* have been associated with bacteraemia; *Leptotrichia goodfellowii* has been isolated in bacteraemia as well as endocarditis and dog bite infection, and *Leptotrichia hofstadii* and *Leptotrichia shahii* have been isolated from oral wounds (Eribe & Olsen, 2008; Eribe et al., 2004; Guiu et al., 2012). *Leptotrichia trevisanii* described initially by Tee et al. (2001). It has rarely been isolated from human clinical specimens, and when reported it has been associated with bloodstream infections in patients with haematological malignancies (Kumagai et al., 2013). Here, we report a case of *L. trevisanii* catheter-related bacteraemia rapidly identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) in a patient with diffuse large B-cell lymphoma, who underwent peripheral blood stem-cell transplantation (PBSCT), and we review other published cases.

**Case report**

A 69-year-old woman with diffuse large B-cell lymphoma stage IIIX-A, International Prognostic Index 3, in progression after two lines of chemotherapy and local radiotherapy, was admitted to the Haematology Service for...
autologous blood progenitor-cell transplantation with BEAM [carmustine (BiCNU), etoposide, cytarabine (Ara C) and melphalan] as the treatment scheme. On day 4 post-transplant aplasia phase, the patient developed a febrile diarrhoeal syndrome associated with negative stool cultures, and an oropharyngeal acute grade III mucositis, and required parenteral nutrition support. Laboratory blood analysis revealed haemoglobin of 95 g dl⁻¹, a platelet count of 33 × 10⁹ l⁻¹, a haematocrit of 27.7 %, a total leucocyte count of 0.09 × 10⁹ l⁻¹, an absolute neutrophil count of 0 and a C-reactive protein level of 39.6 mg l⁻¹. BACTEC Standard/10 Aerobic/F and BACTEC Lytic/10 Anaerobic/F vials from a peripheral and central venous catheter (CVC) were obtained during the initial febrile episode, and were submitted to the Microbiology Service. Moreover, empirical piperacillin-tazobactam plus amikacin therapy was initiated. Due to fever persistence, at day 10 post-transplant, piperacillin/tazobactam was changed to meropenem until 2 weeks of treatment had been completed. Furthermore, the catheter tip was removed and submitted for bacterial culture, which was negative. On day 17 of PBSCT, a progressive clinical improvement of the patient was found with a high neutrophil count and negative control blood cultures, and the mucositis was resolved. All of the follow-up blood cultures were negative. The blood culture bottles were incubated using a BACTEC FX (Becton Dickinson) automated blood culture system. The anaerobic bottle obtained through the CVC became positive at 32 h, 17 h less than that obtained from the peripheral blood (49 h). Neither the aerobic bottles obtained from the CVC nor the peripheral blood cultures showed any growth after 5 days of incubation. Based on the microorganism morphology seen on the routine Gram stain from the positive blood culture, a member of the family Fusobacteriaceae (Fig. 1) was suspected. MALDI-TOF MS direct identification from the sediment of the positive blood culture was performed (Juiz et al., 2012). The isolate was identified as L. trevisanii, with a high log score of 2.131, within 2 h after the positive CVC blood culture was reported. For definitive identification of the isolate, sequence analysis of the 16S rRNA gene was performed according to the method of Simmon et al. (2006). The antimicrobial susceptibility testing was determined by Etest in Mueller–Hinton agar supplemented with 5 % sheep blood, and the plates were incubated at 37 °C in a 5 % CO₂ atmosphere for 48 h. The MICs obtained were as follows (mg l⁻¹): penicillin, 0.016; piperacillin-tazobactam, <0.015; cefotaxime, <0.015; meropenem, 0.02; and vancomycin, 0.016.

**Discussion**

The first recorded bacteraemia due to L. trevisanii was reported in a man with acute myeloid leukaemia by Tee et al. (2001). Since then, seven more cases have been described. All were patients with haematological malignancies, except one, who had an oesophageal squamous cell carcinoma with lung, liver and lymph node metastases (Higurashi et al., 2013). L. trevisanii acts as an opportunist pathogen responsible for bloodstream infections in immunocompromised patients, causing systemic disease (Cooreman et al., 2013).

Neutropenic fever with severe alimentary tract mucositis is an established predisposing factor for the development of sepsis by Leptotrichia spp. (Couturier et al., 2012; Lark et al., 2001). PBSCT recipients who are receiving chemotherapy experience more fever due to severe mucositis, and these lesions are the route of bacterial translocation causing the bacteraemia. In these patients, gastrointestinal tract lesions are the route of bacterial translocation (Herbers et al., 2014; Higurashi et al., 2013). Fever during neutropenia must always be considered as a medical emergency, and as being due to an infection, unless otherwise proven (Castagnola et al., 2013). In our case, the patient presented severe oropharyngeal acute grade III mucositis during the aplasia phase. Table 1 shows the clinical characteristics of patients with L. trevisanii bacteraemia described to date, including the present case.

Identification of Leptotrichia spp. is difficult with the commonly used phenotypic identification systems due to its poor chemical reactivity, with identification by biochemical enzymatic reactions normally being inconclusive (Eribe & Olsen, 2008). For this reason, in all previous cases of L. trevisanii infection reported, the identification was performed by 16S rRNA gene sequencing, which is costly, time-consuming and not always readily available. However, MALDI-TOF MS provides precise, rapid and inexpensive identification of many bacterial isolates that cannot be effectively identified by conventional methods (Rodriguez-Sánchez et al., 2014; Schmitt et al., 2013). We used the method described by Juiz et al. (2012) that

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**Fig. 1.** Gram stain of a colony that grew in 5 % sheep blood agar showing Gram-negative rod-shaped bacilli. Bar, 20 μm; magnification × 1000.
Table 1. Demographic characteristics, comorbidities, risk factors and clinical characteristics of the patients with L. trevisanii bacteraemia

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age ± SD (years)</td>
<td>54.9 ± 18.3</td>
</tr>
<tr>
<td>Male [n/total (%)]</td>
<td>5/9 (55.5%)</td>
</tr>
<tr>
<td>Co-morbidities and risk factors</td>
<td></td>
</tr>
<tr>
<td>Haematological malignancy</td>
<td>8/9 (88.8%)</td>
</tr>
<tr>
<td>Solid tumour</td>
<td>1/9 (11.1%)</td>
</tr>
<tr>
<td>Immunosuppressive therapy</td>
<td>7/9 (77.7%)</td>
</tr>
<tr>
<td>PBSCCT</td>
<td>5/9 (55.5%)</td>
</tr>
<tr>
<td>Clinical presentation</td>
<td></td>
</tr>
<tr>
<td>Neutropenic fever</td>
<td>7/9 (77.7%)</td>
</tr>
<tr>
<td>Mucositis</td>
<td>7/9 (77.7%)</td>
</tr>
<tr>
<td>Gingivitis</td>
<td>1/9 (11.1%)</td>
</tr>
<tr>
<td>White blood cell count (&lt;4000 µl)</td>
<td>5/6 (83.2%)</td>
</tr>
<tr>
<td>Outcome</td>
<td></td>
</tr>
<tr>
<td>Survived</td>
<td>5/6 (83.2%)</td>
</tr>
</tbody>
</table>

allows the identification of micro-organisms isolated directly from blood cultures. With this method, the identification by MALDI-TOF MS was L. trevisanii with a high log score of 2.131 within 2 h after the blood culture bottle from CVC became positive. Positive blood culture bottles were inoculated into 5% Brucella sheep blood agar, Brucella blood agar supplemented with vitamin K₁ and hemin (Oxoid), and chocolate agar plates, and incubated in aerobic, anaerobic and 5% CO₂ atmospheres, respectively. Faster growth was observed in anaerobic conditions, as described previously by Tee et al. (2001). Moreover an on-plate formic acid preparation method published by Schmitt et al. (2013) was performed by MALDI-TOF MS. This method also allowed correct identification to the species level of L. trevisanii with a high log score of 2.532, within 48 h after the culture became positive. Finally, the identification was confirmed by 16S rRNA gene sequencing.

The optimal therapy for L. trevisanii infections has not been established, and in vitro susceptibility testing may not correlate with in vivo efficacy. Most strains are apparently susceptible to penicillin, tetracycline, rifampicin, metronidazole, chloramphenicol, clindamycin and carbapenems (Tee et al., 2001). Although Leptotrichia spp. show in vitro resistance to gentamicin, kanamycin, erythromycin, vancomycin and fluoroquinolones, one patient with L. trevisanii bacteraemia described by Schrimsher et al. (2013) became afebrile after treatment with vancomycin. Our patient was treated empirically with piperacillin-tazobactam for 6 days plus three doses of amikacin. Due to the persistence of fever, piperacillin-tazobactam was changed to meropenem until 2 weeks of treatment had been completed. She also improved clinically after CVC removal, although the culture did not reveal growth of microorganisms. The patient was discharged from the hospital at day 17 post-transplant with a good clinical outcome.

In summary, we have presented the case of a 69-year-old woman diagnosed with diffuse large B-cell lymphoma, who suffered a catheter-related bacteraemia due to L. trevisanii. Correct identification of this opportunistic pathogen should include both phenotypic and molecular methods, as it can be difficult to distinguish between Fusobacteria and Lactobacillus spp. In this report, the identification was done directly on blood culture bottles using MALDI-TOF MS, shortening the time period required for identification from days to a few hours, which contributed to a successful clinical outcome for the patient. MALDI-TOF MS provides an accurate, rapid and cost-effective methodology for aetiological diagnosis, allowing rapid identification of bacterial isolates that have been identified inconclusively by conventional methods, therefore improving the management of invasive bacterial infections, especially in immunocompromised patients.

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**References**


