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RESEARCH ARTICLE

Leptotrichia species Bacteremia in Hematological Malignancies

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ABSTRACT

Here we present four clinical cases of immunocompromised patients experiencing bacteremia caused by *Leptotrichia* species in a few months with no common epidemiological link. *Leptotrichia* species are thin anaerobic gram-negative rods that inhabit multiple areas in the human body, including the oral microbiota. Many infections with *Leptotrichia* species occur in immunocompromised individuals classifying *Leptotrichia* species as opportunistic pathogens. Utilization of standard microbial identification methods of matrix-assisted laser-desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) initially yielded the same identification for all four *Leptotrichia* isolates as *Leptotrichia buccalis*. However, 16S ribosomal RNA sequencing confirmed the identification of only one of the four isolates as *L. buccalis*, while two of the four isolates were identified as *Leptotrichia trevisanii*. These four cases highlight the clinical importance of considering opportunistic infection in immunocompromised patients with unusual organisms considered members of the normal oral flora. *J Microbiol Infect Dis 2022; 12(3):130-135.*

Keywords: *Leptotrichia, bacteremia, immunocompromised*

INTRODUCTION

Leptotrichia species are thin anaerobic gram-negative rods that inhabit multiple areas in the human body, including the oral cavity, gastrointestinal tract, the urogenital system, and the female genital tract [1,2]. *Leptotrichia* species are considered opportunistic pathogens and have been isolated in cases of periodontitis and mucositis in the oral cavity [3], chorioamnionitis in the female genital tract [4,5], and endocarditis [6,7]. A preponderance of reported cases of *Leptotrichia* are cases of bacteremia, and they were mainly reported in neutropenic patients with various forms of pre-disposing conditions such as bone-marrow transplants [1,2].

We present four clinical cases of *Leptotrichia* bacteremia in patients with hematological malignancies within three months in our hospital system. Two patients were confirmed to have *Leptotrichia trevisanii* bacteremia via 16S ribosomal RNA (16S rRNA) sequencing, while a third patient was confirmed to have *Leptotrichia buccalis* bacteremia via 16S rRNA sequencing.

CASE PRESENTATIONS

Case 1

The first patient, a 47-year male designated as patient A, had a history of stage IV mantle cell lymphoma and was admitted to the hospital with the concern of fever in the setting of neutropenia. The patient was otherwise healthy but was recently treated for a perianal abscess which was treated successfully with antibiotics. At the time of admission, the patient had an elevated temperature of 99.7 ^{0}F, a white blood cell count of 0.6 x10^{9}/liter, and a hemoglobin level of 6.9 g/dL. Blood cultures were collected upon admission to the hospital.

The anaerobic blood culture bottle flagged the next day positively, and the direct Gram stain showed the presence of gram-negative bacilli that appeared to be gram-variable in some instances (Figure 1A). Colonies appeared as dry spreading colonies on 5% sheep blood

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agar plate (Figure 2). Identification of the colonies was performed using matrix-assisted laser-desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) technology using the VITEK MS Research Use Only database, and the result was the identification of *Leptotrichia buccalis* (Table 1). We performed 16S rRNA sequencing on this isolate from patient A and received identification of *Leptotrichia trevisanii* with over 99% homology with *L. trevisanii* type strain AY029801 [8] via both NCBI's Basic Local Alignment Search Tool (BLAST) database [9] and Ribosomal Database Project's (RDP) SeqMatch [10] (Table 1).

The antibiotic susceptibility profile showed a pan-susceptible *Leptotrichia trevisanii* (Table 2). For treatment, this patient received Vancomycin, Cefepime, Augmentin, and Piperacillin-Tazobactam combination on admission but was narrowed to just piperacillin/tazobactam combination for the remainder of the hospital stay. The patient was discharged on ceftriaxone. Repeated blood cultures were not positive for this patient, who was discharged six days after the initial admission.

Case 2

The second patient, a seven-year boy designated as patient B, had Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ALL), who was in remission but had a central nervous system relapse. They presented for a planned 8/8 human leukocyte antigen (HLA) matched umbilical cord blood transplant. The transplant went as planned, and the patient was being monitored for any possible post-transplant complications. Around ten days post-transplant, the patient developed mucositis, which caused signification pain. Six days after the development of mucositis, the patient developed a fever of up to 101.6 ^{0}F, which prompted the collection of blood cultures.

The anaerobic bottle became positive a day after the collection, and the gram-negative bacilli showed in the direct Gram stain (Figure 1A). Dry spreading colonies were seen 24 hours after subculture from the blood bottles (Figure 2). The VITEK MS results identified *Leptotrichia buccalis* with confidence scores of 78.4 (Table 1). We performed 16S rRNA sequencing on this isolate from patient B and received identification of *L. trevisanii* (Table 1). Similar to patient A, there was over 99% homology to the *L. trevisanii* strain AY029801 [8].

Antibiotic susceptibility showed another pan-susceptible *L. trevisanii* for patient B (Table 2). This patient was treated with a course of intravenous metronidazole for ten days. Repeat blood cultures for this patient were positive for *Pseudomonas aeruginosa*, but the *L. trevisanii* was not seen again.

Case 3

The third patient, a 17-year male designated as patient C, was a previously healthy individual who presented to the emergency department with fatigue, weight loss, and poor appetite. The patient had elevated liver enzymes with aspartate transaminase (AST) of 11,128 units per liter (U/L) and alanine transaminase (ALT) of 7,438 (U/L) when they had been in the normal range just one month prior. The patient also had an abnormal liver function suggestive of early liver failure. Upon admission, blood cultures were collected due to early liver failure in this, otherwise healthy, patient. Interestingly, this patient was found positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). However, the physicians did not attribute the clinical picture of early liver failure to SARS-CoV-2. More tests were performed, and the patient was diagnosed with B-cell acute lymphoblastic leukemia.

In the laboratory, the anaerobic bottle flagged positively the day after collection, and the direct Gram stain showed the presence of gram-negative bacilli (Figure 1B). In a similar time-frame as the other patients, dry grey colonies were seen on the culture plates. The VITEK MS results identified *Leptotrichia buccalis* with confidence scores of 99.9 (Table 1). 16S rRNA sequencing of this isolate from Patient C had the first hit on NCBI BLAST and RDP SeqMatch, confirming the identification of *L. buccalis* with over 99% homology to the 16S rRNA gene of the type strain *Leptotrichia buccalis* C-1013-B [11].

Again, antibiotic susceptibility showed pan-susceptible *Leptotrichia buccalis* (Table 2). This patient was empirically treated with piperacillin-tazobactam, and then when the patient decompensated, they were transitioned to meropenem and vancomycin. After 72 hours with that treatment plan, the patient was

switched to cefepime due to severe neutropenia.

Case 4

Lastly, our final patient, a 28-year female designated as patient D, was recently diagnosed with acute myeloid leukemia (AML) and presented to the hospital for their first bone marrow transplant. The day after a matched unrelated donor transfer of eight million cells, the patient complained of fatigue, low energy, and slight oral discomfort. This oral discomfort continued until it was the chief complaint nine days post-transplant and was eventually diagnosed as mucositis. The patient became febrile to 100.8 ^{0}F in the afternoon of the post-transplant 10th day, prompting blood culture collection.

The blood cultures were positive on day two of incubation in the anaerobic blood culture bottle. A direct Gram stain revealed gram-negative bacilli (Figure 1). The blood was subcultured, and plates were incubated in an enriched CO2 environment. Twenty-four hours later, colonies appeared that were about 1 mm in size with a dry, fan-like grey appearance, and then at 48 hours of growth, the colonies became more defined grey colonies (Figure 2). Identification of the colonies was performed using matrix-assisted laser-desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) technology using the VITEK MS Research Use Only database, and the results were indeterminately giving confidence scores of 70.2 for *Leptotrichia buccalis* (Table 1). A 16S rRNA sequencing was attempted to identify the species level more definitively. However, due to insufficient growth of this organism, we could not obtain an accurate identification with 16S rRNA sequencing.

Antibiotic susceptibility testing was performed on this *Leptotrichia* isolate, which was found susceptible to all antibiotics tested (Table 2). For treatment, the patient was given one dose of cefepime on the day of blood culture collection and then placed on piperacillin-tazobactam combination infusions until discharge. Then, the patient was put on metronidazole to complete a 14-day treatment course. Repeat blood cultures were not positive for this patient.

DISCUSSION

Leptotrichia species are thin anaerobic gram-negative rods that inhabit multiple mucosal areas on the human body. Infections with these organisms are considered rare but mainly occur in immunocompromised individuals, making them opportunistic pathogens [1,2]. The four cases presented here could be regarded as classical cases of *Leptotrichia* bacteremia. Many reported *Leptotrichia* bacteremia cases to involve patients with hematological malignancies [1,2]. One specific pre-disposing factor for developing sepsis by *Leptotrichia* species is neutropenic fever and mucositis [12]. This disruption of the integrity of the oral cavity caused by mucositis is considered a portal of entry for *Leptotrichia* into the bloodstream [2]. All four of these patients had a hematologic malignancy, and three had a fever in the setting of neutropenia that prompted blood culture collection. Specifically, in patients B and D, there was an indication of mucositis before the elevation of the fevers. In patient C, *Leptotrichia* was seen in the blood without fever and before chemotherapy.

There were no commonalities among these patients to suggest a common bacterial acquisition. There was no overlap in the admission dates of these four patients; therefore, they did not share rooms or units. Three patients underwent bone marrow transplants during their admission, with patients B and D being admitted for the procedure and C having it scheduled after their initial admission. All three of these patients were on different chemotherapeutic agents. Patient A was in remission and not on any chemotherapeutic agents at the time of admission. As a result of no commonalities, we decided whole genome sequencing of these isolates was not necessary to show the relatedness of these organisms. *Leptotrichia* bacteremia in patients with malignancies is generally associated with a dysbiosis of the patient's normal oral flora [12], which is known to be very diverse between individuals [13].

The *Leptotrichia* genus comprises eight species, with *L. buccalis* and *L. trevisanii* comprising most of the reported case studies [1,2]. *Leptotrichia* species are often misidentified using conventional microbiology methods due to their similarity to other anaerobic bacteria. In addition, mass spectrometry technology is limited depending on the system being used [14].

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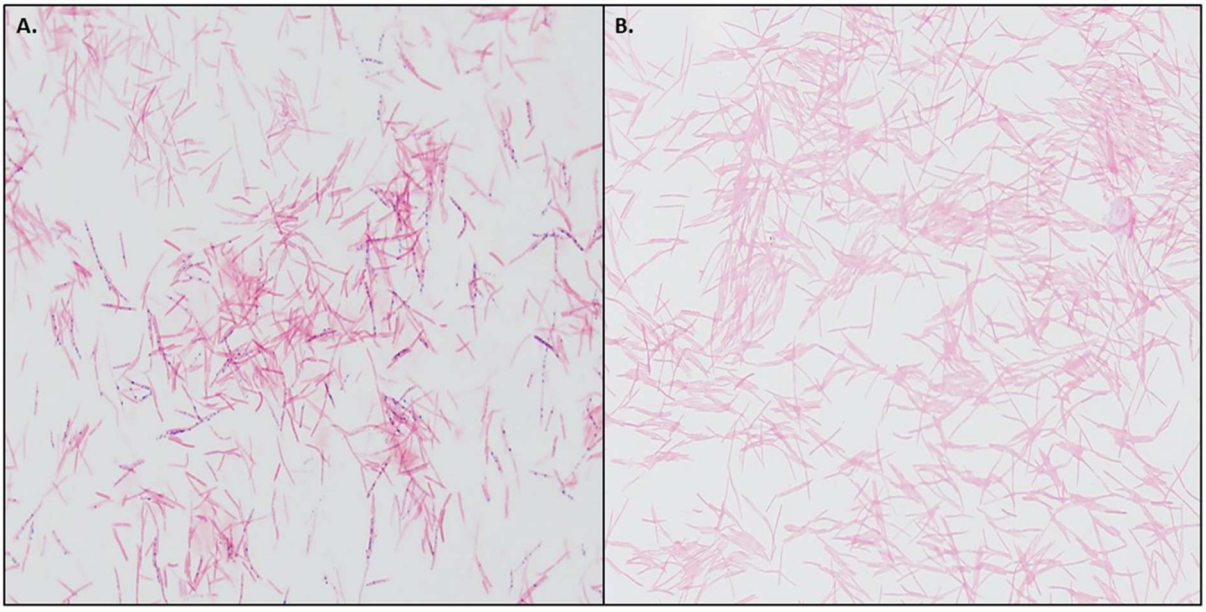

Figure 1. Gram Stain Images of *Leptotrichia* species. A. Image of *L. trevisanii* Gram stain directly from 24 hours of growth on the 5% sheep's blood agar plate. Long thin gram-variable rods can be seen. This is characteristic of fresh cultures of *Leptotrichia* species. B. Image of *L. buccalis* Gram stain from 48 hours of growth on the 5% sheep's blood agar plate. Long thin gram-negative rods can be seen (1000X oil immersion).

Table 1. Patient Demographic Information and *Leptotrichia* Organism Identification.

Demographic Information	**VITEK MS DATABASE**		**NCBI DATABASE**			**RDP SeqMatch**	
	Organism Identification	Confidence Value	Organism Identification (Accession)	Query Score	Percent Identity	Organism Identification (Accession)	Match	Total RDP Sequences
Patient A	*Leptotrichia buccalis*	N/A	*L. trevisanii,* LB11 (AY029801)	100%	99.54%	*L. trevisanii,* LB11 (AY029801)	0.971	1383
Patient B	*Leptotrichia buccalis*	78.4	*L. trevisanii,* LB11 (AY029801)	100%	99.08%	*L. trevisanii,* LB11 (AY029801)	0.940	1383
Patient C	*Leptotrichia buccalis*	99.9	*L. buccalis,* C-1013-b (CP001685)	100%	99.57%	*L. buccalis,* C-1013-b (CP001685)	0.956	1429
Patient D	*Leptotrichia buccalis*	70.2	N/A	N/A	N/A	N/A	N/A	N/A

*Age Range= the patient's age in years falls within that range

Table 2.Antibiotic susceptibility results for *Leptotrichia* species.

	Patient A ***L. trevisanii***		**Patient B** ***L. trevisanii***		**Patient C** ***L. buccalis***		**Patient D** ***L.*** species	
	MIC*	**I****	**MIC***	**I****	**MIC***	**I****	**MIC*)**	**I****
Amoxicillin/Clavulanate	0.032	S	0.125	S	0.125	S	0.50	S
Cefotaxime	0.047	S	0.094	S	0.125	S	0.047	S
Clindamycin	0.016	S	0.023	S	0.023	S	0.023	S
Meropenem	0.032	S	0.064	S	0.047	S	0.094	S
Metronidazole	0.032	S	1.0	S	0.38	S	1.0	S
Penicillin	0.016	S	0.023	S	0.064	S	0.19	S

*MIC=Minimum Inhibitory Concentration (µg/mL), I**= Interpretation

The VITEK MS database only has *L. buccalis*, while the Bruker Biotyper (Bruker Daltonics) contains *L. trevisanii* and *L. wadei* [14]. Our initial identification of the four isolates was *L. buccalis*, but for two of the cases, we were able to obtain 16S rRNA sequencing data (Table 1). We received identification of *L. trevisanii*. Therefore, we would advise reporting the isolate identification as *Leptotrichia* species unless 16S rRNA sequencing can be performed to confirm the species identification. Our system had high confidence scores for all the isolates identified as *L. buccalis*, confirmed in one of the cases with 16S rRNA sequencing. However, two of the four cases initially identified as *L. buccalis* were confirmed to be *L. trevisanii* by sequencing.

Figure 2. *Leptotrichia trevisanii* on Blood Agar Plates-Image is of *L. trevisanii* growing on 5% of sheep's blood agar plates after 48 hours of growth.

Leptotrichia trevisanii was first recognized as a separate species of *Leptotrichia* in 2001 when it was isolated from the blood of a patient with acute myeloid leukemia (AML) [15]. This species of *Leptotrichia* has mostly been detected in blood cultures [1,2]. In a review of the case reports of *Leptotrichia* bacteremia, *L. trevisanii* accounted for 25% of these cases [1, 2]. However, it should be noted that this may be an underestimate since *Leptotrichia buccalis* was considered the only known species of *Leptotrichia* until 1995 [16].

Antibiotic resistance has not been well documented in *Leptotrichia* species, and many of the case reports in the literature have documented *Leptotrichia* being very susceptible to antibiotics [1,2]. Therefore, there are no specific treatment recommendations or guidelines for *Leptotrichia* bacteremia; treatment needs to be tailored to the patient. Our antibiotic susceptibility data reflect what is known in the literature: all four isolates were pan-susceptible to the antibiotics tested (Table 2). Each patient was placed on an antibiotic regimen after the fever had increased and the blood cultures were drawn. In all four cases, the *Leptotrichia* organism was only recovered from one set of blood culture bottles. The remaining blood cultures were negative for patients A, C, and D, while patient B had *P. aeruginosa* in subsequent blood culture bottles.

This appearance of transient bacteremia suggests that *Leptotrichia* species have low pathogenicity. However, oral microbiome studies have correlated a higher presence of *Leptotrichia* in patients with varying dental caries [17]. While no direct studies have been conducted with *L. trevisanii*, *L. buccalis* has been studied experimentally in a rabbit model [18]. The study compared the virulence of an *L. buccalis* endotoxin to other enteric endotoxins and concluded that the *L. buccalis* endotoxin had a potency of 10-20% compared to the *Escherichia coli* endotoxin but was more potent than the *Salmonella* endotoxin [18]. Other than the endotoxin work, transmission electron microscopy images of *L. buccalis* showed protruding structures on the cell surface that may promote attachment to cells [19]. However, the extent of this work was only to show the images, and no further studies were pursued.

CONCLUSION

Here we have four separate cases of *Leptotrichia* bacteremia in one hospital system within three months. There was no common epidemiologic link among these four patients. Of note, two of the four patients had clinical evidence of mucositis. We identified these organisms to the genus level using MALDI-TOF MS, but arriving at the species level required 16S rRNA sequencing. In three of the four cases, we obtained a species identification. Two cases were due to *L. trevisanii*, and the third was due to *L. buccalis*. These four cases highlight that opportunistic infection in immunocompromised patients may

occur from organisms considered members of the normal oral flora and required anaerobic blood culture for detection. In addition, special molecular microbiologic techniques are necessary to confirm the precise species identification of *Leptotrichia.*

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