Case Description: A 4-year old female patient with a history of acute lymphocytic leukemia presented to the Emergency Department with a fever of 100.9°F, vomiting, diarrhea, and complaints of bi-lateral leg pain. A Complete Blood Count (CBC) was performed and blood cultures were collected upon admission to the Emergency Department. CBC results indicated a WBC count of 0.2 K/mcL (normal range 5.5-15.5 K/mcL) with a decreased Absolute Neutrophil Count. Bacteremia was suspected based on the patient’s febrile neutropenia; therefore, piperacillin-tazobactam and vancomycin were administered to the patient while in the Emergency Department. The patient was also being treated with fluconazole for a case of oral thrush. In order to monitor her fever and neutropenia, she was admitted to the Pediatric Intensive Care Unit (PICU) and transferred to the Hematology/Oncology service.

Four days after collection, the anaerobic blood culture bottle flagged as positive in the Becton Dickinson (BD) Bactec System. A Gram-stained smear was examined revealing long, Gram-negative bacilli (Figure 2). The specimen was inoculated onto 5% sheep blood agar, chocolate agar, MacConkey agar and anaerobic blood agar for bacterial isolation. Aerobic plates were all incubated in 5% CO₂ at 35°C. The anaerobic media was incubated in an anaerobe chamber at 35°C. After two days of incubation, gray colonies approximately 1.0 mm in diameter were observed on both the aerobic and anaerobic sheep blood
agar plates, but growth was much heartier in the anaerobic environment (Figure 1). Colonies with a slight pink hue were seen on the chocolate agar, and no growth was observed on the MacConkey agar. Colonies were negative for both catalase and oxidase production. A Remel Rapid ANA II (Thermo Fisher Scientific, Lenexa, KS) was performed on the isolate, following the manufacturer’s procedures, with inconclusive results; repeat testing remained inconclusive (Figures 3 & 4). The isolate was subcultured to anaerobic blood agar and sent to FOCUS Diagnostics reference laboratory (Cypress, CA) for identification. What do you suspect the organism to be?

See page 82 for case followup and discussion.
Case Twenty-Four: Bacteremia Caused by an Uncommon Gram-negative Organism

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The organism was identified as *Leptotrichia* species by FOCUS Diagnostics based upon conventional biochemical reactions and examination of cellular morphology on the Gram-stained smear. Refer to Tables 1 and 2 for phenotypic characteristics and key biochemical reactions used in the identification of *Leptotrichia* spp.

After the initial positive blood culture, subsequent blood cultures were all negative. The patient continued treatment with piperacillin-tazobactam, vancomycin, fluconazole and acetaminophen until being discharged from the hospital eleven days after admission. After discharge, she was reported to be afebrile and was no longer vomiting or experiencing diarrhea.

**Background**

*Leptotrichia* species are typically anaerobic, non-spore-forming, non-motile, highly saccharolytic, Gram-negative bacilli found, primarily, as normal flora in the mouth, but can also be found in the intestinal tract and female genital tract. Upon first isolation, *Leptotrichia* grow better under anaerobic conditions, but some species have been known to grow in the presence of CO₂, especially after repeat subculture. *Leptotrichia* are typically present in dental plaque and can be found orally in approximately 17% of children before tooth eruption and in as many as 71% of children after tooth eruption. Lactic acid production by *Leptotrichia* has been implicated in tooth enamel erosion and is a means of distinguishing *Leptotrichia* from *Bacteroides* and *Fusobacterium*. *Leptotrichia* species have been implicated in bacterial vaginosis, endocarditis, liver abscesses, gingivitis, acute appendicitis, cellulitis, wound infections after dog bites, and in cases of bacteremia in immunocompromised patients, particularly those with lesions of the oral mucosa. Such breaks in the mucosa can allow the organism into the bloodstream, potentially leading to bacteremia. Its pathogenicity can be attributed to both lipopolysaccharide production and protruding structures on the cell surface, possibly aiding in attachment.

*Leptotrichia* belongs to the family *Fusobacteriaceae* and in the phylum *Fusobacteria*. The genus *Leptotrichia* contains six identified species: *L. buccalis*, *L. trevisanii*, *L. goodfellowii*, *L. hofstadii*, *L. shahii* and *L. wadei*. There are 179 16S rRNA sequences of *Leptotrichia* species that have been deposited in the GenBank, the majority of which still have yet to be named.

**Clinical Presentation**

Systemic infections caused by *Leptotrichia* spp. are likely to present as a fever caused by the release of endotoxin or lipopolysaccharide (LPS). Some patients may also present with oral lesions. A number of cases of *Leptotrichia buccalis* bacteremia have been reported naming such portals of entry as diverticulitis, esophageal lesions, and mucositis and noted mucosal disruption as a likely cause for systemic infection. Many patients with reported *Leptotrichia* infections have lowered immune defenses from cancer, diabetes, or cystic fibrosis and may already be on broad spectrum antibiotics. Cases of endocarditis caused by *Leptotrichia* spp. have been reported in patients with no altered immune status. A patient with a history of erosive gastritis presented with fatigue and weight loss, while another patient with periodontal disease presented with fever, chest pain and shortness of breath. Blood cultures from both patients recovered a Gram-negative bacilli identified as *Leptotrichia goodfellowii* by 16s rRNA sequencing.
Bacterial vaginosis caused by *Leptotrichia* spp. has also been reported. One such case described a 24-year-old female presenting with fever, malaise and rigors 5 weeks after an uncomplicated vaginal delivery. The causative organism was identified as *Leptotrichia amnionii* after it was recovered from pus from a renal abscess. Rare cases have been reported in which spontaneous abortion has been associated with isolation of *Leptotrichia amnionii.*

A case of pneumonia was reported with *Leptotrichia* spp. as the potential causative organism in an otherwise healthy subject with no decayed teeth or dental caries observed. The patient presented with a low-grade fever, sore throat, and a non-productive cough. Culture of bronchoalveolar lavage fluid collected from the patient grew mixed oral bacteria along with a predominating Gram-negative bacilli identified as most similar to *Leptotrichia wadei* via 16S rRNA sequencing.

Bacterial arthritis was reported in a 59-year-old male with a history of diabetes and long-term hemodialysis. The patient was admitted to the hospital with swelling and pain in his left knee. A culture of purulent fluid aspirated from the patient’s knee grew a Gram-negative bacilli identified as *Leptotrichia amnionii* by 16S rRNA sequencing.

**Laboratory Identification**

Isolation and identification of *Leptotrichia* spp. can pose a challenge. While most strains grow better under anaerobic conditions, some strains of *Leptotrichia* have been found to grow aerobically in the presence of CO2. Also, cellular morphologies can vary among species. Colonies are typically 0.5mm-3.0mm in diameter on sheep blood agar plates and may appear opaque and dry or grayish brown and irregular. *Leptotrichia* can be difficult to identify in the laboratory, especially when anaerobic growth conditions are not available. Fresh, or newly synthesized, cell walls from young cultures can cause *Leptotrichia* to have a Gram-positive appearance upon staining, so discerning Gram-variability can be challenging. Biochemical reactions and phenotypic characteristics can vary among species of *Leptotrichia* (See Tables 1 and 2). It can also be difficult to distinguish *Leptotrichia* from *Lactobacillus* and *Fusobacterium,* so it is thought that *Leptotrichia* infections are largely underdiagnosed. If necessary, 16S rRNA sequencing can be performed to identify *Leptotrichia* spp.

**Antimicrobial Agents and Therapy**

A number of oral antimicrobial agents can be used for the treatment of infections caused by *Leptotrichia* spp.
Leptotrichia spp. including: clindamycin, rifampin, penicillin, tetracycline, imipenem, and chloramphenicol. Questions have been raised concerning antibiotic prophylaxis and pre-disposing patients to infections with Leptotrichia. Cases have been noted in which patients were treated with vancomycin, ciprofloxacin, and trimethoprim/sulfamethoxazole (antimicrobial agents to which Leptotrichia is resistant) prior to infection.

**Conclusion**

The clinical importance of *Leptotrichia* is not clear due to the difficulty of isolation and identification. While *Leptotrichia* is typically non-pathogenic, clinicians and microbiologists should be aware of *Leptotrichia* as a possible pathogen in immunocompromised patients, particularly those with oral lesions and/or those who have been on therapies of aminoglycosides, vancomycin, or ciprofloxacin prior to infection.

**References**


Questions for STEP Participants

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1. *Leptotrichia* spp. can grow under which conditions?
   A. strictly anaerobic environments
   B. strictly aerobic environments, no CO₂
   C. microaerophilic environments
   D. both A & C

2. *Leptotrichia* spp. are susceptible to which of the following antimicrobials?
   A. vancomycin
   B. ciprofloxacin
   C. clindamycin
   D. trimethoprim/sulfamethoxazole

3. *Leptotrichia* spp. are considered normal flora in which of the following?
   A. nares
   B. oral cavity
   C. skin
   D. stool

4. Gram-variability can be difficult to discern in young cultures of *Leptotrichia* with newly synthesized cell walls.
   A. True
   B. False

5. Production of the following can aid in distinguishing *Leptotrichia* from *Bacteroides* and *Fusobacterium*:
   A. lactic acid
   B. catalase
   C. oxidase
   D. esculin

6. The pathogenicity of *Leptotrichia* spp. can be attributed to:
   A. capsule formation
   B. exotoxin production
   C. motility
   D. endotoxin production

7. Cases of *Leptotrichia* infection have been described in all of the following EXCEPT:
   A. acute appendicitis
   B. diarrhea
   C. bacteremia
   D. liver abscess

8. *Leptotrichia* spp. are NEGATIVE for all of the following EXCEPT:
   A. indole production
   B. Nitrate reduction
   C. catalase production
   D. motility

9. Antibiotic prophylaxis may pre-dispose patients to infections with *Leptotrichia*.
   A. True
   B. False

10. *Leptotrichia* could be the possible causative agent in which of the following cases?
    A. 8-year old with inflammation of the gum-line
    B. 22-year old with a history of acute myelogenous leukemia and a recent tooth extraction with a possible bacteremia
    C. 15-year old cystic fibrosis patient with an infected wound after having been recently bitten by a neighborhood dog
    D. all of the above