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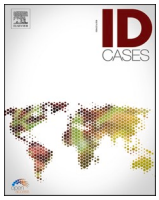

Case report

Eubacterium callanderi bacteremia: A case report

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ABSTRACT

Eubacterium species are a group of obligated anaerobic gram-positive bacilli that are recognized as commensals of the gastrointestinal tract flora. Cases of bacteremia mediated by *Eubacterium* are rare. This report describes a case of bacteremia caused by *Eubacterium callanderi* in an 82-year-old female with a history of a cecal perforation secondary to an obstructing sigmoid stricture. The results showed the utility of using whole genome sequencing to identify the causative agent and underlined the significance to identify anaerobic organisms in diagnostic microbiology practice and to perform antimicrobial susceptibility testing to guide therapy and enhance patient outcomes.

Introduction

Anaerobic bacteria are rare causes of bacteremia when compared to aerobic bacteria [1]. However, with the advanced technologies related to blood culture now utilized in the clinical microbiology laboratory, more anaerobes are isolated and recognized as blood-borne pathogens with *Bacteroides fragilis* group the most common species identified [1–3]. Rarer anaerobes such as *Eubacterium* species, a group of non-spore-forming gram-positive rods, have recently been identified as causes of bacteremia [4–6]. The *Eubacterium* species is considered part of the commensal flora in the gastrointestinal tract, and they can be associated with intra-abdominal infections and abscess formation under opportunistic conditions [7,8]. This report describes a case of *Eubacterium callanderi* bacteremia associated with a cecal perforation secondary to an obstructing sigmoid stricture in an elderly individual.

Case report

An 82-year-old female with a past medical history of stage 3 chronic kidney disease, hypertension, mitral stenosis secondary to rheumatic fever, and chronic constipation presented to the Emergency Department with a 1-week history of diarrhea and worsening abdominal pain. She reported that the pain had initially been crampy and slowly worsening but had become suddenly worse over the past day. Upon an examination, cardiovascular and pulmonary systems were normal while the abdominal examination revealed severe, diffuse tenderness to palpation and significant pain while sitting up or lying flat, consistent with peritonitis. Neurological and musculoskeletal systems were both unremarkable and her skin was warm and dry. On admission, her body temperature was 36.6 °C, she was hypotensive with a blood pressure of 82/46 mmHg, and she had normal heart rates (88–92 beats per minute) and respiratory rates.

A complete blood count revealed a leukopenia of 2300 white blood cells/mm^3 with hemoglobin, hematocrit, and platelet count within normal ranges. A comprehensive metabolic panel revealed a decreased serum potassium of 3.1 mmol/L with normal ranges for blood glucose, total bilirubin, blood urea nitrogen, creatinine, and liver function enzymes. A computed tomography angiography of the chest, abdomen and pelvis was obtained revealing pneumoperitoneum concerning for a hollow viscus perforation. No stool culture was performed.

The patient was taken emergently to the operating room for an exploratory laparotomy. Prior to surgery, two sets of blood cultures were collected by peripheral draw followed by the initiation of piperacillin-tazobactam therapy due to concern for an intraabdominal sepsis. Intraoperatively, the patient remained hemodynamically unstable requiring vasopressor support and she subsequently was taken to the

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surgical intensive care unit post-operatively; intubated and sedated. Her post-operative course was largely uneventful and she was weaned from vasopressors and extubated on post-operative day 1. She was transitioned out of the intensive care unit on post-operative day 3, originally requiring tube feeds via a nasogastric tube, but eventually able to tolerate a general diet. She was discharged from the hospital to a skilled nursing facility on hospital day 16 in stable condition. The etiology of the obstructing sigmoid colon stricture will be investigated endoscopically as an outpatient, with the differentials to include diverticular disease, malignancy, and stercoral colitis.

After 30 h of incubation, the anaerobic blood culture bottles for both sets flagged positive with gram positive rods (Fig. 1). Organism identification via matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) had no hit in the FDA-cleared library (Bruker MALDI Biotyper CA System Reference Library Claim 6) but had matches for *Eubacterium limosum* with scores of 1.96 (isolate 1) and 2.33 (isolate 2) from the research-use-only library. Since MALDI-TOF MS may be unable to distinguish between *E. limosum* and *E. callanderi* [4], whole genome sequencing was performed on isolate 2 to confirm the identification of the organism. Following sequencing, A BLASTN search on the assembled 16S rRNA gene sequence was originally done which matched 100 % with *E. callanderi*. Next, a pangenome analysis was performed using *Eubacterium* genome references from the NCBI genome database (https://www.ncbi.nlm.nih.gov/genome/). The sequence clustered with *E. callanderi* reference genome (Fig. 2A). To confirm this finding and to allow for more gene content to be included in the pangenome analysis, only representative genomes of *E. limosum* and *E. callanderi* were compiled, and the *Eubacterium* isolate clustered with the *E. callanderi* genomes (Fig. 2B). However, the isolate shared most similarity with GCA_018363664 which was a Metagenome-assembled Genome submitted as an *E limosum.* Upon further inspection of the submission, GCA_018363664 failed the taxonomy check and the best match at the time the analysis was run was an *E. callanderi* genome (GCA_03422015) with an average nucleotide identity of 99.14 %, suggesting an incorrect organism designation and that our case isolate was confirmed as *E. callanderi.*

Antimicrobial susceptibilities were performed on both blood culture isolates with each being susceptible to ampicillin, amoxicillin-clavulanate, piperacillin-tazobactam, cefoxitin, meropenem, metronidazole, while resistant to clindamycin, tetracycline, and moxifloxacin (Table 1).

Materials and methods

Blood culture

Two sets of blood cultures collected via peripheral draws (<10 min apart) were performed using the automatic BD BACTEC FX blood culture system (BD, Franklin Lakes, New Jersey) [9]. Both BD BACTEC Plus Aerobic/F Culture Vials and BACTEC Lytic/10 Anaerobic/F Culture Vials bottles were used for each set with a standard incubation time of 5 days. Since only the anaerobic bottles were positive, they were examined via Gram-stain and subcultured onto anaerobic (CDC) blood agar plates (Remel, Lenexa, KS) and incubated at 37 °C anaerobically via the ANOXOMAT III Anaerobic Jar System (Advanced Instruments, Norwood, MA).

Organism identification

The Bruker Microflex LT matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) (Bruker, Billerica, MA) was used for the identification of the organisms [10]. The Bruker MBT Compass software and MALDI Biotyper CA System Reference Library Claim 6 and The MBT Compass reference library (research use only) were used to determine the bacterial species. According to manufacturer's cutoffs, scores of > 2.0 were used for high confidence organism identification, and scores between 1.7 and 2.0 were used for low confidence organism identification. Whole genome sequencing (WGS) was performed on the ClearLabs Dx automated system using the iSeq integrated instrument (Illumina, San Diego, CA) at the Nebraska Public Health Laboratory following the microbial surveillance whole genome sequencing from bacteria isolate method.

Genome assembly and phylogenetic analysis

Illumina paired-end reads were trimmed, and de novo assembled using an in-house pipeline that available at the following GitHub repository: https://github.com/rchapman2000/de-novo-assembly-pipeline. The raw reads and assembly were deposited to NCBI with the accession number PRJNA1107636 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA1107636).

Reference genomes corresponding to the accession numbers in Fig. 2 were downloaded from NCBI Genome. All reference and assembled sample genomes were annotated using Prokka v1.14.6. A pan-genome analysis was subsequently performed on the annotated genomes (GFF files) using Panaroo v1.4.2 [11] with the following selections: MAFFT v7.520 set as the aligner, clean-mode set as moderate, remove-invalid-genes supplied to remove annotations that do not

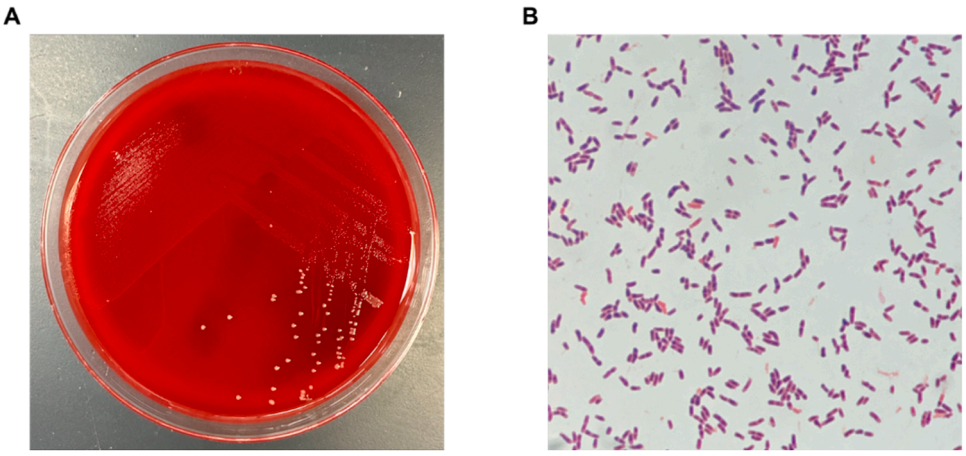

Fig. 1. (A) *Eubacterium callanderi* isolated from the patient's positive blood culture plated on anaerobic blood agar (CDC) incubated anaerobically. (B) Gram stain of *Eubacterium callanderi* from culture 1000X magnification.

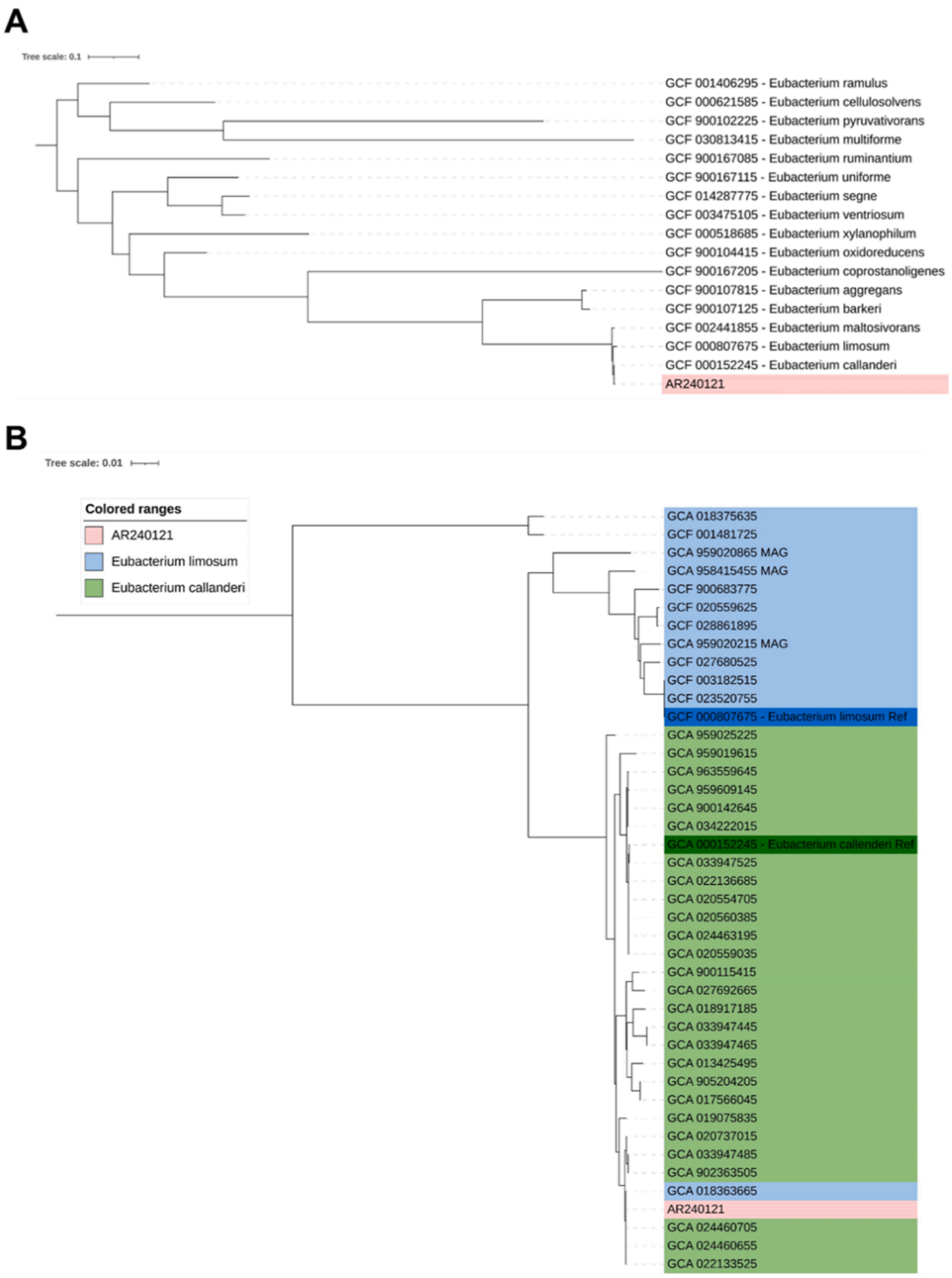

Fig. 2. Phylogenetic analysis of *E. callanderi* isolate 2 (AR240121, light red). (A) Phylogenetic tree of the case isolate compared to reference genomes of *Eubacterium* species to include *E. maltosivorans*, *E. limosum* and *E. callanderi*. The case isolate is genetically most closely related to *E. callanderi*. (B) Phylogenetic tree of the case isolate compared to genomes of *E. limosum* (highlighted in blue) and *E. callanderi* (highlighted in green). The case isolate is genetically most closely related to a cluster of *E. callanderi* strains. Interestingly, another genome (GCA 018363665, is annotated and classified as *E. limosum* but clustered with the *E. callanderi* strains. This is likely due to a misannotation since the genus is not well defined and many strains often undergo reassignment.

Table 1
Antimicrobial susceptibilities of the *Eubacterium callanderi* isolate 2.

Antimicrobial Agent	MIC (μg/ml)	Interpretation[ab]
Ampicillin	0.064	S
Amoxicillin-clavulanate	0.064	S
Piperacillin-tazobactam	0.125	S
Cefoxitin	0.5	S
Meropenem	0.016	S
Clindamycin	> 256	R
Tetracycline	24	R
Metronidazole	0.023	S
Moxifloxacin	> 32	R

[a] Interpretation based on the Clinical Laboratory Standards Institute M100 [14].
[b] S: susceptible, R: resistant.

conform to the expected Prokka format (i.e. premature stop codons, etc.), merge_paralogs, and finally core_threshold set to 0.98 (e.g. the frequency of a gene in a sample required to classify this gene as "core"). The alignment from Panaroo was then passed to IQ-Tree v1.6.12 to reconstruct an evolutionary tree using the ModelFinder setting to determine the best-fit model for the input data [12]. Finally, the phylogenetic tree was visualized and annotated using the Interactive Tree of Life (iTol) v6.9 [13].

Antimicrobial susceptibility testing

The minimum inhibitory concentrations (MICs) of antimicrobial agents were determined via agar dilution method with Etest strips (bioMerieux, Marcy-l'Étoile, France) with 48 h anaerobic incubation at 37 °C. *Bacteroides fragilis* ATCC 25285 was used as a quality control strain. The susceptibilities were determined according to Clinical and Laboratory Standards Institute (CLSI) guidelines [14].

Discussion

Anaerobic blood stream infections account for 0.5 % to 12 % of all bacteremia cases with *Bacteroides fragilis* and *Clostridium perfringens* the most common anaerobes detected [15,16]. Although reports of *Eubacterium* bacteremia cases date back in the 1980s [17], *Eubacterium* species are still considered rare causes of bacteremia.

In this paper, a case of *Eubacterium callanderi* bacteremia from a patient with a cecal perforation caused by an obstructing sigmoid colon stricture is reported. Based on the current study and others, recovery of *Eubacterium* in blood cultures may be indicative of pathology in the gastrointestinal tract (GI), caused by a hollow viscus perforation or colon cancer [5,17]. A case was reported from Thailand of a patient with colon cancer who developed three episodes of bacteremia, and one of those was mediated by *Eubacterium limosum* [5]. A case of *Eubacterium callanderi* bacteremia was also reported from a patient with bladder carcinoma however the gastrointestinal tract was unlikely to be the source [18]. In another case, *Eubacterium moniliforme* was detected in the blood from a women with orthopedic fractures thus not associated with the GI tract as well [19]. It was also suggested that *Eubacterium* bacteremia are likely to be associated with immunocompromised patients [6]. Despite the reports of *Eubacterium* infections, the pathogenesis mechanisms of *Eubacterium* and host-microbe interactions in *Eubacterium* infections are still ill-defined.

In the present study both isolates from the two blood culture bottles were identified via MALDI-TOF MS as *Eubacterium limosum*. However, upon whole genome sequencing, the species was confirmed as *Eubacterium callanderi*. This was not a surprise since *E.limosum* and *E.callanderi* are closely related genetically where MALDI-TOF MS was not able to provide accurate differentiation between these [4]. Our study underscores the usefulness of species identification via comparative genomic analysis. With regards to antimicrobial susceptibilities, the *Eubacterium callanderi* isolates in our case were susceptible to most of the commonly used anti-anaerobe antibiotics tested, including piperacillin-tazobactam which was used to treat our patient. Nonetheless, it is worth noting that our isolates were resistant to clindamycin, tetracycline, and moxifloxacin, which was confirmed by the detection of clindamycin and tetracycline resistance genes (*ermB* and *tetW*) through whole genome sequencing. This is consistent with the results from a retrospective study of *Eubacterium* bacteremia in Sweden, where 12 out of 14 *Eubacterium* isolates were interpreted as resistant to clindamycin [4]. However, in a retrospective study of bacteremia cause by *Eubacterium* in Taiwan, only one *Eubacterium callanderi* isolate was non-susceptible (intermediate) to clindamycin but susceptible to moxifloxacin [6]. This is suggestive of the phenotypic and genetic diversity of *Eubacterium* isolates which causes bacteremia and therefore recognizing the emerging resistance to antimicrobial agents among the *Eubacterium* species is critical.

In conclusion, this report presents a rare case of bacteremia caused by *E. callanderi* and showed the utility of molecular sequencing using the whole genome for species identification and successful treatment with piperacillin-tazobactam.

Ethical approval

The study was identified by the University of Nebraska Medical Center Institutional Review Board (IRB) as "does not require IRB review".

Consent

Consent to publish was not obtained since the case report does not contain any personal identifiers

CRediT authorship contribution statement

Michael R. Wiley: Writing – original draft, Formal analysis, Data curation. **Bennett J. Berning:** Writing – review & editing, Writing – original draft. **Peter C. Iwen:** Writing – review & editing, Supervision. **Paul D. Fey:** Writing – review & editing, Supervision. **Chunyi Zhou:** Writing – original draft, Data curation, Conceptualization, Writing – review & editing. **Casey Schwee:** Writing – original draft. **Ronald E. Matovu:** Data curation, Investigation. **Jessica D. Wiley:** Writing – original draft, Formal analysis, Data curation.

Declaration of Competing Interest

The authors declare no competing interests.

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