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Identification and Antibiotic Susceptibility of *Eggerthella lenta* in Bloodstream Infections

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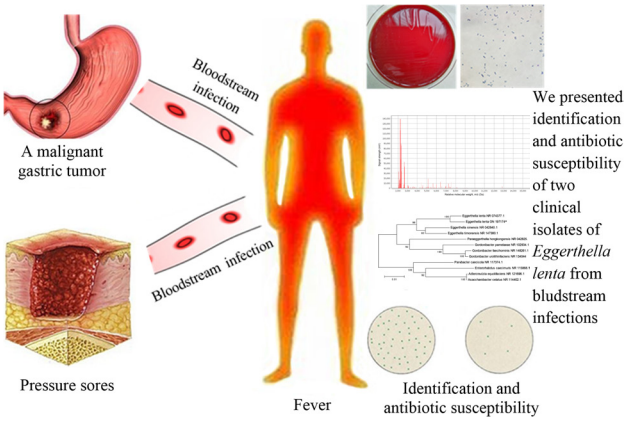
Abstract

The identification and antibiotic susceptibility of two clinical isolates of *Eggerthella lenta* from bloodstream infections were determined. This microorganism is rarely pathogenic, and the findings are presented here to promote the detection and awareness of this infection. The bacteria were obtained from one patient with pressure sores and another with a malignant gastric tumor. Smears were prepared, stained, and examined by microscopy. Single colonies were analyzed by Gram staining, MALDI-TOF MS, and the 16S rRNA gene sequencing. Antibiotic sensitivity was assessed by the agar dilution method. The bacilli were found to be Gram-positive, and the MS results showed 99.8% homology with *E. lenta*. It was confirmed by gene sequencing. Antibiotic susceptibility tests demonstrated that *E. lenta* was sensitive to piperacillin-tazobactam, ampicillin-sulbactam, imipenem, meropenem, metronidazole, clindamycin, and vancomycin. This study could increase awareness of this rare infection.



K e y w o r d s: *Eggerthella lenta*, bacteremia, identification, antibiotic sensitivity

Introduction

Eggerthella lenta (formerly *Eubacterium lentum*) is a non-sporulating, anaerobic, Gram-positive bacillus. It was renamed in 1999 based on the 16S rRNA gene sequencing (Kageyama et al. 1999; Wade et al. 1999). The bacterium grows optimally at 37°C with arginine, and it is a component of the human gut microbiome. Genome sequencing showed that it was closely related to *Paraeggerthella hongkongensis* and *Eggerthella sinensis* (Saunders et al. 2009). Unlike the anaerobes commonly identified in blood cultures, *Bacteroidetes fragile*

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and *Clostridium difficile*, *E. lenta* is relatively rare (Deng et al. 2018; Silva-García et al. 2019; Fatima et al. 2020). As a pathogenic agent, it may cause appendicitis, liver and skin abscesses, and on rare occasions may enter the blood after the primary disease, resulting in bacteremia.

In addition, it has been found that Cgr2, a cardiac glycoside reductase from the gut microbe *E. lenta*, is responsible for reducing and inactivating the widely used cardiac drug digoxin. It was observed that the Cgr2-digoxin interaction primarily involved negatively charged polar amino acids and several hydrophobic residues. The bioactivity of plant-derived lignans is also dependent on *E. lenta,* which is critical for enterolignan production (Kumar et al. 2018; Bess et al. 2020). Few reports describe this bacterium in China (Lee et al. 2014; Woerther et al. 2017; Ugarte-Torres et al. 2018). In addition, over the past four years, only two cases involving *E. lenta* have been identified in blood cultures in our hospital.

Nevertheless, the mortality rate of bacteremia caused by *E. lenta* is high, reaching 22–43%, suggesting that this bacterium should receive more attention (Haiser et al. 2014). In this study, we describe two bacteremia samples positive for *E. lenta.* The samples were cultured, and their characteristics and antibiotic sensitivity were investigated.

Experimental

Materials and Methods

Reagents and instruments. The following reagents and instruments were used: agar powder (Jiangmen Kailin Trading Co. Ltd., China); Ezup Column Bacterial Genomic DNA Extraction Kit SK8255 (Shanghai Shenggong Biological Company, China); SanPrep Column DNAJ Glue Recovery Kit SK8131 (Shanghai Shenggong Biological Company, China); PMD®18-T Vector Connection Kit D101A (TaKaRa Company, Japan); SanPrep Column Plasmid DNA Microextraction Kit SK8191 (Shanghai Shenggong Biological Company, China); DNA Marker and Premix Taq III (TaKaRa Company, Japan); Vitek MS CHCA matrix solution (Bio-Merieux Company, France); MALDI-TOF MS Mass Spectrometer (Bio-Merieux Company, France); synthesized primers (Shanghai Shenggong Biological Company, China); ABI9700 PCR instrument (Applied Biosystems, USA); ABI3730XL sequencing equipment (Applied Biosystems, USA); DNA electrophoresis tank DYCP-31DN (Beijing Liuyi Instrument Company, China); stabilized electrophoresis apparatus (Beijing Liuyi Instrument Company, China); electric thermostatic water tank DK-8D (Shanghai Yiheng Instrument Company, China); Gel Imager System FR980 (Shanghai Furi Instrument Company, China); constant-temperature incubator DHP9162 (Taicang Science and Education Equipment Factory, China); constant-temperature shaker TH2-C (Taicang Experimental Equipment Factory, China); refrigerated high-speed centrifuge HC-2518R (BBI Life Science Corporation, China); Surf series precision single-channel adjustable pipette SP10-1000 (Shanghai Shenggong Biological Company, China).

Isolation and characterization of *E. lenta*. Two *E. lenta* strains were successfully identified from positive anaerobic blood cultures between January 2018 and January 2022 in the Department of Laboratory Medicine, First Affiliated Hospital of Anhui Medical University. The bacteria were cultured anaerobically on the Columbia blood agar at 37°C and 5% CO_2, followed by Gram staining and observation under microscopy.

MALDI-TOF MS identification. One pure colony was selected and added to 10 µl of 70% formic acid; 1 µl of this was then mixed with 0.5 µl of matrix solution, dried, and evaluated by MALDI-TOF MS on a Vitek MS platform. Spectral data were analyzed by comparison with typical spectra.

16S rRNA sequencing and phylogenetic analysis. DNA was extracted with an Ezup Column Bacterial Genomic DNA Extraction Kit, following the provided instructions. PCR amplification of the 16S rRNA gene was performed on an ABI 9700 system (Applied Biosystems) using a reaction volume of 25 µl containing 2.5 µl 10 × Prime-STAR buffer, 1 µl dNTP mixture, 0.2 µl PrimeSTAR HS DNA polymerase, 0.5 µl forward primer, 0.5 µl reverse primer, 10 ng template DNA, made up to volume with ddH_2O. The forward primer was 27F (5'-AGAGTTTGATCATGGCTCAG-3'), and the reverse primer was 1492R (5'-TACGGCTACCTTGTACGACTT-3'). The reaction procedure was as follows: 96°C for 3 min; 96°C for 30 s; 58°C for 30 s; 72°C for 1 min, for 35 cycles, and 72°C for 10 min. The amplified products were sequenced using the ABI3730XL sequencer, and the sequences were compared with those of other bacteria in GenBank. MEGA 7.0 was used for phylogenetic tree construction.

***In vitro* antibiotic sensitivity test.** Antibiotic sensitivity was investigated using the agar dilution method. The results were analyzed in accordance with the 2019 CLSI document (CLSI 2019).

Patient data. Case 1: A 56-year-old man was admitted to the hospital with pressure sores accompanied by fever. He had an axillary temperature of 39.2°C. After anaerobic culture, the responsible organism was identified as *E. lenta*, with a time to positivity of 70.7 h. The patient was treated with meropenem and vancomycin. The patient was discharged after recovery.

Case 2: An 80-year-old man who had experienced breathing difficulties with choking sensations for two

months was brought to the hospital and diagnosed with a malignant gastric tumor and hypertension. At the time of admission, the patient's axillary temperature was 39°C. *E. lenta* was detected in anaerobic cultures, and the time to positivity was 70.0 h. Imipenem-cilastatin sodium and vancomycin were given immediately. The patient passed away because of his serious illness.

Results

Colonies appeared gray, translucent, smooth, and slightly raised after 72 h in culture. The diameters were between 0.25 and 0.75 mm, and hemolytic rings were not apparent (Fig. 1). The bacteria were Gram-positive (Fig. 2). The MALDI-TOF MS results indicated 99.8% homology to *E. lenta* (Fig. 3). The 16S rRNA gene sequence was consistent with that from *E. lenta*. Phylogenetic analysis showed that the isolate was present on the same branch as *E. lenta* (Fig. 4). The isolates were thus identified as *E. lenta* with the GenBank accession number ON 197174 for the 16S rRNA gene sequence. Both strains were sensitive to piperacillin-tazobactam, ampicillin-sulbactam, imipenem, meropenem, metronidazole, clindamycin, and vancomycin. They were resistant to amoxicillin, penicillin, cefotaxime, and ceftriaxone. Table I shows the minimum inhibitory concentrations (MICs) of the antibiotics tested.

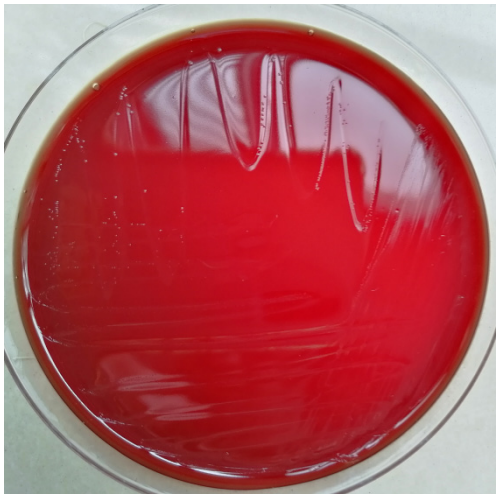

Fig.1. Culture of the pure *E. lenta* isolate after 72 h.

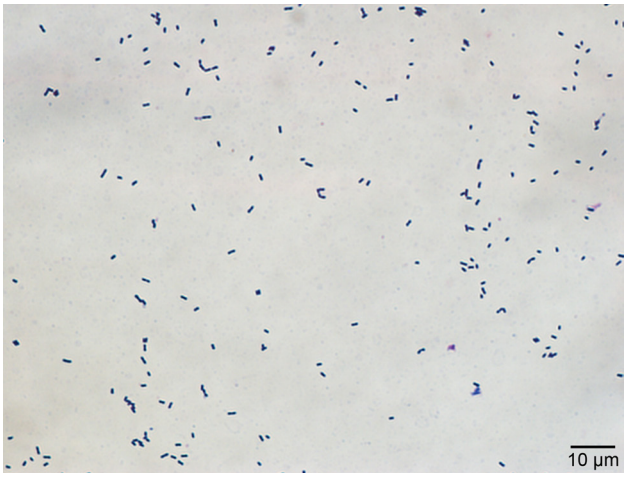

Fig. 2. Gram staining of *E. lenta* isolate.

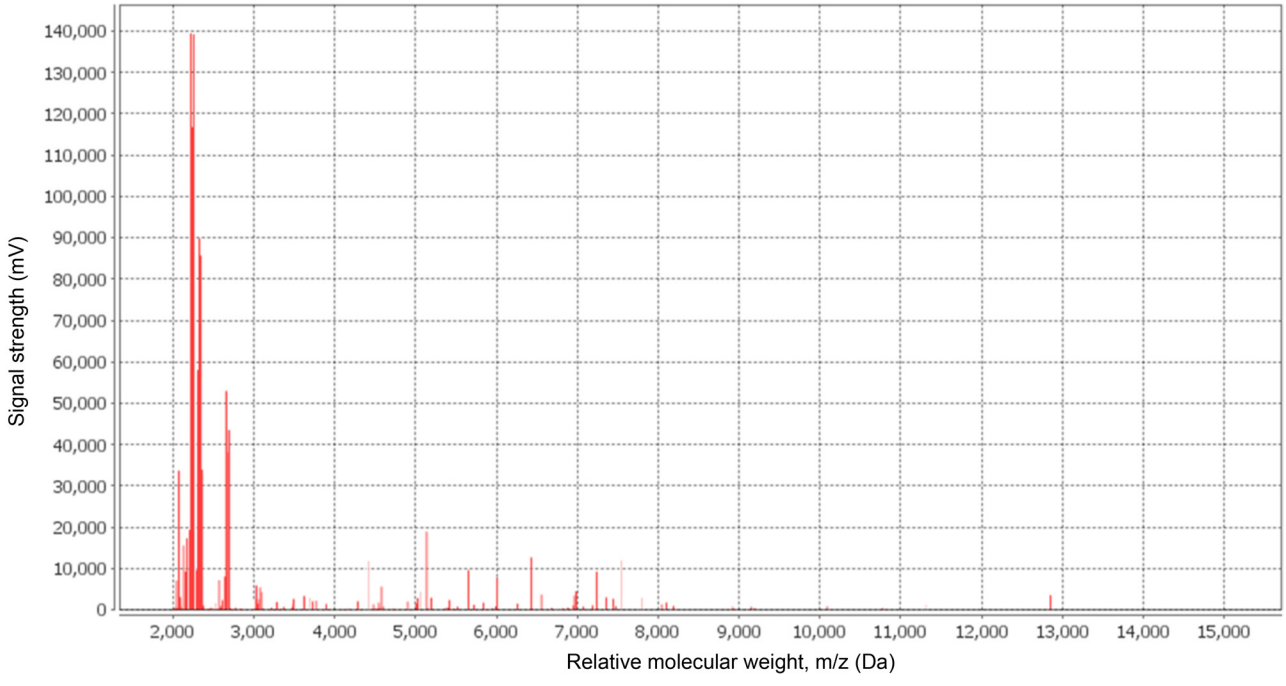

Fig. 3. Mass spectra of *E. lenta* isolates.

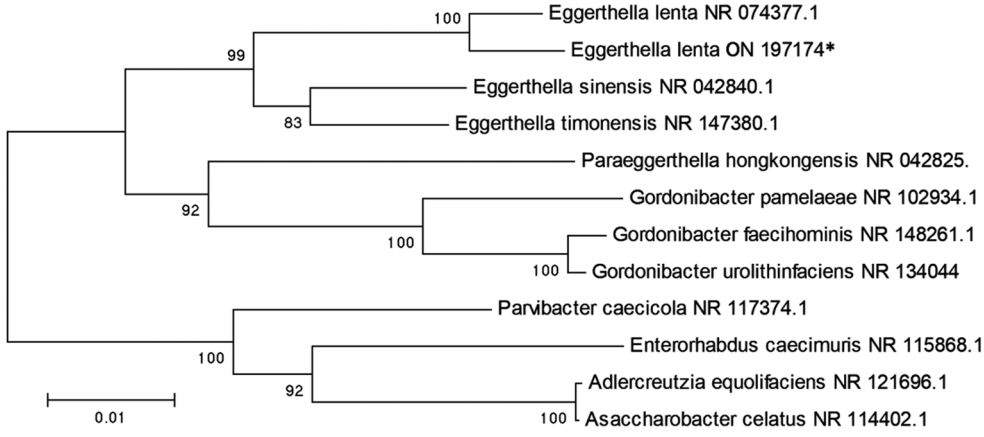

Fig. 4. Neighbor-joining phylogenetic tree of *E. lenta* isolates.

Table I
Antibiotic sensitivity of *E. lenta* isolates.

Antibiotics	MIC (mg/l)	Results
Piperacillin-tazobactam	16	Sensitive
Imipenem	0.5	Sensitive
Meropenem	0.5	Sensitive
Metronidazole	0.5	Sensitive
Clindamycin hydrochloride	1	Sensitive
Vancomycin	2	Sensitive
Amoxicillin	16	Resistant
Penicillin	16	Resistant
Cefotaxime	64	Resistant
Ceftriaxone	64	Resistant

Discussion

It is challenging to identify anaerobic Gram-positive bacilli because many are small, slow-growing, fastidious, and biochemically inert. The isolation of *E. lenta* is traditionally challenging, presenting obstacles to an accurate diagnosis. However, the application of mass spectrometric techniques allows for the rapid and accurate identification of bacterial pathogens (Ugarte-Torres et al. 2018). Here, two isolates were identified as *E. lenta* using the MALDI-TOF MS identification and the 16S rRNA gene sequencing, with phylogenetic analysis confirming the molecular findings. The MALDI-TOF MS and sequencing results were consistent and highly accurate.

Currently, the confirmation of *E. lenta* as the cause of infection presents significant difficulty. The bacillus is found as part of the microbiome in the gastrointestinal tracts of healthy individuals. It has also been identified in other sites such as the frontal sinus, skin, vagina, and liver. Overall, the risk factors for *E. lenta* infection can be classified into three categories: a compromised immune system, digestive disorders, and potential predisposing conditions. Infections are usually associated with clinical conditions, such as pressure sores, malignancies, diabetes mellitus, trauma, surgery, gastrointestinal pathology, and hepatobiliary diseases. The *E. lenta* infections were associated with pressure sores and malignancy in our study. The first patient had severe pressure sores, and his skin barrier was likely broken, allowing the bacteria to access the bloodstream. The patient in case 2 had a malignant gastric tumor, and the bacteria might access the bloodstream through damaged areas of the stomach. Bacteremia due to *E. lenta* should be managed cautiously as it has been found to have a poor prognosis. Unfortunately, few studies on the efficacy of antimicrobial treatment and outcomes could guide the management of these infections. In terms of drug resistance, Stinear et al. (2001) identified a *vanB* locus in the *E. lenta* genome, suggesting that the bacillus could develop resistance to vancomycin similarly to enterococci with vancomycin resistance (Venugopal et al. 2012; Stinear et al. 2018).

However, another study by Gardiner et al. (2015) concluded that while *E. lenta* was susceptible to various antibiotics, including amoxicillin-clavulanate and meropenem; it did not appear to be resistant to vancomycin. The present study found that both isolates were sensitive to vancomycin, piperacillin-tazobactam, imipenem, meropenem, metronidazole, and clindamycin hydrochloride. Studies have demonstrated that treating bacteremia caused by *E. lenta* empirically with piperacillin-tazobactam or ceftriaxone monotherapy may increase mortality. Ceftriaxone resistance is likely due to the presence of the β-lactamase gene identified in most *E. lenta* genomes (Buttimer et al. 2022). Antibacterial treatment with amoxicillin-clavulanate, cefoxitin, or the carbapenems has been recommended as a first-line treatment for *E. lenta* bacteremia (Gardiner et al. 2015; Liderot et al. 2016). In the first patient, meropenem and vancomycin were chosen. In contrast, imipenem-cilastatin sodium and vancomycin

were given immediately to cure the infection in the second patient, and both treatments resulted in an excellent clinical response.

Conclusions

In conclusion, *E. lenta* was isolated from two clinical samples and was identified by Gram staining, MALDI-TOF MS, and the 16S rRNA gene sequencing. These are rapid and accurate detection methods and may be completed within 24 h. The isolates were sensitive to piperacillin-tazobactam, ampicillin-sulbactam, imipenem, meropenem, metronidazole, clindamycin, and vancomycin. The above demonstration of rapid identification and antibiotic sensitivity may increase awareness of the rare infection with *E. lenta*.

Ethical statement

The patients' clinical data were collected in accordance with the Medical Ethics committee of the First Affiliated Hospital of Anhui Medical University (Quick-PJ-2021-09-12).

Acknowledgments

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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