



Clinical Importance and Laboratory Diagnosis of *Eikenella corrodens*: Literature Update

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ABSTRACT

Objective: Review the clinical importance of infections caused by *Eikenella corrodens* as well as gather and synthesize the main identification methods from the genesis of its discovery to the present day. **Method:** Between July and September 2022, a literary survey was carried out for this review in scientific books as well as through the electronic search of scientific publications indexed in the Scielo (Scientific Electronic Library), PubMed and Science Direct databases, which allow the use of common terminology in Portuguese, English, French and Spanish. The following descriptors in Portuguese were used *Eikenella corrodens* , pathology, microbiology, diagnosis, laboratory tests. **Literature Review:** *Eikenella corrodens* is associated with dental and periodontal infections, osteomyelitis, endocarditis, septic arthritis, meningitis, eye infections and post-surgical infections. Gram staining, cultural examination and the use of appropriate identification methods can be decisive to obtain a quick result. Biomolecular techniques have revolutionized the identification of microorganisms compared to more conventional techniques that require more time. **Conclusion:** It is imperative to follow the evolution of microbial identification methods that are emerging in order to ensure that a result is given as quickly as possible for clinical guidance.

Descriptors: *Eikenella corrodens*-Pathology- Microbiology-Diagnosis-Laboratory Tests

I. INTRODUCTION

The history of the discovery of *Eikenella corrodens* begins in 1948, when Henriksen discovered a slow-growing anaerobic bacterium that produced a small depression in the culture medium, which he called corrosive Bacillus .¹ In 1958, Eiken isolated a gram-negative anaerobic bacillus from a human abscess capable of adapting to aerobic growth after several subcultures, classifying it within the genus *Bacteroides* of *Bacteroides corrodens*.² Years later, it was discovered that this facultative anaerobic bacterium had a different guanine-cytosine percentage than the genus *Bacteroides* and that it grew aerobically in

a culture medium containing hemin (factor X).² These new discoveries led to the appearance of a new genus in 1972, the genus *Eikenella* , with *Eikenella corrodens* being the first species identified.^{2,3}

Eikenella corrodens is part of the microbiota of the oral cavity, upper respiratory tract and probably the gastrointestinal tract, presenting an opportunistic pathogenic behavior.^{4,5} This bacteria is associated with dental and periodontal infections, osteomyelitis, endocarditis, septic arthritis, meningitis, eye infections and post-surgical infections.^{5,6}

A more in-depth and up-to-date knowledge of the characteristics of *Eikenella corrodens* is important, as this bacterium appears as a rare isolate in routine culture tests, sometimes requiring several days of incubation to be identified.

The main objective of this review is to review the clinical importance of infections caused by *Eikenella corrodens* as well as to gather and synthesize the main methods of identification from the genesis of its discovery to the present day.

II. METHODS

To carry out the review, a microorganism, *Eikenella corrodens* , was initially selected . Because it is a fastidious and slow growing microorganism, it became pertinent to clarify its clinical importance and the evolution in laboratory diagnostic methods for its isolation and identification. Between July and September 2022, a literary survey was carried out for this review in scientific books as well as through the electronic search of scientific publications indexed in the Scielo databases (Scientific Electronic Library), PubMed and Science Direct , which allow the use of common terminology in Portuguese, English, French and Spanish. The following descriptors were used in Portuguese *Eikenella corrodens* , pathology, microbiology, diagnosis, laboratory tests, as well as their translations in English, French and Spanish. We found 25 relevant works on the subject among reviews, original articles and reports. After reading the abstracts, they were



evaluated in their entirety and cited throughout the work.

III. LITERATURE REVIEW

1.1 Taxonomy

The genus *Eikenella* initially included isolates considered to be facultative strains of *Bacteroides* *corrodens*, an anaerobic gram-negative bacillus. Later studies based on genotypic and phylogenetic characteristics changed the species epithet *Bacteroides* *corrodens* to

Bacteroides *ureolyticus* (urease-positive microorganism), and the facultative isolates were grouped in the genus *Eikenella*, the only known species being *Eikenella* *corrodens*.² With the study of 16S rRNA sequencing and rRNA-DNA hybridization, it was concluded that *Eikenella* *corrodens* was related to the *Neisseria* species, being included in the *Neisseriaceae* family, a beta subgroup of proteobacteria.^{4,7} In Table 1 below we can find the taxonomic hierarchy of *Eikenella* *corrodens*.

Table 1 : Taxonomic hierarchy of *Eikenella* *corrodens*^{5,7}

Taxonomic Hierarchy	
Kingdom	Bacterium
subkingdom	Negibacteria
Phylum	proteobacteria
Class	Betaproteobacteria
Order	neisseriales
Family	Neisseriaceae
Genre	<i>Eikenella</i>
Species	<i>eikenella</i> <i>corrodens</i>

Currently, new species of the genus *Eikenella* have been described based on phenotypic, biochemical and genetic characteristics. As of 2019, four species of the genus *Eikenella* have been described: *Eikenella* *laexigua*, *Eikenella* *halliae*, *Eikenella* *farinosa* and *Eikenella* *glucosivorans*.⁸⁻¹⁰

1.2 Phenotypic characteristics

The phenotype (observable characteristics) of a bacterium provides important information that

can be used to differentiate species (Table 2). Some of the important phenotypic characteristics in the taxonomy are: morphology, motility, metabolism, physiology, cellular chemistry, among other characteristics.^{4,5} As one of the main objectives of clinical microbiology is the identification of microorganisms, and speed can be a critical step, the use of a set of well-defined characteristics is essential to arrive more quickly at the identification of the microorganism.⁴

Table 2 : Main phenotypic characteristics of *Eikenella* *corrodens*^{4,5}

Phenotypic Characteristics	Results
Hemolysis on agar - sheep blood - reduction of nitrite to nitrogen gas - catalase - indole - urease - esculin - o-nitrophenyl-β-D-galactopyranoside - deoxyribonuclease - gaseous glucose Acid production from: glucose - maltose - fructose - sucrose - lactose - xylose - mannitol - mannose - galactose - trehalose - raffinose - sorbitol	Negative
Oxidase - reduction of nitrate to nitrite - ornithine decarboxylase	Positive

2. CLINICAL IMPORTANCE

Eikenella *corrodens* is a bacterium that is part of the normal flora of the oral cavity and upper respiratory tract.^{5,11} This opportunistic agent can cause infections in immunosuppressed patients or patients with diseases or trauma in the oral cavity, and it can often be isolated from wounds caused by human bites and injuries caused by physical confrontation.^{3,8} In the oral cavity, this

microorganism may be responsible for some periodontal diseases.^{8,11} There is a high prevalence of *Eikenella* *corrodens* in subgingival plaque samples from patients diagnosed with periodontal disease compared to patients without this pathology.¹¹ This microorganism is part of the mixed bacterial microbiota isolated from gingival and periodontal samples, nasal duct infections and periapical abscesses.^{8,9}



Isolated from a wide variety of infections and clinical specimens, *Eikenella corrodens* isolates have been reported in head and neck infections, including ocular infections (tear abscesses, periorbital cellulitis, corneal ulcers, endophthalmitis), submandibular and mastoid abscesses and abscesses.¹²⁻¹⁴

This bacterium can also be an agent of pleuropulmonary infections, with lung abscesses and empyema being described in some cases.^{11,15}

Eikenella corrodens isolates have been identified in some Central Nervous System (CNS) infections.¹⁶ From periodontal infections, previous otitis media or infections of the paranasal sinuses, this bacterium can affect the CNS causing meningitis, cerebral or paraspinal abscesses and subdural empyemas and osteomyelitis.¹⁷⁻¹⁹

Belonging to the HACEK group (*Haemophilus parainfluenzae*, *H. aphrophilus* and *H. paraphrophilus*, *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella kingae*) *Eikenella corrodens* is one of the opportunistic microorganisms that can cause infective endocarditis, usually in people with prosthetic valves or dependent on intravenous drugs.^{20,21}

As a primary causal agent or in association with other bacteria, *Eikenella corrodens* has been isolated in cases of osteomyelitis, head and neck infections in drug addicts with complications in cases of abscesses in the upper extremities.^{22,23}

Cellulitis and osteomyelitis in the hand are two of the complications that *Eikenella corrodens* can cause as a result of nail biting, human bites and physical confrontations where injuries occur with clenched fists. The presence of this bacterium in the subcutaneous tissue can lead to an extension of the infection to bones and joints, which may cause osteomyelitis and/or septic arthritis.²³

In various types of wound infections and abscesses, *Eikenella corrodens* can be isolated from pure or mixed cultures, including, for example, cases of necrotizing fasciitis.²⁴ *Eikenella corrodens* can survive in adverse conditions such as those existing in the gastrointestinal tract, and can be isolated from abdominal, splenic, hepatic and pancreatic abscesses, peritoneal infections, as well as from the facultative and anaerobic intestinal microbiota.²⁵

Cases have been described where bacteremia due to localized wound infections can lead to hematogenous infections and abscesses in sites such as joint spaces, vertebrae and the female genital tract.²⁶

Gynecological infections by the infectious agent *Eikenella corrodens* have been described in cases of vulvar abscess, chorioamnionitis, endometritis, amniotic fluid infection and cervicitis associated with in situ colonization of intrauterine devices.²⁷ Several predisposing factors for *Eikenella corrodens* infections have already been described: individuals with periodontitis and smoking; HIV-positive people; Down syndrome, Papillon-Lefèvre and Behçet's disease; diabetes; human death; vascular prostheses; intravascular drug addicts; immunosuppressed patients and diagnostic procedures are some of the most described factors.²⁴

3. LABORATORY DIAGNOSIS

3.1 Direct Examination – Microscopic

3.1.1 Gram staining

Gram stain is one of the most used and relevant staining techniques in the field of clinical microbiology.²⁸ The Danish bacteriologist Hans Christian Gram developed in 1882 this staining technique that became known by his name.²⁹ The purpose of this staining is to separate the bacteria into two large groups: gram positive and gram negative. The method principle of this staining technique is based on the difference in the composition of the bacterial cell wall and the ability of the walls to retain the dyes used.²⁹ Gram positive bacteria stain violet while gram negative bacteria stain red. The observation of the stained material under the microscope is extremely important to monitor the isolation of samples of the infectious agent, as well as to determine and guide antimicrobial therapy in different infections.²⁸

Eikenella corrodens is a coccobacillus, gram negative, facultative anaerobe, fastidious growth, 0.3 to 0.4 by 1.5 to 4 µm.^{3,4,29} Its main microscopic characteristics in terms of morphology, size and gram staining are shown in Table 3. Figure 1 (A) shows an example of gram staining of *Eikenella corrodens* from a 48 hour growth culture.²⁸

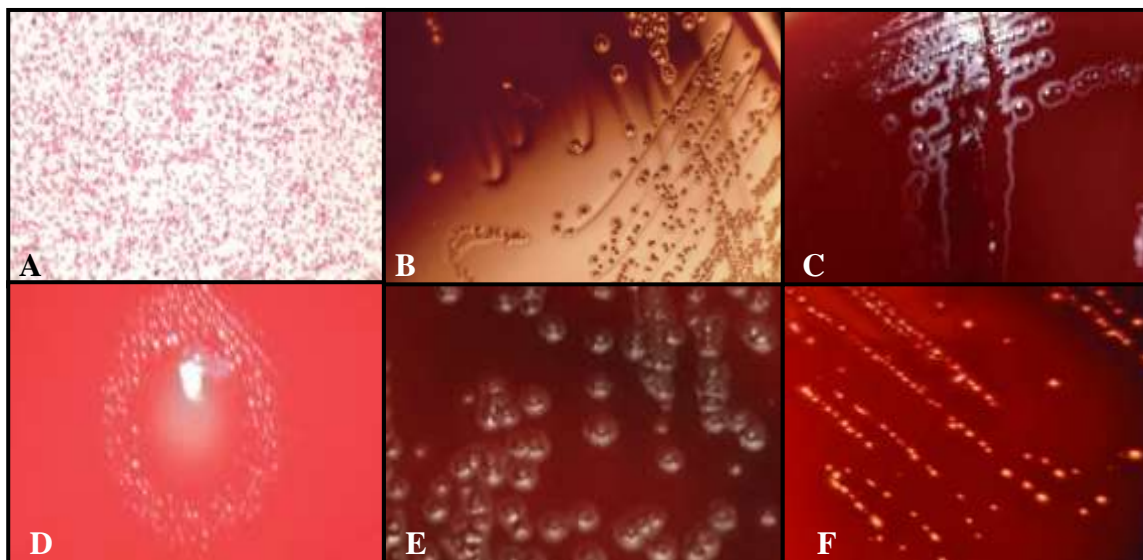
**Table 3** : Main microscopic characteristics of *Eikenella corrodens* ^{3,4,29}

Morphology	Pleomorphic and unbranched bacilli . Filaments short, united and straight with a rounded end. No flagellates, spores or capsule.
Size	0.3 to 0.4 by 1.5 to 4 mm
gram coloring	Negative

3.2 Cultural exam

Eikenella corrodens grows on chocolate agar and blood agar (rich culture medium), not showing growth on MacConkey agar (selective and differential culture medium for gram- negative bacilli). ^{1,2,24} Colonies are small, measuring approximately 0.5 to 1.0 mm, with rough, circular or irregular borders, gray, translucent, non-hemolytic. ^{2,4} About half of *Eikenella corrodens* isolates can, during growth, penetrate the agar, causing a depression in the culture medium, and perforating and non-perforating strains of the culture medium can be observed during growth in the same culture (Figure 1 (B,C,D and E) ^{28,29} The presence of a pale yellow pigment is generally characteristic of these colonies (this characteristic pigmentation is evidenced when colonies are grown on chocolate agar). Most colonies have a characteristic bleach odor (sodium hypochlorite),

similar to the smell of a heavily chlorinated swimming pool. ^{28,30} These bacteria show fastidious growth, and their growth can be observed after 48 hours of incubation in a rich medium, their growth being favored with an atmosphere of 5% to 10 % CO₂ , 5 to 25µg of hemin/mL. ⁴ Aerobic or facultative anaerobic, this microorganism grows at a pH of 7.2-7.4 and a temperature of 34°C. and culture, *Eikenella corrodens* strains have the same biochemical characteristics. ⁴ On chocolate agar medium, strains that do not perforate the medium can be confused with *Haemophilus* and, if tested with X and V disks, grow around the X^{disk} and do not grow around the V disk . HACEK group, *Eikenella corrodens* has a characteristic that differentiates it from other microorganisms, as it is non-saccharolytic. ⁴ Table 4 shows the main characteristics of the colonies and requirements for the growth of *Eikenellacorrodens*.

Figure 1: *Eikenella corrodens*: Gram staining and cultural characteristics.

A: Gram stain of 48 h culture of *Eikenellacorrodens* ²⁸

B: Growth of *Eikenellacorrodens* on chocolate agar after 72 hours of incubation at 35° to 37°C with 5 to 7% CO₂ . In this image, depressions are visible on the agar surface, typical of most strains of this species ⁴

C: Culture of *Eikenellacorrodens* on blood agar after 72 hours of incubation ²⁸

D: Isolated colony of *Eikenellacorrodens* on blood agar after 48 hours of incubation ²⁸

E: Perforation in blood agar medium of colonies of *Eikenellacorrodens* on blood agar after 48 hours of incubation ³⁰



F: Morphology of Eikenellacorrodens colonies in blood agar culture after 48 hours of incubation at

35°C in an atmosphere of 5% CO₂³⁰

Table 4: Main characteristics of the colonies and requirements for the growth of Eikenellacorrodens (Adapted from EIKEN, 1972)⁴

colonies	Colonies are small, bordered, circular or irregular, gray, translucent, non-hemolytic. Characteristic odor of lye and can penetrate the agar during growth.
Growth	Growth after 48 hours of incubation in rich medium, its growth being favored with an atmosphere of 5% to 10% CO ₂ , 5 to 25µg of hemin/mL5. Aerobic or facultative anaerobic this microorganism grows at pH 7.2-7.4 and temperature of 34°C
Biochemical tests	Consult Table 2 (phenotypic characteristics of Eikenellacorrodens)

3.3 Identification methods in commercial kit systems

The clinical microbiology revolution in the field of bacterial identification began with the launch of a range of standardized galleries that combined high quality, ease of use and reliability of the results obtained.⁴ The bioMérieux API® galleries (Profile Analytical Index) are a worldwide reference technique, in addition to being routinely used, they are also used to evaluate the performance of other identification methodologies.³¹ With different chemical reactions in its various wells, combined with a large identification database currently available on the market, the API® line makes bacterial identification reliable and accurate.³¹ The API® calculation method resulting from the numerical identification of the wells, combined with the software, makes this identification easier and more accurate.^{4,28} Responding to current needs and requirements' apiweb™ was developed, an advanced technology that is essential for manual identification. Used by everyone, everywhere, this easy-to-use, high-throughput web platform contains all the API® gallery databases, enabling the identification of 700 species of bacteria and fungi, presenting a comprehensive biochemical profile and providing a comprehensive report. complete for each ID.⁴

The API® test kit consists of assimilation tests of enzymatic elements and carbon compounds, the number of which varies according to the type of API® test kit used.⁴ For the identification of Eikenella corrodens, two API® galleries can be used: API®20E (bioMérieux) and API®20NE (bioMérieux), the latter requiring the use of complementary tests to identify the species of Eikenella corrodens.³¹

3.4 Identification methods in automatic and semi-automatic systems

In recent decades, automatic and semi-automatic systems have been developed for the identification of microorganisms with the aim of providing a faster response (2 to 7 hours) compared to traditional methods (15 to 24 hours).³² The best known systems that identify the species of Eikenella corrodens are:

- **Vitek ® system (Legacy) (bioMérieux):** introduced in the 1980s to perform identification and antimicrobial susceptibility tests, it was widely used in clinical microbiology with good acceptance (BARON, 2001). The Vitek ® 2 system (bioMérieux) is presented as an integrated modular system composed of a sealed filling unit, an incubator-reader, a computerized control module, a data terminal and a printer.³² This system features several technical advances that have automated some procedures that are performed manually on the Vitek ® Legacy system.³³ Vitek ® 2 detects bacterial growth and metabolic changes in the microchambers of thin plastic cards, using fluorescence technology, introducing a colorimetric device from 2004 onwards.^{32,33} For the identification of Eikenellacorrodens, bioMérieux developed the table of fastidious identification of microorganisms Vitek ® 2 NH that identifies more than 25 microorganisms.³² Using this card is practical and safe.
- **MicroScan Systems wa96, wa plus wa-4-oe autoscan :** The fully automated Walkaway (Beckman Coulter , West Sacramento, CA) incubates any combination of up to 96 conventional or Rapid panels MicroScan ® simultaneously, automatically adding reagents to conventional panels if needed.³⁴ Reads and



interprets test results and prints results without operator intervention.³⁵ In addition to conventional

MicroScan® panels, fast fluorescence panels are also available for use with Walkaway kits that use fluorescently labeled compounds and require only 2 hours of incubation to perform bacterial identification.³⁶ In the case of this system, the panel indicated for the identification of *Eikenella corrodens* is the NH

- **Sensititre® Automated Readout and Incubation System (ARIS):** The Sensititre® Automated Readout and Incubation System (ARIS) (Thermo Fisher Scientific Inc., Waltham, MA) is an automated system that uses fluorescence to detect bacterial growth and activity. This system consists of 32 newly formulated biochemical tests as well as revamped classic media to emit fluorescence signals.³² Each plate is designed to identify up to 3 different microorganisms. Self-identification tests are read on the Sensititre® AutoReader which detects the presence or absence of fluorescence.⁴ The results are thus transmitted to a compartment for analysis and identification. Results can be read after 5 hours, however if results are not satisfactory these plates can be incubated overnight.⁴ The advantage of this equipment is an additional incubation period for the most demanding and slow-growing microorganisms, such as *Eikenella corrodens*.
- **Phoenix® System:** The Phoenix® Automated Microbiology System (Becton Dickinson Microbiology Systems, Sparks, MD) is also one of the systems developed for antimicrobial identification and susceptibility testing. This system consists of disposable panels that combine identification and antimicrobial susceptibility tests, together with equipment that performs automatic readings every 20 minutes during the incubation period.⁴ This kit can also identify *Eikenella corrodens*.

3.5 Identification methods based on molecular systems

Biomolecular techniques came to revolutionize clinical microbiology. These fast and effective techniques allow the identification of fastidious microorganisms in minutes. The big downside is the price. The most frequent biomolecular techniques to investigate *Eikenella corrodens* are:

- **Matrix-Assisted Laser Desorption/Ionisation Time-Of-Flight (MALDI-TOF):** The identification of microorganisms by MALDI-TOF is performed by comparing the mass

spectrum of peptides of an unknown microorganism with existing standards in a database or by combining the masses biomarkers of unknown organisms with the proteome database.³⁷ Although culture conditions can affect the morphology and physiology of microorganisms, they do not influence microbial identification by MALDI-TOF.³⁸ Two examples of equipment that use this methodology are the Microflex LT™, by BrukerDaltonics/BD Diagnostics, and that of bioMérieux, VITEK MS™.

- **Genetic sequencing of 16S ribosomal RNA (rRNA):** A quick method, more precise than conventional ones, which allows the identification of strains that are difficult to grow in the laboratory. Strain differentiation at the molecular level allows discrimination between phenotypically identical bacteria. 16S rRNA binds to a complex of 19 proteins to form a 30S subunit of the bacterial ribosome.³⁹ It is encoded by the 16S rRNA gene, which is present and highly conserved in all bacteria due to its essential role in ribosome assembly; however, it also contains variable regions that can serve as fingerprints for certain species.⁴⁰ These characteristics made the 16S rRNA gene an ideal genetic fragment to be used in the identification, comparison and phylogenetic classification of bacteria.³⁹ Genetic sequencing of 16S rRNA is based on polymerase chain reaction (PCR) followed by DNA sequencing.⁴⁰

IV. CONCLUSION

The identification of fastidious microorganisms such as *Eikenella corrodens*, which is not always easy to isolate and diagnose, represents a challenge for the clinician. It is necessary to deepen knowledge and provide all the clinical information that supports the investigation and isolation of this bacterium, adapting this investigation to the reality of the laboratory. Biomolecular techniques have revolutionized the identification of microorganisms in clinical microbiology compared to more conventional techniques that require more time. However, the cost of acquiring expensive equipment cannot always be supported by the laboratory, which sees added value for the future in these biomolecular techniques.

Sometimes there is not much information about *Eikenella corrodens* and what is available is very scattered. This review made it possible to collect information on the main characteristics of this pathogenic microorganism in oral and non-oral



infections, which have an opportunistic character and are generally common in association with other microorganisms, as well as the existing laboratory means that allow its identification.

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