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Original Article

Epidemiological Insights into HACEK Bacteria: A Seven-year Retrospective Analysis at a Tertiary Care Center in Istanbul

HACEK Bakterilerine Yönelik Epidemiyolojik Bulgular: Istanbul'daki Bir Üçüncü Basamak Merkezde Yedi Yıllık Retrospektif Analiz

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ABSTRACT

Objective: HACEK bacteria (*Haemophilus parainfluenzae, Aggregatibacter* spp., *Cardiobacterium hominis, Eikenella corrodens,* and *Kingella kingae*) represent a group of fastidious organisms implicated in endocarditis and a range of opportunistic infections. Despite their clinical importance, epidemiological data on HACEK infections remain limited, particularly in Türkiye.

Methods: This retrospective analysis investigated 30 cases of HACEK infections diagnosed at a tertiary care hospital in Istanbul over a seven-year period (2017-2023). Data were collected from electronic medical records and laboratory databases.

Results: Patients ranged in age from 0 to 76 years, with isolates derived from a variety of clinical specimens. *Cardiobacterium hominis* was notably absent among the identified species. Polymicrobial growth was documented in 20 cases, predominantly involving Gram-positive cocci, particularly *Streptococcus* spp. Antimicrobial susceptibility testing was performed for four isolates, revealing significant challenges in interpretation due to the absence of standardized guidelines for HACEK pathogens. None of the cases received pathogen-specific therapy; all were managed with empirical antimicrobial regimens. Clinical outcomes were favorable in all but one patient, who succumbed to complications of coronavirus disease-2019. No cases of recurrent HACEK infection or infective endocarditis were observed during follow-up.

Conclusions: These findings underscore the diagnostic challenges associated with HACEK infections and the potential underestimation of their prevalence. Prospective, multicenter studies are needed to clarify the epidemiological and clinical significance of these organisms. Moreover, the development of standardized antimicrobial susceptibility testing protocols and evidence-based therapeutic strategies is essential to optimize patient management and improve clinical outcomes.

Keywords: HACEK group, infectious disease epidemiology, opportunistic infections, polymicrobial infections, microbial sensitivity tests

ÖZ

Amaç: HACEK grubu bakteriler (*Haemophilus parainfluenzae, Aggregatibacter* spp., *Cardiobacterium hominis, Eikenella corrodens* ve *Kingella kingae*), endokardit ve çeşitli fırsatçı enfeksiyonlara neden olabilen, zor üreyen mikroorganizmalardır. Klinik önemlerine rağmen, Türkiye'de HACEK enfeksiyonlarına ilişkin epidemiyolojik veriler oldukça sınırlıdır.

Yöntemler: Bu retrospektif çalışmada, Istanbul'daki bir üçüncü basamak hastanede 2017-2023 yılları arasında tanı konan 30 HACEK enfeksiyonu olgusunu incelemiştir. Veriler elektronik hasta kayıtları ve mikrobiyoloji laboratuvarı veritabanlarından elde edilmiştir.

Bulgular: Hastaların yaşları 0 ile 76 arasında değişmekte olup, izolatlar çeşitli klinik örneklerden elde edilmiştir. Tanımlanan türler arasında *Cardiobacterium hominis* saptanmamıştır. Yirmi olguda çoklu mikroorganizma üremesi görülmüş, en sık eşlik eden bakteriler Gram-pozitif koklar, özellikle *Streptococcus* türleri olmuştur. Antimikrobiyal duyarlılık testi yalnızca dört izolat için uygulanabilmiş, HACEK patojenlerine yönelik standart test kılavuzlarının bulunmaması yorumlamayı güçleştirmiştir. Tüm olgular ampirik antimikrobiyal rejimlerle tedavi edilmiş, hiçbir hasta özel olarak HACEK hedefli tedavi almamıştır. Takip sürecinde sadece bir hasta koronavirüs hastalığı-2019 komplikasyonları nedeniyle yaşamını yitirmiş, diğer hastalarda klinik seyir olumlu olmuştur. Nüks HACEK enfeksiyonu ya da enfektif endokardit olgusu gözlenmemiştir.

Sonuçlar: Bu bulgular, HACEK enfeksiyonlarının tanısal zorluklarını ve gerçek prevalanslarının muhtemel olarak düşük tahmin edildiğini göstermektedir. Bu mikroorganizmaların epidemiyolojik ve klinik etkilerini daha iyi anlayabilmek için çok merkezli prospektif çalışmalara ihtiyaç vardır. Ayrıca, standardize edilmiş antimikrobiyal duyarlılık test protokolleri ve kanıta dayalı tedavi yaklaşımlarının geliştirilmesi, hasta yönetiminin optimize edilmesi ve klinik sonuçların iyileştirilmesi açısından büyük önem taşımaktadır.

Anahtar kelimeler: HACEK grubu, enfeksiyon hastalıkları epidemiyolojisi, fırsatçı enfeksiyonlar, polimikrobiyal enfeksiyonlar, mikrobiyal duyarlılık testleri

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INTRODUCTION

The HACEK group, comprising *Haemophilus parainfluenzae, Aggregatibacter* spp.*, Cardiobacterium hominis, Eikenella corrodens (E. corrodens),* and *Kingella kingae (K. kingae),* is recognized for its role in infective endocarditis and various opportunistic infections, such as septic arthritis, osteomyelitis, and abscesses[1,2]. Predisposing factors for infections caused by these organisms include immunosuppression due to neutropenia, malignancies, and cancer chemotherapy[2].

These organisms are part of the normal flora in the oropharynx, respiratory tract, and gastrointestinal system, but often evade detection in routine microbiological workflows due to their fastidious growth requirements and extended incubation periods[3]. Beyond infective endocarditis, isolating HACEK bacteria from other clinical specimens presents challenges in distinguishing true pathogens from contamination.

While traditional culture methods for HACEK organisms yield low success rates, advancements in Matrix-assisted laser desorption/Ionization-time of flight (MALDI-TOF) mass spectrometry (MS) have significantly improved the rapidity and accuracy of bacterial identification; MALDI-TOF MS achieves species-level identification success rates of 66% with Microflex LT (Bruker) and 93% with Vitek MS (bioMérieux), and genus-level success rates of 88% and 95%, respectively[4]. Additionally, molecular diagnostic methods, such as polymerase chain reaction (PCR), enable the detection of *K. kingae*, a notoriously challenging bacterium to culture, using in-house developed protocols[5]. However, the clinical application of molecular tests for HACEK bacteria remains underdefined, emphasizing the need for clinical suspicion to guide testing.

While the pathogenicity of HACEK bacteria is typically limited, they are responsible for 1-3% of infective endocarditis cases. Notably, *K. kingae* is a leading cause of septic arthritis and osteomyelitis in children under three years of age[4-6]. Mortality rates for infective endocarditis caused by HACEK organisms vary by species, ranging from 5% to 18%[4]. However, data on the clinical outcomes of other HACEK-related infections are limited.

Diagnostic challenges associated with HACEK bacteria are further exacerbated by the absence of robust guidelines for antimicrobial susceptibility testing (AST). The clinical and laboratory standards institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) provide limited recommendations for HACEK pathogens, resulting in a significant gap in standardized testing protocols and complicating treatment decision-making[7,8].

In Türkiye, the literature on HACEK infections is scarce, primarily consisting of isolated case reports. Some abscess infections caused by *E. corrodens* have been documented[9-11]. A four-year multicenter study identified *Aggregatibacter actinomycetemcomitans* as the causative agent in one of 50 infective endocarditis cases[12]. Another multicenter study of 75 children with septic arthritis found *K. kingae* to be PCR-positive in three cases[13]. Beyond these studies, comprehensive epidemiological data on HACEK infections in Türkiye remain limited. This lack of information hampers a thorough understanding of their epidemiological and clinical impact in the region.

This study aims to fill the knowledge gap by retrospectively analyzing cases of HACEK infections diagnosed at a tertiary care center in Istanbul over a seven-year period (2017-2023). The findings are intended to provide valuable epidemiological data and offer insights into the clinical and microbiological characteristics of HACEK infections in the region.

MATERIALS and METHODS

A total of 30 cases of HACEK infections diagnosed over a seven-year period (2017-2023) at a tertiary care hospital in Istanbul were retrospectively analyzed. Data were obtained from electronic medical records and microbiology laboratory databases. The study was conducted in accordance with the Declaration of Helsinki and ethics committee approval for the study was obtained Istanbul Medeniyet University, from the Non-Interventional Ethics Committee (reference number: 2025/04-36, date: 05.03.2025).

Clinical specimens collected between 2017 and 2023 were cultured on 5% sheep blood agar, chocolate agar, and chromogenic agar (bioMérieux, France), under appropriate aerobic or microaerophilic conditions. Specimens submitted via swabs following abscess drainage or surgical procedures were processed as wound cultures. Bacterial species identification was performed using MALDI-TOF MS (Vitek MS, bioMérieux, France), in accordance with the manufacturer's protocols.

AST was performed for only four isolates due to the fastidious nature of HACEK organisms and the absence of standardized testing protocols, which limited routine AST implementation in clinical practice during the study period. AST was conducted using E-test strips (bioMérieux, France). Bacterial suspensions were adjusted to a 0.5 McFarland turbidity standard

and inoculated onto Mueller-Hinton Fastidious (MH-F) agar (bioMérieux, France). Minimum inhibitory concentrations (MICs) were interpreted according to the applicable versions of the EUCAST Clinical Breakpoint Tables (versions 9.1-13.1, corresponding to the study years 2019-2023), using pharmacokinetic/pharmacodynamic non-species-related breakpoints.

Inclusion criteria consisted of patients with confirmed HACEK infections in which the organism was isolated and clinically determined to be the causative agent by the treating physicians. Exclusion criteria included isolates considered clinically insignificant or likely contaminants based on specimen type, clinical context, and physician assessment. In cases of polymicrobial infection, HACEK organisms were deemed causative only when supported by clinical correlation and judgment by attending clinicians. Only one isolate per patient was included in the analysis.

Statistical Analysis

Given the descriptive nature of the study, no advanced statistical analyses were performed. Descriptive statistics were used to summarize patient demographics, infection types, specimen sources, isolated HACEK species, and the proportion of polymicrobial infections with co-isolated microorganisms. Continuous variables were reported as means, median, and range, while categorical variables were expressed as frequencies and percentages. The interquartile range (IQR) was calculated as the difference between the 25th and 75th percentiles of the patient age distribution. All analyses were conducted using Microsoft Excel for Windows (Microsoft 365; Microsoft Corp., Redmond, WA, USA). The findings are descriptive and should be interpreted with caution due to the limited sample size.

RESULTS

Over the 7-year study period, 30 cases of HACEK infections were identified in patients. The isolates were obtained from various clinical specimens, reflecting the diverse clinical presentations of HACEK infections (Table 1).

Of the patients, 20 (66.7%) were male and 10 (33.3%) female, with a median age of 37 years (IQR: 18-59 years; range: 0-76 years). *E. corrodens* was the most frequently isolated species, identified in 25 cases (83.3%) and associated with the broadest spectrum of infections, including 12 wound cultures. Other isolates included *H. parainfluenzae* (n=2, 6.7%), *Aggregatibacter segnis* (n=1, 3.3%), *Aggregatibacter aphrophilus* (n=1, 3.3%), and *K. kingae* (n=1, 3.3%). No cases of *C. hominis* or *Aggregatibacter actinomycetemcomitans* were detected. Clinical specimens were most frequently obtained from wound cultures (n=13, 43.3%), followed by blood (n=5, 16.7%), tissue (n=4, 13.3%), peritoneal fluid (n=4, 13.3%), abscesses (n=2, 6.7%), sputum (n=1, 3.3%), and urine (n=1, 3.3%).

Polymicrobial growth was observed in 20 cases (66.7%), predominantly involving *Gram-positive bacteria* (Table 1). A total of 39 co-isolated non-HACEK organisms were identified, most frequently *Streptococcus species* (30.7%, n=12), followed by *anaerobes* (23%, n=9), including *Parvimonas micra* (n=3), *Prevotella* spp. (n=3), *Fusobacterium* spp. (n=1), and *Gemella* spp. (n=2), and then *Staphylococcus species* (15.3%, n=6).

Bacteremia was documented in five patients: *H. parainfluenzae* in a child with congenital dyserythropoietic anemia; *E. corrodens* in two patients with pulmonary infections and one with acute cholecystitis; and *K. kingae* in a 2-year-old with chronic diarrhea without additional comorbidities.

AST results were available for only four isolates (Table 2), reflecting the challenge posed by the lack of standardized guidelines for HACEK pathogens. None of the patients received targeted therapy for HACEK infections due to the absence of specific treatment protocols; instead, they were managed with empirical therapy containing cephalosporins or ciprofloxacin. Clinical outcomes were favorable in all but one patient, who died of COVID-19 related complications (*A. segnis* isolated from sputum). No mortality directly attributable to HACEK infections was observed. Follow-up cultures showed no recurrence of HACEK growth or subsequent infective endocarditis in any patient.

Table 1. Characteristics of HACEK group infections in patients.

Species	Specimen	Gender	Age	Comorbidity	Polymicrobial infections
Haemophilus parainfluenzae (n=2)	Abscess (n=1)	F	44	Crohn's abscess	*Candida albicans*
	Blood (n=1)	M	7	Congenital dyserythropoietic anemia	*Streptococcus mitis/oralis, Leuconostoc lactis*
Aggregatibacter segnis (n=1)	Sputum (n=1)	M	61	Lung cancer, COVID-19 pneumoniae	-
Aggregatibacter aphrophilus (n=1)	Wound (n=1)	F	8	-	*Staphylococcus epidermidis, Staphylococcus hominis, Streptococcus constellatus*
Eikenella corrodens (n=25)	Wound (n=12)	8 M, 4 F	7-72	• Stomach cancer • Crohn's abscess • Necrotizing fasciitis • Mandibular abscess (n=2) • Cervical lymphadenopathy after cat scratch • Cellulitis after paronychia • Furuncle/carbuncle • Pilonidal cyst abscess • Branchial cleft cyst • Laryngocutanous fistula • Liver abscess	Polymicrobial (n=6); detected with one or more of the following: *S. constellatus (n=3), Streptococcus sanguinis, Streptococcus parasanguinis, Parvimonas micra (n=2), Atopobium parvulum, Fusobacterium nucleatum, Prevotella nigrescens, Prevotella denticola, Klebsiella pneumoniae*
	Tissue (n=4)	2 M, 2 F	13-71	• Insulin-related cellulitis • Skin abscess • Paronychia abscess • Osteomyelitis	Polymicrobial (n=3); detected with one or more of the following: *Staphylococcus aureus, S. epidermidis, Staphylococcus haemolyticus, Streptococcus intermedius, S. anginosus, S. constellatus, Corynebacterium tuberculostearicum*
	Peritoneum (n=4)	3 M, 1 F	4-63	• Perforated appendicitis (n=3) • Colon cancer (n=1)	Polymicrobial (n=4); detected with one or more of the following: *Streptococcus pneumoniae, Enterobacter aerogenes, Pseudomonas aeruginosa, Gemella haemolysans*
	Blood* (n=3)	3 M	44-76	• Bronchial cancer and lung abscess • Acute cholecystitis • Bronchiectasis, empyema	Polymicrobial (n=3); detected with one or more of the following: *S. aureus, S. anginosus, Gemella morbillorum, Prevotella* spp., *Actinomyces odontolyticus, Parvimonas micra, Citrobacter freundii, Klebsiella oxytoca*
	Abscess (n=1)	M	26	Submandibular sialadenitis	-
	Urine (n=1)	M	0	Hydronephrosis, nephrostomy	*Candida parapsilosis, Stenotrophomonas maltophilia*
Kingella kingae (n=1)	Blood (n=1)	F	2	Chronic diarrhea	-

*: In addition to blood samples, *Eikenella corrodens* was also identified in the pleural fluid of one patient and in bronchoalveolar lavage samples from another.

Data include species, specimen type, patient demographics, comorbidities, and co-isolated organisms in polymicrobial infections.

"Polymicrobial infections" indicate other microorganisms isolated from the same sample.

Age is given in years; M: Male, F: Female

Table 2. Antimicrobial susceptibility testing results for HACEK isolates.

Bacteria	Specimen	Year	Antimicrobial	Result*
Eikenella corrodens	Wound	2019	Ampicillin, ceftriaxone	Susceptible
Eikenella corrodens	Aspirate	2022	Penicillin, ampicillin	Susceptible
Eikenella corrodens	Wound	2023	Ampicillin, ceftazidime, ertapenem, imipenem, meropenem, ciprofloxacin, levofloxacin, linezolid	Susceptible
Haemophilus parainfluenzae	Blood	2023	Ceftriaxone, cefotaxime, cefuroxime, ertapenem, meropenem, imipenem, ciprofloxacin, levofloxacin, trimethoprim-sulfamethoxazole	Susceptible except trimethoprim-sulfamethoxazole

*Antimicrobial susceptibility testing was performed and interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines using pharmacokinetic/pharmacodynamic (PK/PD) breakpoints. Results are reported as "susceptible" for isolates showing *in vitro* sensitivity to the listed antibiotics. *H. parainfluenzae* isolate demonstrated resistance to trimethoprim-sulfamethoxazole.

DISCUSSION

C. hominis is a low-virulence organism primarily associated with infective endocarditis, and its isolation from specimens other than blood cultures is exceedingly rare[2]. Consistent with this characteristic, *C. hominis* was not reported in our study, further supporting the infrequent nature of infections caused by this organism in clinical settings.

K. kingae colonizes the upper respiratory tract of infants starting at 6 months, with peak colonization rates occurring between 6 months and 2 years of age[4]. It is a rare cause of bacteremia and endocarditis, particularly in children with underlying conditions, and has also been reported in immunocompetent adults following dental procedures[2].

In our study, we documented a case of *K. kingae* bacteremia in a 2-year-old child, with no identifiable underlying predisposing conditions. While the potential association between a recent dental procedure and the onset of infection remains speculative, it highlights the need for further research to better understand possible predisposing factors. Although *K. kingae* is recognized as a causative agent of septic arthritis in children, arthritis was not observed in this case. The role of *K. kingae* PCR testing in diagnosing arthritis and other infections remains uncertain, and there is no consensus on when to apply PCR in suspected infections. Moreover, the availability of PCR testing is not consistent across all centers, complicating its widespread use. In Türkiye, there is a lack of data regarding oropharyngeal carriage and infection rates of *K. kingae*. As surveillance data become more comprehensive, clearer diagnostic and therapeutic guidelines are expected to emerge. Additionally, the role of dental procedures in *K. kingae* bacteremia warrants further investigation, especially in immunocompetent individuals.

E. corrodens has been implicated in a wide array of infections, including head and neck infections such as ocular, mastoid, submandibular, and thyroid abscesses, as well as pleuropulmonary infections like lung abscesses and empyema. These infections are particularly common in individuals with immunosuppression, a predisposition for pulmonary aspiration, or underlying lung disease. *E. corrodens* has also been isolated in both pure and mixed cultures from various wound infections, including necrotizing fasciitis[2]. In our study, *E. corrodens* was the most frequently isolated species, associated with bacteremia in two patients with pulmonary infections and one with acute cholecystitis.

The ability of *E. corrodens* to cause a diverse range of abscesses, including polymicrobial infections, complicates the identification of the pathogen versus contamination, particularly in mixed cultures. This highlights the challenge of distinguishing between colonization and true infection. Additionally, the pathogenesis of *E. corrodens* remains poorly understood, and further research is needed to elucidate the mechanisms by which this organism contributes to infection. Given its potential as both a pathogen and part of the normal flora, it is clear that more studies are required to better define its role in infections and refine diagnostic criteria. The clinical relevance of polymicrobial infections involving *E. corrodens* further underscores the importance of thorough clinical evaluation and microbiological analysis when identifying the causative agents.

Due to the fastidious nature of HACEK organisms, AST is challenging and is not commonly performed in many centers. Consequently, treatment is often empirically guided by published reports and clinical guidelines. β-lactamase-producing strains may be encountered, and therefore, the use of penicillin or ampicillin is not recommended for invasive infections, such as infective endocarditis. HACEK bacteria are generally susceptible to third-generation cephalosporins and fluoroquinolones,

with ceftriaxone being the first-line treatment and ciprofloxacin serving as an alternative to β-lactams[3,4,14]. The CLSI and the EUCAST provide limited recommendations for HACEK pathogens, resulting in gaps in standardized testing protocols and complicating treatment strategies. Since 2017, EUCAST v.7.1 has included disk diffusion and MIC breakpoints for *K. kingae*, MH-F broth, and for agar. However, guidelines for *Haemophilus* species are restricted to *H. influenzae*[8]. The CLSI guideline M45-ED3 outlines MIC breakpoints for *Aggregatibacter, Cardiobacterium, E. corrodens*, and *Kingella,* using cation-adjusted mueller-hinton broth/laked-horse-blood[7]. Both guidelines cover susceptibility testing for β-lactams, macrolides, fluoroquinolones, tetracyclines, rifampin, and trimethoprim-sulfamethoxazole, and they include a recommendation for β-lactamase testing. Despite these frameworks, significant challenges persist in testing fastidious HACEK bacteria due to their stringent growth requirements and limited data, resulting in the unmet need for standardized protocols across all HACEK group members. Furthermore, the lack of robust resistance surveillance data hampers our ability to monitor trends in antimicrobial resistance, especially in regions where surveillance systems are underdeveloped.

In our data, MIC values were reported for three *E. corrodens* strains and one *H. parainfluenzae* isolate. All four samples were susceptible to β-lactams, while *H. parainfluenzae* exhibited resistance to trimethoprim-sulfamethoxazole. These findings highlight the need for further research into the resistance patterns of HACEK organisms, as both local and global surveillance data on antimicrobial resistance remain limited. Aside from β-lactamase production, the mechanisms underlying resistance to other antibiotic classes remain largely unknown. Despite this, the empirical use of cephalosporins remains a reasonable and effective alternative for treating infections caused by these organisms. The scarcity of comprehensive resistance surveillance data, coupled with the fastidious nature of HACEK bacteria, underscores the need for standardized testing protocols and broader research to better understand the evolving resistance landscape of this group. As more data become available, these findings will contribute to improving treatment strategies and guiding clinical decision-making for infections caused by HACEK organisms.

Study Limitations

This study has several limitations. Its retrospective and single-center design may limit the generalizability of the findings and carry an inherent risk of missing or incomplete data. The relatively small sample size, with only 30 culture-positive cases, restricted the ability to perform detailed subgroup comparisons and limited the statistical power. The absence of standardized AST guidelines for HACEK pathogens posed additional challenges in interpreting susceptibility data. Furthermore, the high proportion of polymicrobial infections complicated the assessment of the pathogenic role of HACEK organisms as the primary causative agents.

CONCLUSION

The prevalence of HACEK infections is likely underestimated due to their demanding growth requirements and limited research, particularly in Türkiye. Future research should prioritize prospective, multicenter studies to better define the epidemiology and clinical significance of the condition. The role of HACEK organisms in polymicrobial infections, especially in immunocompromised hosts, warrants further study to fully elucidate their pathogenic potential. Developing standardized AST protocols and evidence-based treatment recommendations will be crucial for improving patient care and outcomes in HACEK-related infections.

Ethics

Ethics Committee Approval: The study was conducted in accordance with the Declaration of Helsinki and ethics committee approval for the study was obtained Istanbul Medeniyet University, from the Non-Interventional Ethics Committee (reference number: 2025/04-36, date: 05.03.2025).

Informed Consent: This is a retrospective study.

Footnotes

Author Contributions

Concept: M.E.K., T.O., Design: M.E.K., T.O., Data Collection and/or Processing: T.D., Z.H., Analysis or Interpretation: T.D., Z.H., Literature Search: T.D., Writing: T.D.

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