https://doi.org/10.31925/farmacia.2022.5.8 **ORIGINAL ARTICLE**

CURRENT DATA ABOUT THE AETIOLOGY AND TREATMENT OF INFECTIVE ENDOCARDITIS

DANA CARMEN ZAHA [1], MARIA CLAUDIA JURCA [1]*, CRISTIAN DAINA [2], VICTOR VLAD BABEȘ [3], CODRUȚA DIANA PETCHESI [1], ALEXANDRU DANIEL JURCA [1], COSMIN VESA [1], IOANA CORALIA CODREANU [1], EMILIA ELENA BABEȘ [3]

[1] *Department of Preclinical Disciplines University of Oradea, Faculty of Medicine and Pharmacy, Oradea, Romania*
[2] *Department of Surgical Disciplines University of Oradea, Faculty of Medicine and Pharmacy, Oradea, Romania*
[3] *Department of Medical Disciplines University of Oradea, Faculty of Medicine and Pharmacy, Oradea, Romania*

**corresponding author: claudiajurca70@yahoo.com*

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Abstract

The aim of this study was to provide current data on the incidence, the aetiology and the treatment of infective endocarditis (IE) in a group of patients from one centre over a period of ten years. Our study includes data from patients over the age of 18, diagnosed with bacterial endocarditis, over a period of 10 years (January 2010 - December 2019). The data analysed pertains to clinical presentation, lab results, medical imaging, treatment and outcome during hospitalization. A total of 92 cases were included, with an age average of 63.80 ± 13.45 years, and a male/female ratio of 1.13. Most patients had endocarditis on native valves. Native valve IE was more frequent on the mitral valve, while in cases with prosthetic valves, the aortic valve was more frequently affected. A large number of patients had comorbidities and healthcare-associated IE. Negative blood cultures were recorded in 30.43% of the cases, probably due to the administration of antimicrobials before blood cultures were drawn. Gram-positive pathogens were predominant in terms of aetiology. *Staphylococcus aureus* and coagulase negative staphylococci (CoNS) were the most commonly isolated microorganisms, a large number of which were methicillin-resistant. The Charlson comorbidity index was higher in IEs caused by *Enterococcus spp.*, and in those cases the patients were also older. Seven patients had positive cultures for *Streptococcus spp.,* less frequently isolated than *Staphylococcus spp.,* but showing the best antibiotic susceptibility rates. The main etiologic agent involved in healthcare-associated infective endocarditis was *Staphylococcus aureus,* followed by CoNS. Despite the best diagnostic and treatment options, the mortality rate remained high (33.7%). An increased incidence of *Staphylococcus spp.* as an etiological agent was found and this was correlated with complications and increased mortality.

Rezumat

Scopul acestui studiu a fost de a furniza date actuale despre incidența, etiologia și tratamentul endocarditei infecțioase (EI) la un grup de pacienți dintr-un singur centru timp de zece ani. Studiul nostru include date de la pacienți cu vârsta peste 18 ani diagnosticați cu endocardită bacteriană pe o perioadă de 10 ani (ianuarie 2010 - decembrie 2019). Au fost analizate datele privind aspectele clinice, de laborator, imagistice, tratamentul și evoluția din timpul spitalizării. Un număr total de 92 de cazuri au fost incluse cu o vârstă medie a grupului de 63.80 ± 13.45 ani, cu un raport bărbați/femei de 1.13. Majoritatea pacienților au avut endocardită pe valva nativă. Endocardita infecţioasă pe valvă nativă a fost mai frecventă pe valva mitrală iar dintre valvele protetice cea aortică a fost mai frecvent afectată. Un număr mare de pacienți au avut comorbidități și EI asociată asistenței medicale. Hemoculturi negative au fost înregistrate la 30.43% dintre pacienți, probabil din cauza administrării de antimicrobiene înainte de recoltarea hemoculturilor. Etiologia a fost dominată de germenii Gram pozitivi. *Staphylococcus aureus* și stafilococii coagulazo negativi (CoNS) au fost cel mai frecvent izolați, un număr mare fiind rezistenți la meticilină. Indicele de comorbiditate Charlson a fost mai mare în EI cauzate de *Enterococcus spp.*, unde și pacienții au fost mai vârstnici. Șapte pacienți au avut culturi pozitive pentru *Streptococcus spp.*, mai puțin izolat decât *Staphylococcus spp.,* dar prezentând cele mai bune rate de susceptibilitate la antibiotice. Agentul etiologic cel mai frecvent implicat în endocardita infecţioasă asociată asistenței medicale a fost *Staphylococcus aureus,* urmat de CoNS. În ciuda celor mai bune opțiuni diagnostice și terapeutice, mortalitatea a rămas ridicată (33.7%). S-a constatat o incidență crescută a *Staphylococcus spp.* ca agent etiologic și aceasta s-a corelat cu complicații și mortalitate crescută.

Keywords: endocarditis, aetiology, evaluation, mortality

Introduction

Despite over a century's worth of studies and recent advances in diagnosis and treatment, infective endocarditis (IE) remains an incompletely understood disease, with high morbidity and mortality rates [29]. Endocarditis is a multisystem disease, and it refers to an acute or a subacute infection of the endocardial surface of the heart, usually involving the heart valves, the endocardium or intracardiac devices. The patients are relatively young – the

average age is around 50-60 – and the incidence rates reported cover a wide range: from 1 to 15 cases per 100,000 per year [62]. The mortality rate reported also covers a wide spectrum, from 4% to 48%, without a decrease in mortality rate over time [8, 22]. This contrasts with sustained improvements in both diagnosis and in the medical and surgical treatment of endocarditis.

The development of IE requires the presence of the etiologic agent in the blood. IE usually occurs at the site of a predisposing cardiac lesion, triggering the adhesion of platelets, followed by thrombus formation. These sites then provide favourable conditions for microorganisms to adhere and form vegetations. In the case of IE on prosthetic valves, early IE occurs within twelve months of valve placement, and it is usually the result of intraoperative or postoperative contamination of the prosthesis. Late IE occurs at least one year (a minimum of 12 months) after placement of the prosthesis, and it involves microbes and entry portals similar to those of native valve endocarditis. Prosthetic valve IE could cause perivalvular invasion and the extension of the infection to the adjacent tissue, potentially resulting in myocardial abscess, pericarditis, or conduction system disorders, including atrioventricular blocks.

The diagnosis of IE is based on complex clinical, laboratory, and imaging evaluations. Over-diagnosis and under-diagnosis of IE may often occur. Over-diagnosis is followed by unnecessary antibiotic treatment and the development of antimicrobial resistance, while under-diagnosis could be as fatal as delaying diagnosis [10].

The clinical presentation of IE is variable and the evolution difficult to predict. The risk factors described are: age (over 60), gender (male), congenital heart disease, history of valvular disease, haemodialysis patients, injection drug use, history of prior IE, poor dentition or dental procedures, presence of a prosthetic valve or intracardiac device. On the other hand, advances in cardiology and cardiac surgery contribute to a change in the patients' risk factor profile [36, 57]. Healthcare-associated infection has become increasingly common, representing about 30% of all endocarditis cases [30, 56]. Community-acquired IE is diagnosed at the time of admission or within 48 hours of admission in a patient not meeting the criteria for healthcare-associated infection. Healthcare-associated IE manifests itself after more than 48 hours from admission into the hospital or is acquired during an invasive procedure performed in the 6 months before diagnosis. In community hospitals, numerous cases of endocarditis recorded in the last decade were nosocomial, with important regional variations. This type of IE often affects elderly patients and is the result of bacteraemia associated with a hospital procedure. Infected intravascular devices represented the risk factor for at least half of these cases. Other sources include genitourinary or gastrointestinal tract procedures, as well as the infection of the surgical wound. Risk groups include immunosuppressed patients, patients with central venous catheters and patients undergoing haemodialysis.

Endocarditis should be suspected in any patient displaying the following unexplained symptoms: fever, night sweats, signs of systemic illness, especially in the presence of risk factors such as previous cardiac disease, congenital heart disease, prosthetic heart valve, recent history of invasive procedures such as haemodialysis, pneumonia, wound, intravenous drug use, previous therapies (antibiotic or surgical). Clinical signs consistent with IE include both cardiac lesions and evidence for the source of bacteraemia.

The treatment of endocarditis requires antimicrobial therapy, while infected valves frequently require surgical removal and replacement. Valve surgery itself is a risk factor for infection, especially in the first year, after which that risk decreases to about 1%. The patient's progress and successful treatment depends on the quick identification of the etiological agent. Antibiotic treatment of IE is determined by the causative microorganism and its antibiotic susceptibilities [43, 46, 65]. The most frequently isolated germs included Gram-positive cocci: streptococci (especially *S. gallolyticus*), staphylococci, especially *Staphylococcus aureus*, *Enterococcus spp.* [9, 47]. Fungal infections are rare, usually in intravenous drug users and patients with severe underlying conditions [49]. The incidence of methicillin-resistant *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS), vancomycin-resistant enterococci and multidrug-resistant Gram-negative bacteria has increased in recent years. The consequence is a increased number of patients for whom empirical antibiotic therapy is ineffective. Other cases are caused by HACEK organisms and enterococci and less often pneumococci, Gram-negative bacilli, and polymicrobial organisms [25]. HACEK organisms are typically oropharyngeal commensals, and they are also a group of fastidious Gram-negative coccobacillary organisms (*Haemophilus* species, *Aggregatibacter* species, *Cardiobacterium hominis*, *Eikenella corrodens*, and *Kingella* species) that account for approximately 5-10% of community-acquired native-valve endocarditis cases in patients who do not use intravenous drugs.

Blood culture is the gold standard investigation for the detection of microorganisms in the blood and a major diagnostic criterion for IE. It is also important in the diagnosis of prosthetic device infections and bloodstream infections, conditions

associated with pneumonia and osteomyelitis. Unfortunately, the identification of etiologic agents is often unsuccessful because of several factors: antibiotic administration prior to culturing, improper collection and culturing techniques, fastidious pathogens or other issues.

Most studies on IE have been limited by its relative low frequency. Some of these studies consisted of case reports or single-centre studies, which limits the scope and statistical power necessary for definitive conclusions. Moreover, the lack of multinational studies has impaired the understanding of geographic differences in terms of risk factors, patient characteristics, management and progress of patients with IE. Knowing the prevalence of pathogens frequently involved in the pathogenesis of endocarditis in different categories of patients and the susceptibility of those pathogens can help with initial empirical therapy until the etiological agent is identified or in patients with culture-negative endocarditis.

The aim of the study is to describe the incidence, the microbiological and clinical profile, the treatment, and the progress of infective endocarditis in our centre.

Materials and Methods

This retrospective single-centre study includes data from all patients over 18 with clinical suspicion of bacterial endocarditis admitted in the Cardiology Department of the Clinical County Emergency Hospital Oradea, Romania, over a 10-year period (2010-2019). Data pertaining to demographic and clinical characteristics, type of endocarditis (native valve, prosthetic valve), echocardiographic and laboratory investigations, surgical and healthcare-associated procedures six months prior to admission, and hospitalization progress charts were retrieved. Patients were enrolled upon being diagnosed with IE by use of the modified Duke criteria [29, 40] and Charlson's comorbidity index [13]. Laboratory evaluation was performed using inflammation tests, complete blood count (CBC), erythrocyte sedimentation rate, C-reactive protein and biochemical parameters.

Aetiology was highlighted *via* blood cultures and serological tests. At least two sets of special vials were collected at 30 - 60 minute-intervals before the initiation of antibiotic treatment or before the next dose. With IE, collecting blood for culture is not related to a certain time of day because of continuous bacteraemia. The samples were immediately transported to the lab, where they were loaded into an automated blood culture detection system. When the blood samples are detected as positive, they are removed from the system and cultured in accordance with CLSI guidelines for identification and susceptibility testing. The identification of isolates was achieved using biochemical tests and Maldi Biotyper, while antibiotic sensitivity was ascertained using Vitek-2 Compact Systems and the Kirby–Bauer disk diffusion method. The isolate was classified as susceptible, intermediate, or resistant based on the Clinical Laboratory Standards Institute (CLSI) criteria [16]. *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, *E. faecalis* ATCC 29212 were used as quality control strains to check the quality of culture media, as well as antimicrobial cards and disks.

Transthoracic echocardiography and transesophageal echocardiography were performed and analysed. Complications during hospitalization, antibiotic treatment, evolution of patients, and in-hospital mortality rates were also recorded.

Statistical analysis was performed by using SPSS statistical package version 25. Categorical data is expressed as frequencies and percentages. Continuous data is expressed as mean ± SD. The relationship between quantitative variables was analysed using the Pearson bivariate correlation test, and the relationship between categorical variables was expressed using the Spearman bivariate correlation test. For intergroup comparisons, the Kruskal-Wallis test was used for categorical data and the one-way Anova test for continuous data, with post-hoc analysis, in order to determine which of these groups differ from the others. A $p < 0.05$ was considered statistically significant.

The study was approved by the institutional ethics committee and all patients included signed the informed consent form upon admission.

Results and Discussion

During the evaluated period, there were 116 cases of IE admitted in our hospital, but only 92 of these cases had definite IE. The rest were possible IE cases, according to the modified Duke criteria, and they were excluded. We noted an average number of 9.2 ± 4.51 patients admitted *per* year with definite IE. The smallest number of patients diagnosed with IE was recorded in 2010 and the largest in 2018 (Figure 1).

The average age of the group was 63.80 ± 13.45 years. The male/female ratio is 1.13 (49/43), with half of them being over the age of 65. Most patients (70 patients, 76.08%) were admitted from the start to the Cardiology Department and ICU (5 patients, 5.43%), while the rest were initially admitted to other departments (Internal Medicine, Neurology, Infectious Diseases, Pneumology, Diabetology, and Neurosurgery).

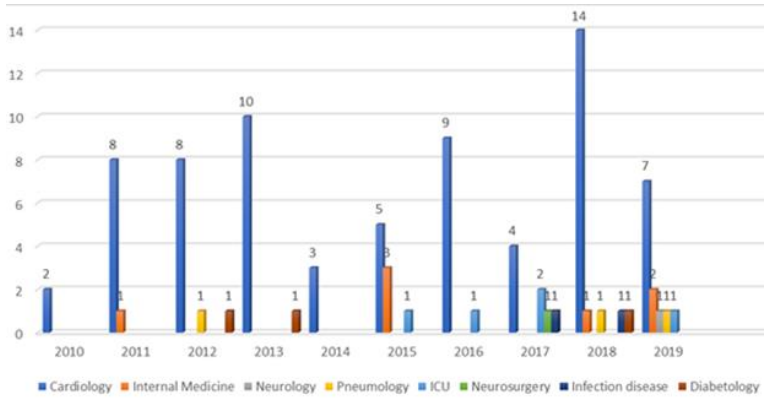

Figure 1.
Patients diagnosed with IE – admission year and department

Most patients (66 patients, 71.7%) had native valve endocarditis and only 26 of them (27.2%) had prosthetic valve endocarditis. Only one patient had a congenital heart disease, surgically treated Fallot tetralogy. The native valve most frequently affected was the mitral valve, followed by the aortic valve, while in the case of prosthetic valves, the most frequently affected was the aortic mechanic valve, followed by the mitral mechanic valve. Multiple valves were affected in only four patients (Figure 2 and Figure 3).

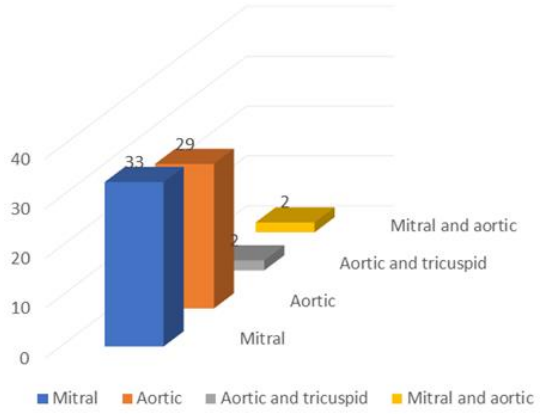

Figure 2.
Endocarditis localization – native valve

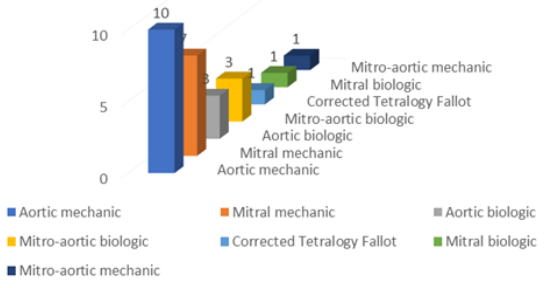

Figure 3.
Endocarditis localization – prosthetic valve

Healthcare-associated endocarditis occurred in 30 patients (32.6%) – a condition related to an invasive procedure performed less than six months prior to being diagnosed with endocarditis. The majority of these cases (24 patients) had nosocomial IE and 6 patients had out-of-hospital interventions (non-nosocomial healthcare-associated endocarditis).

A number of 77 patients (83.69%) had comorbidities, and 37 of them (40.22%) had more than one associated disease. Charlson's comorbidity index value was 3.53 ± 2.029. The most common associated conditions were: coronary heart disease (31, 33.7%), atrial fibrillation (17, 18.5%), older stroke (17, 18.47%), heart failure (13, 14.13%), diabetes mellitus (10, 10.86%), chronic renal failure (9, 9.78%). More than half of the patients had previous cardiovascular diseases (valvular heart disease).

Blood samples were collected from all patients to ascertain the etiological agent, but 28 patients (30.43%) had negative blood cultures. Administration of antimicrobials before blood collection was noted in 54.35% of these cases. The microorganisms isolated from blood cultures in patients with IE are shown in Figure 4.

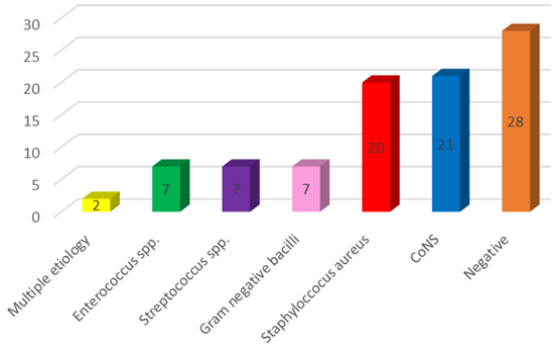

Figure 4.
The aetiology of IE

Gram-positive organisms predominated in the list of isolated strains (55, 58.69%), with *Staphylococcus aureus* accounting for 20 patients

(21.73% of all infections) – about the same rate as CoNS (21 patients, 22.82%). Half of the isolated *Staphylococcus aureus* strains were resistant to methicillin, showing a high resistance profile (Figure 5). These strains were susceptible to aminoglycoside, glycopeptide, and linezolid.

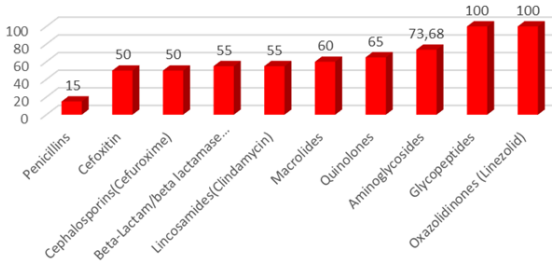

Figure 5.
Susceptibility rates for isolated *Staphylococcus aureus*

In the CoNS group, *Staphylococcus haemolyticus*, *Staphylococcus hominis* and *Staphylococcus epidermidis* were isolated, and 15 of the 21 microorganisms were resistant to methicillin (Figure 6).

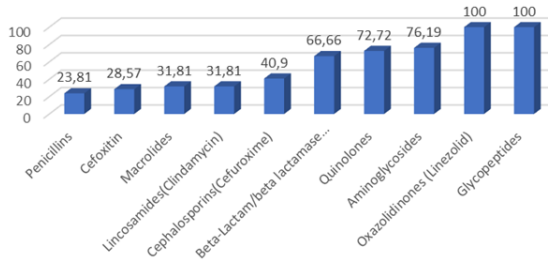

Figure 6.
Susceptibility rates for isolated CoNS

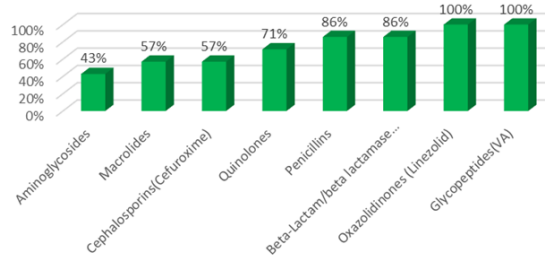

Figure 7.
Susceptibility rates for isolated *Enterococcus spp.*

Enterococcus spp. were isolated in seven patients and showed good antibiotic susceptibility (Figure 7), while two strains were *Enterococcus faecium*. The only therapeutic options for *Enterococcus faecium* were vancomycin or linezolid. Charlson's comorbidity index values were greater in the IE caused by *Enterococcus spp.*, where patients were also older.

Seven patients had positive cultures for *Streptococcus spp.* (7.6%), part of viridans group (two strains) and group D (five strains of *Streptococcus gallolyticus*). These strains showed the best antibiotic susceptibility rates (Figure 8).

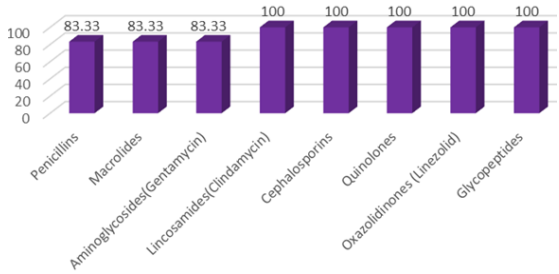

Figure 8.
Susceptibility rates for *Streptococcus spp.*

Other seven patients' blood cultures tested positive for Gram-negative bacilli (7.6%), one with *Haemophilus influenzae* and six with non-HACEK group: *Escherichia coli* (three patients), *Klebsiella pneumoniae* (two patients) and *Pseudomonas aeruginosa* (one patient). All these patients had native valve IE and had undergone vascular catheterization the previous week. These strains showed good rates of antibiotic susceptibility, except for tested cephalosporins (cefuroxime, ceftriaxone). In two patients, multiple germs (polymicrobial growth) were identified in the blood cultures.

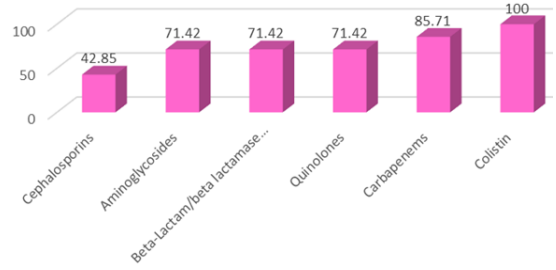

Figure 9.
Susceptibility rates of Gram-negative bacilli

Among patients with healthcare-associated infection, *Staphylococcus aureus* was the main etiologic agent of IE, followed by CoNS. In most cases of community-acquired IE, the blood cultures were negative, and in a quarter of them CoNS were isolated (Table I).

Table I

The aetiology of healthcare-associated and community-acquired IE

Healthcare-associated	Number (%)	Community- acquired	Number (%)
S. aureus	10 (33.33%)	***S. aureus***	10 (16.12%)
CoNS	6 (20%)	**CoNS**	15 (24.19%)
Enterococcus spp.	5 (16.66%)	***Enterococcus spp.***	2 (3.22%)
Streptococcus spp.	2 (6.67%)	***Streptococcus spp.***	5 (8.06%)
GN bacilli	3 (10%)	**GN bacilli**	4 (6.45%)
Multiple	0	**Multiple**	2 (3.22%)
Negative	4 (13.33%)	**Negative**	24 (38.7%)
TOTAL	**30**		**62**

Serological tests can help in the diagnosis of organisms that do not grow in routine bacterial cultures (*Coxiella burnetti*) or are fastidious (*Bartonella spp.*). For this purpose, special aetiology serological tests were performed in a number of 22 patients, more frequently in the last five years. Serology for *Coxiella burnetti* is the best-established serologic test for IE diagnosis and it is included as a major criterion in the modified Duke criteria. We had no positive tests for *Coxiella*, but three patients were positive for *Chlamydia pneumoniae* and two for *Mycoplasma pneumoniae*.

All patients were subjected to complete blood count and biochemical tests, some of which showed inflammatory syndrome. Some of these parameters, such as white blood cells count (WBC), haemoglobin (HGB), platelets count (PLT), erythrocytes sedimentation rate (ESR), C-reactive protein (CRP), creatinine – were investigated in order to establish a correlation with the aetiology (Table II). There was an inverse correlation between the *Staphylococcus aureus* aetiology and the platelets count (r = - 0.289, p = 0.005), but there was no statistical difference between aetiology groups.

Table II

Etiological correlation with biological data

Biological data	WBC	HGB	PLT	ESR	CRP	Creatinine
S. aureus	10.554 ± 3.89	11.94 ± 2.76	168.15 ± 147.80	41.47 ± 32.89	14.38 ± 11.21	1.58 ± 0.95
CoNS	14.630 ± 5.67	12.32 ± 1.35	205.58 ± 83.25	50.15 ± 25.28	11.28 ± 9.71	1.25 ± 0.95
Enterococcus spp.	11.410 ± 2.68	11.43 ± 2.51	243.37 ± 85.52	55.75 ± 29.12	7.16 ± 4.02	1.18 ± 0.41
Streptococcus spp.	11.821 ± 3.81	10.68 ± 1.38	212.38 ± 73.99	57 ± 7.97	7.92 ± 4.27	0.92 ± 0.29
GN bacilli	14.436 ± 10.16	10.62 ± 2.01	206.09 ± 129.77	46.66 ± 24.68	7.81 ± 6.28	1.31 ± 0.64
Multiple	10.630 ± 1.47	10.69 ± 1.57	389.56 ± 145.62	68.66 ± 20.55	10.7 ± 11.02	0.68 ± 0.097
Negative	12.340 ± 6.15	11.41 ± 2.09	245.36 ± 127.60	49.76 ± 25.79	8.40 ± 7.13	1.66 ± 1.66
p	0.421	0.401	0.088	0.65	0.305	0.581

Echocardiography was commonly used (99% of patients). More than half (59%) of the patients had both transthoracic and transesophageal echocardiography. Vegetations are a major criterion for diagnosis, and they were identified in 82 cases (89.13%). A significant proportion (39 patients) presented vegetations larger than 10 mm and intracardiac abscesses were found in 14 patients (15.21%). A third of the patients (28.6%) developed acute valvular regurgitation and seven patients (7.6%) presented prosthetic valve dysfunction.

Most patients presented in-hospital follow-up complications (64 patients, 69.6%). Heart and acute renal failure, cardiogenic shock or acute pulmonary oedema, and embolic events were the most frequently encountered complications. Embolic events occurred in 19 patients, most of them being cerebral, and they were associated with *Staphylococcus spp.* aetiology (r = 0.209, p = 0.046). *Staphylococcus aureus* infections were correlated with congestive heart failure, acute renal failure and embolic events, but upon intergroup comparison, the difference was not statistically significant. Abscess was the most common paravalvular complication (15.2% of patients), while 7/26 (26.9%) of patients with prosthetic valve IE showed evidence of a prosthetic valve complication, such as dehiscence or new paravalvular regurgitation. Abscess formation was correlated with *Staphylococcus aureus* infections, and prosthetic valve dysfunction was correlated with *Staphylococcus spp*, with CoNS aetiology in bivariate analysis (Table III). Using multivariate logistic regression analysis, CoNS aetiology was found as an independent predictor of prosthetic valve dysfunction (p = 0.015, OR = 7.385, 95%CI = 1.477 - 36.916). In multivariate logistic regression, an independent predictor of congestive heart failure was the *Staphylococcus aureus* infection (p = 0.035, OR = 3.324, 95%CI = 1.086 - 10.175). Septic shock was correlated with *Staphylococcus aureus* and Gram-negative bacilli aetiology in

bivariate analysis, but the correlation didn't persist in multiple regression analysis.

The mortality rate was 33.7% and was correlated with *Staphylococcus aureus* aetiology in univariate Cox regression analysis (p = 0.04, OR = 2.207, 95% CI = 1.036 - 4.700).

Table III

Etiological correlation with in-hospital evolution

Complications Number (%)	***S. aureus***	**CoNS**	***Enterococcus spp.***	***Streptococcus spp.***	**GN rods**	**Multiple**	**Negative**	**p**
Total 64/92 (69.6)	15/18 (83.33)	13/17 (76.47)	6/8 (75%)	4/7 (57.14%)	¾ (75)	1/3 (33.33)	19/31 (61.29)	0.329
Embolic events 19 (20.7)	6 (33.3)	5 (29.4)	0	2 (28.6)	2 (25)	0	4 (12.9)	0.34
Ischemic **s**troke and TIA 12 (13)	4 (22.2)	1 (5.9)	0	3 (42.9)	1 (12.5)	0	3 (42.9)	0.092
Cardiogenic shock/Pulmonary oedema 18 (19.6)	2 (11.1)	3 (17.6)	3 (37.5)	2 (28.6)	3 (37.5)	0	5 (16.1)	0.502
Congestive heart failure 27 (29.3)	9 (50)	5 (29.4)	2 (25)	1 (14.3)	2 (25)	0	8 (25.8)	0.43
Septic shock 13 (14.1)	4 (22.2)	1 (5.9)	0	0	3 (37.5)	0	5 (16.1)	0.202
Acute renal failure 27 (29.3)	7 (38.9)	7 (41.2)	2 (25)	1 (14.3)	3 (37.5)	0	7 (22.6)	0.567
Abscess 17 (18.5)	12 (66.7)	3 (17.6)	0	0	0	0	2 (6.5)	< 0.01
Prosthetic dysfunction 7 (7.6)	3 (16.7)	4 (23.5)	0	0	0	0	0	0.04
AV Block 6 (6.5)	1 (5.6)	3 (17.6)	0	1 (14.3)	0	0	1 (3.2)	0.43

Regarding antibiotic treatment, we observed that glycopeptides were most frequently used in *Staphylococcus aureus* IE (72.22%) and a significant bivariate correlation was found between *Staphylococcus aureus* EI and treatment with glycopeptides (p = 0.001, r = 0.334), teicoplanin (p = 0.001, r = 0.332), and vancomycin (p = 0.001, r = 0.262), respectively. Glycopeptides were mostly associated with clindamycin (3/18, 16.66%) and cefuroxime (2/18, 11.11%). All patients with Gram-negative bacilli IE were treated with beta-lactams. In bivariate correlation, there were significant associations between Gram-negative IE and treatment with imipenem (p = 0.001, r = 0.339), imipenem plus gentamicin (p = 0.001, r = 0.339), and cefepime (p = 0.001, r = 0.340). For *Streptococcus spp.* IE, the antibiotic therapy most frequently prescribed consisted of gentamicin (6/7, 85.71%) and amoxicillin/clavulanate (3/7, 42.85%). There was a significant bivariate correlation between *Streptococcus spp.* IE and the combination of these two antibiotics (p = 0.033, r = 0.225). The most frequently used antibiotics in *Enterococcus* EI were amoxicillin/clavulanate (4/8, 50%) and ciprofloxacin (4/8, 50%). A significant bivariate correlation was found between *Enterococcus* IE and amoxicillin/clavulanate plus ciprofloxacin (p = 0.034, r = 0.225), and amoxicillin/clavulanate plus linezolid (p = 0.001, r = 0.340). Patients with CoNS IE were treated most often with beta-lactams (12/17, 70.58%), aminoglycosides (gentamicin) (13/17, 76.47%) and glycopeptides (10/17, 58.82%). Beta lactams were used always in association with aminoglycoside (7/17, 41.17%) or quinolone (6/17, 35.29%).

Despite over a century's worth of studies and recent advances in diagnosis and treatment, IE remains an incompletely understood disease, with high morbidity and mortality rates. The lack of progress is partly due to the fundamental difficulties inherent to studying this type of disease. By necessity, most studies are derived from case reports or small case series recorded in single sites, with few large cohort studies or randomized trials. A shift in approach is necessary in order to further the understanding of endocarditis and to conclusively study therapeutic choices.

Elderly patients face a higher risk in terms of incidence of bacterial endocarditis [29, 50]. The age of patients has increased because the survival rate among patients suffering from rheumatic and congenital heart disease has increased too, and degenerative valve disease and therapeutic procedures are frequent in the elderly [30]. 50% of patients included in our survey were aged 65 or over, and the finding above is consistent with the outcomes observed in other research papers [9, 47, 62]. In our research, the distribution of patients by gender is roughly balanced i.e., 1/1, but it differs from the gender distribution reported by other

studies, where male subjects are predominant (male/female ratio ranging from 1 to 3:1) [29]. Despite the reports about disease incidence being higher in male patients, outcomes seem to be worse in female patients, a fact also confirmed by our study [1, 2].

Most patients in our survey presented associated comorbidities, with a calculated Charlson's comorbidity index of 3.53 ± 2.029. Overall, patients facing IE are nowadays older and suffering from several comorbidities. The number of IE cases treated in healthcare facilities has been increasing and new groups of people at-risk have emerged, such as patients with implanted prosthetic heart valves and intracardiac devices [25]. The incidence of healthcare-associated IE has increased from 7% to 34% according to many authors [3, 41, 63]. That can be explained by the rising number of invasive diagnostics tests and therapeutic procedures and techniques. In our study, 32.6% of patients had healthcare-associated IE and 80% had nosocomial IE, as reported in a previous study [18, 33, 54]. The infection was of vascular origin in most patients, similar to the findings of other reports [41].

It is worth mentioning that previous studies reported a lower prevalence of prosthetic valve endocarditis, of 25% and 21%, respectively [48, 56]. Recent studies have shown an increasing prevalence of prosthetic valve endocarditis (PVE), between 26 and 30%, and the same increased frequency has also been reported in 27.2% of the cases in our survey [30, 60].

Our study shows that aortic and mitral valves were affected in almost equal measure, similar to the cases reported in the EURO-ENDO registry [30]. Thus, the mitral valve was affected in 35.86% of patients and the aortic valve was affected in 31.52% of our patients. Multi-valvular endocarditis was present in 4.4% of patients.

Pathogen identification is essential in the diagnosis and treatment of IE, and failure to identify the etiologic agent is most frequently related to previous antibiotic treatment. Other studies also highlighted the consequences of antibiotic treatment prior to drawing blood for culturing purposes. Blood cultures were performed in all cases, but they were negative in one third of the patients (33.7%). The incidence of blood culture-negative endocarditis in EURO-ENDO is 21% higher than that reported in the 2002 French Survey (14%) and in 2009 in the International Collaboration on Endocarditis-Prospective Cohort Study (11%) [30, 48, 60]. On the other hand, a study reported 46.7% of patients with negative blood cultures and this finding is consistent with other studies conducted in developing countries [1, 37]. The rate of negative cultures was 11% in the study performed by Barrau *et al*., which was comparable to the findings of other reports and was limited mainly to patients with previous antibiotic treatment [7].

Among culture-positive patients, the *Staphylococcus* group was the most frequently encountered: *Staphylococcus aureus* (21.73%) and CoNS infections (22.82%). *Enterococcus spp, Streptococcus spp.*, and Gram-negative bacilli were less commonly identified, but in the same proportion. Only 69.6% of patients had positive cultures. In 2.7% patients, polymicrobial growth was found in the blood cultures. Polymicrobial infective IE is an uncommon entity, ranging from 1% to 6.8%, a fact also confirmed by our results [1, 7]. In various research it appears to be more frequent in patients with underlying diseases, mostly diabetics, with previous cardiac surgery and prosthetic valves. Different studies reported particular IE etiology related trends. The most frequently isolated pathogen was *Staphylococcus spp.* (44.1%), followed by *Streptococcus spp.* and *Enterococcus spp.* in the EURO-ENDO registry [30]. In a recent study, *Staphylococcus spp.* was reported as the dominant aetiology, although rheumatic heart disease was the major condition for cardiac disease [7]. An international collaborative study carried out in 25 countries by Murdoch *et al*. reported that *Staphylococcus* aetiology was dominant in IE, except in South America, where *Streptococcus* is the dominant aetiology because of rheumatic heart disease [48]. In the *Staphylococcus* IE group, a high incidence of CoNS was noticed to an extent similar to the results observed in our work [19, 26, 66].

Our study found that patients with prosthetic valve endocarditis presented an increased rate of infection with *Staphylococcus aureus*, as reported in other studies [12, 14, 28]. Another study reported that *Streptococcus bovis* was the pathogen most isolated in prosthetic valve endocarditis, while *Staphylococcus spp.* came next in terms of frequency rate [7]. In the same group of *Staphylococcus spp.*, CoNS were also frequently isolated pathogens, causing more than 10% of IE cases [35, 39], involving between 25 and 48% of all prosthetic valve IE cases [27, 32, 39, 53]. Other authors report that CoNS account for no more than 5% of native valve endocarditis cases and they are associated with the hospital environment [15, 44, 52]. Our study reported an increased frequency of CoNS infections, affecting 26.92% of patients with prosthetic valve related IE, while being frequently associated with prosthetic valve dysfunction.

In the EURO-ENDO registry, positive blood tests for *Streptococcus spp.* were found in 19% of patients, a lower percentage than in other studies [30]. The Euro Heart Survey reported a prevalence of 28% [60], the 2008 French registry reported

20.6% [56] and the International Collaboration on Endocarditis-Prospective Cohort Study reported 29% [48]. Our study reports even lower percentages of streptococcal infections (7.6%). Among *Streptococcus spp.*, the most frequently isolated was *Streptococcus gallolyticus*, all those patients developed strokes and their imagistic investigations showed large vegetations. This is consistent with observations made by other authors who outlined a high embolic potential due to its ability to form large vegetations [51, 55]. Endocarditis due to *Streptococcus gallolyticus*, previously included in the *Streptococcus bovis* designation, affects most frequently the aortic valve, followed by the mitral valve [6, 42]. Several studies reported multiple valve involvement [23, 38]. In our study, 66.66% of patients with a *Streptococcus gallolyticus* infection had aortic valve involvement and 33.33% of patients had multiple aortic and tricuspid valve involvement.

Enterococcus spp. is the causative agent in approximately 5 to 10% of bacterial endocarditis cases reported in several series [52, 57]. A higher frequency of enterococcal infection may be the third leading cause of IE, as observed in the EURO-ENDO registry (15.8%), which some authors believe may be related to increasing age [30]. In our study, the *Enterococcus* aetiology was found to be associated with an increased Charlson's index of comorbidities.

Coxiella burnetti was identified in 0.8% of patients in the EURO-ENDO registry and in significantly higher percentages in the study performed by Barrau *et al*. (9%) [7, 30]. In our study and in other studies no cases were found [24].

Non-HACEK Gram-negative (GN) infective endocarditis (IE) is a relatively rare condition associated with significant morbidity and mortality rates [20, 21, 24]. The incidence of IE due to non-HACEK Gram-negative bacilli was 3.9% in a multicentric Italian study conducted by Falcone *et al*., slightly higher than that previously recorded in the ICE study (1.8%) [21, 48]. The frequency of IE caused by Gram-negative bacilli is increasing especially in old or immunocompromised patients with central catheter infections. Gram-negative bacilli were identified in a lower percentage (7.6%) of patients in our study, 85.71% of them from the non-HACEK group.

The medical imaging diagnosis method most commonly used is echocardiography [34]. In our study, a transthoracic echocardiogram was performed on all patients, while a transesophageal echocardiogram was performed on only 35 of them (38%). Vegetations are a primary diagnostic criterion, and they were reported in most patients. The EURO-ENDO registry and other studies found vegetations in 72.7% of patients and cardiac abscesses in 17.8% of patients [30]. Similar percentages were found in our clinical trial and *Staphylococcus aureus* aetiology was found to be associated with large vegetations and intracardiac abscesses. Given the increasing incidence and antimicrobial resistance of *Staphylococcus aureus*, including to vancomycin, this germ is emerging as a potentially lethal pathogen [11, 31, 45, 58, 64].

The EURO-ENDO registry reported that stroke occurred in 6.8% of patients [30]. In our study, a smaller percentage of patients presented strokes at admission, and they were managed initially in the neurology department. Heart failure and acute kidney failure were the complications we noticed most often in our clinical trial. Congestive heart failure was reported in one third of patients and was correlated with the *Charlson Comorbidity Index* and *Staphylococcus aureus* aetiology.

Acute kidney failure was reported in one third of patients enrolled in our study, a significantly higher proportion than the one reported in the EURO-ENDO registry (17.7%) [30]. The same registry reported embolic events as the most frequent complication incurred by up to 40% of patients, 44.1% of whom presented cerebral localization. Our study observed a smaller rate of embolic events, i.e. 20.7%, which is similar to the share reported in the ICE cohort study and in the Euro Heart Survey, 23% and 18%, respectively [48, 60]. Our clinical study reported cerebral manifestations such as strokes in 63.15% of patients, with embolic events correlated with *Staphylococcus* aetiology and large vegetations despite proper therapy [3, 4].

Early identification of patients at risk of developing septic shock is mandatory in order to improve the outcome. Septic shock is a rare condition and was correlated with Gram-negative bacilli aetiology, especially *Pseudomonas aeruginosa* [61]. Gram-negative bacilli were isolated in the same number as *Streptococcus spp.*, and a large proportion were resistant to cephalosporins.

Several inflammatory biomarkers that predict outcomes in IE have been identified. Leukocytosis, elevated C-reactive protein (CRP) levels and hypoalbuminemia were previously identified as early predictors of in-hospital mortality in IE cases. At the same time, leukocytosis and elevated procalcitonin are independent predictors of complications [5, 17]. Other haematological and coagulation parameters reported as useful predictors of outcome are brain-type natriuretic peptide levels, neutrophil-to-lymphocyte ratio, D-dimer levels, ESR and mean platelet volume levels, but our study failed to show a significant difference when they are explored by aetiology.

Regarding antibiotic therapy, the most frequently prescribed in *Staphylococcus aureus* IE were glycopeptides, in accordance with the high

susceptibility rates to glycopeptides registered, and in compliance with the ESC guidelines and other current research recommendations [29, 46]. Patients with Gram-negative IE were most commonly treated with beta-lactams and imipenem, in accordance with the high susceptibility to carbapenems and beta-lactams registered. Most patients with *Streptococcus* IE were treated with amoxicillin plus gentamicin, which is in compliance with the ESC guidelines and recommendations [30], and also with the susceptibility rates registered. Amoxicillin was frequently used in enterococcus IE, in compliance with the same guidelines and recommendations, and also with the high susceptibility rate (86%), and was frequently associated with linezolid, in accordance with the high susceptibility rate. Almost 60% of patients with CoNS IE were treated with glycopeptides, in accordance with the susceptibility rates recorded. Approximately 40% of patients with CoNS IE were treated with beta lactams combined with aminoglycosides.

Our clinical trial reported a mortality rate of 33.7%, higher than that reported by some authors, mortality rates ranging between 15 and 30% [29, 30].

The study lacked long-term follow-up, thereby limiting our ability to analyse outcomes beyond initial hospitalization. The precise timing of all complications was not recorded and that fact may affect our ability to ascertain the clinical significance of some findings.

Conclusions

IE remains a serious disease, despite recent advances in diagnosis and therapy. The identified aetiology depends on the diagnostic methods and strategies used. With more standardized and diversified tests, the rate of IE without an identified etiologic agent will decrease. The microbial aetiology of IE revealed a higher incidence rate of staphylococcal IE and a lower incidence rate of streptococcal IE, correlated with an increased frequency of negative blood cultures. These results suggest that the etiologic changes in the spectrum of organisms causing bacterial endocarditis reported by other researchers are also present in our community. When it comes to IE caused by microorganisms that are difficult to culture, geographic differences could reflect a variation in the threshold for performing additional diagnostic tests. Large vegetations, intracardiac abscesses, congestive heart failure, embolic events and death are associated with the *Staphylococcus aureus* aetiology. Prosthetic valve IE complicated by prosthetic dysfunction was associated with the CoNS aetiology. Healthcare–associated IE accounts for approximatively 30% of cases. Antibiotic treatment followed the recommendations in the guidelines, but was also adapted to susceptibility rates observed in isolated germs.

These changes in terms of both patients and pathogens have important implications for the diagnosis and management of IE.

Conflict of interest

The authors declare no conflict of interest.

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