Clinical and microbiological features of bacteremia caused by *Enterococcus faecalis*

Mónica Ceci¹, Gastón Delpech², Mónica Sparo², Vito Mezzina³, Sergio Sánchez Bruni⁴⁵, Beatriz Baldaccini³

¹ Center for Biochemical Studies, Tandil, Argentina
² School of Medicine, Universidad Nacional del Centro de la Provincia de Buenos Aires, Olavarría, Argentina
³ Ramón Santamarina Hospital, Tandil, Argentina
⁴ Faculty of Veterinary Medicine, Universidad Nacional del Centro de la Provincia de Buenos Aires, Tandil, Argentina
⁵ CONICET, Ciudad Autónoma de Buenos Aires, Argentina

Abstract

Introduction: *Enterococcus faecalis* is a frequent etiologic agent of invasive infections in hospitalized patients. The aim of this study was to analyze clinical and microbiological features of bacteremia caused by *E. faecalis*.

Methodology: Between 2011 and 2013, significant bacteremia caused by *E. faecalis* in hospitalized patients was studied. Patient characteristics, comorbid conditions, and 14-day mortality were recorded. Virulence genes *esp*, *gelE*, and *cylA*; opsonophagocytosis resistance; resistance to bactericidal effect of normal serum; beta lactamase production; and susceptibility to ampicillin, vancomycin, teicoplanin, gentamicin, and streptomycin were investigated.

Results: *E. faecalis* strains were recovered from 33 bacteremic patients. Polymicrobial bacteremia was diagnosed in 2 patients; 10 patients died. Virulence genes were found in strains from both deceased patients and survivors. Sources of bacteremia included urinary tract infections (36.4%), vascular catheters (15.1%), abscesses (9.1%), and unknown (48.5%). Underlying diseases included cancer (30.3%), diabetes (36.4%), cirrhosis (6.1%), renal (36.4%), and chronic obstructive pulmonary disease (2.0%). Co-morbidities included alcohol use (26.1%); glucocorticoid therapy (19.0%); prior antibiotic therapy (60.6%); and central venous (21.2%), arterial (12.1%), and urinary (63.6%) catheters. Also, 57.6% of patients came from the intensive care unit (ICU); 33.3% had mechanical ventilation. Significant mortality-associated conditions included polymicrobial bacteremia, oncological disease, APACHE II score ≥ 20, ICU stay, renal disease, central venous catheter, and mechanical ventilation.

Conclusions: Outcome of patients was associated with their status and not with the presence of virulence genes in *E. faecalis* strains. A significant percentage of bacteremia had undetermined origin. An alternate origin may be the gastrointestinal tract, through translocation.

Key words: bacteremia; *E. faecalis*; hospital; virulence determinants; co-morbidity; mortality.


(Received 14 January 2015 – Accepted 08 April 2015)

Copyright © 2015 Ceci et al. This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

The genus *Enterococcus* belongs to a group of microorganisms that colonize diverse niches, such as human organs and tissues, due to their ability to survive under adverse environmental conditions [1].

In humans, enterococci are part of intestinal microbiota; they can be found in smaller proportions in secretions (oropharyngeal and vaginal) and on skin. The wide spectrum of clinical infections caused by these bacteria can include urinary tract and intra-abdominal infections, endocarditis, and bacteremia. In the last two decades, enterococcal species have emerged as opportunistic pathogens in severe human infections [2].

Nowadays, more than 50 species are considered to be members of this genus [3], but only a few cause clinical infections in humans. *Enterococcus faecalis* and *Enterococcus faecium* are the most prevalent species cultured from humans, accounting for more than 90% of clinical enterococcal isolates. However, *E. faecalis* is the most common species causing invasive infections in hospitalized patients. The increasing incidence of enterococcal infections associated with healthcare is the result of a combination of bacterial features such as expression and transfer of genetic material, which increases their antimicrobial resistance and pathogenicity [4-6].

The carriage of genetic determinants and expression of their products (virulence factors) that
contribute to colonization, transmission, and invasiveness of these bacteria were detected in human *E. faecalis* strains. The *gelE* gene encodes gelatinase, an extracellular metalloendopeptidase that can hydrolyze bioactive proteins such as gelatin, hemoglobin, and endothelin-1. This enzyme is involved in the degradation of polymerized fibrin and may facilitate the spread of enterococci to the host [7]. The enterococcal surface protein (*esp*) gene product is a cell-wall-associated surface protein, which is linked to adhesion, colonization, evasion of immune response and biofilm formation, and is believed to contribute to antimicrobial resistance. The nature of biofilm structure confers an inherent resistance to antimicrobial agents. Mechanisms responsible for resistance may be delayed penetration of the antimicrobial agent through the biofilm matrix, altered growth rate of biofilm microorganisms, and other physiological changes due to the biofilm mode of growth [4,8]. The *cylA* gene synthesizes a protein that leads to activation of cytolysin, a bacterial toxin produced by *E. faecalis* strains. Lytic action of cytolysin on various cell types has been proven, along with its contribution to virulence in animal models of enterococcal infections [9]. Cytolysin is widely spread into the environment; hemolysin-producing *E. faecalis* strains have been recovered from food, animal, and human samples [10].

Little is known about the traits that contribute to the ability of enterococci to cause human infection. Although many putative virulence factors of *E. faecalis* have been described, their association with an adverse clinical outcome is still not widely accepted [11-13].

Nowadays, few antimicrobials are active against enterococcal species. Enterococci have an intrinsic resistance to several clinically used antimicrobials agents, making them important nosocomial pathogens. They are intrinsically resistant to cephalosporins and present low-level resistance to clindamycin as well as to aminoglycosides. Enterococci can absorb folate from the environment, which allows them to bypass the inhibition of folate synthesis, resulting in resistance to trimethoprim-sulfamethoxazole [5].

*E. faecalis* is prone to acquire resistance, possibly related to its ability to participate in various forms of conjugation, which can result in the spread of genes through conjugative transposons, pheromone-responsive plasmids, or broad-host-range plasmids [6,10].

Worldwide, most of the studies about risk factors of mortality and enterococci were conducted using vancomycin-resistant strains [14]. In Argentina, research about severe enterococcal infection has been focused on antimicrobial-resistant strains [15]. However, locally, there is scarce information about the prevalence of bacteremia caused by *E. faecalis* as well as the factors of co-morbidity and mortality associated with these clinical episodes.

The aim of this study was to analyze clinical and microbiological features of bacteremia caused by *E. faecalis*.

**Methodology**

**Study setting**

This was a non-experimental, prospective, and longitudinal study.

**Patients**

From January 2011 to December 2013, bacteremic patients attending Ramón Santamarina Hospital at Tandil City, Argentina, were analyzed. When the etiological agent was characterized as *E. faecalis*, clinical features of patients, co-morbid conditions, and 14-day mortality were recorded. All patients received an initial empirical antimicrobial treatment (ampicillin-gentamicin) that was adjusted according to the results of susceptibility testing.

**Ethics**

Ethical legislation (the last version of the Helsinki Declaration and the Argentinian Bill for Personal Data Protection) was applied. Results were analyzed in an anonymous fashion, ensuring for patients absolute confidentiality of obtained information and the exclusive use of the data for research.

**Sample collection**

Blood specimens for culture were collected from adult patients suspected of having nosocomial bacteremia. All samples were collected when clinically indicated as part of routine care. Up to 20 mL of blood was obtained from each patient. BacT/Alert 3D automated system (bioMérieux, Buenos Aires, Argentina) was used to process blood cultures. For each blood sample, one aerobic and one anaerobic bottle were inoculated with 10 mL of blood and incubated at 35°C for five days or until they signaled positive for growth.

**Phenotypic and genotypic characterization of *E. faecalis***

Phenotypic characterization was carried out according to Facklam *et al.* [16].
Genotypic characterization was done using polymerase chain reaction (PCR). Genus confirmation was performed through amplification of the *tuf* gene. Oligonucleotide sequences were used as primers: Ent1, TACGTGAAAACATTCAATGATG and Ent2, AACTTGGTCAACCAAGCGGAAC [17]. For species confirmation, amplification of the *sodA* gene was done using sequences FL1, ACTTATGTAACAACTTTACC and FL2, TAATGGTAATCTTGGTTGG [18].

**Antimicrobial susceptibility testing**

Antimicrobial susceptibility of *E. faecalis* strains was investigated using qualitative (agar diffusion) and quantitative (minimum inhibitory concentration [MIC], agar dilution) techniques according to the Clinical and Laboratory Standards Institute (CLSI)’s recommendations [19]. The following antimicrobials were tested: ampicillin, vancomycin, teicoplanin, gentamicin, and streptomycin. Type strains *E. faecalis* ATCC 29212 and *E. faecalis* ATCC 51299 were used for quality control.

**Molecular detection of high-level gentamicin resistance genes**

PCR for amplification of gentamicin resistance genes was carried out according to the protocol described by Sparo *et al.* [10].

**Beta-lactamase production**

Beta-lactamase production was studied by the nitrocefin disk method (BD BBL, Franklin Lakes, NJ, US), according to manufacturer’s instructions. The type strains used for reaction control were *S. aureus* ATCC 29213 (positive control) and *E. faecalis* CECT7121 (negative control).

**Molecular detection of virulence genes**

PCR for detection of virulence genes *cylA*, *esp*, and *gelE* was performed.

After incubating *E. faecalis* strains into brain-heart infusion (BHI) broth for 18 hours at 35°C, DNA extraction was done. Aliquots (10 mL) of each fresh culture were taken and centrifuged at 11,430 g for 10 minutes at 4°C. The pellet was re-suspended into 500 µL of sterile water (MilliQ, Billerica, MA, US) and incubated at 100°C for 30 minutes. The obtained supernatant was stored at -20°C until processing.

PCR was done using a DNA thermal cycler (PTC-100) in a final volume of 25 µL containing 1.0 µL of DNA as template, Tris-HCl 10 mM (pH 8.4), KCl 50 mM, MgCl₂ 1.5 mM, 50 pmol of each primer, 200 µM of each dNTP, and 2.0 U of Taq DNA polymerase. The cycles were as follows: 5 minutes at 94°C (initial denaturalization) followed by 30 cycles of 45 seconds at 94°C (denaturalization), 1 minute (annealing temperature, 54°C [gelE], 62°C [esp], and 57°C [cylA]), 1 minute at 72°C (extension), and a final extension cycle of 3 minutes at 72°C. Oligonucleotide sequences used as primers were as follows: *cylA*, TE17 and TE18 [20]; *esp*, Esp11 and Esp12 [21]; and *gelE*, gelEF1 and gelER1 [22].

PCR products were resolved by electrophoresis on a 1.0% agarose-Tris-borate-EDTA (pH 8.0) gel containing ethidium bromide and molecular weight markers (New England Biolab, Ipswich, MA, US). Interpretation of bands was performed with an UV transilluminator. Reference strains *E. faecalis* MMH 594 (*cylA+, esp+*), *E. faecalis* ATCC 29212 (*gelE+*) and *E. faecalis* CECT 7121 (*cylA+, esp−*, *gelE−*) were used for quality control [23].

**Opsonophagocytosis resistance (OPR)**

Resistance to human polymorphonuclear leukocytes (PMNs) phagocytosis was studied using an opsonophagocytic assay, according to the protocol of Hufnagel *et al.* [24]. For the assay, 100 µL of the viable studied strain (2.0 × 10⁷ colony-forming units [CFU] mL⁻¹, confirmed by viable counts), 100 µL of a PMN suspension (2.6 × 10⁶ mL⁻¹), 100 µL of pooled normal rabbit serum (1:500 dilution in saline solution), and 100 µL of adsorbed normal human serum diluted 1/20 in saline solution were mixed. The mixture was incubated at 37°C for 90 minutes. Samples were plated at time 0 and after 90 minutes. Killing percent was calculated by comparing viable colony counts in the inoculum (T₀) with the viable count after incubating for 90 minutes (T₉₀). *E. faecalis* DS16 (Gilmore Collection, Oklahoma, USA) was used as a positive control (resistance to opsonophagocytosis by human PMNs), and *E. faecalis* CECT7121 was used as a negative control (sensitive to opsonophagocytosis by human PMNs).

**Resistance to bactericidal effect of normal serum**

The bactericidal effect of human normal serum on enterococcal strains was assessed following the protocol of Pelkonen and Finne [25]. A pool of human normal serum was obtained by heating at 56°C for 30 minutes and adding ethylenglycol 10 mM as well as MgCl₂ 5 mM in order to inactivate the classical complement pathway. The pooled serum was kept at 4°C until use. Each bacterial strain was incubated at 35°C for 18 hours and diluted 1/10 in fresh BHI broth.
After incubation at 37°C for 90 minutes, it was centrifuged at 1,500 g for 15 minutes at 4°C. The pellet was re-suspended in phosphate buffer saline (PBS; pH 7.4), diluting until a final concentration of 10^8 CFU mL^-1 was reached. A 96-well micro-plate was used. To perform the assay, 175 µL of PBS, 175 µL of each strain suspension, and 100 µL of pooled normal human serum were added to each well. After being mixed for 30 seconds in a mechanical agitator, it was incubated at 37°C. Absorbance (630 nm) at 30, 60, 90, 120, and 180 minutes was measured. Viable cells counts were carried out at each reading time. Strains were classified as sensitive, intermediate, or resistant to bactericidal effect of normal serum, based on the work of Taylor [26]. Serum-resistant strain *E. faecalis* DS16 and serum-sensitive strain *E. faecalis* CECT 7121 were used for quality control [27].

Statistical analysis

Fisher’s exact test for dichotomous variables was applied and a multivariate logistic regression analysis was performed. The Statistical Package for Social Sciences (SPSS) version 11.5 was used. P values < 0.05 were considered to be statistically significant.

Results

During the period of this study, *E. faecalis* strains were recovered from 33 of 292 bacteremic patients (11.3%). Sources of bacteremia were unknown (48.5%), urinary tract infections (36.4%), vascular catheters (15.1%), and abscesses (9.1%).

Epidemiological characteristics of deceased patients and survivors with bacteremia caused by *E. faecalis* are shown in Table 1. Ten of the patients with enterococcal bacteremia died (30.3%). In two of those patients, polymicrobial bacteremia was diagnosed. Acute physiology and chronic health evaluation (APACHE II) score was between 8 and 29. In bacteremic patients, the following underlying diseases were detected: renal (39.4%), diabetes (36.4%), oncologic disease (30.3%), cirrhosis (6.1%), and obstructive pulmonary chronic disease (OPCD) (3.0%). The following co-morbid conditions were observed: urinary catheter (63.4%), previous antimicrobial use (60.6%), abuse of alcohol (27.3%), central venous catheter (21.2%), glucocorticoid therapy (18.2%), and arterial catheter (12.1%). Hospitalization in the intensive care unit (ICU) was reported for 57.6% (19/33) of the patients, while 33.3% (11/33) needed mechanical ventilation (MV). Mortality-associated conditions included (p < 0.05) polymicrobial bacteremia, oncologic disease, APACHE II score ≥ 20, ICU stay, renal disease, central venous catheter, and MV (Table 1).

In the analyzed enterococcal strains, carriage of virulence genes was proven. In *E. faecalis* isolated

### Table 1. Clinical characteristics of patients with bacteremia caused by *E. faecalis*

<table>
<thead>
<tr>
<th>Patients</th>
<th>Deceased (N = 10)</th>
<th>Survivors* (N = 23)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epidemiologic factors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Years (mean)</td>
<td>62.4</td>
<td>64.8</td>
<td>NS</td>
</tr>
<tr>
<td>Male sex</td>
<td>8</td>
<td>16</td>
<td>NS</td>
</tr>
<tr>
<td>APACHE II ≥ 20</td>
<td>9 (90.0)</td>
<td>1 (4.4)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><strong>Comorbid conditions</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abuse of alcohol</td>
<td>3 (30.0)</td>
<td>6 (26.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Previous use of ATM</td>
<td>6 (60.0)</td>
<td>14 (60.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Glucocorticoid therapy</td>
<td>2 (20.0)</td>
<td>4 (17.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Central venous catheter</td>
<td>6 (60.0)</td>
<td>1 (4.4)</td>
<td>0.0023</td>
</tr>
<tr>
<td>Arterial catheter</td>
<td>2 (20.0)</td>
<td>2 (8.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Urinary catheter</td>
<td>6 (60.0)</td>
<td>15 (65.2)</td>
<td>NS</td>
</tr>
<tr>
<td>MV</td>
<td>10 (100)</td>
<td>1 (4.4)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>ICU</td>
<td>9 (90.0)</td>
<td>10 (23.0)</td>
<td>0.0005</td>
</tr>
<tr>
<td><strong>Underlying disease</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oncologic</td>
<td>9 (90.0)</td>
<td>1 (4.4)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>4 (40.0)</td>
<td>8 (34.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>1 (10.0)</td>
<td>1 (4.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Renal</td>
<td>8 (80.0)</td>
<td>5 (21.7)</td>
<td>0.0161</td>
</tr>
<tr>
<td>COPD</td>
<td>0 (0%)</td>
<td>1 (4.4)</td>
<td>NS</td>
</tr>
</tbody>
</table>

* At 14 days; NS: P-value > 0.05 (not statistically significant); APACHE II: acute physiology and chronic health evaluation II; ATM: antimicrobials; MV: mechanical ventilation; ICU: intensive care unit; COPD: chronic obstructive pulmonary disease.
from outsourced patients, gelE (69.6%), cylA (43.5%), esp (21.7%), gelE + cylA, (26.1%), and gelE + esp (17.4%) were detected. In addition, a lower frequency of virulence gene carriage was observed in deceased patients: gelE (60.0%), cylA (30.0%), esp (20.0%), while a higher prevalence of gelE + cylA (30.0%) and gelE + esp (20.0%) was detected in this latter group (Table 2). No significant association (p > 0.05) was found between the prevalence of virulence determinants and mortality of patients.

However, in all patients, *E. faecalis* isolates showed resistance to opsonophagocytosis and serum bactericidal power.

With regard to antimicrobial resistance, only two isolates showed high-level resistance to gentamicin (MICs > 500 mg/L), associated with the presence of *aac(6’)-le-aph(2’’)-la* (Figure 1). In addition, one of the isolates from a patient who died presented a cylA determinant. None of the isolates were resistant to ampicillin (MICs < 8.0 mg/L), vancomycin (MICs < 4.0 mg/L), teicoplanin (MICs < 8.0 mg/L), or streptomyacin (MICs < 2,000 mg/L). Production of beta-lactamase was not detected in any of the studied *E. faecalis* isolates.

**Discussion**

*Enterococci* are opportunistic pathogens that affect elderly patients with underlying diseases and other immunocompromised patients who have been hospitalized for long periods, treated with invasive devices, or who have received broad-spectrum antimicrobials. In the United States of America, enterococci are common nosocomial pathogens, accounting for 10.0% of hospital-acquired infections. This genus ranks second or third among the most common bacterial agents of urinary tract infections, wound infections, and bacteremia in hospitals. Enterococci are responsible for about 16.0% of nosocomial urinary tract infections [28].

*Enterococci* can be carried on the hands of healthcare workers and be transferred from one patient to another. The transmission from healthcare workers’ hands to patients can take place upon contact with patients’ intravenous or urinary catheters [29]. Therefore, transmission of *E. faecalis* between patients is the most common way to cause infections by this species in a hospital or long-term care facility.

Bacteremia caused by *E. faecalis* has significant relevance for public health since it is linked with an increase of a subsequent endocarditis, one of the most severe enterococcal infectious diseases [30]. In this study, virulence traits of blood *E. faecalis* isolates along with the comorbidity and mortality factors of bacteremic patients were analyzed. Enterococcal isolates and epidemiological data were obtained from patients attending a public hospital in a medium-sized city from Argentina over a two-year period.

**Table 2.** Characteristics of *E. faecalis* isolated from patients with bacteremia

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Deceased (N = 10)</th>
<th>Survivors (N = 23)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATM resistance</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>NS</td>
</tr>
<tr>
<td>HLRG</td>
<td>1 (10.0)</td>
<td>1 (10.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Opsonophagocytosis resistance</td>
<td>10 (100)</td>
<td>23 (100)</td>
<td>NS</td>
</tr>
<tr>
<td>RBENS</td>
<td>10 (100)</td>
<td>23 (100)</td>
<td>NS</td>
</tr>
<tr>
<td>gelE</td>
<td>6 (60.0)</td>
<td>16 (69.6)</td>
<td>NS</td>
</tr>
<tr>
<td>cylA</td>
<td>3 (30.0)</td>
<td>10 (43.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Esp</td>
<td>2 (20.0)</td>
<td>5 (21.7)</td>
<td>NS</td>
</tr>
<tr>
<td>gelE + cylA</td>
<td>3 (30.0)</td>
<td>6 (26.1)</td>
<td>NS</td>
</tr>
<tr>
<td>gelE + esp</td>
<td>2 (20.0)</td>
<td>4 (17.4)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*At 14 days; *N*: number of isolates; ATM: antimicrobials; NS: p value > 0.05 (not statistically significant); HLRG: high-level resistance to gentamicin; RBENS: resistance to bactericidal effect of normal serum.
This investigation showed that, during 2011 and 2013, 11.3% of the included patients had bacteremia caused by *E. faecalis*. In other studies that included patients from a more populated Argentinian city, lower prevalence (2.1% and 4.2%) of *E. faecalis* as the etiological agent of bacteremia were reported, highlighting the existence of differences of enterococcal bacteremia features among regions within the same country [15,31].

The results of this study indicate that the frequency of enterococcal bacteremia found in the analyzed region should not be overlooked. In a four-year prospective study conducted at the United States, *E. faecalis* ranked fourth among the leading pathogens causing nosocomial bacteremia, with a similar prevalence to the one found in this investigation (10.4%). In Denmark, trends in microbiological agents of nosocomial and community-acquired bacteremia were investigated. Through a nine-year period, the incidence of enterococcal bacteremia increased to 13.0%, mainly due to an increase of *E. faecium*-associated episodes, rising from the fourth-most to the third-most frequent agents of nosocomial bacteremia [32,33].

In this research, most of the bacteremic episodes were monomicrobial, while a low prevalence of polymicrobial cases was registered. Other authors have found a variety of frequencies as well as a predominance of mono and polymicrobial bacteremia.

In a Spanish study, in patients with enterococcal bacteremia due to *E. faecalis*, most of the bacteremia were polymicrobial (52.4%). Conversely, in a very elderly Spanish population, a lower prevalence (5.8%) of polymicrobial bacteremia was observed [34,35].

In this study, episodes of bacteremia were mainly without identifiable source, followed by documented sources such as urinary tract infections, intravascular catheters, and abscesses. Lark *et al.* [32] observed bacteremia without documented origin to a lesser extent (7.0%) and at different incidences; in that study, catheters were linked in 47.0% of the episodes, while urinary infections had a lower incidence (11.0%) after intra-abdominal sites, pneumonia, and skin or soft-tissue infections. However, a previous study conducted in Argentina reported a similar frequency (42.0%) to that of this study of bacteremia of unknown source, followed by respiratory, urinary, skin, and abdominal origin [31].

In the patients in the present study with documented *E. faecalis* bacteremia, renal pathologies, diabetes, cancer, cirrhosis, and chronic obstructive pulmonary disease were found to be underlying diseases. In Spanish bacteremic patients, diabetes was the first-ranked underlying disease (31.0%), followed by cancer (18.0%), chronic pulmonary disease (17.0%), and renal insufficiency (13.0%). Similar outcomes were observed in nosocomial and healthcare-associated bacteremia episodes in Spain as well as in nosocomial bacteremic patients from Argentina, since diabetes and cancer were the two most common underlying diseases among the analyzed patients [31,34,35].

In the present study, urinary catheterization and previous antimicrobial therapy were reported as the most frequent comorbid conditions, followed by alcoholism, central venous catheter, glucocorticoid therapy, and arterial catheter. Surgical procedures such as vascular catheterization are associated with episodes of bacteremia. In a study performed at an acute-care hospital to determine the epidemiology of bacteremia, it was observed that vascular catheterization preceding the onset of bacteremia was performed on 23.8% of patients with subsequent enterococcal bacteremia [36]. Other authors have also investigated the impact of arterial catheters on patients with bacteremia. A prospective study that aimed to delineate the pathogenesis of arterial catheter-related bloodstream infections concluded that catheters caused bacteremia, even though at a lower frequency (1.3%) than that observed in the patients of the present study [37]. In a collaborative investigation carried out in an elderly population, 43.0% of the bacteremic patients had urinary catheters, while in 38.0% of the episodes, previous antimicrobial use was registered [35].

A recent report about the impact of bacteremia over a specific type of infectious disease stated that abuse of alcohol is considered a comorbid condition. In France, after a seven-year period, 14.0% of the patients had alcohol issues, a smaller incidence than that observed in this investigation [38]. In a study about clinical features of enterococcal bacteremia, immune system suppressors were applied as non-antimicrobial therapy in patients (25.6%) with bacteremia caused by *E. faecalis* strains [34].

In the present study, 57.6% of the patients with bacteremia caused by *E. faecalis* were hospitalized in the ICU. This prevalence of ICU patients is higher than that reported by other researchers. In a survey conducted at an American hospital, 33.9% of bacteremic episodes were found to be associated with patients hospitalized in an ICU [32]. In a prospective multicenter cohort study, it was shown that 10.0% of very elderly patients with bacteremia were admitted to the ICU. Furthermore, in 28.0% of enterococcal
bacteremia linked with strains of *E. faecalis*, patients were hospitalized in the ICU [34,35].

Seven predictors for 14-day mortality of bacteremia caused by *E. faecalis* were detected: polymicrobial bacteremia, malignancy, APACHE II ≥ 20, hospitalization in ICU, renal disease, central venous catheter, and MV. A study carried out in Korea also detected risk factors for 7-day mortality in deceased patients and survivors with enterococcal bacteremia. Malignancy, ICU, renal disease, and MV were not considered to be significant risk factors. However, an APACHE II score ≥ 20 was a significant mortality risk factor despite its p value (0.001) being higher than that estimated in this investigation (p < 0.0001). Polymicrobial bacteremia and central venous catheters were not analyzed as risk factors for enterococcal bacteremia in the Korean research [39].

In this study, virulence traits of the enterococcal strains recovered from patients with bacteremia were investigated. Resistance to ampicillin, vancomycin, and teicoplanin, as well as high-level resistance to streptomycin, were not detected in any of the strains of *E. faecalis*. In a deceased patient, the presence of *cylA* determinant and high-level resistance to gentamicin were observed. This association in *E. faecalis* strains may determine the clinical and microbiological resolution of severe infectious diseases in patients with these strains [40].

Other researchers have not concurred with these results, since resistance to one or more of the assayed antimicrobials have been reported in enterococcal strains associated with bacteremia. High-level aminoglycoside-resistant *E. faecalis* have been recovered from bacteremic patients in different countries, with a wide prevalence range. In a European university hospital, 70.0% of *E. faecalis* isolates from blood expressed high-level resistance to gentamicin, while lower frequencies for streptomycin and gentamicin/streptomycin were observed [41]. Lower prevalence rates of high-level gentamicin resistance were reported in hospitalized bacteremic patients from France (6.9%), Spain (6.2%), and Korea (32.0%). However, in the latter studies, streptomycin resistance was not investigated [13,42,43]. In this study, all high-level gentamicin resistant isolates carried the *aac(6′)-le-aph(2′′)-la* gene. In Spanish patients, this genetic determinant was one of the most frequently found in enterococcal strains showing high-level gentamicin resistance [41]. Resistance to glycopeptides and ampicillin has not been detected in *E. faecalis* from French and Spanish patients with bacteremia. Nevertheless, in a recent population-based study carried out in Canada, bloodstream *E. faecalis* isolates showed a very low prevalence of ampicillin (0.4%) and vancomycin (1.0%) resistance [13,42,44].

All *E. faecalis* strains included in this study showed resistance to opsonophagocytic killing. This resistance may be a factor that contributes to persistence of enterococci in the bloodstream [45]. In a comparative opsonization assay carried out with clinical *E. faecalis*, as well as with a probiotic strain, resistance to opsonic killing in *E. faecalis* from blood cultures was proven [27]. This trait seems to be more expressed in clinical enterococcal isolates than in non-human *E. faecalis*. Previous studies have shown that clinical enterococci had lower PMN-killing values than did non-clinical isolates [24,46].

A similar trend of higher resistance to the bactericidal effect of normal serum has been observed in human enterococcal strains. In this study, *E. faecalis* strains recovered from patients with nosocomial bacteremia were resistant to the activity of normal serum. It is known that bacteria associated with septicemia are frequently resistant to serum, since the main role of the serum bactericidal system is to prevent microorganisms from invading and persisting in blood [47]. It is noteworthy that in a recent investigation conducted in the same region of this study, the sensitivity to bactericidal effect of serum in *E. faecalis* isolates recovered from non-human samples [48] was found.

Carriage of three virulence genes (*gelE*, *cylA*, and *esp*) in bacteremia *E. faecalis* strains was investigated. Overall, the most frequent virulence genes was *gelE*, followed by *cylA* and *esp*. Other investigations have shown that in *E. faecalis* strains isolated from blood or other clinical samples, the most prevalent virulence genes are *gelE* or *esp* with a lower frequency of the *cylA* gene. In France, the carriage of *gelE* (65.5%), *esp* (55.2%), and *cylA* (27.6%) was detected among patients with bacteremia caused by *E. faecalis*. In strains recovered from Australian patients, the *esp* gene was found to be the most prevalent (67.8%), with a slightest lower detection of *E. faecalis* harboring *gelE* (64.4%) gene and, to a lesser extent (10.0%), the *cylA* gene [13,49].

Also, in this study, combinations of two virulence genes, *gelE* + *cylA* (27.3%) or *gelE* + *esp*, were detected. In a study carried out to assess the distribution of virulence genes among European *Enterococcus* isolates from different origins, the occurrence of two virulence determinants in clinical *E. faecalis* strains was observed. These authors reported the joint carriage of *gelE* + *cylA* (28.5%) and *gelE* +
esp (20.0%), the frequencies in the same order as those presented in the present study [50].

In this study, there was no clinical impact of virulence features from *E. faecalis* in deceased or surviving patients. None of these traits were found to be significant mortality factors for nosocomial bacteremia episodes. In line with these results, in a study conducted by Vergis et al. [12] on enterococcal bacteremia, the relationship of virulence factors such as hemolysin production, gelatinase production, and presence of the *esp* determinant to patient mortality was analyzed. The researchers concluded that the virulence factors were not associated with increased mortality rates among patients with bacteremia. However, it was previously observed that in bacteremic episodes caused by *E. faecalis*, the expression of virulence factors was linked to an increase of mortality risk in those patients [51].

**Conclusions**

This research has shown that the outcomes of patients with bacteremia caused by *E. faecalis* were associated with their clinical status and not with the presence of one or more virulence genes and/or the lack of expression of antimicrobial resistance in the isolated enterococcal strains. None of those features were considered significant factors of mortality for enterococcal bacteremic episodes. There was a significant percentage of bacteremia whose origin was not determined. A possible origin may be the gastrointestinal tract, through the mechanism of translocation. We analyzed the clinical and microbiological features of bacteremia caused by *E. faecalis*, seeking connections between the patients and the etiologic agent, in order to gain a better understanding of these episodes and their impact on public health.

**Acknowledgements**

Authors thank the financial support provided by Center for Biochemical Studies in order to carry out this study.

**References**


**Corresponding author**

Mónica Sparo
School of Medicine, Universidad Nacional del Centro de la Provincia de Buenos Aires.
4375 Pringles Av. ZIP: 7400. Olavarría, Argentina.
Phone: +54-2284-426382
Email. msparo@vet.unicen.edu.ar

**Conflict of interests:** No conflict of interests is declared.