

Two decades of changing Enterococcus epidemiology and antimicrobial susceptibility in a Brazilian tertiary university hospital, 2002–2023

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Abstract

Background

Enterococcus spp. are major opportunistic hospital pathogens characterised by remarkable environmental persistence, genomic plasticity, and the ability to acquire antimicrobial resistance determinants. This study aimed to evaluate the occurrence, temporal dynamics, clinical distribution, and phenotypic antimicrobial susceptibility trends of *Enterococcus* spp. clinical isolates in a tertiary teaching hospital in southern Brazil over a 22-year period (2002–2023).

Methods

A retrospective, observational study based on positive clinical cultures from hospitalised patients. After applying the inclusion and exclusion criteria, unique isolates were analysed for species, clinical specimens, hospital ward, hospital outcome, and antimicrobial resistance. The study was divided into two periods: January 2002 through March 2019, and April 2019 through December 2023.

Results

A total of 6,283 isolates were included, comprising 4,881 (77.7%) *E. faecalis* and 1,286 (20.5%) *E. faecium*. Urine was the primary clinical specimen for both species. An increase in the frequency of isolates was observed in recent years, peaking in 2021 for *E. faecalis* and in 2022 for *E. faecium*. *E. faecium* maintained high resistance rates, whereas in *E. faecalis*, vancomycin resistance increased from 6.6% to 25.2% between the analysed periods. Linezolid resistance remained low in both species.

Conclusions

Although *E. faecalis* predominated throughout the period, *E. faecium* was disproportionately associated with bloodstream infections, Intensive Care Unit (ICU) admissions, deaths, and antimicrobial resistance. The significant increase in antimicrobial resistance among both species underscores the importance of continuous microbiological surveillance and species-specific analysis, as well as the need to strengthen infections prevention and control measures.

Background

Enterococcus spp. are commensal microorganisms of the human gastrointestinal tract that frequently act as opportunistic pathogens in healthcare facilities, driven by their remarkable ability to adapt to the environment and their prolonged persistence on hospital surfaces and devices [1].

Among the species of the genus, *Enterococcus faecalis* and *Enterococcus faecium* stand out as the leading causes of healthcare-associated infections, including those affecting the urinary tract, bloodstream, surgical sites, and intra-abdominal sites. In the hospital setting, the clinical impact of these pathogens is amplified in intensive care units, where the severity of patient's conditions, length of hospital stay, use of invasive procedures, and intense selective pressure from prior antimicrobial use increase the susceptibility to serious infections, particularly in vulnerable populations, such as the elderly and immunocompromised individuals [2, 3].

The high genomic plasticity of the genus, combined with its intrinsic resistance to various classes of antimicrobials, facilitates the acquisition and spread of resistance determinants. In this context, the emergence and spread of vancomycin-resistant enterococci (VRE) pose a serious global epidemiological and therapeutic challenge, significantly limiting treatment options. Therefore, longitudinal laboratory surveillance and continuous monitoring of phenotypic susceptibility profiles are essential for understanding the local dynamics of these microorganisms, guiding therapeutic decisions, and supporting microbiological surveillance efforts [1, 4, 5].

In Brazil, long-term institutional studies describing the distribution of *Enterococcus* spp. in clinical samples and their phenotypic trends in antimicrobial susceptibility remain scarce. A temporal analysis of these isolates, combined with characterisation by species, clinical specimen type, hospital department, and patient demographic characteristics, can contribute to a better understanding of the local epidemiology and laboratory trends of these microorganisms over time. Long-term analyses are particularly valuable for identifying temporal changes in species distribution and antimicrobial resistance patterns.

Considering this, the present study aimed to evaluate the occurrence, epidemiological distribution, and phenotypic profile of antimicrobial susceptibility of *Enterococcus* spp. isolated from clinical specimens at a tertiary university hospital in southern Brazil over a 22-year period (2002–2023).

Methods

2.1. Study design and setting

This is a retrospective observational study conducted at the University Hospital of the State University of Londrina (Hospital Universitário da Universidade Estadual de Londrina [HU/UEL]), a regional tertiary teaching centre in Londrina, Paraná, Brazil. Clinical cultures showing growth of *Enterococcus* spp. isolated from inpatients between January 2002 and December 2023 were evaluated.

The research was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee on Research Involving Human Subjects at the State University of Londrina (Comitê de Ética em Pesquisa Envolvendo Seres Humanos [CEP-UEL]; approval No. 6,906,258). The requirement for informed consent was waived by the CEP-UEL due to the retrospective nature of the study.

2.2. Inclusion and exclusion criteria

During the study period, 11,758 cultures positive for the genus *Enterococcus* spp. were initially identified. Clinical isolates obtained from hospitalised patients, with species-level identification and available antimicrobial susceptibility testing profiles, were included. Clinical, demographic, and laboratory data were electronically extracted from the hospital's laboratory information system (Labhos® Aghata Healthcare).

The following were excluded: (i) isolates not identified as the species level; (ii) records without antimicrobial susceptibility testing or without vancomycin susceptibility testing; (iii) screening/epidemiological surveillance cultures and outpatient specimens; (iv) samples from non-biological specimens; (v) records with incomplete data; (vi) duplicate cultures. Duplicates were defined as isolates of the same species obtained from the same patient, from the same biological material, and on the same collection date. In such cases, only one isolate per species was considered for analysis.

After rigorously applying the eligibility criteria, a total of 6,283 unique clinical isolates were identified. The detailed inclusion and exclusion flowchart is shown in Additional file 1.

2.3. Variables analysed

The anatomical sites of the samples were grouped into the following categories: urine, blood, skin and soft tissues, bone fragments, body cavity fluids, and secretions from surgical wounds and abscesses. The demographic and clinical variables analysed included: age (categorised into 10-year age groups: 0–10, 11–20, 21–30, ..., up to ≥ 71 years old), sex, hospital unit at the time of specimen collection (categorised as Intensive Care Unit [ICU] versus general wards/ other hospital departments), *Enterococcus* species, phenotypic antimicrobial susceptibility profile, date of admission, and hospital outcome (discharge, death or transfer/discharge against medical advice).

2.4. Microbiological identification and antimicrobial susceptibility

Species identification and determination of antimicrobial susceptibility profiles were routinely performed by the clinical microbiology laboratory using the automated VITEK® 2 system (bioMérieux, Marcy-l'Étoile, France) throughout the study period. Confirmatory testing was conducted using the Kirby-Bauer disk diffusion method according to standard laboratory protocols.

Interpretive criteria for antimicrobial susceptibility followed Clinical Laboratory Standards Institute (CLSI) guidelines from January 2002 to May 2022, and the Brazilian committee on Antimicrobial Susceptibility Testing (BrCAST) guidelines from June 2022 to December 2023, applying the corresponding annual editions in effect at the time of testing.

The antimicrobial resistance profile was evaluated separately for *E. faecalis* and *E. faecium*, the species most frequently isolated from the samples. Based on the marked increase in the isolation frequency of

these microorganisms starting in 2019, the 22-year timeframe was divided into two periods: 1) January 2002 through March 2019, and 2) April 2019 through December 2023.

2.5. Data analysis

Data analysis was conducted using a descriptive statistical approach. Categorical variables were described by absolute (n) and relative (%) frequencies. Clinical and demographic variables (site of infection, sex, age group, hospital ward, and outcome) were calculated based on the total number of isolates of each species as the denominator. For antimicrobial resistance rates, the denominator represented exclusively the number of isolates tested for each specific agent in each period, disregarding missing data. Temporal trends between the two periods were quantified by the absolute difference in percentage points. Data tabulation and processing were performed using the software Microsoft® Excel®, version 2608, build 16.0.20326.20072. This study has some limitations inherent to its retrospective, single-centre design. Molecular epidemiology analyses (such as pulsed-field gel electrophoresis, multilocus sequence typing (MLST), or detection of vanA/vanB determinants) were not performed.

Results

3.1. General characterisation of the isolates

Following the application of inclusion and exclusion criteria, 6,283 unique clinical isolates of *Enterococcus* spp. were included in the epidemiological analysis. Among these, *E. faecalis* was the most frequently identified species, accounting for 4,881 isolates (77.7%), followed by *E. faecium*, with 1,286 isolates (20.5%). The remaining species comprised 116 isolates (1.8%), including *E. avium* (n = 40), *E. gallinarum* (n = 32), *E. casseliflavus* (n = 18), *E. hirae* (n = 13), *E. durans* (n = 10) and *E. raffinosus* (n = 3) (Additional file 2).

3.2. Temporal distribution of *Enterococcus* spp. over the study period

The temporal analysis demonstrated the continued predominance of *E. faecalis* over *E. faecium* throughout the 22 years evaluated, with annual fluctuations in the isolation frequency of the two species (Fig. 1). In the early years of the timeframe, a low number of isolates and sporadic variations were observed. Starting in 2014, there was a gradual increase in the number of *Enterococcus* spp. positive cultures, which became more evident between 2020 and 2022. *E. faecalis* was more frequent in 2021 (n = 745), while *E. faecium* peaked in 2022 (n = 247). In 2023, a decrease in the prevalence of both species was observed, with 588 isolates of *E. faecalis* and 176 of *E. faecium*.

The less common species of the genus showed low occurrence throughout the entire period, with a slight increase in the most recent years of the historical series, notably *E. avium* and *E. gallinarum* (Additional file 3).

3.3. Isolate distribution according to clinical specimen

Isolate distribution varied across clinical specimens and is shown in Table 1. Urine was the most common biological source for both species, representing 2,793 isolates (57.2%) of *E. faecalis* and 730 isolates (56.8%) of *E. faecium*. Blood cultures ranked second, accounting for 813 isolates (16.7%) of *E. faecalis* and 259 isolates (20.1%) of *E. faecium*.

Among the remaining specimens, *E. faecalis* exhibited higher relative proportions in skin and soft tissues (671; 13.7%) and bone fragments (186; 3.8%). Conversely, *E. faecium* showed higher relative frequencies in secretions (89; 6.9%) and body cavity fluids (86; 6.7%).

Table 1
Distribution of *E. faecalis* and *E. faecium* by clinical specimen.

Clinical specimen	<i>E. faecalis</i> n (%)	<i>E. faecium</i> n (%)
Urine	2793 (57.2)	730 (56.8)
Blood	813 (16.7)	259 (20.1)
Skin and soft tissues	671 (13.7)	96 (7.5)
Secretions	230 (4.7)	89 (6.9)
Cavity fluid	188 (3.9)	86 (6.7)
Bone fragment	186 (3.8)	26 (2.0)
Total	4881 (100.0)	1286 (100.0)

3.4. Demographic and hospital characteristics by isolated species

Differences in demographic and clinical distribution were observed between the two main species. *E. faecalis* was more frequently recovered from male patients (58.6%), whereas *E. faecium* presented a balanced sex distribution (50.4% male vs. 49.6% female). Regarding age groups, both species predominated in older age categories, particularly in the 61–70 years (18.1% for *E. faecalis* and 23.3% for *E. faecium*) and ≥ 71 years strata (25.1% and 29.8%, respectively).

Regarding hospital unit at the time of specimen collection, *E. faecium* showed a higher proportion of isolates from patients hospitalised in intensive care units (ICUs – 46.4%) compared to *E. faecalis* (29.0%). In-hospital mortality was notably higher among patients with *E. faecium* isolates (48.9%), whereas hospital discharge was more frequent among *E. faecalis* isolates (70.1%). Transfer/discharge against medical advice and other outcomes accounted for a minor proportion in both species, precluding the evaluation of discharge or mortality in these cases. The complete distribution of demographic and hospital characteristics according to species is presented in Table 2.

Table 2
Demographic and hospital characteristics by *Enterococcus* species.

Variable	Category	<i>E. faecalis</i> n (%)	<i>E. faecium</i> n (%)
Sex	Female	2022 (41.4)	638 (49.6)
	Male	2859 (58.6)	648 (50.4)
Age group	0–10 years	566 (11.6)	33 (2.6)
	11–20 years	183 (3.7)	38 (3.0)
	21–30 years	357 (7.3)	82 (6.4)
	31–40 years	403 (8.3)	113 (8.8)
	41–50 years	598 (12.3)	117 (9.1)
	51–60 years	665 (13.6)	220 (17.1)
	61–70 years	884 (18.1)	300 (23.3)
	≥ 71 years	1225 (25.1)	383 (29.8)
Hospital unit at the time of specimen collection	ICU	1416 (29.0)	597 (46.4)
	General wards	3465 (71.0)	689 (53.6)
Hospital outcome	Discharge	3424 (70.1)	630 (49.0)
	Death	1309 (26.8)	629 (48.9)
	Transfer/ discharge against medical advice	148 (3.0)	27 (2.1)
Total		4881 (100.0)	1286 (100.0)

Note: Values are expressed as absolute and relative frequencies, calculated within each species. ICU: intensive care unit. The “General wards” category includes hospital units not classified as ICUs at the time of data collection. “Transfer/ discharge against medical advice” preclude ascertainment of the final hospital outcome.

3.5. Phenotypic antimicrobial resistance profiles

The phenotypic profile of antimicrobial resistance was evaluated separately for *E. faecalis* and *E. faecium*, considering the two periods: January 2002 through March 2019, and April 2019 through December 2023 (Table 3).

E. faecalis showed increased resistance to most of the antimicrobials evaluated, with the highest levels observed for high-level gentamicin, levofloxacin, vancomycin, teicoplanin, and high-level streptomycin. Resistance to linezolid remained low in both periods.

E. faecium resistance to ampicillin and levofloxacin was high in both periods. The largest absolute increases were observed for high-level gentamicin, high-level streptomycin, and levofloxacin. A slight decrease in *E. faecium* resistance was observed for vancomycin and teicoplanin in the second period evaluated, while resistance to linezolid and tigecycline remained low for both species.

Distinct vancomycin resistance patterns were observed between species: while *E. faecium* sustained high resistance rates (63.7% in period 1 and 55.0% in period 2), *E. faecalis* experienced an approximate fourfold increase, rising from 6.6% to 25.2%, respectively.

Table 3
Antimicrobial resistance profiles by species and study period.

Species	Antimicrobial agent	January 2002 – March 2019	April 2019 – December 2023	Absolute difference ^d
		n R ^b /n tested ^c (%)	n R ^b /n tested ^c (%)	
<i>E. faecalis</i>	Ampicillin	76/2369 (3.2)	28/2511 (1.1)	-2.1
	Streptomycin ^a	899/2354 (38.2)	1535/2505 (61.3)	+ 23.1
	Gentamicin ^a	821/2359 (34.8)	1545/2507 (61.6)	+ 26.8
	Levofloxacin	766/2370 (32.3)	1405/2510 (56.0)	+ 23.7
	Linezolid	6/1864 (0.3)	7/2511 (0.3)	-0.04
	Teicoplanin	157/2045 (7.7)	634/2511 (25.2)	+ 17.6
	Tigecycline	4/1857 (0.2)	88/2506 (3.5)	+ 3.3
	Vancomycin	157/2370 (6.6)	634/2511 (25.2)	+ 18.6
<i>E. faecium</i>	Ampicillin	479/559 (85.7)	654/726 (90.1)	+ 4.4
	Streptomycin ^a	108/546 (19.8)	242/713 (33.9)	+ 14.2
	Gentamicin ^a	63/552 (11.4)	157/719 (21.8)	+ 10.4
	Levofloxacin	479/558 (85.8)	658/727 (90.5)	+ 4.7
	Linezolid	3/480 (0.6)	3/726 (0.4)	-0.2
	Teicoplanin	356/542 (65.7)	399/726 (55.0)	-10.7
	Tigecycline	1/479 (0.2)	11/724 (1.5)	+ 1.3
	Vancomycin	356/559 (63.7)	400/727 (55.0)	-8.7

Note: The values represent percentages of phenotypic resistance reported in routine laboratory testing. Susceptibility testing was interpreted according to the criteria in effect during the analysis period: CLSI through May 2022 and BrCAST beginning in June 2022. ^a High-level gentamicin and streptomycin resistance. ^b Values expressed as the number of resistant isolates. ^c Number of isolates evaluated and percentage of resistance. Antimicrobial discs not evaluated due to unavailability were excluded from the denominator used to calculate the percentages. ^d The absolute variation was calculated in percentage points between the periods analysed.

Discussion

This study evaluated the temporal, clinical and phenotypic distribution of *Enterococcus* spp. in clinical specimens over a 22-year period at a tertiary university hospital. The primary findings were the persistent predominance of *E. faecalis*, the substantial burden of *E. faecium*, urine as the leading clinical specimen,

a notable rise in isolate numbers in recent years, the disproportionate involvement of *E. faecium* in blood cultures, ICUs, and fatal outcomes, and a marked recent surge in vancomycin resistance among *E. faecalis*.

Enterococcus spp. are opportunistic microorganisms that represent a major challenge in hospital settings. In hospitalised patients, particularly those with prolonged lengths of stay, invasive devices, previous antimicrobial exposure, immunosuppression, and ICU admission, these bacteria play an important clinical role in difficult-to-treat infections [1, 6].

E. faecalis and *E. faecium* were the predominant species isolated from the clinical samples, consistent with the existing literature. A retrospective Italian study similarly documented *E. faecalis* as the prevailing enterococcal species with comparable proportions. In addition, historical surveillance data consistently confirm that *E. faecalis* represents the majority of clinical enterococcal isolates in routine diagnostic laboratories, while *E. faecium* accounts for a smaller fraction [7, 8].

Non-*faecalis* and non-*faecium* species accounted for only 1.8% of isolates, including *E. avium*, *E. gallinarum*, *E. casseliflavus*, *E. hirae*, *E. durans* and *E. raffinosus*. Although uncommon, accurate species-level identification remains essential, as these species may also exhibit resistance mechanisms, transfer plasmids, and cause infections [9].

The temporal series showed a steady increase in isolation numbers from 2014, followed by a sharp elevation between 2020 and 2022. This surge coincided with the COVID-19 pandemic, a period characterised by healthcare overburden, higher patient acuity, extended hospitalisations, and intensive use of invasive devices and broad-spectrum antimicrobials. The subsequent decline in 2023 coincided with the easing of pressure of the healthcare system following the most critical phase of the pandemic, although the retrospective nature of this study does not allow for the establishment of a direct causal relationship.

Urine was the leading clinical specimen for both species with comparable proportions, followed by blood cultures, in which *E. faecium* exhibited a higher relative frequency than *E. faecalis*. These findings are clinically relevant, as enterococcal bloodstream infections can originate from diverse primary foci, notably the urinary tract. Previous studies have highlighted *Enterococcus* urinary tract infections (UTIs) as a significant source of secondary bacteraemia, particularly in hospitalised patients undergoing invasive urinary catheterization [3, 10].

Although *Enterococcus* species are not the predominant uropathogens overall in community settings, their presence is particularly relevant in complicated and healthcare-associated UTIs. In retrospective analyses of uropathogens, *E. faecium* and *E. faecalis* rank among the most frequent pathogens isolated from hospital-acquired urinary infections, consistent with broader surveillance data [11–13].

Enterococcal bloodstream infections (BSIs) are associated with substantial morbidity and mortality among hospitalised patients. In studies evaluating nosocomial bacteraemia, a higher 30-day mortality

has been consistently reported for *E. faecium* compared with *E. faecalis*. Similarly, other investigations have demonstrated elevated mortality rates in patients with *E. faecium* bacteraemia. These findings align closely with our data, in which *E. faecium*, despite being less frequent overall, accounted for a disproportionately higher burden in blood cultures, ICU admissions, and in-hospital mortality [3, 14].

Prolonged hospitalisation, particularly in intensive care units, markedly heightens patient vulnerability to opportunistic infections, including those caused by *Enterococcus* spp. This increased risk is driven by advanced age, indwelling invasive devices, prolonged broad-spectrum antimicrobial therapy, baseline comorbidities, and invasive procedures [6, 15].

In cohorts examining enterococcal bacteraemia, *E. faecium* episodes have been strongly associated with nosocomial acquisition and prior antimicrobial exposure, whereas *E. faecalis* bacteraemias more frequently stem from urinary tract sources. Although our study did not evaluate independent predictors nor was it restricted exclusively to bloodstream infections, our results similarly demonstrate notable clinical distinctions between species: *E. faecalis* predominated overall with high urinary frequency, whereas *E. faecium* was disproportionately associated with blood cultures, ICU stay, fatal outcomes, and extensive antimicrobial resistance [16].

The distribution across the remaining clinical specimens also differed between the species: *E. faecalis* exhibited higher relative frequencies in skin and soft tissues and bone fragment samples, whereas *E. faecium* was proportionally more frequent in purulent secretions and cavitory fluids.

Regarding demographic characteristics, a progressive concentration of isolates was observed in older age groups for both species, particularly among patients aged 61–70 years and ≥ 71 years. This pattern is well-documented in the literature and reflects the heightened clinical vulnerability of the elderly population, driven by immunosenescence, multiple underlying comorbidities, frequent hospitalisations, and cumulative antimicrobial exposure [17]. In our cohort, *E. faecalis* was notably more frequent in male patients, whereas *E. faecium* displayed an even sex distribution.

The analysis of inpatient units and clinical outcomes revealed striking differences between the two species. While *E. faecalis* predominated in general medical/ surgical wards and was predominantly associated with hospital discharge, *E. faecium* accounted for a substantially higher proportion of ICU admissions and fatal outcomes.

The critical role of *E. faecium* in severely ill patients is well-supported by multicentre investigations and ICU cohorts, which consistently highlight this species as a major pathogen in nosocomial monomicrobial bacteraemias, frequently associated with multidrug-resistant phenotypes and higher 30-day mortality compared with *E. faecalis* [18–20]. This unfavorable clinical course reflects the complex environment of long-term ICU stay, where patients are exposed to compounding risk factors including indwelling medical devices, invasive procedures, and intense selective pressure from broad-spectrum antimicrobial regimens [6, 15].

Antimicrobial susceptibility profiling revealed pronounced differences between *E. faecalis* and *E. faecium*, highlighted by a notable surge in resistance among *E. faecalis* isolates during the most recent study period (April 2019 to December 2023). Historically, although *E. faecalis* accounts for most clinical isolates, it typically displays higher susceptibility rates compared with *E. faecium*. Conversely, *E. faecium* maintained consistently high baseline resistance rates across both evaluated intervals, exhibiting less pronounced temporal fluctuations, in line with the well-established multidrug-resistant nature of this species [4].

Regarding aminoglycosides, enterococci exhibit intrinsic-level resistance to this class; however, high-level aminoglycoside resistance to gentamicin or streptomycin is a critical clinical concern. The emergence of high-level aminoglycoside resistance abolishes the synergistic bactericidal activity achieved by combining aminoglycosides with cell-wall-active agents, a cornerstone therapeutic regimen for invasive enterococcal infections [21].

Resistance to linezolid remained negligible in both species across the entire 22-year timeframe. Tigecycline resistance was likewise uncommon, although a modest increase was observed in the recent period, particularly among *E. faecalis* isolates. Consequently, both antimicrobial agents remain vital therapeutic options in complex clinical scenarios with restricted alternatives, especially against glycopeptide-resistant enterococci [22].

The temporal dynamics of VRE revealed one of the most critical findings of this study. Among *E. faecalis* isolates, vancomycin resistance exhibited a substantial and alarming increase between the two study periods, escalating from 6.6% (January 2002-March 2019) to 25.2% (April 2019-December 2023). In contrast, vancomycin resistance in *E. faecium* remained persistently elevated throughout both intervals, with a modest decrease from 63.7% to 55.0%.

In the present study, *E. faecium* exhibited high levels of vancomycin resistance during both study periods. However, the most relevant finding was the marked increase in resistance among *E. faecalis*, the species responsible for most of the clinical isolates. This finding partially contrasts with the pattern most frequently described in the literature, in which vancomycin resistance is predominantly concentrated in *E. faecium*, and reinforces the importance of local surveillance of VRE, with species-stratified analysis.

In a study of hospital clinical specimens, vancomycin resistance was observed in 2.4% of *E. faecalis* and 7.6% of *E. faecium* isolates. Similarly, another study reported a substantially higher proportion of vancomycin resistance in *E. faecium* than in *E. faecalis*. In addition, epidemiological studies conducted in hospitals in India between 2013 and 2021 and a 10-year analysis performed at a tertiary-care hospital in Mexico indicated that most VRE strains belonged to the species *E. faecium* [23–26].

This study demonstrates the persistent relevance of *Enterococcus* spp. as opportunistic pathogens in a tertiary university hospital over a 22-year period, characterised by the predominance of *E. faecalis* and a substantial contribution of *E. faecium*. Comparative analysis revealed marked differences between both

species regarding specimen source, hospital ward, clinical outcome, and antimicrobial resistance profiles.

Conclusions

These findings highlight the critical importance of species-stratified surveillance for *E. faecalis* and *E. faecium* in hospital microbiology. Although *E. faecium* consistently exhibited higher baseline resistance levels and poorer clinical outcomes, the substantial surge in vancomycin resistance among *E. faecalis* isolates represents a major institutional epidemiological shift.

Consequently, continuous longitudinal monitoring of *Enterococcus* spp. is essential to detect local microbiological trends, guide antimicrobial stewardship, and reinforce infection prevention and control strategies.

Abbreviations

BrCAST

Brazilian Committee on Antimicrobial Susceptibility Testing

BSI

bloodstream infection

CEP

UEL—Ethics Committee on Research Involving Human Subjects, State University of Londrina

CLSI

Clinical and Laboratory Standards Institute

HU/UEL

University Hospital of the State University of Londrina

ICU

intensive care unit

MLST

multilocus sequence typing

VRE

vancomycin-resistant enterococci

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee on Research Involving Human Subjects at the State University of Londrina (CEP-UEL; approval No. 6,906,258). The requirement for informed consent was waived by the CEP-UEL due to the retrospective nature of the study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request due to institutional data privacy regulations.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SLC contributed to the conceptualization, data curation, formal analysis, investigation, methodology, project administration, and writing of the original draft, and participated in reviewing and editing the manuscript. APO, DMLOA, and EROJ contributed to the investigation and project administration. LCAA, STC, MAJ, and EHWS contributed to data curation, methodology, and review and editing of the manuscript. ECV and SFYO contributed resources and validation. MREP contributed to the conceptualization, data curation, formal analysis, methodology, project administration, supervision, validation, visualization, and review and editing of the manuscript. All authors read and approved the final manuscript.

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Use of artificial intelligence

The authors declare that no artificial intelligence (AI) tools, machine learning-based text generators, or large language models (LLMs) were used in the conception, study design, data collection, analysis, drafting, or editing of this manuscript.

References

1. García-Solache M, Rice LB. The Enterococcus: a model of adaptability to its environment. Clin Microbiol Rev. 2019;32:e00058–18. <https://doi.org/10.1128/CMR.00058-18>.
2. Blot S, Ruppé E, Harbarth S, Asehnoune K, Poulakou G, Luyt CE, et al. Healthcare-associated infections in adult intensive care unit patients: changes in epidemiology, diagnosis, prevention and

- contributions of new technologies. *Intensive Crit Care Nurs.* 2022;70:103227. <https://doi.org/10.1016/j.iccn.2022.103227>.
3. Zerbato V, Pol R, Sanson G, Suru DA, Pin E, Tabolli V, et al. Risk factors for 30-day mortality in nosocomial enterococcal bloodstream infections. *Antibiot (Basel).* 2024;13:601. <https://doi.org/10.3390/antibiotics13070601>.
 4. Hollenbeck BL, Rice LB. Intrinsic and acquired resistance mechanisms in *Enterococcus*. *Virulence.* 2012;3:421–33. <https://doi.org/10.4161/viru.21282>.
 5. Zaidi S-Z, Zaheer R, Zovoilis A, McAllister TA. Enterococci as a One Health indicator of antimicrobial resistance. *Can J Microbiol.* 2024;70:303–35. <https://doi.org/10.1139/cjm-2024-0024>.
 6. Krawczyk B, Wityk P, Gałęcka M, Michalik M. The many faces of *Enterococcus* spp.: commensal, probiotic and opportunistic pathogen. *Microorganisms.* 2021;9:1900. <https://doi.org/10.3390/microorganisms9091900>.
 7. Boccella M, Santella B, Pagliano P, De Filippis A, Casolaro V, Galdiero M, et al. Prevalence and antimicrobial resistance of *Enterococcus* species: a retrospective cohort study in Italy. *Antibiot (Basel).* 2021;10:1552. <https://doi.org/10.3390/antibiotics10121552>.
 8. Treitman AN, Yarnold PR, Warren J, Noskin GA. Emerging incidence of *Enterococcus faecium* among hospital isolates (1993 to 2002). *J Clin Microbiol.* 2005;43:462–3. <https://doi.org/10.1128/JCM.43.1.462-463.2005>.
 9. Mullally CA, Fahriani M, Mowlaboccus S, Coombs GW. Non-*faecium* non-*faecalis* enterococci: a review of clinical manifestations, virulence factors, and antimicrobial resistance. *Clin Microbiol Rev.* 2024;37:e00121–23. <https://doi.org/10.1128/cmr.00121-23>.
 10. Ali GA, Goravey W, Najim MS, Shunnar KM, Ibrahim SI, Daghfal J, et al. Epidemiology, microbiological and clinical characteristics of *Enterococcus* species bloodstream infections: a 10-year retrospective cohort study from Qatar. *Ann Med Surg (Lond).* 2022;80:104258. <https://doi.org/10.1016/j.amsu.2022.104258>.
 11. Zhang K, Fang X, Zhang Y, Chao M. A retrospective study of uropathogen and its antibiotic resistance among children with urinary tract infection from a single center in China. *Heliyon.* 2024;10:e31902. <https://doi.org/10.1016/j.heliyon.2024.e31902>.
 12. Yang J, Du M, Duan Z, Cai M, Xie H, Wu W, et al. Epidemiological characteristics and antibiotic resistance of pathogens in urinary tract infection in Chengdu 2019–2023, China. *BMC Microbiol.* 2026;26:124. <https://doi.org/10.1186/s12866-026-04719-z>.
 13. Broughton E, Bektas M, Colosia A, Kuper K, Fernandez MM, Al-Taie A, et al. A systematic literature review of the epidemiology of complicated urinary tract infection. *Infect Dis Ther.* 2025;14:1157–81. <https://doi.org/10.1007/s40121-025-01149-8>.
 14. Rinaldi M, Rancan I, Malerba F, Gatti M, Ancillotti L, Tazza B, et al. *Enterococcus faecium* bacteraemia: a multicentre observational study focused on risk factors for clinical and microbiological outcomes. *J Antimicrob Chemother.* 2025;80:2247–56. <https://doi.org/10.1093/jac/dkaf197>.

15. Rogers R, Rice LB. State-of-the-art review: persistent enterococcal bacteremia. *Clin Infect Dis*. 2024;78:e1–11. <https://doi.org/10.1093/cid/ciad612>.
16. Scharloo F, Cogliati Dezza F, López-Hernández I, Martínez Pérez-Crespo PM, Goikoetxea Aguirre AJ, Pérez-Rodríguez MT, et al. Clinical characteristics, predisposing factors and outcomes for *Enterococcus faecalis* versus *Enterococcus faecium* bloodstream infections: a prospective multicentre cohort study. *Eur J Clin Microbiol Infect Dis*. 2024;43:2011–22. <https://doi.org/10.1007/s10096-024-04917-5>.
17. Theodorakis N, Feretzakis G, Hitas C, Kreouzi M, Kalantzi S, Spyridaki A, et al. Antibiotic resistance in the elderly: mechanisms, risk factors, and solutions. *Microorganisms*. 2024;12:1978. <https://doi.org/10.3390/microorganisms12101978>.
18. Herbel S, Dumas G, Chantelot L, Massol J, Nagle S, Lecronier M, et al. Daptomycin is associated with higher treatment failure rates than alternatives for *Enterococcus faecium* bloodstream infections in critically ill patients: a multicentric retrospective cohort. *Crit Care*. 2025;29:505. <https://doi.org/10.1186/s13054-025-05733-9>.
19. Jin Y, Yi Z, Hu Y, Huang M. Epidemiology, clinical characteristics, and outcome of enterococcal bloodstream infections in critically ill patients in China: a single-center retrospective 10-year analysis. *Microbiol Spectr*. 2025;13:e01067–25. <https://doi.org/10.1128/spectrum.01067-25>.
20. Zimmermann V, Fourré N, Senn L, Guery B, Papadimitriou-Olivgeris M. Predictors of mortality of enterococcal bacteraemia and the role of source control interventions; a retrospective cohort study. *Infection*. 2025;53:2149–58. <https://doi.org/10.1007/s15010-025-02561-5>.
21. Chow JW. Aminoglycoside resistance in enterococci. *Clin Infect Dis*. 2000;31:586–9. <https://doi.org/10.1086/313949>.
22. O'Driscoll T, Crank CW. Vancomycin-resistant enterococcal infections: epidemiology, clinical manifestations, and optimal management. *Infect Drug Resist*. 2015;8:217–30. <https://doi.org/10.2147/IDR.S54125>.
23. Bulut D, Karakoç Parlayan HN, Şencan İ. Comprehensive comparative analysis of *Enterococcus faecalis* versus *Enterococcus faecium* strains isolated from clinical samples: a retrospective observational study. *FLORA*. 2024;29:280–8. <https://doi.org/10.5578/flora.2024021037>.
24. Liu Y, Zheng X, Wu B. In vitro activity of vancomycin and other commonly used antibiotics against clinical isolates of *Enterococcus faecalis* and *Enterococcus faecium*: data from the Antimicrobial Testing Leadership and Surveillance (ATLAS) program from 2019 to 2023. *J Glob Antimicrob Resist*. 2026;46:254–63. <https://doi.org/10.1016/j.jgar.2025.12.018>.
25. López-Luis BA, Sifuentes-Osornio J, Lambraño-Castillo D, Ortiz-Brizuela E, Ramírez-Fontes A, Tovar-Calderón YE, et al. Risk factors and outcomes associated with vancomycin-resistant *Enterococcus faecium* and ampicillin-resistant *Enterococcus faecalis* bacteraemia: a 10-year study in a tertiary-care centre in Mexico City. *J Glob Antimicrob Resist*. 2021;24:198–204. <https://doi.org/10.1016/j.jgar.2020.12.005>.

26. Iqbal F, Alocious A, Joy SC, Stanly EAR, Rajesh V, Unnikrishnan MK, et al. Vancomycin-resistant enterococci: a rising challenge to global health. Clin Epidemiol Glob Health. 2024;28:101663. <https://doi.org/10.1016/j.cegh.2024.101663>.

Figures

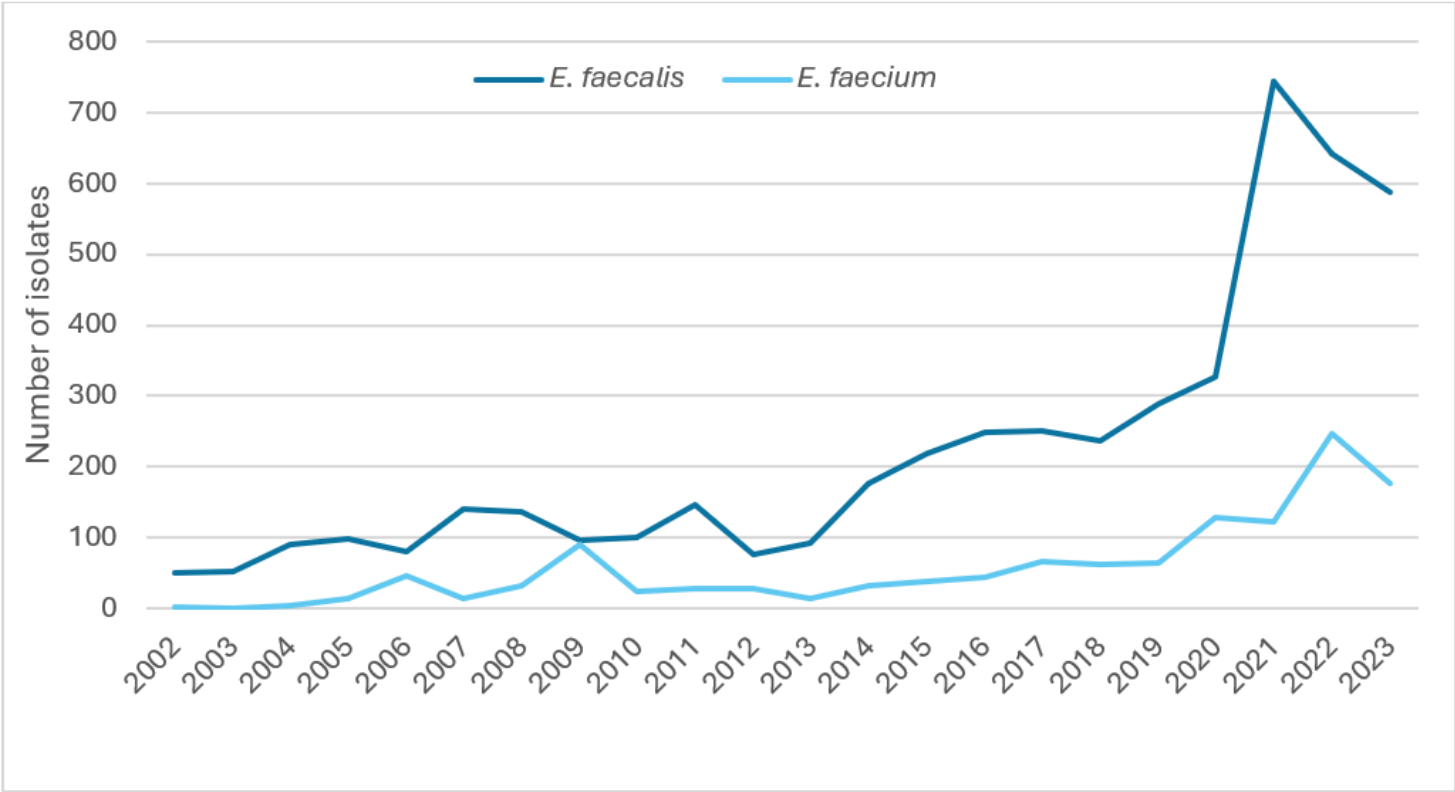


Figure 1
Annual frequency of *E. faecalis* and *E. faecium*, 2002–2023

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