

# Genomic Characterization of Antibiotic-Resistant *Enterococcus* Spp. Strains in Clinical Settings of Yichang, China

Hong Yu<sup>1–3</sup>, Peng Xiong<sup>2,3</sup>, HuiNa Zhang<sup>2,3</sup>, Zhi Lu<sup>2,3</sup>, XinLu Chen<sup>2,3</sup>, RuHua Yan<sup>2,3</sup>, Lei Wang<sup>2,3</sup>, Long Yu<sup>2,3</sup>, Qingyong Zhang<sup>4,5</sup>, Lili Zou<sup>2,3</sup>, Lu Wang<sup>2,3</sup>

<sup>1</sup>Department of Medical Ultrasound, Tongji Hospital, Tongji Medical College, and State Key Laboratory for Diagnosis and Treatment of Severe Zoonotic Infectious Diseases, Huazhong University of Science and Technology, Wuhan, Hubei, 430030, People's Republic of China; <sup>2</sup>Hubei Key Laboratory of Tumor Microenvironment and Immunotherapy, College of Basic Medical Sciences, China Three Gorges University, Yichang, Hubei, 443002, People's Republic of China; <sup>3</sup>Institute of Infection and Inflammation, College of Basic Medical Sciences, China Three Gorges University, Yichang, Hubei, 443002, People's Republic of China; <sup>4</sup>The First College of Clinical Medical Science, China Three Gorges University, Yichang, Hubei, 443002, People's Republic of China; <sup>5</sup>Department of Clinical Laboratory of Yichang Central People's Hospital, Yichang, Hubei, 443002, People's Republic of China

Correspondence: Lu Wang, Email wanglou@ctgu.edu.cn

**Objective:** Antimicrobial-resistant *Enterococcus* species are important causes of hospital-acquired infections, yet genomic surveillance data from Yichang, China, are lacking.

**Methods:** Between February 2023 and December 2024, 150 antibiotic-resistant *Enterococcus* isolates were collected from five hospitals in Yichang. Antimicrobial susceptibility testing was performed according to CLSI standards. Fifty-nine representative isolates underwent whole-genome sequencing for species identification, multilocus sequence typing (MLST), antimicrobial resistance (AMR) gene detection, virulence profiling, and phylogenetic analysis.

**Results:** *E. faecalis* (46.7%) and *E. faecium* (42.7%) were the predominant species, mainly isolated from urine specimens. High multidrug resistance rates were observed, particularly in *E. faecium*, which showed extensive resistance to  $\beta$ -lactams and fluoroquinolones. MLST analysis identified *E. faecium* ST78 and *E. faecalis* ST16 as the dominant clones, both corresponding to internationally reported epidemic lineages. Importantly, two novel *E. faecalis* sequence types were identified, each resulting from single-nucleotide substitutions in housekeeping genes, indicating ongoing local genetic evolution. Resistome analysis revealed enrichment of vancomycin, tetracycline, aminoglycoside, and lincosamide resistance genes in Yichang isolates compared with reference genomes. In addition, *E. faecalis* carried a broader repertoire of resistance and virulence genes than *E. faecium*.

**Conclusion:** Our findings demonstrate that whole-genome sequencing is not only a powerful tool for characterizing dominant epidemic clones but also an effective strategy for surveillance of atypical and emerging *Enterococcus* species. The detection of two novel sequence types and region-specific resistance patterns highlights the value of genomic monitoring in guiding infection-control policies, antimicrobial stewardship, and early warning of multidrug-resistant strain dissemination in hospital settings.

**Keywords:** antimicrobial-resistant *Enterococcus*, whole-genome sequencing, multilocus sequence typing, resistance gene, virulence gene

## Introduction

*Enterococcus* spp. are Gram-positive cocci that are ubiquitous in the environmental-including soil, water, and the gastrointestinal tracts of humans and animals.<sup>1</sup> These opportunistic pathogens are responsible for a wide spectrum of infections, such as urinary tract infections, intra-abdominal infections, and infective endocarditis.<sup>2–4</sup> Enterococci currently account for approximately 10–15% of hospital-acquired infections worldwide and pose particular risks to elderly, immunocompromised, and critically ill patients, especially those undergoing invasive procedures or prolonged hospitalization.<sup>5,6</sup> In these high-risk populations, infections are associated with increased morbidity, mortality, and healthcare burden.

Over recent decades, extensive use of broad-spectrum antibiotics and horizontal gene transfer have driven the emergence of multidrug-resistant (MDR) *Enterococcus* strains.<sup>7,8</sup> Particularly concerning are resistances to glycopeptides and aminoglycosides, which considerably limit treatment options and increase mortality risk. Horizontal gene transfer further accelerates the evolution of multidrug-resistant (MDR) strains, complicating infection management in hospital environments.<sup>9</sup> Recognizing the growing threat of antimicrobial resistance, the World Health Organization (WHO) classified vancomycin-resistant *Enterococcus faecium* (VRE) as a Priority 2: High pathogen in its Global Priority Pathogens List (initially released in 2017 and reaffirmed in 2024).<sup>10</sup> This highlights the urgent need for improved surveillance and infection control strategies against VRE and other resistant *Enterococcus* lineages that are increasingly reported in clinical settings worldwide.<sup>11</sup> However, focusing exclusively on clinically defined resistant subsets such as VRE may overlook the broader evolutionary landscape of resistance accumulation within *Enterococcus* populations.<sup>12</sup> Resistance determinants often emerge progressively through clonal expansion and gene acquisition before reaching defined clinical thresholds.<sup>12</sup> Therefore, comprehensive genomic surveillance of antibiotic-resistant *Enterococcus* spp., rather than restriction to VRE alone, is essential for early detection of high-risk clones and evolving resistance traits.

The advent of whole-genome sequencing (WGS) has revolutionized pathogen surveillance by enabling high-resolution insights into species diversity, virulence factors, and antimicrobial-resistance (AMR) determinants. Multilocus sequence typing (MLST), a foundational approach in molecular epidemiology, continues to provide a robust framework for strain classification.<sup>13</sup> Comparative-genomic analyses have even revealed novel *Enterococcus* species, emphasizing the genus's adaptability and evolutionary potential.

Although several regional studies in China have investigated the molecular epidemiology of *Enterococcus*,<sup>14,15</sup> genomic data from Yichang remain scarce. As an important regional healthcare hub in central China, Yichang serves a large hospitalized and referred patient population. The lack of local genomic surveillance limits understanding of resistance evolution, clonal dissemination, and potential emergence of atypical or high-risk *Enterococcus* lineages in this region. Therefore, this study conducted comprehensive whole-genome sequencing of antimicrobial-resistant *Enterococcus* isolates collected from clinical settings in Yichang. By integrating phenotypic resistance profiles, MLST typing, and genomic analyses of AMR and virulence genes, we aimed to establish region-specific molecular epidemiological evidence to support antimicrobial stewardship and infection-control strategies.

## Methods

### Isolate Collection and Ethical Statement

Between February 2023 and December 2024, a total of 150 antibiotic-resistant *Enterococcus* isolates were collected from five hospitals in Yichang, Hubei Province, China—Yichang Central People's Hospital, Yichang Second People's Hospital, Yichang Youfu Hospital, Zigui County People's Hospital, and Yidu First People's Hospital. These institutions were selected to represent both plain and mountainous regions according to the city's geomorphological distribution. Suspected *Enterococcus* colonies were preliminarily identified using the Autof MS 1000 MALDI-TOF MS system (Autobio Diagnostics, China), and confirmed isolates were stored at  $-80^{\circ}\text{C}$  for subsequent analyses.

All isolates were obtained as residual anonymized materials from routine clinical diagnostic procedures, with no additional sampling or patient intervention conducted for this study. The use of these fully anonymized bacterial isolates and associated de-identified clinical data (specimen type, ward, susceptibility profile) was reviewed and approved for exemption of ethical approval by the Ethics Committee of Yichang Central People's Hospital (the lead institution). The exemption was granted in accordance with national regulations and international guidelines (eg., the Declaration of Helsinki) for retrospective studies that utilize irreversibly anonymized specimens and pose no risk to patient privacy or welfare.

### Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing (AST) was performed using the VITEK 2 Compact automated system (bioMérieux, France), and minimum inhibitory concentrations (MICs) were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2023). The antimicrobial panel included routinely tested agents such as

aminoglycosides, penicillins, vancomycin, and linezolid. Resistance phenotypes were determined to guide selection of representative isolates for genomic analysis.

## Multilocus Sequence Typing (MLST)

MLST was carried out following the *Enterococcus* schemes available in the PubMLST database (<https://pubmlst.org/data>) using the MLST software package. Validated seven-locus schemes were applied for *E. faecium* (adk, atpA, ddl, gdh, gyd, pstS, and purK) and *E. faecalis* (aroE, gdh, gki, gyd, pstS, xpt, and yqiL). Allele assignment and sequence type (ST) designation were performed using custom Python scripts. Phylogenetic relationships were inferred from concatenated allele sequences. The distribution of STs was analyzed to identify dominant or high-risk clones and compared with previously reported global lineages.

## AMR Gene and Virulence Gene Analysis

Fifty-nine representative isolates, selected based on their antimicrobial-resistance phenotypes, were subjected to WGS by Magigene Biotechnology (Guangdong, China). Genomic DNA was extracted using a standard phenol–chloroform method, and sequencing libraries were prepared according to the manufacturer's protocols. Raw reads were quality-trimmed and assembled with SPAdes v3.15.5. Genome annotation was performed using Prokka v1.14.6.

## Whole-Genome Sequencing and Assembly

Sequencing libraries were prepared using the Illumina DNA Prep kit and sequenced on an Illumina NovaSeq 6000 platform with 150-bp paired-end reads. Raw reads were quality-filtered using fastp (v0.23.2), removing adapters, and reads with Phred scores <20. Assemblies were generated using SPAdes (v3.15.5), and assembly quality was assessed by QUAST (v5.2.0). The average genome coverage depth was approximately 150×, with N50 values ranging from 29,159 to 1,485,635 bp.

Antimicrobial-resistance and virulence factors were identified via local BLAST searches against the CARD and VFDB databases, respectively. AMR genes were identified using the Comprehensive Antibiotic Resistance Database (CARD, version 3.2.7) with a minimum identity threshold of 90% and coverage  $\geq 70\%$ . Virulence factors were detected by BLASTp against the Virulence Factor Database (VFDB, version 5.0) under the same criteria. Mobile genetic elements were filtered out by cross-referencing with IslandViewer 4 predictions. The resulting genomic features were summarized and visualized.

## Phylogenetic Reconstruction

Two complete reference genomes per species were retrieved from the NCBI database. Single-copy core genes shared among reference and study isolates were identified using OrthoFinder v2.5.5. Core-gene alignments were generated with MAFFT v7 (G-INS-i algorithm), concatenated, and used to infer a maximum-likelihood phylogeny in IQ-TREE v2.2.2 with 1,000 bootstrap replicates. *Lactobacillus casei* (GenBank accession: GCA\_000309565.2) served as the outgroup.<sup>12</sup> The distribution of resistance and virulence genes was mapped onto the phylogenetic tree, which was visualized in iTOL v6.

The best-fit substitution model was selected using ModelFinder implemented in IQ-TREE. Phylogenetic inference was performed using the GTR+F+I+G4 model with 1,000 ultrafast bootstrap replicates (-bb 1000). Core genes were defined as single-copy orthologs present in  $\geq 95\%$  of isolates. SNPs were extracted from concatenated alignments using custom Python scripts.

## Statistical Analysis

Descriptive statistics, including means and standard deviations, were calculated using R software (v4.3.2). Principal component analysis (PCA) of resistance and virulence gene profiles was performed using the `prcomp` function in R. No formal hypothesis testing or inferential statistical comparisons were conducted.

## Results

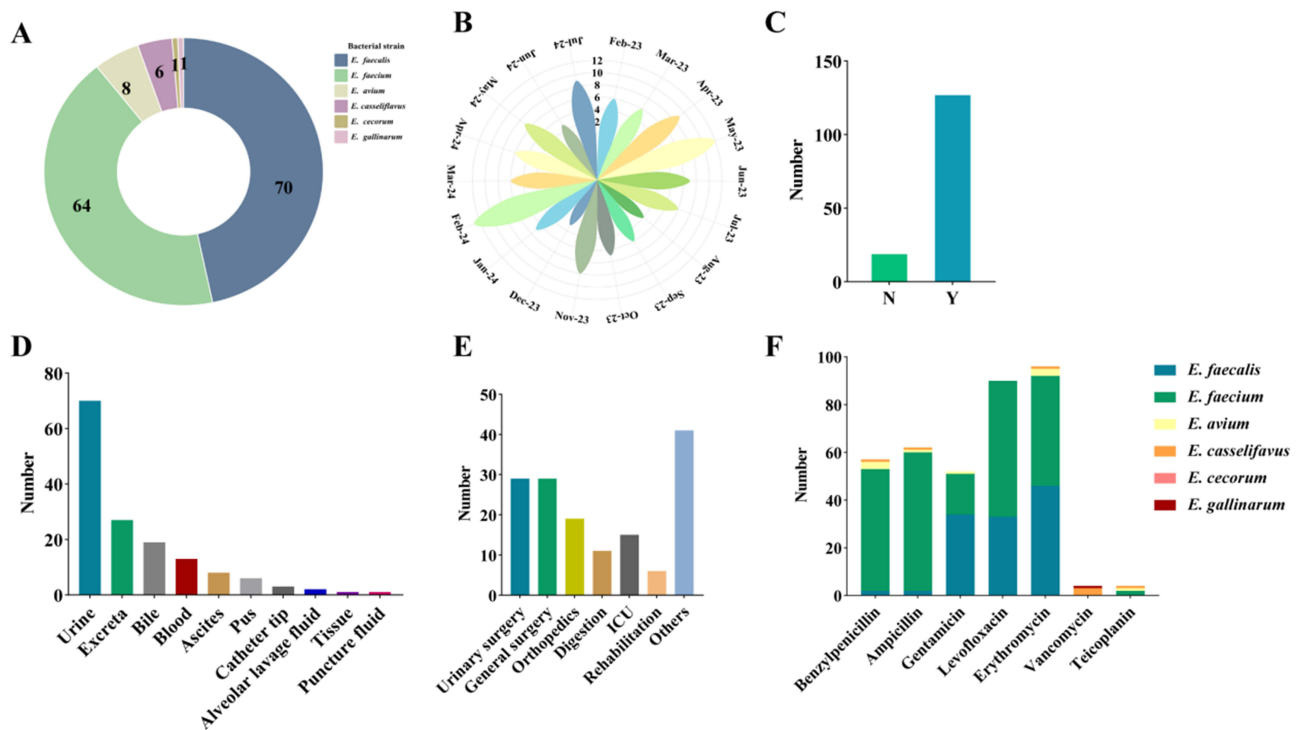
### Epidemiological Distribution and Clinical Characteristics

A total of 150 antimicrobial-resistant *Enterococcus* isolates were recovered, including 64 *E. faecium*, 70 *E. faecalis*, 8 *E. avium*, 6 *E. casseliflavus*, and one each of *E. gallinarum* and *E. cecorum*. Among them, *E. faecalis* and *E. faecium* were the predominant species (Figure 1A). Most isolates were obtained during the winter-to-spring period (Figure 1B). Notably, 84% of patients had undergone invasive procedures, indicating that instrumentation was a major predisposing factor; however, infections also occurred in non-surgical cases (Figure 1C). The majority of isolates were recovered from urine and blood samples, reflecting the well-known tendency of *Enterococcus* to cause urinary tract and bloodstream infections and its ability to persist in hospital environments (Figure 1D). Correspondingly, most cases were concentrated in surgical wards, particularly urology, where urinary tract infections are common (Figure 1E).

Antimicrobial susceptibility testing revealed widespread multidrug resistance among the 150 clinical isolates. High resistance rates were observed for aminoglycosides, tetracyclines, and macrolides (Figure 1F). Both *E. faecalis* and *E. faecium* exhibited strong resistance to macrolides and fluoroquinolones, but their overall resistance profiles differed. *E. faecalis* showed notably high resistance to aminoglycosides—73.0% to erythromycin, 50.0% to levofloxacin, and 49.3% to gentamicin—whereas *E. faecium* demonstrated predominant resistance to  $\beta$ -lactams, with rates of 91.1% for penicillin G and 92.1% for ampicillin, along with 82.1% resistance to erythromycin, 24.6% to gentamicin, and 91.9% to levofloxacin. Collectively, these findings indicate that *E. faecium* exhibited a broader and more robust antimicrobial resistance phenotype than *E. faecalis* (Supplementary Table S1).

### Genomic Characteristics and Assembly Quality of the Sequenced Isolates

A total of 59 *Enterococcus* isolates underwent whole-genome sequencing, including 28 *E. faecium*, 22 *E. faecalis*, 4 *E. avium*, 3 *E. casseliflavus*, and one isolate each of *E. gallinarum* and *E. cecorum*. Genome sizes ranged from 2.43 to 4.55 Mb, with GC contents between 36.58% and 42.55%. The average sequencing depth was approximately 150 $\times$ .



**Figure 1** Summary of Clinical Isolate Information. (A) Species composition of antimicrobial-resistant *Enterococcus* isolates; (B) Temporal distribution of sample collection; (C) History of invasive procedures including surgery; (D) Specimen sources of isolates; (E) Distribution of isolates across hospital wards; (F) Antimicrobial resistance profiles of the 150 clinical isolates representing six *Enterococcus* species.

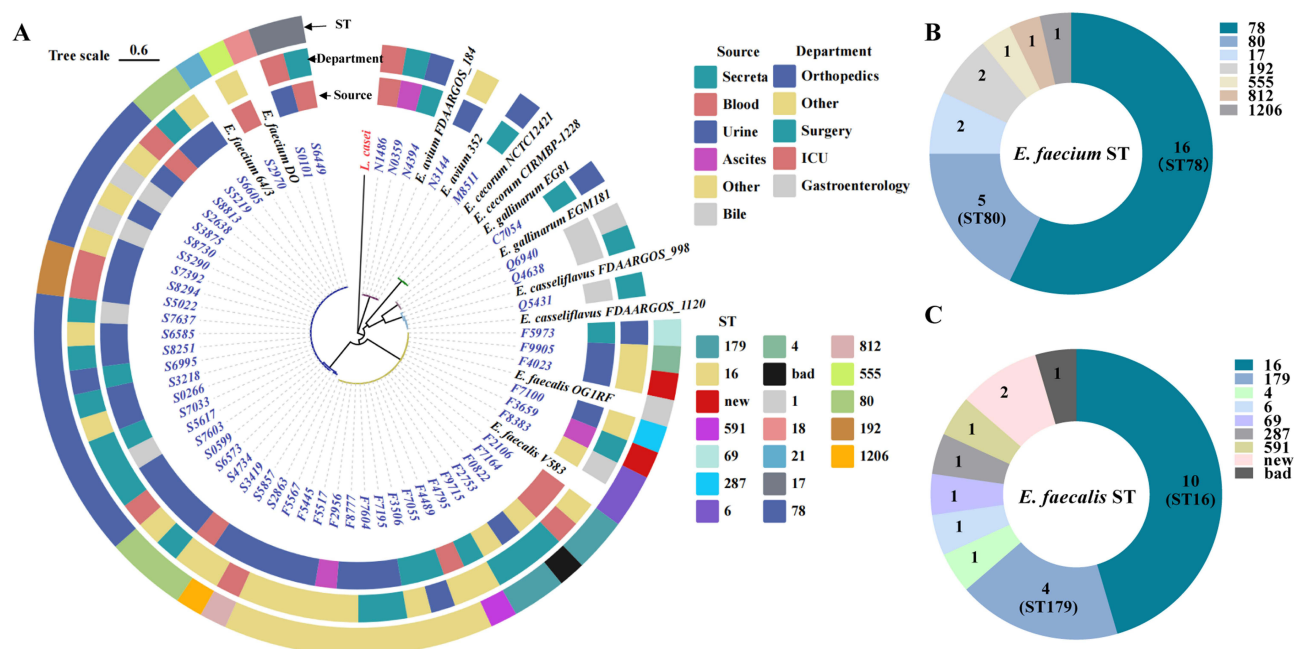
Assembly N50 values ranged from 29,159 to 1,485,635 bp. Detailed assembly statistics are provided in [Supplementary Table S2](#), and all genome data have been deposited in the NCBI database under the accession numbers listed in [Supplementary Table S2](#).

## Species-Level Genomic Features

Species-specific genomic summaries are presented in [Supplementary Table S3](#). The mean genome sizes of *E. faecium* and *E. faecalis* were  $2.95 \pm 0.12$  Mb and  $3.00 \pm 0.12$  Mb, respectively, with GC contents of  $37.70 \pm 0.19\%$  and  $37.27 \pm 0.18\%$ . *E. avium* exhibited a larger average genome size ( $4.45 \pm 0.10$  Mb), whereas *E. casseliflavus* and *E. gallinarum* displayed higher GC contents ( $>40\%$ ). The single *E. cecorum* genome measured 2.43 Mb with a GC content of 36.58%. The numbers of predicted genes, rRNA operons, and tRNAs varied among species.

## MLST Typing Characteristics

Based on the 59 clinical *Enterococcus* genomes sequenced in this study and two complete reference genomes per species for *E. faecium*, *E. faecalis*, *E. avium*, *E. casseliflavus*, *E. cecorum*, and *E. gallinarum* retrieved from NCBI (12 references in total), together with *Lactobacillus casei* as an outgroup, we identified 509 452 SNPs across 679 single-copy core genes and constructed a maximum-likelihood phylogenetic tree. STs for *E. faecium* and *E. faecalis* were assigned via the PubMLST scheme, and the resulting ST information was merged with epidemiological metadata to generate a transmission network. In [Figure 2A](#), isolates sharing the same ST cluster tightly within the same phylogenetic branch, confirming their close genetic relatedness. Several regional patterns emerge. *E. faecium* exhibited a distinct epidemiological imprint, with most isolates derived from urine, and ST78 predominates (16/28, 57.1%). In contrast, *E. faecalis* was more genetically heterogeneous, displaying wider ward and specimen distributions and a dispersed ST profile, although ST16 remains the leading type (10/22, 45.5%). Notably, isolates within the same phylogenetic branch did not consistently originate from the same department or specimen category, implying multiple independent transmission routes within the study hospitals. The dominance of ST78 in *E. faecium* and the expanding assortment of *E. faecalis* lineages—particularly the ascendant ST16—highlight the dynamic population structure of *Enterococcus* in this region.



**Figure 2** Phylogeny and MLST profiles of *Enterococcus* isolates. (A) Maximum-likelihood phylogenetic tree of clinical isolates constructed from core-genome SNPs; (B) Sequence-type distribution of sequenced *E. faecium* isolates (n = 28), ST78 is the predominant type (16 isolates); (C) Sequence-type distribution of sequenced *E. faecalis* isolates (n = 22), ST16 is the predominant type (10 isolates).

Among the 28 sequenced *E. faecium* isolates, seven STs were identified (Figure 2B): ST78 (16 isolates, 57.1%), ST80 (5, 17.9%), ST17 (2, 7.1%), ST192 (2, 7.1%), and the rarer ST555, ST812, and ST1206 (one isolate each, 3.6%). A survey of 23,616 publicly available *E. faecium* genomes in NCBI showed that these STs account for 3.51%, 17.77%, 7.03%, 1.88%, 0.89%, 0.03%, and 0.01% of typed genomes, respectively, confirming ST78 and ST80 as globally prevalent lineages. The 22 *E. faecalis* isolates likewise yielded seven STs (Figure 2C): ST16 (10 isolates, 45.5%), ST179 (4, 18.2%), and single representatives of ST4, ST6, ST69, ST287, and ST591 (each 4.5%). Among 9071 *E. faecalis* genomes in NCBI, these STs occur at frequencies of 8.11%, 6.05%, 1.06%, 8.02%, 0.10%, 0.60%, and 0.15%, respectively, highlighting ST16 as both the local and an international epidemic clone.

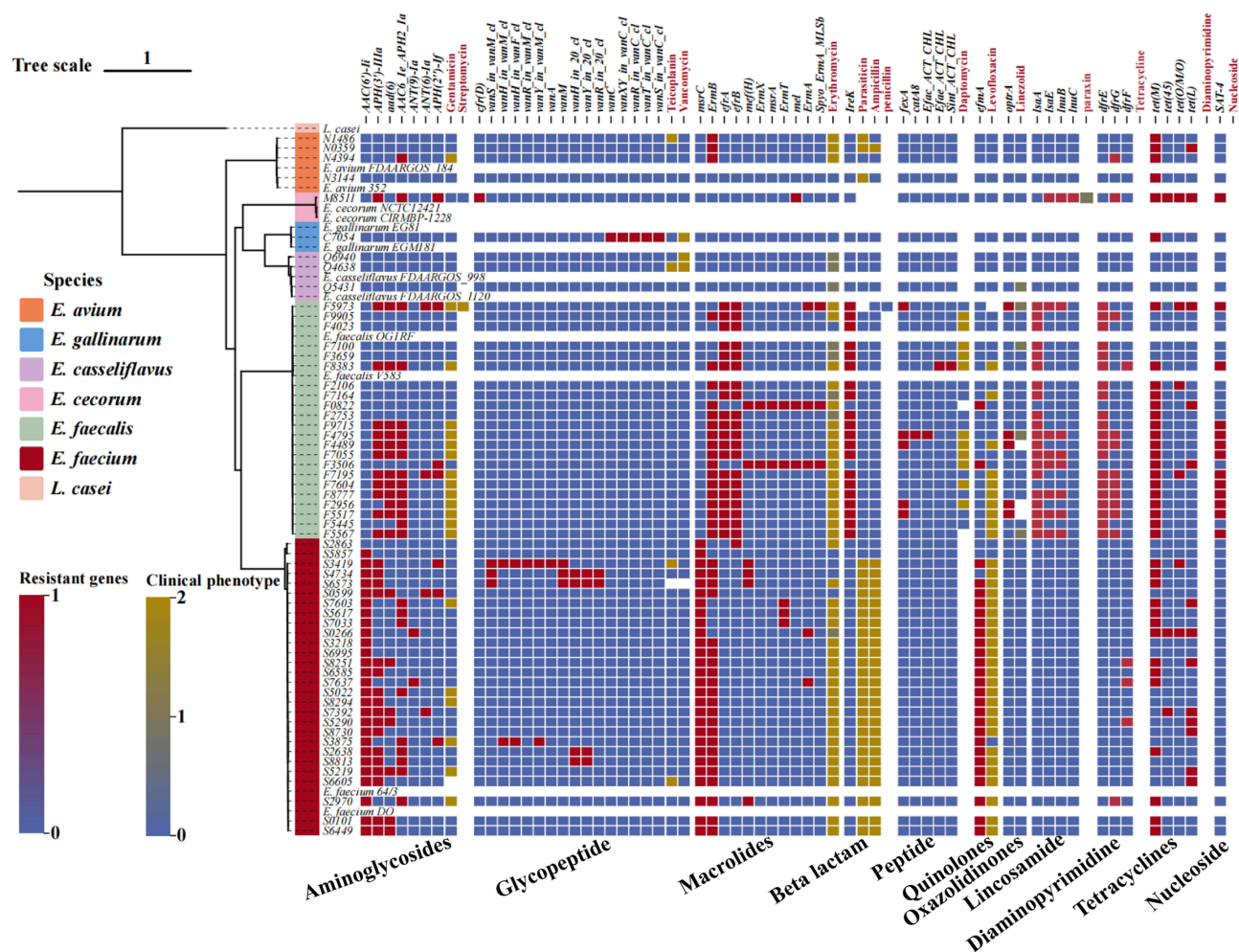
Yichang’s dominant resistant clones, *E. faecium* ST78 and *E. faecalis* ST16, correspond to globally prevalent lineages, whereas rare types such as *E. faecium* ST1206 and ST812 were also present locally. During ST assignment, three *E. faecalis* isolates (F0822, F3659, and F4023) did not match any known profiles: F0822 lacked one allele due to a sequencing gap, while F3659 and F4023 each harboured a single-nucleotide change that generated novel STs (Figure 3 and Supplementary Table S4). The emergence of these new STs indicates ongoing local evolution and dissemination, potentially driven by adaptation, strain importation, or the risk of future outbreaks.

### Resistance - Gene Profile Analysis

CARD screening detected 11 classes of AMR genes among the clinical *Enterococcus* isolates, and their distribution differed markedly by species (Figure 4). Among the rarer taxa, *E. avium* (n = 4) carried between one and four AMR genes per genome, whereas *E. casseliflavus* (n = 3) harboured none of the surveyed determinants. The single *E. gallinarum* isolate contained six AMR genes, including five vancomycin-resistance loci, while the lone *E. cecorum* genome encoded 14 genes that mainly conferred resistance to aminoglycosides, lincosamides, and tetracyclines. In the predominant species, *E. faecium* (n = 28) carried 2–12 AMR genes, principally targeting aminoglycosides, β-lactams, macrolides, tetracyclines, and fluoroquinolones; notably, six isolates possessed vancomycin-resistance genes. *E. faecalis* (n = 22) encoded 5–20 AMR genes, predominantly harbors resistance genes against macrolides, quinolones, tetracyclines, and aminoglycosides, and also carries smaller numbers of resistance genes for diaminopyrimidines, lincosamides, nucleosides, and cephalosporins. Thus,



**Figure 3** Identification of two novel *E. faecalis* sequence types. (A) Isolate F3659 carries a single-nucleotide substitution in the housekeeping gene *pstS* (allele *pstS\_17*; g.277 C→T), as indicated by the red arrow; (B) Isolate F4023 carries a single-nucleotide substitution in the housekeeping gene *aroE* (allele *aroE\_1*; g.127 C→A), as indicated by the red arrow. Asterisks (\*) indicate conserved positions in the alignment.



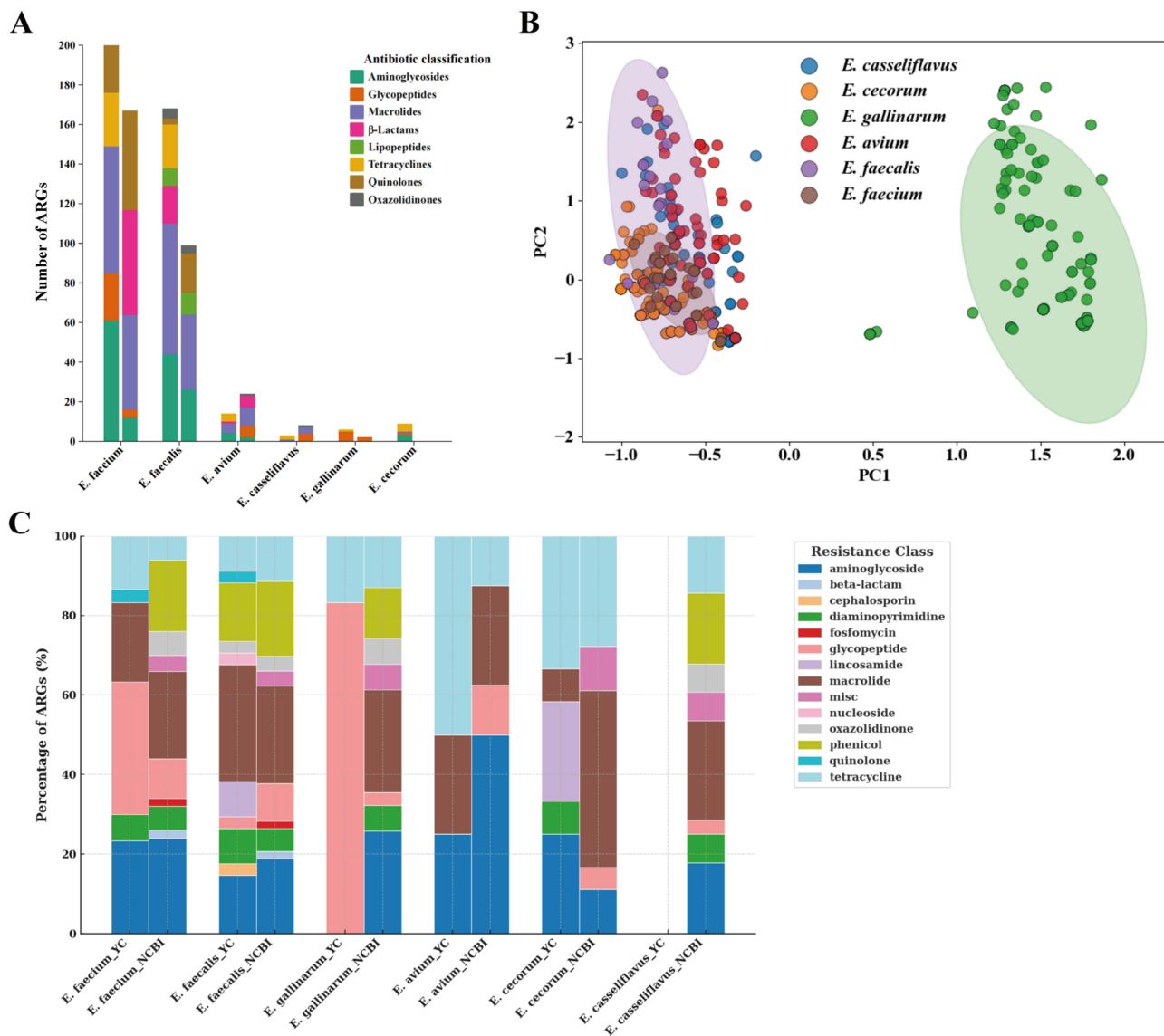
**Figure 4** Correlation between resistome and phenotype in *Enterococcus* isolates. Eleven classes of antimicrobial-resistance genes were identified across the study isolates. On the x-axis, black labels denote individual resistance genes, while red labels indicate the corresponding antibiotic classes tested phenotypically. Blank cells signify either the absence of that gene class or the lack of susceptibility data for the matching drug. Resistant genes: 0=gene absent; 1=gene present. Clinical phenotype: 0=susceptible; 1=intermediate; 2=resistant.

although the rarer species generally harboured fewer resistance genes, the *E. gallinarum* isolate exhibited extensive vancomycin resistance and the *E. cecorum* isolate possessed a broad profile of aminoglycosides, lincosamides, and tetracyclines. The large and varied resistomes of *E. faecium* and *E. faecalis* underscore their clinical importance, especially given the presence of vancomycin-resistant *E. faecium* strains circulating in the Yichang region.

Analysis of the 59 sequenced *Enterococcus* isolates revealed heterogeneous distributions of antimicrobial resistance genes across species (Figure 5A). The most prevalent resistance determinants included *ermB* (macrolide resistance), *tetM/tetL* (tetracycline resistance), and *aac(6')-aph(2'')* (high-level aminoglycoside resistance). Six *E. faecium* isolates and the single *E. gallinarum* isolate carried vancomycin-resistance genes (*van* cluster). Notably, although >90% of *E. faecium* isolates were phenotypically resistant to ampicillin and penicillin G, no acquired  $\beta$ -lactamase genes were detected, consistent with intrinsic PBP-mediated resistance. Principal component analysis further demonstrated inter-species clustering based on resistome composition (Figure 5B). Compared with NCBI reference genomes, Yichang isolates were enriched in vancomycin-, tetracycline-, aminoglycoside-, and lincosamide-resistance genes (Figure 5C).

### Virulence Gene Profiles

Comparative analysis using the VFDB revealed widespread distribution of key virulence determinants (Figure 6). Prominent adhesins included *esp*, *ace*, and *efaA*, supporting strong colonization potential. Several isolates, particularly

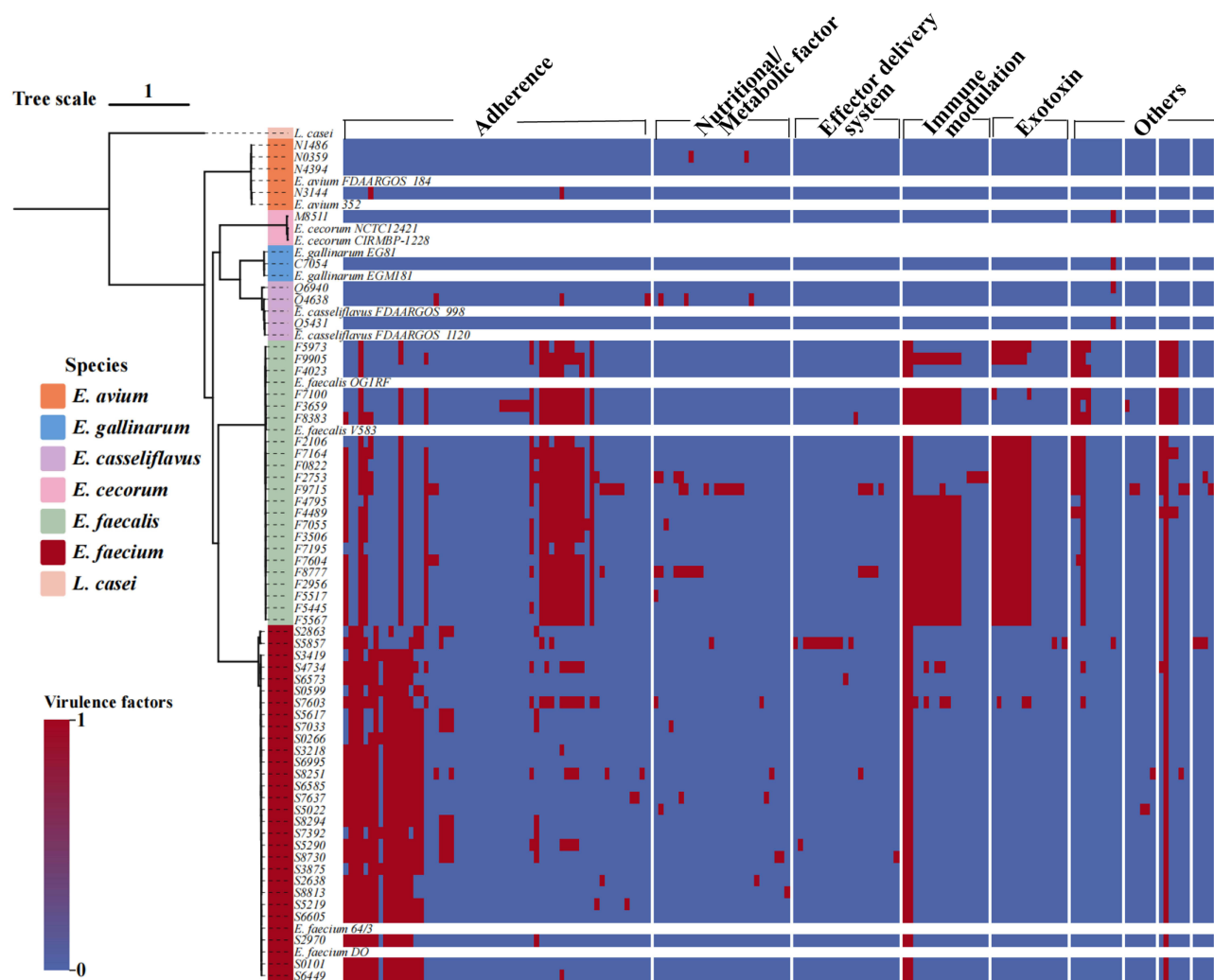


**Figure 5** Analysis of Antimicrobial Phenotypes and Resistance Genes. **(A)** Stacked bar plot showing the distribution and counts of antibiotic resistance genes (ARGs) across six *Enterococcus* species; **(B)** Principal component analysis (PCA) of ARG profiles among six *Enterococcus* species; **(C)** Comparative distribution of ARG classes between Yichang isolates and reference genomes from the NCBI database.

*E. faecalis*, harbored cytolysin-associated genes (eg., *cylA*) and gelatinase (*gelE*), along with genes involved in immune evasion and effector secretion systems. Overall, *E. faecalis* exhibited a broader and denser virulence-gene repertoire than *E. faecium*, consistent with enhanced host adaptation and pathogenic potential.

A quantitative comparison of virulence determinants showed that *E. faecalis* harboured a broader set of virulence factors than *E. faecium* (Figure 7A). Principal component analysis further highlighted clear interspecies separation based on virulence-gene repertoires (Figure 7B). Compared with six reference *Enterococcus* species from the NCBI database, Yichang isolates exhibited reduced prevalence of adherence genes but enrichment in exoenzymes, nutrient/metabolism factors, and effector secretion systems, yielding a more diverse virulence profile (Figure 7C).

Resistance traits correlate with both the number of acquired AMR genes and the burden of virulence factors (Figure 8). Isolates belonging to high-prevalence sequence types—*E. faecalis* ST16 and ST179, and *E. faecium* ST78, ST192, and ST80—exhibit the densest clustering of virulence and resistance genes, reflecting their tight evolutionary relatedness. Although *E. avium* and *E. casseliflavus* carry comparatively few virulence or resistance genes, they are nonetheless isolated with appreciable frequency in clinical practice. Conversely, the rare species *E. gallinarum* and



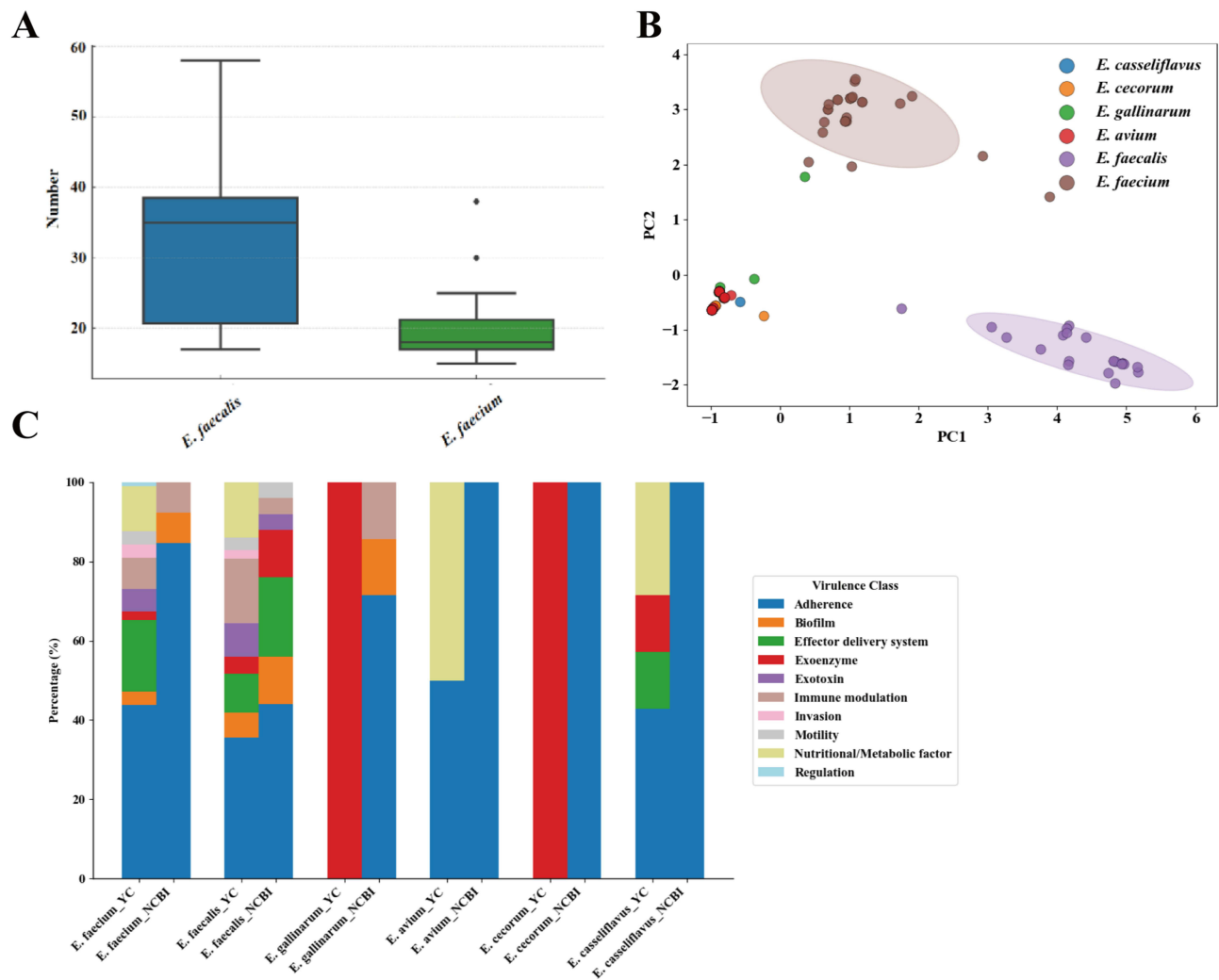
**Figure 6** Virulence gene profiles of sequenced *Enterococcus* isolates. Nine categories of virulence genes were detected, with factors from *E. faecium* and *E. faecalis* predominating. Black squares indicate the presence of a gene (1), and white squares indicate absence (0).

*E. cecorum* display extensive resistomes yet fewer virulence determinants. Notably, *E. cecorum*—mostly reported in poultry outbreaks—was recovered from a wound exudate in a trauma patient in this study. Given the zoonotic nature of enterococcal infections, clinicians should remain alert to the outbreak potential of such uncommon species.

## Discussion

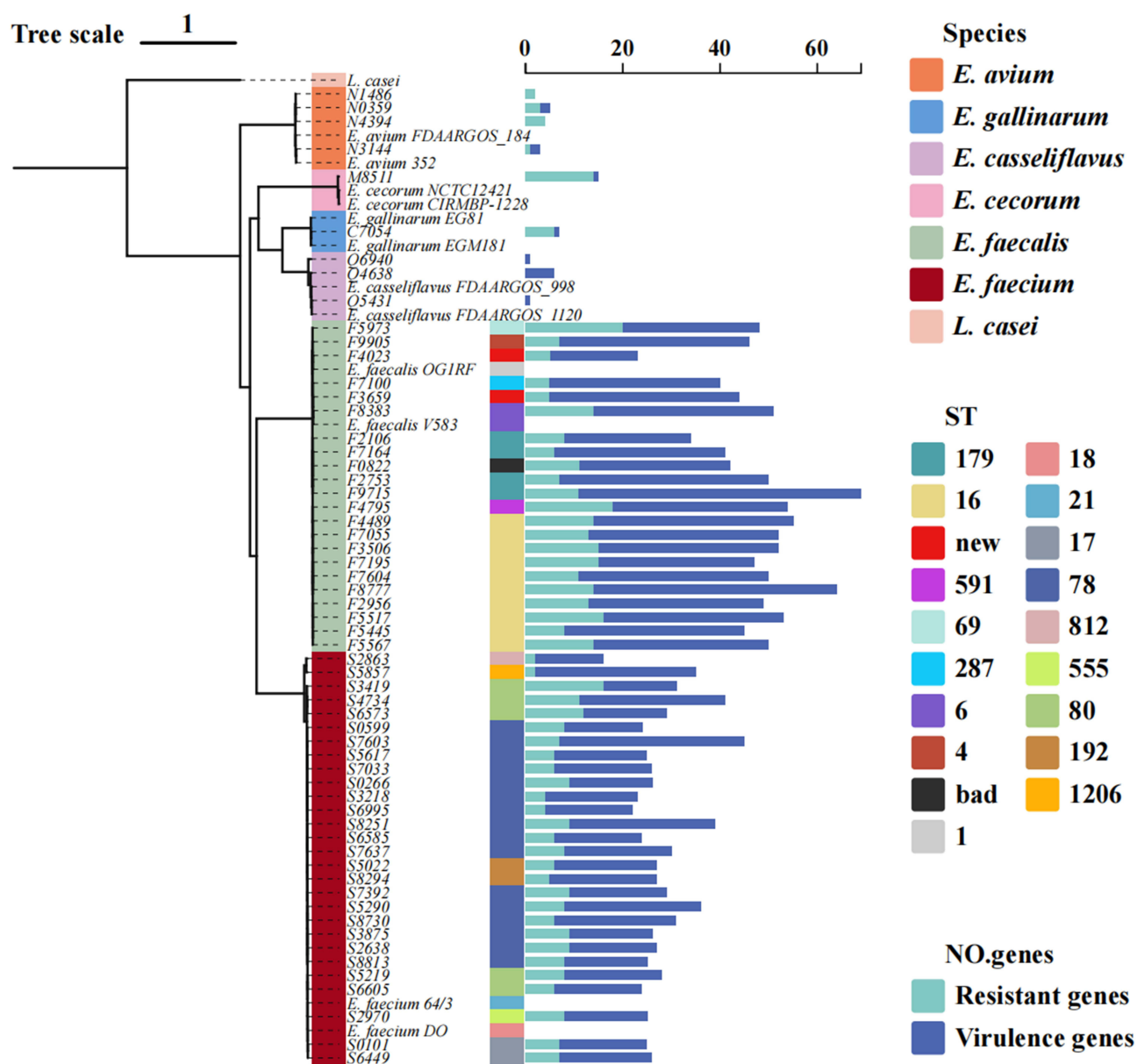
*Enterococcus* species are ubiquitous opportunistic pathogens and have become major contributors to hospital-acquired infections worldwide. Their ability to acquire multidrug resistance through horizontal gene transfer and adaptive genome evolution has resulted in escalating therapeutic challenges.<sup>16,17</sup> The increasing incidence of enterococcal infections—particularly among elderly, immunocompromised, and critically ill patients—reflects both selective antibiotic pressure and the ecological resilience of these organisms.<sup>18–21</sup> Their resistance spectrum is broad, encompassing  $\beta$ -lactams, aminoglycosides, macrolides, tetracyclines, and other classes.<sup>22,23</sup>

Through WGS, our study provides the first comprehensive genomic characterization of antibiotic-resistant *Enterococcus* isolates from Yichang. Consistent with national and global epidemiological patterns, *E. faecalis* and *E. faecium* were the predominant species, accounting for the majority of infections, most frequently originating from urine specimens in surgical and urology wards. This distribution aligns with previous reports highlighting the urinary tract as a common source of enterococcal infection in hospitalized patients.<sup>24</sup> MLST analysis identified *E. faecium* ST78



and *E. faecalis* ST16 as dominant lineages, both of which correspond to internationally disseminated epidemic clones. The detection of two novel *E. faecalis* sequence types, each arising from single-nucleotide substitutions in housekeeping genes, provides evidence of ongoing local microevolution. Although further functional investigation is warranted, the emergence of these novel STs suggests adaptive diversification within regional *Enterococcus* populations under antimicrobial selection pressure. The coexistence of epidemic clones and newly emerging lineages highlights the dynamic nature of resistance evolution in this setting.

Comparative genomic profiling revealed that Yichang isolates display a distinct antimicrobial resistance landscape, characterized by enrichment of vancomycin- and tetracycline-resistance genes and relative reduction in phenicol and oxazolidinone determinants compared with publicly available reference genomes. Notably, six *E. faecium* isolates and one *E. gallinarum* isolate harbored vancomycin-resistance genes, underscoring the importance of vancomycin-variable *Enterococcus* as a potential reservoir for resistance dissemination.<sup>25</sup> In parallel, virulence profiling demonstrated that *E. faecalis* possessed a broader repertoire of virulence determinants than *E. faecium*, particularly in effector secretion and immune-evasion modules, suggesting enhanced host adaptation potential.<sup>26</sup> Although *E. gallinarum* and *E. cecorum* were infrequently isolated, their relatively extensive resistomes indicate that atypical species may still pose clinical risk. The recovery of *E. cecorum*, a species commonly associated with poultry outbreaks, from a human wound infection further supports the potential zoonotic interface of enterococcal infections.<sup>27</sup>



**Figure 8** Comprehensive analysis of *Enterococcus* genomic data in conjunction with phylogenetic tree analysis. Bar chart summarising, for each ST, the numbers of resistance and virulence genes detected in the sequenced isolates.

Collectively, these findings delineate a region-specific adaptive pattern among *Enterococcus* isolates in Yichang. The convergence of multidrug resistance, virulence diversification, and emerging sequence types underscores the necessity of sustained genomic surveillance to monitor atypical species and prevent dissemination of high-risk clones in clinical settings.

## Conclusions

Whole-genome sequencing of 59 *Enterococcus* isolates from Yichang revealed that *E. faecalis* and *E. faecium* remain the dominant species, with ST16 and ST78 as prevailing epidemic lineages. Two novel *E. faecalis* sequence types indicate ongoing microevolution under local antibiotic pressure. The regional resistome is marked by enrichment of vancomycin, tetracycline, aminoglycoside, and lincosamide genes, whereas virulence profiles shift from adhesion factors toward exoenzymes and secretion systems, reflecting adaptive diversification.

These findings support the implementation of sustained genomic surveillance, strengthened infection-control measures, and targeted monitoring of atypical *Enterococcus* species to limit dissemination of multidrug-resistant and emerging high-risk clones in clinical settings.

## Data Sharing Statement

The genome datasets presented in this study can be found in online NCBI database (<https://www.ncbi.nlm.nih.gov/>). Detailed information on clinical strains can be found in the [Supplementary Material](#).

## Acknowledgments

Special thanks to the teachers in the clinical laboratory of relevant hospitals in Yichang City for providing the strains required for the experiment (Yichang Second People's Hospital, Yichang Youfu Hospital, Zigui County People's Hospital, and Yidu First People's Hospital).

## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

## Funding

This research was supported by the National Natural Science Foundation of China (32201986), the Natural Science Foundation of Hubei Province (2022CFB743, 2024AFD145), Open Fund of Hubei Provincial Clinical Research Center for Precise Prevention and Treatment of Elderly Gastrointestinal Cancer (2024EGC-01), Open Foundation of Hubei Province Key Laboratory of Tumor Microenvironment and Immunotherapy (2024ZLKF2-51).

## Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## References

1. Ramos S, Silva V, Dapkevicius MDLE, Igrejas G, Poeta P. Enterococci, from harmless bacteria to a pathogen. *Microorganisms*. 2020;8(8):1118. doi:10.3390/microorganisms8081118
2. McDonald JR, Olaison L, Anderson DJ, et al. Enterococcal endocarditis: 107 cases from the international collaboration on endocarditis merged database. *Am J Med*. 2005;118(7):759–766. doi:10.1016/j.amjmed.2005.02.020
3. Zhu Z, Du W, Yang Y, et al. Enterococci independently increase the risk for initial antibiotic treatment failure and prolonged hospitalization in adult patients with complicated urinary tract infection: a retrospective cohort study. *Infection*. 2025;53(1):307–315. doi:10.1007/s15010-024-02372-0
4. Prosty C, Sorin M, Katergi K, et al. Revisiting the evidence base that informs the use of adjunctive therapy for *Enterococcus faecalis* endocarditis: a systematic review and meta-analysis. *Clin Infect Dis*. 2024;79(5):1162–1171. doi:10.1093/cid/ciae379
5. Lu Z, McInnes RS, Allen F, Gadar K, van Schaik W. Resistance to last-resort antibiotics in enterococci. *FEMS Microbiol Rev*. 2025;49:fuaf057. doi:10.1093/femsre/fuaf057
6. Cattoir V. The multifaceted lifestyle of enterococci: genetic diversity, ecology and risks for public health. *Curr Opin Microbiol*. 2022;65:73–80. doi:10.1016/j.mib.2021.10.013
7. Wei L, Li M, Xia F, et al. Phosphate transport system mediates the resistance of *Enterococcus faecalis* to multidrug. *Microbiol Res*. 2021;249:126772. doi:10.1016/j.micres.2021.126772
8. Werner G, Neumann B, Weber RE, et al. Thirty years of VRE in Germany - “expect the unexpected”: the view from the national reference centre for staphylococci and Enterococci. *Drug Resistance Updates*. 2020;53:100732. doi:10.1016/j.drup.2020.100732
9. Yoshino Y. *Enterococcus casseliflavus* infection: a review of clinical features and treatment. *Infect Drug Resist*. 2023;16:363–368. doi:10.2147/IDR.S398739
10. Yu X, Yu Z, Huang L, et al. Global epidemiology and genomic perspectives on vancomycin-resistant *Enterococcus faecium*: rising antimicrobial resistance and transmission risks within the one health framework. *Sci total environ*. 2025;1002:180595. doi:10.1016/j.scitotenv.2025.180595
11. El Haddad L, Hanson BM, Arias CA, et al. Emergence and transmission of daptomycin and vancomycin-resistant Enterococci between patients and hospital rooms. *Clin Infect Dis*. 2021;73(12):2306–2313. doi:10.1093/cid/ciab001
12. Akter T, Haque MN, Ehsan R, et al. Virulence and antibiotic-resistance genes in *Enterococcus faecalis* associated with streptococcosis disease in fish. *Sci Rep*. 2023;13(1):1551. doi:10.1038/s41598-022-25968-8

13. Yan S, Jiang Z, Zhang W, et al. Genomes-based MLST, cgMLST, wgMLST and SNP analysis of salmonella typhimurium from animals and humans. *Comp Immunol Microbiol Infect Dis.* 2023;96:101973. doi:10.1016/j.cimid.2023.101973
14. Chen W, Wang Q, Wu H, et al. Molecular epidemiology, phenotypic and genomic characterization of antibiotic-resistant enterococcal isolates from diverse farm animals in Xinjiang, China. *Sci total environ.* 2024;912:168683. doi:10.1016/j.scitotenv.2023.168683
15. Zhao J, Liu R, Sun Y, Yang X, Yao J. Tracing enterococci persistence along a pork production chain from feed to food in China. *Anim Nutr.* 2022;9:223–232. doi:10.1016/j.aninu.2022.01.005
16. Cunha S, Miranda C, Martins A, et al. Analysis of antibiotic-resistant and virulence genes of *Enterococcus* detected in calf colostrum—one health perspective. *Animals.* 2023;13(12):1900. doi:10.3390/ani13121900
17. Shahveh M, Tajbakhsh E, Momtaz H, Ranjbar R. Molecular characterization of *Enterococcus faecalis* and *Enterococcus faecium* isolated from a meat source in Shahrekord local markets, Iran. *Arch Razi Inst.* 2023;78(4):1387–1396. doi:10.32592/ARI.2023.78.4.1387
18. Das S, Bombaywala S, Srivastava S, et al. Genome plasticity as a paradigm of antibiotic resistance spread in ESKAPE pathogens. *Environ Sci Pollut Res Int.* 2022;29(27):40507–40519. doi:10.1007/s11356-022-19840-5
19. Goh HMS, Yong MHA, Chong KKL, Kline KA. Model systems for the study of Enterococcal colonization and infection. *Virulence.* 2017;8(8):1525–1562. doi:10.1080/21505594.2017.1279766
20. Song H, Yoo JS, Unno T. Discerning the dissemination mechanisms of antibiotic resistance genes through whole genome sequencing of extended-spectrum beta-lactamase (ESBL)-producing *E. coli* isolated from veterinary clinics and farms in South Korea. *Sci total environ.* 2024;926:172068. doi:10.1016/j.scitotenv.2024.172068
21. Torres C, Alonso CA, Ruiz-Ripa L, et al. Antimicrobial resistance in *Enterococcus* spp. of animal origin. *Microbiol Spectr.* 2018;6(4). doi:10.1128/microbiolspec.arba-0032-2018
22. Khan A, Miller WR, Axell-House D, Munita JM, Arias CA, Humphries RM. Antimicrobial susceptibility testing for enterococci. *J Clin Microbiol.* 2022;60(9):e00843–21. doi:10.1128/jcm.00843-21
23. Wang J, Cassone M, Gibson K, et al. Gut microbiota features on nursing home admission are associated with subsequent acquisition of antibiotic-resistant organism colonization. *Clin Infect Dis.* 2020;71(12):3244–3247. doi:10.1093/cid/ciaa662
24. Farsi S, Salama I, Escalante-Alderete E, Cervantes J. Multidrug-resistant enterococcal infection in surgical patients, what surgeons need to know. *Microorganisms.* 2023;11(2):238. doi:10.3390/microorganisms11020238
25. Sun L, Chen Y, Hua X, et al. Tandem amplification of the *vanM* gene cluster drives vancomycin resistance in vancomycin-variable enterococci. *J Antimicrob Chemother.* 2020;75(2):283–291. doi:10.1093/jac/dkz461
26. Ko KKK, Chng KR, Nagarajan N. Metagenomics-enabled microbial surveillance. *Nat Microbiol.* 2022;7(4):486–496. doi:10.1038/s41564-022-01089-w
27. Rhoads DD, Pummill J, Alrubaye AAK. Molecular genomic analyses of *Enterococcus cecorum* from sepsis outbreaks in broilers. *Microorganisms.* 2024;12(2):250. doi:10.3390/microorganisms12020250

## Infection and Drug Resistance

### Publish your work in this journal

Infection and Drug Resistance is an international, peer-reviewed open-access journal that focuses on the optimal treatment of infection (bacterial, fungal and viral) and the development and institution of preventive strategies to minimize the development and spread of resistance. The journal is specifically concerned with the epidemiology of antibiotic resistance and the mechanisms of resistance development and diffusion in both hospitals and the community. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/infection-and-drug-resistance-journal>

**Dovepress**  
Taylor & Francis Group