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Genomic analysis elucidates characteristics and possible origins of high-risk antimicrobial resistance genes in *Enterococcus faecium* from a global perspective

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ABSTRACT

Under the One Health framework, it is crucial to undertake a comprehensive analysis of antimicrobial resistance (AMR) across various countries and regions. High-risk ARGs pose a severe threat to human health, yet systematic research on them is scarce. This study developed a high-risk ARGs database using the existing risk assessment system and explored a genome-based investigation workflow for high-risk ARGs. We investigated *Enterococcus faecium*, a common clinical pathogen, to understand the epidemiological characteristics of high-risk ARGs, including their primary sources and destinations. Results revealed that high-risk ARGs are widespread in *E. faecium*, with *tet(M)* being the most abundant and *ermB* the most widely distributed. The combination of vancomycin_ARGs (*vanA*, *vanYA*, *vanYB*, *vanYM*) -*tet(M)*-*ermB* is the most prevalent. ST1579 harbors the most high-risk ARGs, and the top five STs carrying high-risk ARGs are all from the hospital-specific CC17 clone lineage (cladeA1). Similarly, *tet(M)*-, *ermB*-, and vancomycin_ARGs-positive strains also belong to the nosocomial infection-related lineage cladeA1. Oxazolidinones_ARGs (*optrA*, *cfr(D)*, *cfrA*)-positive strains are mainly from the cladeA2 lineage associated with animals. *OptrA*, a last-resort antibiotics ARG with potential outbreak risk, requires particular attention. Additionally, plasmids, transposons (Tn), Insertion sequence (IS), and integrative conjugative elements (ICE) show varying preferences for encoding high-risk ARGs, with *tet(M)*, *ermB*, *APH (3 ')-IIIa*, *vanA*, *vanYA*, and *vanYB* being more readily carried by these MGEs. The USA, China, and Belgium are key origin regions for high-risk ARGs in *E. faecium*, while Australia, France and Netherlands are significant introduction regions. This study provides essential data for tackling the global AMR crisis.

1. Introduction

The spread of antimicrobial resistance (AMR) presents a serious global challenge that demands coordinated international efforts [1]. Numerous studies have demonstrated that antimicrobial resistance genes (ARGs) are widespread in various environmental compartments, including water [2], soil [3], air [4], plants [5], animals and human intestines [6,7], as well as in permafrost [8]. Despite their widespread presence, most ARGs in the environment are naturally occurring, and only a subset that can be transferred to pathogens poses a significant risk to human health. The transfer of ARGs involves overcoming several challenges, including the transferability of the ARGs, ecological connectivity, the founder effect, and fitness costs [6]. ARGs that cannot surmount these transferability barriers do not pose a health risk, regardless of their abundance. Consequently, high-risk ARGs have become a focal point for research. It is generally accepted that high-risk ARGs exhibit the following characteristics [9,10,11]: 1) they can accumulate in significant quantities in human environments, and 2) they can be transferred to and among human-associated pathogens via mobile genetic elements (MGEs) through horizontal gene transfer (HGT).

Enterococcus faecium is a prevalent opportunistic pathogen in clinical and healthcare settings [12,13]. The genome of *E. faecium* is highly plastic, allowing it to rapidly acquire ARGs through HGT, thereby presenting a significant risk in clinical treatment [14]. Notably, vancomycin-resistant *E. faecium* (VREfm) has been responsible for outbreaks in multiple countries [15,16,17], leading to the clinical failure of

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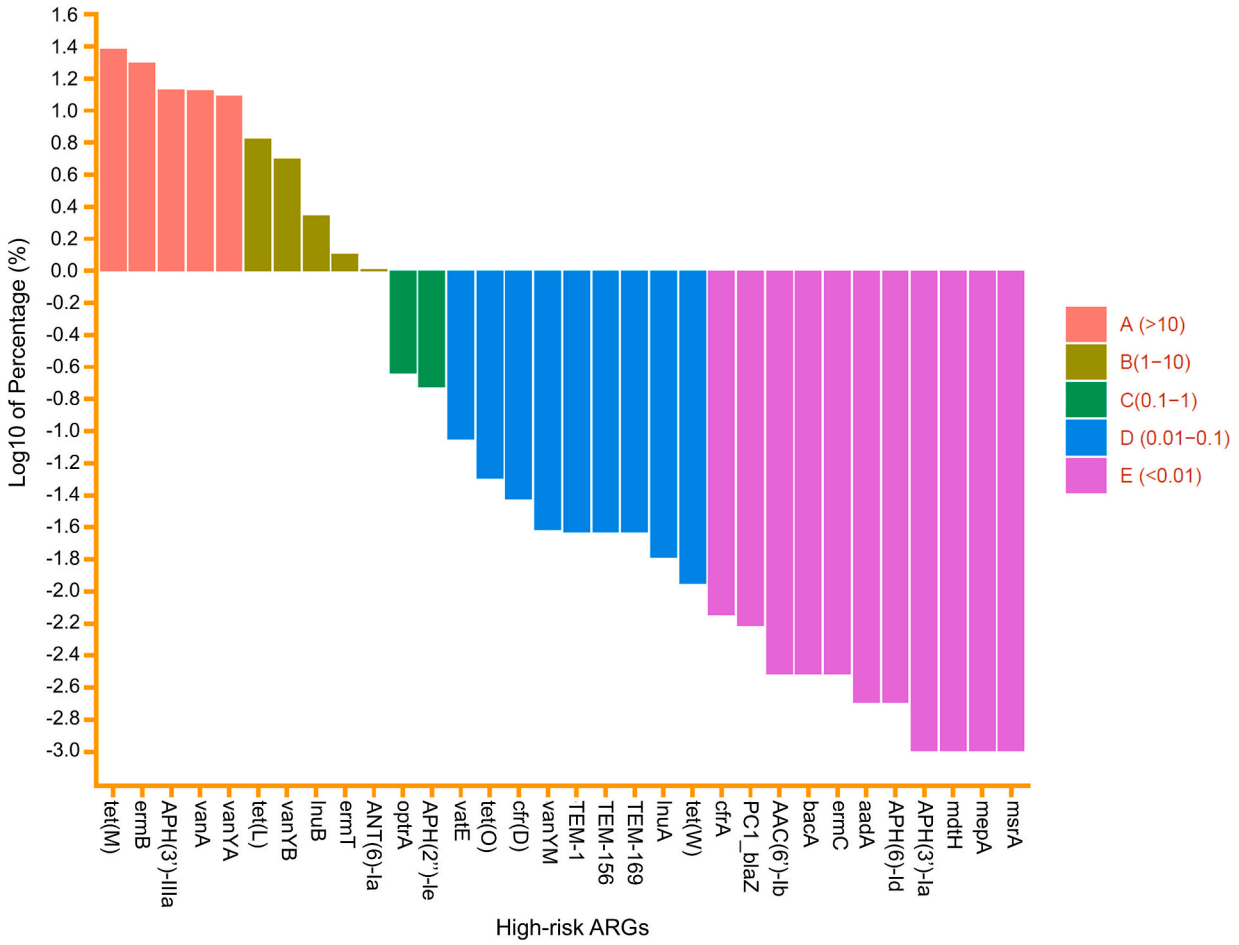

Fig. 1. Abundance of high-risk ARGs in *Enterococcus faecium*. Different colors represent varying ranges of abundance values: A, B, C, D, and E denote ARGs with abundance values >10 %, 1 %–10 %, 0.1 %–1 %, 0.01 %–0.1 %, and < 0.01 %, respectively.

vancomycin, which is a last-resort antibiotic for treating *E. faecium* infections. Additionally, a specific multidrug-resistant lineage of *E. faecium*, known as clonal complex 17 (CC17) [18], has emerged in hospital settings and is associated with the majority of hospital-acquired infections, including VREfm, resulting in severe outcomes [19]. Due to these concerns, *E. faecium* is classified as one of the five major clinical pathogens in the ESKAPE group [20], and VREfm has been designated by WHO as a high-priority pathogen, emphasizing the urgent need to address AMR [21]. Researchers are increasingly focused on the AMR of *E. faecium*. Beyond vancomycin ARGs, other high-risk ARGs, such as those conferring resistance to tetracyclines and macrolides antibiotics, have been frequently reported [22]. Furthermore, resistance to other last-resort antibiotics against VRE, including linezolid, tigecycline, and daptomycin [23,24,25], is also becoming more common.

Given the severity of AMR in *E. faecium*, it is crucial to systematically investigate the characteristics of high-risk ARGs at both the species level and from a global perspective. This approach will enhance the implementation of AMR surveillance in *E. faecium*. Advances in omics technologies have provided a wealth of data that supports this research. By integrating the list of high-risk ARGs identified by the WHO with those identified through omics data analysis, and considering the rise and rapid spread of last-resort antibiotic ARGs in recent years, we have identified 106 high-risk ARGs and developed a high-risk ARGs database. We acquired global genomic data from 13,324 *E. faecium* strains from genomic databases and used this information to create a genome-based workflow for high-risk ARGs research. This study aims to address the following questions: 1) The prevalence of high-risk ARGs in *E. faecium*; 2) The global distribution and molecular epidemiological characteristics of high-risk ARGs; 3) The potential sources and destinations of high-risk ARGs. Our findings are expected to contribute valuable insights for monitoring AMR in *E. faecium* and inform antimicrobial agent stewardship efforts, providing a data foundation for addressing the global AMR crisis.

2. Materials and methods

2.1. Construction of the high-risk ARGs database and collection of *E. faecium* genomic sequences

In this study, we screened 106 high-risk ARGs based on criteria from previous literature [10], including: 1) Predominant enrichment in human-associated environments; 2) Presence on mobile genetic elements, indicating a propensity for dissemination; 3) Occurrence in ESKAPE pathogens (known for their pathogenicity). Following the established criteria, 99 high-risk ARGs were identified through comprehensive analysis of omics big data [10]. To detect emerging threats, we augmented the identification of high-risk antimicrobial resistance genes (ARGs) through a systematic literature review and integration of the Comprehensive Antibiotic Resistance Database (CARD). Specifically, we included the fosfomycin resistance gene (*fosA3*) [26,27,28] and six critical last-resort antibiotic resistance genes that have demonstrated rapid proliferation in recent years: the tigecycline resistance genes (*tet(X3)*, *tet(X4)*, and *tet(X6)*), as well as the oxazolidinone resistance genes (*optrA*, *cfr(A)*, and *cfr(D)*) [29,30,31,25]. Nucleic acid sequences for these ARGs were obtained from the CARD resistance gene database and formatted as fasta files in the high-risk

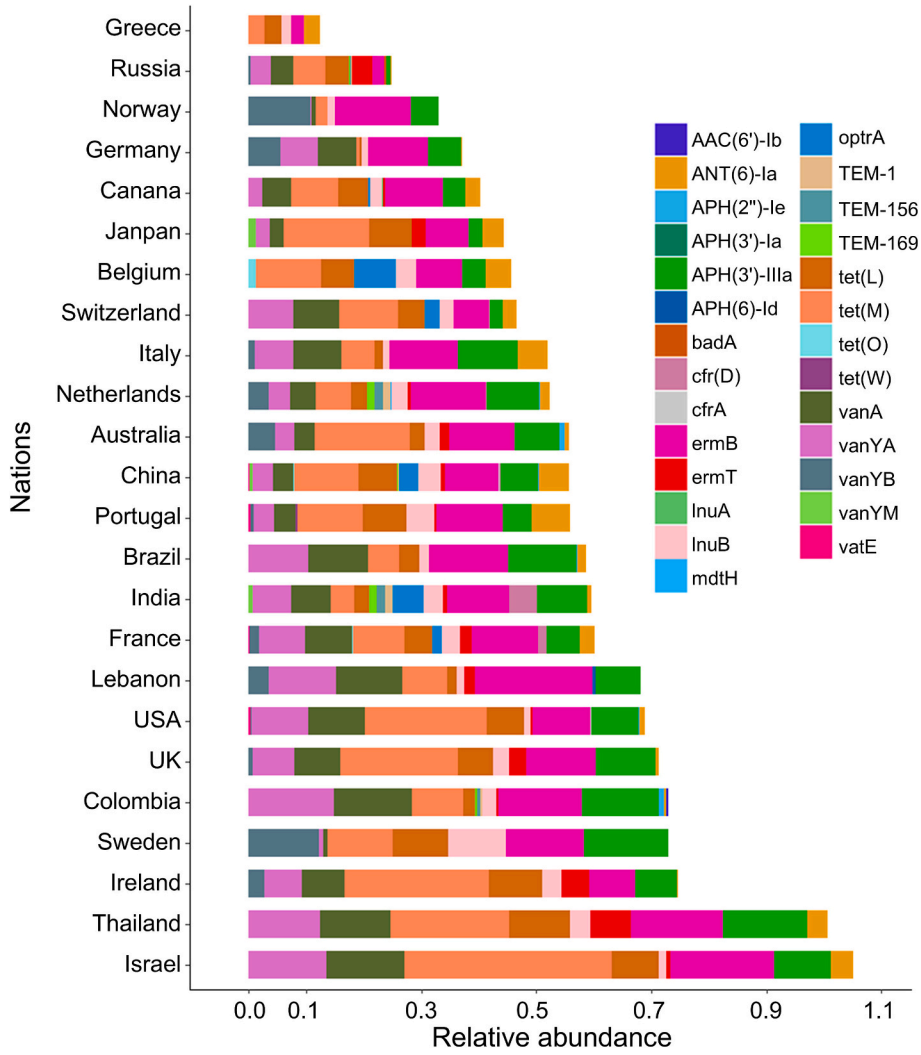

Fig. 2. Relative abundance of high-risk ARGs in *Enterococcus faecium* across different countries.

ARGs database named hrargdb_v1, with sequence information provided in Attachment 1.

Genome sequences of *E. faecium* were retrieved from two publicly accessible repositories: NCBI GenBank and BV-BRC (Bacterial and Viral Bioinformatics Resource Center). All sequences downloaded were limited to those published or updated up to February 2024 to ensure temporal relevance. To eliminate redundancy, sequences sharing identical accession numbers were consolidated, retaining only one representative entry per unique strain. This yielded an initial dataset of 14,269 genomes. The dataset comprised 218 genomes assembled to chromosome-level completeness and 14,051 genomes at the contig level. Genome integrity and contamination were rigorously assessed using CheckM2 v1.0.2 and sequences with integrity ≤75 % and contamination ≥10 % were excluded [32]. Post-filtering, 13,324 high-quality genomes were retained for downstream analyses. Detailed metadata for all sequences—including accession numbers, assembly levels, geographic origins, ST types and release dates—are comprehensively documented in Table S1.

2.2. Multilocus sequence typing and detection of high-risk ARGs and MGEs

Multilocus sequence typing (MLST) was performed using the MLST software (v2.0.9) with the *E. faecium* MLST database (version 2023-03-20) (https://cge.food.dtu.dk/services/MLST/) [33]. Cluster analysis of MLST data was conducted using the goeBURST algorithm in Phyloviz software (v2.0) [34]. High-risk ARGs were identified using blastn (blast 2.12.0+) against the hrargdb_v1 database. Sequences with more than 90 % identity, an e-value ≤1e-10, and alignment length ≥ 100 bp were classified as high-risk ARGs. To investigate the colocalization of ARGs with mobile genetic elements (MGEs), fragments containing high-risk ARGs were examined for MGEs. For complete genome sequences, if ARGs were located on chromosomes, Seqkit v2.8.0 [35] was used to extract sequence fragments 3000 bp upstream and downstream of the ARGs. Based on the extracted fragments, MobileElementFinder v1.1.2 [36] was used to detect insertion sequences (IS), transposons (Tn), and integrative conjugative elements (ICE) with an identity ≥90 % and an e-value ≤1e-5. If ARGs were located on plasmids, plasmid sequences were directly extracted using Seqkit 2.8.0 [35], and ISs, Tns, and ICEs were detected by MobileElementFinder v1.1.2 [36]. For genome sequence assembly at the contig level, contigs carrying ARGs were extracted using Seqkit v2.8.0 [35], and then MobileElementFinder v1.1.2 [36] was used to detect ISs, Tns, and ICEs, while plasmids were detected using Mlplasmids v2.1.0 [37] with a probability ≥0.8.

2.3. Tracing high-risk ARGs

To trace the source and destination of high-risk ARGs in *E. faecium* across different countries, high-risk ARGs with an abundance greater than 10 % and last-resort antibiotic ARGs were analyzed. Ten significant

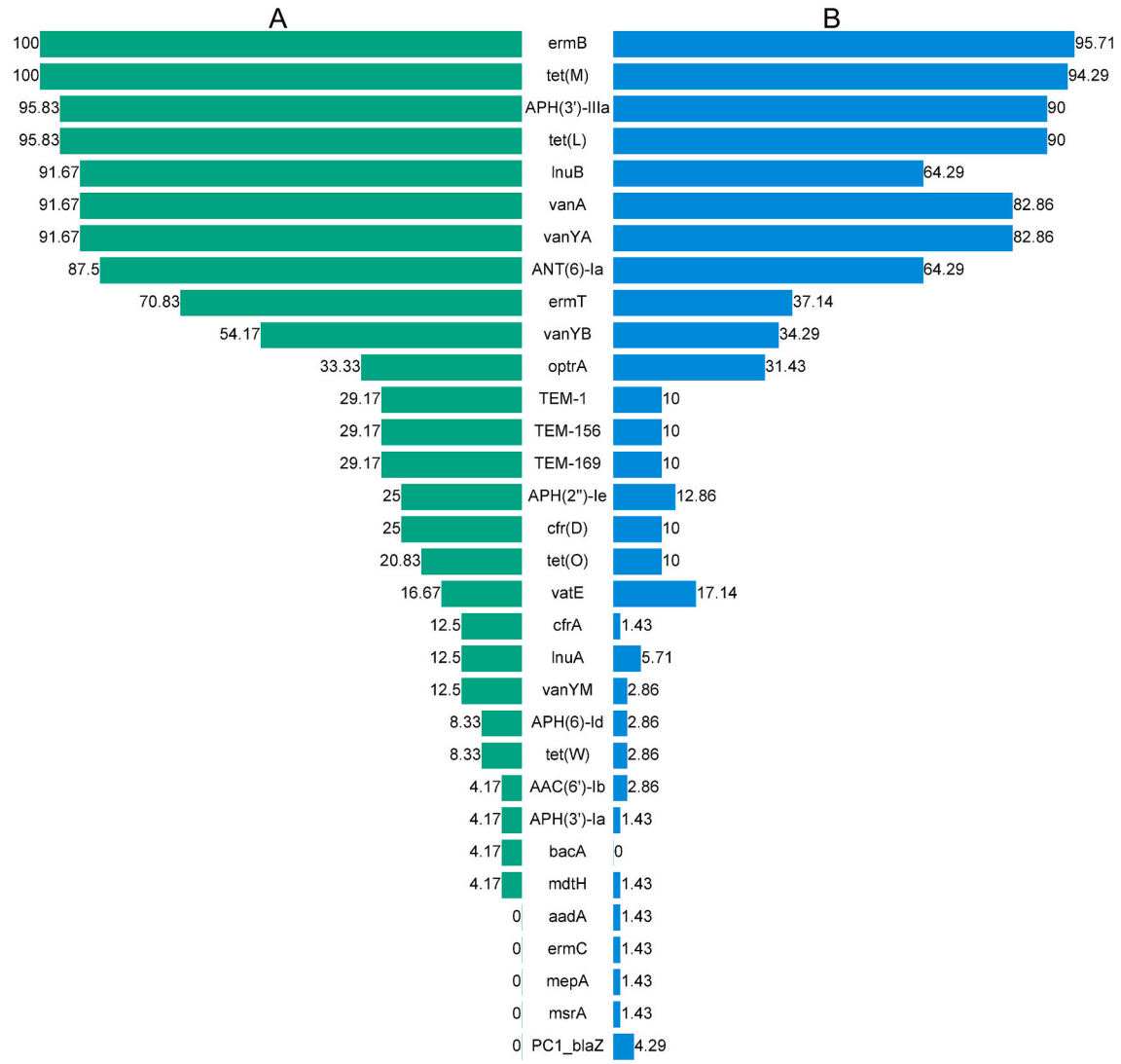

Fig. 3. Detection frequency of high-risk ARGs in *Enterococcus faecium* from different countries and sequence types (STs). Panel A shows the percentage of high-risk ARGs detected in various countries, while Panel B depicts the percentage detected in different STs.

high-risk ARGs were investigated: *tet(M)*, *ermB*, *APH(3′)-IIIa*, *vanA*, *vanYA*, *vanYB*, *vanYM*, *optrA*, *cfr(D)*, and *cfrA*. Sequences of different ARGs were extracted by country, and Vsearch v2.15.2 [38] was used to cluster ARGs sequences per country. Representative sequences were output for each class, with sequences showing ≥97 % consistency considered identical. The BEAST analyses [39] were conducted to estimate the divergence times of high-risk ARGs across various countries, using Relaxed Clock Exponential model coupled with a Yule speciation model. The Markov Chain Monte Carlo (MCMC) simulations were executed for 200 million generations, with sampling every 1000 generations. The first 50 % samples were discarded as burn-in to ensure the removal of any influence from the starting values. The convergence of the MCMC runs was evaluated using Tracer v1.7.2 [40]. Subsequently, TreeAnnotator version 2.7.7 [39] was utilized to summarize the post-burn-in trees and their associated parameters, resulting in a maximum clade credibility chronogram, with 95 % highest posterior density (HPD) intervals.

2.4. Data analysis

To correct for sampling quantity deviation, this study standardized the abundance of high-risk ARGs across different STs and countries to determine the relative abundance of ARGs. Relative abundance is calculated as follows: $I_{RA} = (ARG_i/ARG_t)/(S_i/S_t)$, where I_{RA} represents the relative abundance; ARG_i is the number of ARGs identified in different STs or countries; ARG_t is the total number of ARGs identified in all samples; S_i is the number of samples in different STs or countries; and S_t is the total number of samples. Data processing was performed using R Studio 222.07.1 (based on R 4.3.3), with figures generated using R Studio 222.07.1 and additional drawing platforms [41,42,43].

3. Results

3.1. Distribution of high-risk ARGs in E. faecium

Genome sequences were compared against the database of 106 high-risk ARGs using blastn software, detecting 32 high-risk ARGs, which account for 30.19 % of the total number of high-risk ARGs (Fig. 1, Table S2). Among these, five ARGs had an abundance exceeding 10 %, nine had an abundance of 1 %–10 %, two had an abundance of 0.1 %–1 %, and 11 had an abundance of less than 1 %. The *tet(M)* gene was the most abundant, with a prevalence of 24.19 %, followed by *ermB* and *APH(3′)-IIIa* at 19.77 % and 13.51 %, respectively (Fig. 1, Table S2). *ermB* was the most widely distributed high-risk ARG, with a detection frequency of 77.26 %, followed by *tet(M)*, *APH(3′)-IIIa*, *vanA*, and *vanYA* with detection frequencies of 65.38 %, 55.40 %, 54.56 %, and 51.67 %,

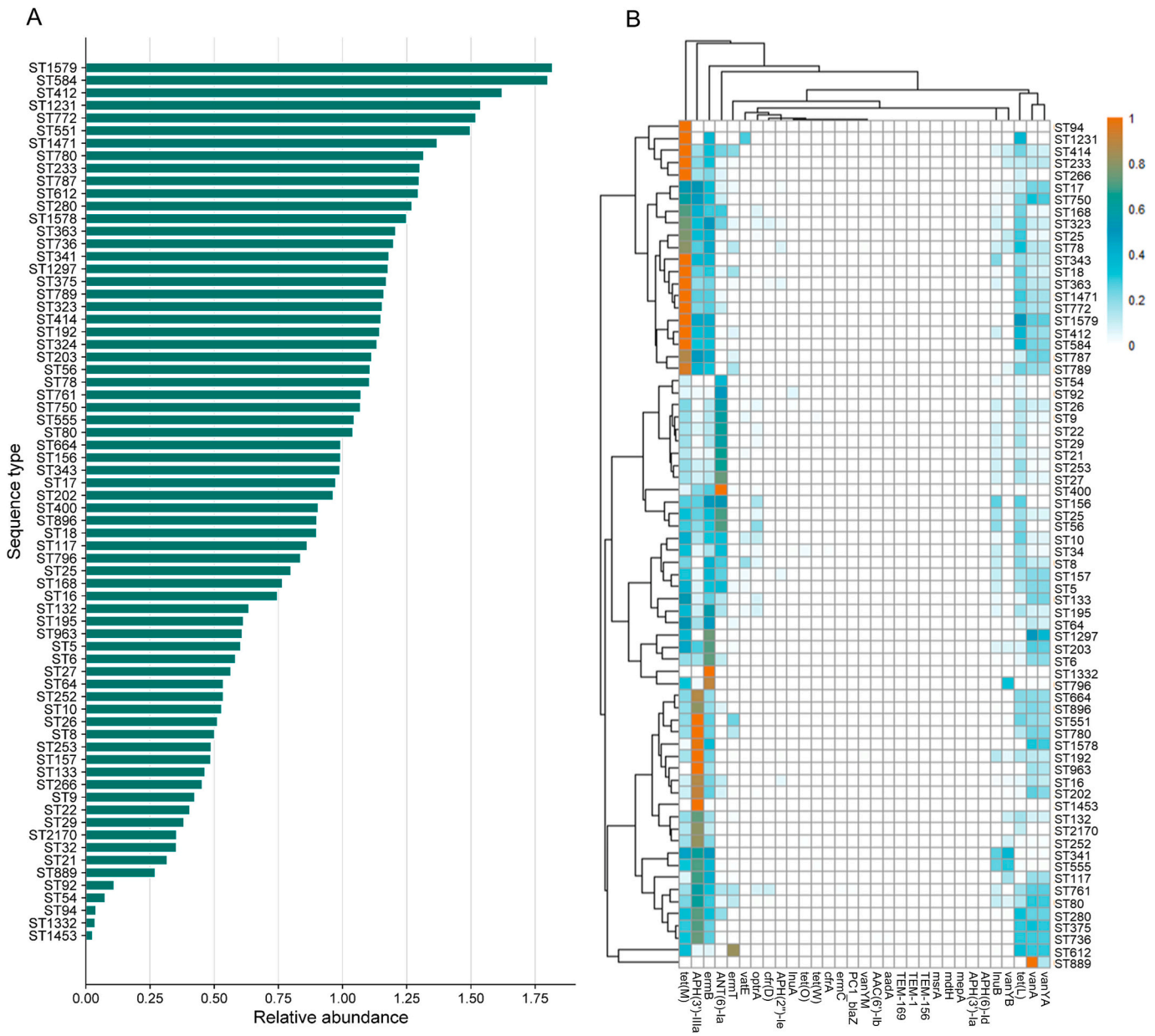

Fig. 4. Relative abundance and heatmap analysis of high-risk ARGs in *Enterococcus faecium* strains of different STs.

respectively (Table S3). Additionally, *vanA-tet(M)-ermB* was the most prevalent ARG combination in VRE, accounting for 52.61 % of all VRE strains (data not shown). The vancomycin_ARGs (*vanA*, *vanYA*, *vanYB*, *vanYM*) *-tet(M)-ermB* combination was also the most common among *E. faecium* strains, representing 50.51 %. Other last-resort antibiotics ARGs, including *optrA*, *cfr(D)*, *cfrA*, and *vanYM*, have been detected, albeit at lower abundance (0.0071 % - 0.2296 %) (Fig. 1, Table S2). Despite their low abundance, these ARGs confer resistance to last-resort antibiotics like linezolid, tedizolid, and vancomycin, warranting close monitoring.

3.2. Distribution of high-risk ARGs in different countries

The 13,324 *E. faecium* genome sequences were analyzed based on metadata (Table S1). Most sequences (7828) were from a single sequencing project (bioproject number PRJNA514245), which used SKESA for sequence assembly [44]. Due to the inability to determine the actual source of these sequences, they were excluded from the analysis. The remaining 5496 genome sequences spanned 57 countries. To ensure representative analysis of high-risk ARGs, we established a minimum sample threshold of 10 sequences per country. Countries below this threshold ($n = 30$) collectively contributed only 0.24 % of identified high-risk ARGs, demonstrating their negligible influence on the overall antimicrobial resistance pattern. For robust statistical analysis, subsequent ARGs abundance assessments focused on 24 countries meeting the minimum sample criterion. These countries cover 99.7 % of the valid data, minimizing the interference of small sample bias on the results. It is shown that Israel had the highest relative abundance at 1.05, followed by Thailand (1.01) and Ireland (0.75). Greece had the lowest relative abundance at 0.12 (Fig. 2).

The detection frequency of high-risk ARGs varied, with *ermB* and *tet (M)* being the most widely detected at 100 %. Other frequently detected ARGs included *APH(3′)-IIIa* (98.83 %), *tet(L)* (95.83 %), *lnuB* (91.67 %), *vanA* (91.67 %), *vanYA* (91.67 %) and *ANT(6)-Ia* (87.5 %) (Fig. 3A). Last-resort antibiotics ARGs *vanYB*, *optrA*, *cfr(D)*, *cfrA*, and *vanYM* also showed notable frequencies of 54.17 %, 33.33 %, 25 %, 12.5 %, and 12.5 %, respectively (Fig. 3A), indicating potential global spread. Notably, 29 strains carrying both oxazolidinones and vancomycin ARGs

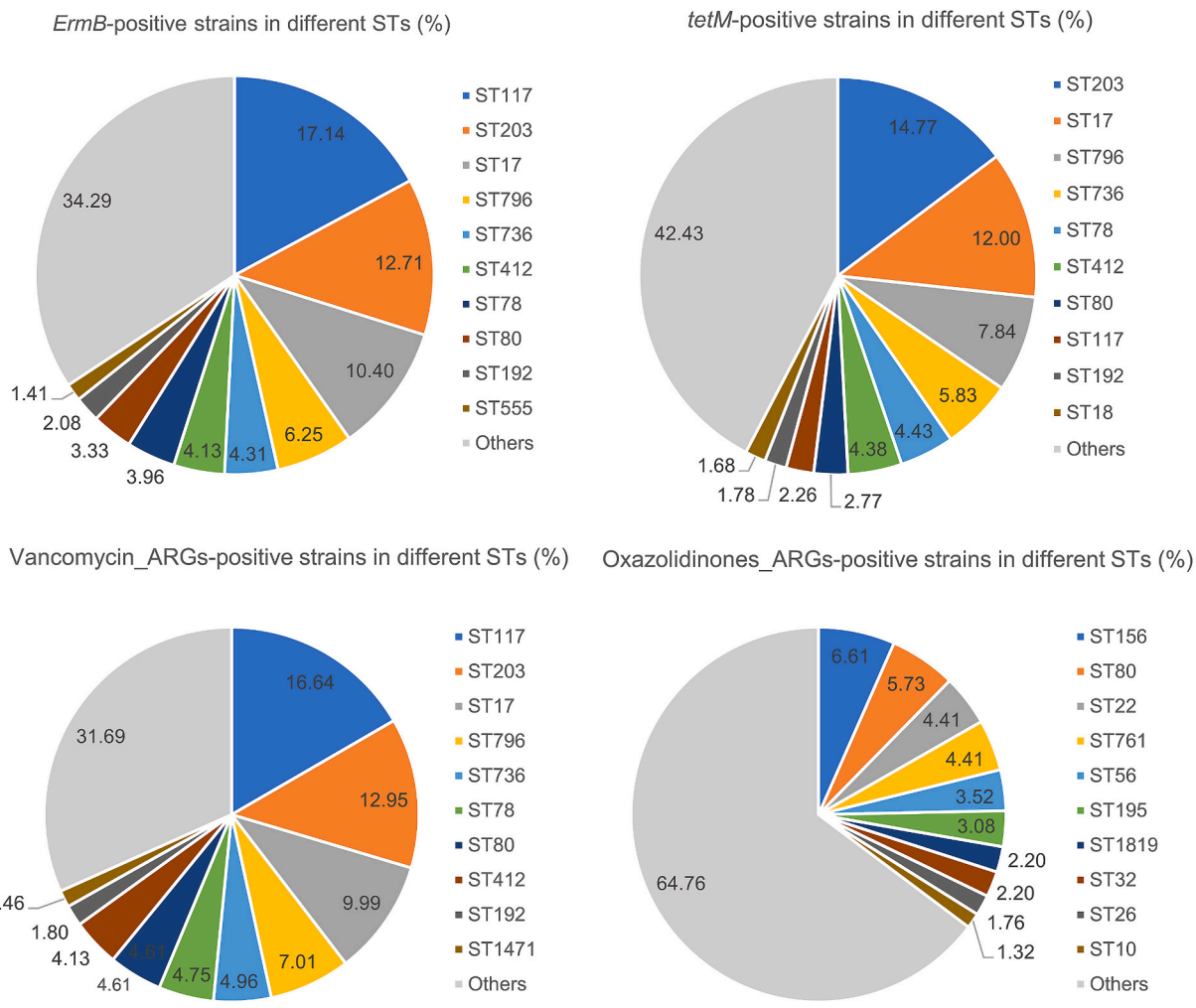

Fig. 5. Distribution of high-risk ARGs in *Enterococcus faecium* of different STs.

were identified, primarily from France and India (13 and 7 strains, respectively), with one strain from the USA, and one each from China and the Netherlands. Six additional strains were from NCBI, with undetermined sources (Table S4).

3.3. Epidemiological characteristics of high-risk ARGs distribution

The 13,324 genome sequences were categorized into 560 sequence types (STs), with 448 STs carrying high-risk ARGs (Table S5). Among these, 70 STs had more than 10 sequences (Fig. 4). The abundance of high-risk ARGs in these STs was normalized to explore their epidemiological patterns in *E. faecium*. ST1579 showed the highest relative abundance of ARGs at 1.82, followed by ST584 (1.80), ST412 (1.62), ST1231 (1.54), and ST772 (1.52), all within cladeA1. ST1453 exhibited the lowest relative abundance at 0.027.

Through heatmap analysis, it was observed that *ermB*, *tet(M)*, *tet(L)*, *lnuB*, *APH(3′)-IIIa*, *vanA*, *vanYA*, and *ANT(6)-Ia* eight high-risk ARGs had higher relative abundances among all STs (Fig. 4B). These ARGs had a detection frequency of over 50 % across various countries and STs (Fig. 3), suggesting strong transmission potential. Notably, while the *ermT* gene is less common among STs (37.14 %), its detection frequency across different countries is high (70.83 %) (Fig. 3), implying possible host specificity for STs. Among the remaining five last-resort antibiotics ARGs (*vanYB*, *optrA*, *cfr(D)*, *cfrA*, *vanYM*), *vanYB* and *optrA* also showed high detection frequencies in STs, at 34.29 % and 34.43 %, respectively (Fig. 3). The two ARGs are widely distributed across countries, with detection frequencies of 54.17 % and 33.33 % (Fig. 3). Conversely, *cfr(D)*, *cfrA*, and *vanYM* were less frequently detected in STs, at 10 %, 1.43 %, and 2.86 %, respectively (Fig. 3). The primary STs associated with *tet(M)*, *ermB*, and vancomycin_ARGs include ST117, ST17, ST192, ST203, ST412, ST736, ST78, ST796, and ST80 (Fig. 5), all from Clade A1 [12,13] (Fig. S1), suggesting a potential association between these STs and the vancomycin_ARGs-*tet(M)-ermB* combination. In contrast, STs associated with Oxazolidinones_ARGs differ significantly from those linked to the aforementioned three genes (except ST80), primarily including ST10, ST156, ST1819, ST195, ST22, ST26, ST32, ST56, ST761, and ST80 (Fig. 5), most of which belong to Clade A2 [12,13] (Fig. S1), indicating distinct transmission pathways for Oxazolidinones_ARGs in *E. faecium*. The detection frequencies of the key ARGs across different branches further support the above findings. Specifically, *tet(M)*, *ermB*, *aph(3')-IIIa*, and vancomycin_ARGs are dominant in Clade A1, while *ant(6)-Ia*, *lunB*, and oxazolidinones_ARGs are more prevalent in Clade A2 (Fig. S2).

3.4. Co localization analysis of high-risk ARGs and MGEs

To assess the transferability potential of high-risk ARGs, MGEs detection were conducted on all genome sequences, including plasmids, transposons (Tn), insertion sequences (IS), and integrated mobile elements (ICE), and examined their co-localization with high-risk ARGs. As anticipated, plasmids are the primary vectors for high-risk ARGs, carrying 63, 702 ARGs (Fig. 6). Tn follows with 16, 216 ARGs, IS with 6162, and ICE with the fewest, 2423 ARGs. Notably, different MGEs preferentially carry specific ARGs: plasmids predominantly transport *ermB*,

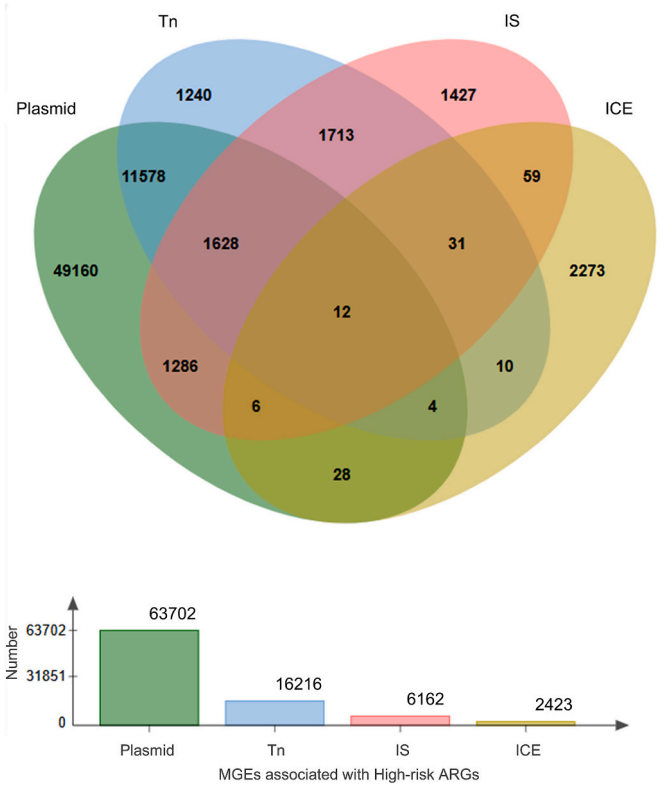

Fig. 6. Number of high-risk ARGs encoded by different MGEs. Tn represents transposons, IS represents insertion sequences, and ICE represents integrative conjugative elements.

vanA, *APH(3′)-IIIa*, and *vanYA*; Tn favors *APH(3′)-IIIa*, *ermB*, and *vanYB*; IS primarily carries *vanYB*, *tet(M)*, and *ermB*; and ICE almost exclusively carries *tet(M)* (Fig. 7). Further analysis revealed that five high-risk ARGs were simultaneously co-located with four types of MGEs, distributed across 12 strains. Among these, seven strains carried *tet(M)* and two carried *ermB* (Table S6). These results confirm that the association with MGEs may be a primary factor contributing to their high abundance.

3.5. Traceability of high-risk ARGs in E. faecium

We traced the origins of 10 significant high-risk ARGs using BEAST analysis. We investigated the origins and destinations of three high-abundance ARGs and seven last-resort antibiotics ARGs; the corresponding divergence time estimation are displayed in Fig. S3-S12. The results indicated that the USA is the principal source of high-risk ARGs in *E. faecium*, with nine possible transmission routes, followed by China and Belgium, with seven and five transmission routes (Fig. 8). France, Netherlands and Australia emerge as the primary introduction sites for high-risk ARGs, with each having three introduction paths (Fig. 8). Most ARGs have multiple origins and destinations, reflecting the complexity of high-risk ARGs transmission. For instance, *tet(M)* primarily originated in Belgium and later spread to UK, Lebanon, Russia, Japan; *ermB* originated in the USA and China and has since disseminated to Australia, Sweden, and Lebanon. Two critical last-resort antibiotics ARGs include *optrA*, which has two sources (the USA and Canada) and was transferred to France, Switerland, India and Belgium; and *vanA*, which originated in the USA and New Zealand and eventually spread to France, Netherlands, South Korea, and Denmark. These findings suggest a potential risk for outbreaks of oxazolidones_ARGs-positive *E. faecium* and VRE in these regions. Overall, these ten significant high-risk ARGs have been identified across 25 countries.

4. Discussion

This study investigated a total of 13,324 *E. faecium* strains for high-risk ARGs at the genomic level. Out of 106 high-risk ARGs examined, 30.19 % of the genes were detected. *tet(M)* emerged as the most abundant high-risk ARG in *E. faecium*. Originally identified in the plasmid pCF10 in *E. faecalis* [45], *tet(M)* has a broad host range, including many Gram-positive and Gram-negative bacteria. In addition to conferring tetracycline resistance to *E. faecium*, recent studies have shown that high expression of *tet(M)* can lead to resistance to the last-resort antibiotic tigecycline [46]. The widespread prevalence of *tet(M)* in *E. faecium* is a concerning indicator that warrants attention. *ermB* is the most extensively distributed high-risk ARG in *E. faecium*, conferring resistance to macrolides, lincosamides, and streptogramins antibiotics [47]. Previous studies based on analysis of small sample data (246 genome sequences) have highlighted *tet(M)* and *ermB* as the two most prevalent ARGs in *E. faecium* [22], and our study validates this observation from a big data perspective. *APH(3′)-IIIa*, an ARG against aminoglycoside antibiotics, is the third most abundant after *tet(M)* and *ermB*. Despite *E. faecium*'s inherent resistance to aminoglycoside antibiotics [48], the investigation of aminoglycoside ARGs like *APH(3′)-IIIa* remains significant because broad-range MGEs can transfer these ARGs from *E. faecium* to Gram-negative bacteria [49], complicating clinical treatment. Consequently, this study also explored *APH(3′)-IIIa* in detail. Furthermore, our results reveal that the *vanA-tet(M)-ermB* combination is the most common in VRE, with strains carrying this ARG combination constituting 52.61 % of all VRE strains, which is corroborated by a PCR-based survey [50]. Additionally, the vancomycin_ARGs-*tet(M)-ermB* combination was found to be the most prevalent in *E. faecium*, accounting for 50.51 %. These findings suggest potential links in the development of antibiotic resistance to vancomycin, tetracyclines, and macrolides antibiotics in *E. faecium*. One possible reason for the observed patterns is that tetracyclines and macrolides antibiotics have a long history of use in treating *E. faecium* infections, whereas vancomycin has been typically reserved as a last-resort antibiotic for cases resistant to conventional antibiotics. *E. faecium*, which already possesses ARGs for tetracyclines and macrolides antibiotics, can develop vancomycin resistance when exposed to this antibiotic. This results in the emergence of strains carrying the vancomycin_ARGs along with tetracycline resistance (*tet(M)*) and macrolides antibiotics resistance (*ermB*). Another significant finding is that 29 genomes were found to harbor both vancomycin and oxazolidinones antibiotics ARGs (Table S4). Oxazolidinones antibiotics, such as linezolid, represent a newer class of last-resort treatments for vancomycin-resistant strains. However, linezolid resistance began to emerge within just a year of its introduction [51], highlighting the remarkable adaptability of *E. faecium*, as previously reported [52]. Fortunately, the proportion of oxazolidinones_ARGs (*optrA*, *cfr (D)*, *cfrA*) identified in this study remains relatively low, suggesting that although new AMR is developing, it remains within a manageable range. Additionally, the absence of the *tet(X4)*, *tet(X5)*, and *tet(X6)* ARGs, which confer resistance to tigecycline, a widely accepted last-resort antibiotic, indicates that this line of defense remains relatively strong.

Multilocus Sequence Typing (MLST) is crucial for studying *E. faecium* epidemiology, which is part of high-risk clonal complexes (HiRCCs) due to its pathogenicity and transmission potential [53,54].*E. faecium* is divided into Clade A (hospital-associated, including VREfm) and Clade B (community-associated). Clade A is further split into Clade A1 (hospital-associated, CC17 lineage) and Clade A2 (animal and livestock -associated) [12]. However, the division of these two subclades remains debated [13], and some studies suggest that Clade A2 is not a monophyletic group [55]. Our results support these findings. The cluster analysis (Fig. S1) indicates that Clade A1 corresponds to the typical CC17 clone, a hospital-associated lineage derived from ST17. In contrast, Clade A2 consists of multiple parallel branches, including those originating from ST5, ST10, ST21, ST26, ST32, and ST150. The distribution of high-risk ARGs is uneven between the two subclades (Fig. S2).

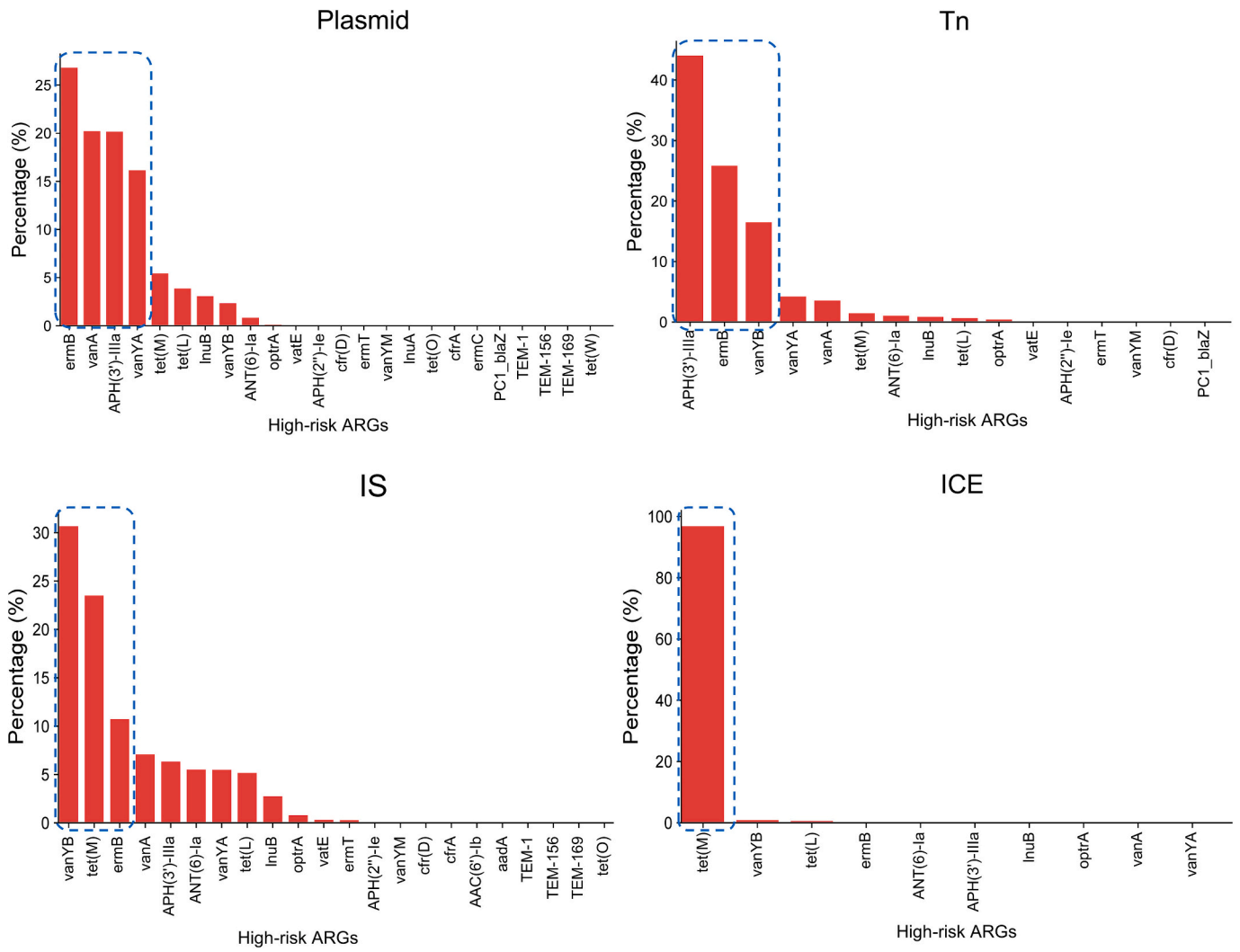

Fig. 7. Preference of MGEs for high-risk ARGs. Tn represents transposons, IS represents insertion sequences, and ICE represents integrative conjugative elements.

As a hospital-associated lineage, Clade A1 is closely linked to VRE outbreaks, and thus, vancomycin ARGs are more prevalent in this clade. The five sequence types (STs) with the highest relative abundance of ARGs are all from Clade A1, highlighting that hospital-associated high-risk ARGs are a major source of AMR in *E. faecium*. Fortunately, these STs have not yet evolved into new branches (Fig. S1). Additionally, we observed dominant *tet(M)* and *ermB* genes in Clade A1, supporting the conclusion that the combination of vancomycin_ARGs-*tet(M)-ermB* is predominant in VREfm [50]. The prevalence of oxazolidinones_ARGs in Clade A2 suggests agricultural antimicrobial use as an evolutionary driver. Nonetheless, the increase in oxazolidinones AMR should be closely monitored. In epidemiological investigations, attention should be given to the five STs with high relative abundance of high-risk ARGs; second, focus should be on VRE-related STs (Clade A1); and third, attention should be paid to STs (Clade A2) associated with oxazolidinones AMR.

MGEs-mediated horizontal gene transfer (HGT) represents a key feature of high-risk ARGs. HGT has driven the evolution of *E. faecium* and significantly contributed to its rapid emergence as a prominent hospital-associated pathogen [56]. This study reveals that different MGEs preferentially facilitate the carriage of high-risk ARGs. For instance, *tet(M)* is predominantly carried by ICE and IS, while *vanYB* is mainly associated with Tn and IS. *VanA* and *vanYA* are primarily carried by plasmids, *APH(3′)-IIIa* is mainly encoded by plasmids and Tn, and *ermB* is favored by plasmids, Tn, and IS. Understanding these MGEs preferences can enhance efforts to control ARGs transmission based on their specific characteristics. The preference of MGEs for certain ARGs also elucidates why these ARGs are more prevalent and widely distributed across various countries and STs. However, some ARGs, such as *ermT*, *tet(L)*, *lnuB*, and *ANT(6)-Ia*, are less frequently encoded by MGEs but still show wide distribution, necessitating further investigation. It is possible that, in addition to MGE-mediated spread, large-scale microbial migration driven by human international travel and global trade has significantly contributed to the global proliferation of high-risk ARGs [57]. Over the past century, human activities have facilitated the extensive migration of microorganisms, further driving the spread of these high-risk ARGs.

Regarding the regional distribution of high-risk ARGs, notable spread is observed in many countries. The relative abundance of ARGs in Israel and Thailand significantly exceeds that in other countries, with the highest contributions still coming from MGEs_preferred ARGs: *tet(M)*, *ermB*, *APH(3′)-IIIa*, *vanA*, and *vanYA* (Fig. 2). These countries may need to improve antimicrobial agent management to control high-risk ARGs. It is also noteworthy that, besides vancomycin ARGs in *E. faecium*, oxazolidinones_ARGs have shown varying degrees of spread across different countries and STs, with *optrA* exhibiting the most severe spread, detected in over 30 % of samples from various regions. *optrA* was first identified on plasmids in enterococci in 2015 [25] and has since been found in diverse MGEs [58,59], across various hosts [60,61], and in multiple countries [62,63,58]. Although the overall abundance of *optrA*

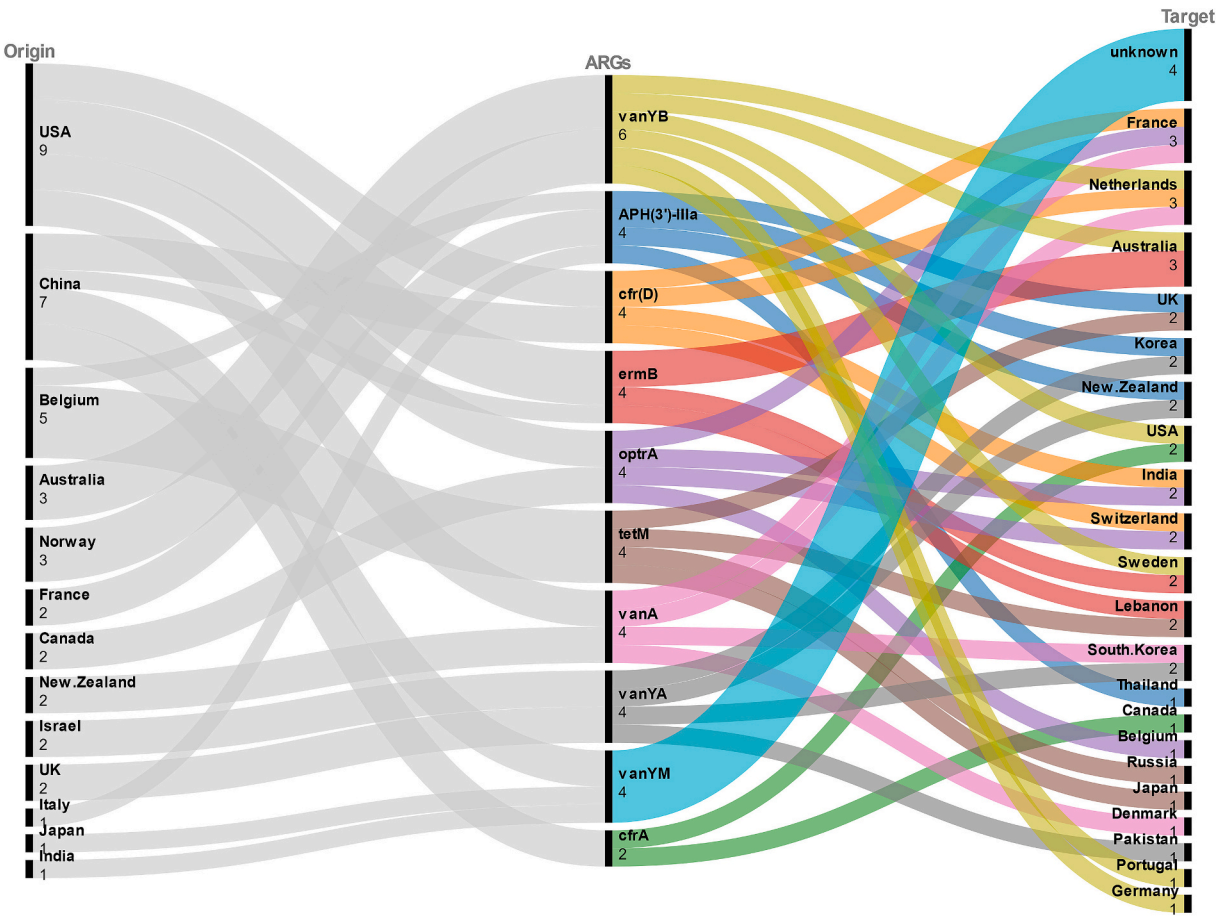

Fig. 8. Origins and destinations of ten significant high-risk ARGs in *Enterococcus faecium*.

in our data is relatively low (0.2296 %), its distribution across different STs and regions is higher than most other high-risk ARGs, indicating its significant transmission potential. We hypothesize that *optrA* may contribute to new outbreaks of antimicrobial-resistant bacteria, similar to vancomycin-resistant enterococci (VRE). Further analysis suggests that *optrA* originated in the USA, and Canada, eventually spreading to France, Switerland, India and Belgium. Understanding the origin of ARGs is crucial, but recognizing their destinations is equally significant. These destinations represent the ultimate evolutionary forms of ARGs and may signal the onset of future AMR outbreaks. It is important to note that the sources of ARGs identified in this study align with the initial sources of ARGs and ARGs outbreaks reported in the literature. For example, vancomycin resistance in *E. faecium* was first observed in the UK [64], before spreading to the USA, Europe, and Australia [15,65]. The origins of the four vancomycin ARGs identified in this study include the UK, the USA, Norway, and Australia, which further supports the reliability of the traceability method used here. Additionally, we identified the origins of vancomycin ARGs in other regions, such as New Zealand, Israel, Japan, India, and China, with the potential spread of these ARGs extending to 12 countries, thereby confirming the global prevalence of VREfm.

Our findings highlight critical One Health implications in antimicrobial resistance dissemination. The distinct resistance profiles between hospital-associated Clade A1 (*van*_ARGs-*tet(M)-ermB*) and livestock-linked Clade A2 (*optrA, cfrA, cfr(D)*) reflect antibiotic usage patterns in clinical versus agricultural settings. Additionally, the ST80 strains harbored both oxazolidinone ARGs and vancomycin_ARGs-*tet(M)-ermB*, with 59 % (63702) of the total ARGs co-located with plasmid. This highlights the potential for the transfer of high-risk ARGs among human, animal, and environmental enterococci. These observations emphasize the necessity for integrated surveillance strategies to address multidrug-resistant *E. faecium*, such as clinical monitoring of high-risk ARGs in epidemic STs (Clade A1), prudent management of last-resort antibiotics in livestock farming, and monitoring of water pollution from livestock farming and clinical wastewater, etc.

Finally, it is important to recognize that due to uneven sampling, regional comprehensive resistance data on *E. faecium* are lacking. Thus, more extensive data collection and in-depth analysis are required, underscoring the need for routine genomic surveillance of pathogens.

5. Conclusion

This study investigated the distribution characteristics of high-risk ARGs in clinically significant *E. faecium* using genome sequences. We assessed the distributions, epidemiological features, and primary origins of these high-risk ARGs. The workflow employed facilitates rapid genome-based investigation and surveillance of high-risk ARGs. As sequencing costs decrease, we recommend that local disease control centers or hospitals develop genomic databases for major pathogens. This approach will enhance clinical antimicrobial treatment and stewardship.

CRediT authorship contribution statement

Chunge Mu: Formal analysis, Data curation. **Shimeng Wang:** Data curation. **Ailan Wang:** Resources, Conceptualization. **Weiwei Li:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author used ChatGPT tool in order to enhance the accuracy of English expression. After using this tool, the author reviewed and edited the content as needed and takes full responsibility for the content of the published article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2025.101054.

Data availability

No data was used for the research described in the article.

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