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Research Article

Genomic and Bioinformatic Insights Into *Enterococcus faecalis* From Retail Meats in Nigeria

Charles Ayodeji Osunla,[1,2] **Ayorinde Akinbobola**,[1] **Arif Elshafea**,[3] **Esther Eyram Asare Yeboah**,[4] **Olayemi Stephen Bakare**,[5] **Aderonke Fayanju**,[1] **Dorcas Oladayo Fatoba**,[6,7] **Bright Boamah**,[8,9] **and Daniel Gyamfi Amoako**[3,10]

[1]*Department of Microbiology, Adekunle Ajasin University, Akungba Akoko, Nigeria*
[2]*School of Public Health, University of Saskatchewan, Saskatoon, Canada*
[3]*Department of Pathobiology, University of Guelph, Guelph, Canada*
[4]*Department of Pharmaceutical Sciences, Central University, Accra, Ghana*
[5]*School of Biological and Behavioural Sciences, Queen Mary University of London, London, UK*
[6]*Health Research Incorporated, Wadsworth Center, New York State Department of Health, Albany, New York, USA*
[7]*The University of Tennessee Health Science Center (UTHSC), Memphis, Tennessee, USA*
[8]*Toxicology Centre, University of Saskatchewan, Saskatoon, Canada*
[9]*Canadian Food Inspection Agency, Ottawa, Canada*
[10]*Antimicrobial Research Unit, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa*

Correspondence should be addressed to Charles Ayodeji Osunla; charles.osunla@aaua.edu.ng and Daniel Gyamfi Amoako; amoakodg@gmail.com

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Enterococcus faecalis is a commensal and opportunistic pathogen increasingly recognized for its antimicrobial resistance (AMR) and zoonotic potential. This study employs whole-genome sequencing (WGS) to characterize *E. faecalis* isolates from retail meat samples, focusing on antimicrobial resistance genes (ARGs), virulence determinants, mobile genetic elements, and phylogenomic relationships. Fifty raw meat samples, including chicken ($n = 18$), beef ($n = 17$), and turkey ($n = 15$), were collected from retail markets in Akungba-Akoko, Nigeria. Confirmed isolates underwent antimicrobial susceptibility testing and WGS-based genomic analysis. Ten *E. faecalis* isolates were recovered, predominantly from chicken. All exhibited resistance to clindamycin, erythromycin, and tetracycline. Dominant AMR genes included *aac(6′)-aph(2″)*, *ant(6)-Ia*, *lsa(A)*, *erm(B)*, *tet(M)*, and *tet(L)*. Plasmid replicons *rep9c* and *repUS43* were associated with sequence types ST477 and ST16, respectively. MGEs such as IS3, IS6, IS256, and IS1380 colocalized with resistance and virulence genes. Phylogenomic analysis revealed two major lineages (ST477 and ST16) and indicated geographic clustering across African isolates. The co-occurrence of multidrug resistance, virulence factors, and MGEs in foodborne *E. faecalis* poses a public health concern due to the risk of horizontal gene transfer and zoonotic spread. These findings support the need for strengthened genomic surveillance and AMR control strategies in food systems, particularly within low- and middle-income countries.

Keywords: antimicrobial resistance; *Enterococcus faecalis*; Nigeria; plasmids; retail meat; virulence; whole-genome sequencing

1. Introduction

Enterococcus faecalis is a commensal bacterium of the gastrointestinal tracts of humans and animals, yet it has emerged as a notable opportunistic pathogen, especially in healthcare settings where multidrug-resistant (MDR) strains contribute to severe and difficult-to-treat infections [1]. Beyond clinical contexts, its presence in the food system, particularly in raw meats, raises significant concerns about its role in the dissemination of antimicrobial resistance genes

(ARGs) and virulence factors via the food chain [2]. The species' adaptability is bolstered by its remarkable capacity to acquire and transfer mobile genetic elements (MGEs), which facilitates horizontal gene transfer and complicates therapeutic strategies [3]. These characteristics collectively pose a dual threat to both food safety and public health, necessitating a comprehensive understanding of its genomic architecture across diverse ecological niches.

Globally, there has been an increase in genomic studies aimed at unraveling the molecular epidemiology, resistance mechanisms, and evolutionary dynamics of *E. faecalis* from various reservoirs [4, 5]. However, low- and middle-income countries (LMICs), including those in sub-Saharan Africa, remain underrepresented in such efforts [6]. This is particularly concerning in Nigeria, where retail meat remains a critical dietary staple, yet formal surveillance of foodborne pathogens is limited. Notably, existing studies in Nigeria have primarily focused on phenotypic AMR profiling, offering limited insights into the genomic context of resistance, virulence, and mobility [7]. Additionally, the widespread and often unregulated use of antibiotics in agriculture continues to drive selection pressure, creating hotspots for the emergence of MDR organisms within the food chain [8].

Nigeria's current surveillance infrastructure lacks the integration of standardized genomic frameworks necessary for early detection of high-risk clones. Consequently, the molecular epidemiology of *E. faecalis* in Nigerian food systems remains poorly understood. Previous studies have shown that genomic traits linked to hospital-adapted *E. faecalis* lineages, such as those conferring resistance to vancomycin, β-lactams, and fluoroquinolones, can also be found in strains from animals and food [9, 10], suggesting a potential interface between clinical and agricultural reservoirs. Moreover, Akungba-Akoko, the study area, is a growing semiurban commercial center in southwestern Nigeria with a bustling meat market [11]. It represents a relevant model for understanding the food safety challenges in similar underserved communities across the country. However, no previous genomic studies have been conducted in this locality to assess the burden or genetic characteristics of *E. faecalis* in meat products.

This study addresses this knowledge gap by performing a comprehensive genomic characterization of *E. faecalis* isolates recovered from retail meat in Akungba-Akoko. Utilizing whole-genome sequencing (WGS) and bioinformatic approaches, we aim to (1) assess the prevalence and diversity of ARGs, including those conferring resistance to critically important antimicrobials; (2) characterize virulence determinants associated with adhesion, biofilm formation, and immune evasion; and (3) investigate the MGEs facilitating gene exchange. These findings will contribute to our understanding of the genomic plasticity of *E. faecalis* in Nigeria's food systems and inform mitigation strategies to reduce the public health risks posed by this emerging foodborne pathogen.

2. Materials and Methods

2.1. Sample Collection and Study Site. The study was carried out over a 3-month period between April and June 2022: 50 samples of raw retail meat, including chicken ($n = 18$), beef ($n = 17$), and turkey ($n = 15$), from the Ibaka market (7.473500° N, 5.736250° E) in Akungba Akoko, Nigeria (Figure 1). The Ibaka market is a rural periodic day market located in Akungba, which is the host community of Adekunle Ajasin University.

2.2. Isolation and Identification of Enterococcus faecalis Isolates. In the laboratory, each meat sample was aseptically homogenized. Smears of the homogenates were prepared and subjected to Gram staining to identify gram-positive cocci arranged in pairs or short chains, which are characteristic of *Enterococcus* species. For bacterial isolation, aliquots of the homogenized samples were inoculated onto blood agar plates and incubated aerobically at 37°C for 24–48 h. Colonies exhibiting typical *Enterococcus* morphology were selected for further testing. Presumptive *Enterococcus* isolates were identified on the basis of their Gram staining characteristics and ability to grow on blood agar. Biochemical tests were performed to confirm that the isolates were *Enterococcus* species. These tests included the Voges–Proskauer (VP) test for detecting acetoin production and the potassium tellurite (PT) test to assess the ability to reduce tellurite. Additionally, fermentation tests for glucose, lactose, and sucrose were conducted to evaluate the carbohydrate utilization profiles of the isolates. *Staphylococcus aureus* ATCC 29213 and *E. faecalis* ATCC 29212 served as negative and positive controls, respectively. Confirmed *E. faecalis* isolates were preserved by storing them in brain heart infusion broth (Difco) supplemented with 20% glycerol at −70°C for long-term storage.

2.3. Antibiotic Susceptibility Testing of Enterococcus faecalis Strains. The antibiotic resistance profiles of *E. faecalis* isolates were determined via the disk diffusion method on Mueller–Hinton agar (MHA), following the guidelines of the Clinical and Laboratory Standards Institute [12]. Overnight cultures of the isolates were used to prepare bacterial suspensions adjusted to a turbidity equivalent to a 0.5 McFarland standard. A sterile cotton swab was dipped into each suspension and evenly streaked across the entire surface of the MHA plates to ensure a uniform bacterial lawn. Commercial antibiotic disks (Hi-Media, India) were placed onto the inoculated plates via sterile forceps. The antibiotics used and their corresponding disk concentrations were as follows: tetracycline (30 μg), chloramphenicol (30 μg), streptomycin (10 μg), kanamycin (30 μg), erythromycin (15 μg), vancomycin (30 μg), clindamycin (2 μg), and tobramycin (10 μg). The plates were incubated aerobically at 37°C for 18–24 h. After incubation, the diameters of the inhibition zones around each antibiotic disk were measured in millimeters. *Staphylococcus aureus* ATCC 25923 was used as the control. The results were interpreted according to CLSI guidelines [12], categorizing the isolates as susceptible, intermediate, or resistant to each antibiotic tested.

2.4. DNA Extraction, WGS, and Assembly of Enterococcus faecium Strains. Genomic DNA was extracted from *E. faecalis* isolates via the MasterPure Gram Positive DNA Purification Kit (Lucigen, Middleton, Wisconsin, United States)



FIGURE 1: Satellite view of Akungba-Akoko, Ondo State, Southwestern Nigeria, captured via Google Earth Web (https://earth.google.com/web, retrieved on July 15, 2025). The red pin marks the central coordinates of the study site (7.473500° N, 5.736250° E). Red boundaries delineate the urban extent of Akungba-Akoko.

according to the manufacturer's instructions. The quality and concentration of the extracted DNA were assessed via a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, United States). The genomic DNA libraries were prepared via the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, California, United States) following the manufacturer's protocol. Sequencing was performed on an Illumina NovaSeq 6000 system (Illumina, San Diego, California, United States) to generate paired-end reads. The raw sequence reads were assembled into contigs via the Shovill pipeline Version 1.0.4, which incorporates Trimmomatic Version 0.38 for read trimming and quality control. Genome annotation was conducted via Prokka Version 1.13.3.

2.5. Identification of Resistance Genes, Virulence Genes, Plasmids, and Multilocus Sequence Typing (MLST). ARGs were identified via ResFinder Version 4.6.0 (http://genepi.food.dtu.dk/resfinder). Virulence genes were detected via VirulenceFinder Version 2.0 (https://cge.food.dtu.dk/services/VirulenceFinder/). Plasmid types were determined by analyzing the assembled genome sequences with PlasmidFinder Version 2.1 (https://cge.food.dtu.dk/services/PlasmidFinder/). MLST was performed via the MLST tool Version 2.0 (https://cge.food.dtu.dk/services/MLST/) to assign sequence types (STs) to the *E. faecalis* isolates.

2.6. Phylogenomic Analysis and Metadata Insights. The de novo assembled contigs of the *E. faecalis* isolates were submitted to CSI Phylogeny Version 1.4 (https://cge.cbs.dtu.dk/services/CSIPhylogeny-1.4), an online tool that identifies single nucleotide polymorphisms (SNPs) from WGS data, filters and validates SNP positions, and infers phylogeny on the basis of concatenated SNP profiles. The *Enterococcus faecalis* ATCC BAA-2128 strain (Accession Number: NAQY00000000.1) was used as an outgroup to root the tree, facilitating the assessment of phylogenetic relationships among the *E. faecalis* strains. The phylogenetic tree was visualized and annotated with isolate metadata, including demographic information, STs, resistome profiles, and MGEs, via ITOL Version 7 (https://itol.embl.de). Additionally, whole-genome sequences of *E. faecalis* isolates from Africa, curated from public databases such as PATRIC (https://www.patricbrc.org/) and NCBI between 2013 and 2023, were downloaded and included in the phylogenomic analysis to provide epidemiological and evolutionary context (Table S1). Isolates belonging to the same STs are highlighted with the same color, and isolates from the same geographical regions are labeled with the same text color to facilitate visual interpretation.

2.7. Nucleotide Sequence. The sequences of the *E. faecalis* strains analyzed in this study were deposited in the National Center for Biotechnology Information GenBank database under BioProject Number PRJNA928459.

3. Results

3.1. Prevalence and Antibiotic Resistance Patterns of E. faecalis in Retail Meats. From the 50 retail meat samples analyzed, a total of 10 *Enterococcus faecalis* isolates were recovered, corresponding to an overall prevalence of 20%. The isolates were unevenly distributed among the meat types: chicken accounted for the highest number of isolates ($n = 6$, 60%), followed by beef ($n = 2$, 20%) and turkey ($n = 2$, 20%). Antimicrobial susceptibility testing revealed consistent resistance profiles across the isolates. All strains exhibited complete resistance (100%) to clindamycin, erythromycin, and tetracycline, antibiotics commonly used in veterinary and clinical settings. Moderate resistance was observed against streptomycin (80%) and tobramycin (80%). In contrast, resistance to chloramphenicol was comparatively lower (20%), and no resistance to vancomycin was detected among any of the isolates. The distribution and co-occurrence of resistance phenotypes are illustrated in Figure 2 using an Upset plot.

3.2. ARG Profiles and MGEs. Genomic analysis identified a diverse repertoire of ARGs among the *E. faecalis* isolates, many of which confer resistance to critically important antimicrobials. The most frequently detected ARGs included *aac(6′)-aph(2″)* and *ant(6)-Ia* (aminoglycoside resistance), *lsa(A)* (lincosamide resistance), *erm(B)* (macrolide resistance), and *tet(M)* and *tet(L)* (tetracycline resistance). Notably, *dfrG* (trimethoprim resistance) and *catA8* (chloramphenicol resistance) were detected exclusively in two isolates recovered from retail chicken, suggesting lineage-specific acquisition or exposure to unique selective pressures.

Across meat sources, the core resistome was largely conserved; however, isolates NigeriaC1 and NigeriaC11 (from chicken) harbored a broader range of ARGs, reflecting potential differential antibiotic exposure in poultry production systems (Table 1). Analysis of MGEs revealed lineage- and source-specific patterns. The plasmid replicon *rep9c*was the most prevalent, detected in all isolates regardless of meat source and consistently associated with ST ST477. Conversely, *repUS43* was uniquely found in ST16 isolates from retail chicken, indicating a possible plasmid-lineage specificity.

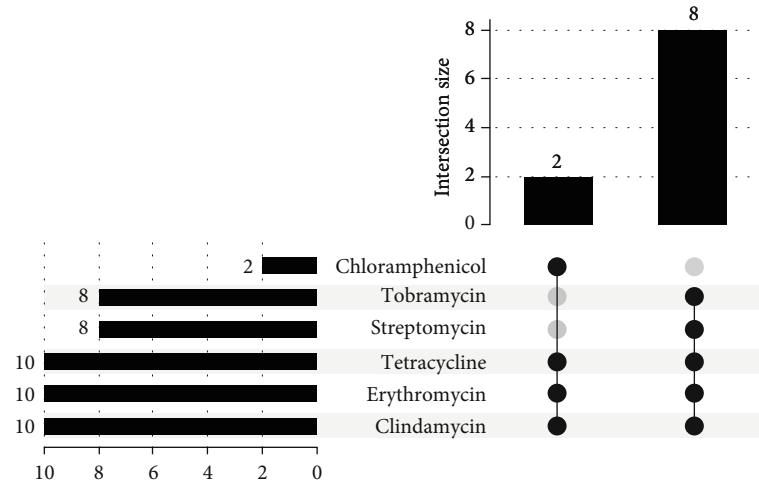


Figure 2: The Upset plot illustrates the distribution of antimicrobial resistance patterns across the isolates. The top bar chart quantifies unique combinations of antibiotic resistance among the isolates, while the horizontal bar chart on the left shows the number of isolates resistant to individual antibiotics. Coresistance to clindamycin, erythromycin, tetracycline, and aminoglycosides (streptomycin and tobramycin) was common. Vancomycin was excluded from the visualization due to the absence of resistance. The arrangement highlights both dominant and infrequent coresistance profiles across the dataset.

Insertion sequences (ISs) played a prominent role in the resistome architecture. The IS6 family was frequently identified in ST477 isolates from all meat types. A unique combination of IS elements, *IS3*, *IS6*, *IS110*, *IS256*, and *IS1380*, was observed only in ST16 isolates, colocalized with *dfrG* and *catA8*. Further analysis identified a resistance gene cassette comprising *aac(6′)-aph(2″)*, *ant(6)-Ia*, and *tet(M)* embedded within a genomic region enriched with MGEs, including *IS1380* and *IS6* family transposases, as well as plasmid recombinase family proteins (Figure 3).

3.3. Virulence Factors, STs, and Phylogenetic Insights. Genome analysis revealed that *E. faecalis* isolates harbored an extensive repertoire of virulence factors associated with colonization, tissue invasion, and immune evasion. Conserved genes across all isolates included *ace* (collagen adhesion), *efaAfs* (endocarditis antigen), *ElrA* (adhesion), *SrtA* (anchoring protein), and the biofilm-associated pili genes *ebpA*, *ebpB*, and *ebpC*. Additionally, all isolates carried *cCF10* and *cOB1*, encoding aggregation substances that enhance horizontal gene transfer and biofilm development.

The distribution of virulence genes displayed clear ST-specific patterns. ST477, the most prevalent lineage, was recovered from beef, chicken, and turkey, and consistently carried *fsrB*, *gelE* (gelatinase), and *hylB* (hyaluronidase), key factors implicated in biofilm formation, extracellular matrix degradation, and immune modulation. In contrast, ST16 isolates (NigeriaC1 and NigeriaC11), found exclusively in chicken, exhibited a distinct virulence profile. These isolates harbored *agg*, *cylA*, *cylB*, *cylL*, and *cylM* genes encoding the cytolysin toxin complex, which contributes to tissue damage and enhanced pathogenicity. ST16 also possessed *hylA*, an alternative hyaluronidase variant, and *camE* (calcium-binding protein).

Plasmid replicons showed strong lineage associations. *rep9c* was universally detected in ST477 isolates and co-occurred with the *fsrB–gelE–hylB* virulence gene set. Conversely, *repUS43* was exclusive to ST16 and linked to the presence of cytolysin genes and *hylA*. ISs were also associated with virulence profiles. ST477 isolates commonly carried *IS6*, while ST16 harbored a broader array of MGEs, including *IS3*, *IS110*, *IS256*, and *IS1380*, possibly facilitating the mobilization of cytolysin and adhesion genes. Phylogenetic reconstruction using SNP-based analysis revealed two well-defined clades corresponding to ST16 and ST477 lineages (Figure 4). ST16 isolates clustered together and were uniquely associated with poultry, distinct ARGs, and virulence factors. ST477 formed a separate, more diverse clade encompassing isolates from all meat types and displaying a conserved resistome–virulome profile.

3.4. Comparative Phylogenomic Analysis and Metadata Insights of E. faecalis Isolates From Africa. To contextualize the Nigerian *E. faecalis* isolates within broader regional dynamics, we conducted a comparative phylogenomic analysis of 149 publicly available African genomes collected between 2013 and 2023. South Africa contributed the highest number of isolates ($n = 60$), followed by Tanzania ($n = 33$) and Ghana ($n = 20$), reflecting the uneven distribution of genomic surveillance efforts across the continent. Nigeria, the focus of this study, accounted for 10 isolates, while Cameroon, Zimbabwe, Tunisia, and Egypt contributed fewer (Figures 5 and 6a).

Temporally, most isolates were obtained between 2017 and 2021, with peaks in 2017 ($n = 42$) and 2021 ($n = 57$). Earlier years (2013–2016) were underrepresented, limiting historical comparisons but indicating a growing interest in enterococcal genomics in recent years (Figure 6b). ST analysis identified 47 distinct STs across the dataset, with both shared and country-specific lineages. ST16 was the most widely distributed, detected in South Africa, Tanzania, Cameroon, and Ghana (Figure 6c). In contrast, ST477, found

TABLE 1: Genomic and phenotypic characteristics of *E. faecalis* isolates.

Isolate ID	Isolation source	Antibiogram	Resistance genes	Insertion sequences	Plasmid replicons	MLST	Virulence factors
NigeriaB142	Beef	STP-TOB-TET-ERY-CLI	*aac(6′)-aph(2″), ant(6)-Ia, lsa(A), erm(B), tet(M), tet(L)*	IS6	rep9c	ST477	*ElrA, SrtA, ace, cCF10, cOB1, cad, camE, ebpA, ebpB, ebpC, efaAfs, fsrB, gelE, hylB, tpx*
NigeriaB242	Beef	STP-TOB-TET-ERY-CLI	*aac(6′)-aph(2″), ant(6)-Ia, lsa(A), erm(B), tet(M), tet(L)*	IS6	rep9c	ST477	*ElrA, SrtA, ace, cCF10, cOB1, cad, came, ebpA, ebpB, ebpC, efaAfs, fsrB, gelE, hylB, tpx*
NigeriaC1	Retailed chicken	TET-ERY-CLI-CHL	*lsa(A), erm(B), catA8, tet(M), dfrG*	IS3, IS6, IS110, IS256, IS1380	repUS43	ST16	*ElrA, SrtA, ace, agg, cCF10, cOB1, cad, came, cylA, cylB, cylL, cylM, ebpA, ebpB, ebpC, efaAfs, hylA, tpx*
NigeriaC11	Retailed chicken	TET-ERY-CLI-CHL	*lsa(A), erm(B), catA8, tet(M), dfrG*	IS3, IS6, IS110, IS256, IS1380	repUS43	ST16	*ElrA, SrtA, ace, agg, cCF10, cOB1, cad, came, cylA, cylB, cylL, cylM, ebpA, ebpB, ebpC, efaAfs, hylA, tpx*
NigeriaC2	Retailed chicken	STP-TOB-TET-ERY-CLI	*aac(6′)-aph(2″), ant(6)-Ia, lsa(A), erm(B), tet(M), tet(L)*	IS6	rep9c	ST477	*ElrA, SrtA, ace, cCF10, cOB1, cad, camE, ebpA, ebpB, ebpC, efaAfs, fsrB, gelE, hylB, tpx*
NigeriaC24	Retailed chicken	STP-TOB-TET-ERY-CLI	*aac(6′)-aph(2″), ant(6)-Ia, lsa(A), erm(B), tet(M), tet(L)*	IS6	rep9c	ST477	*ElrA, SrtA, ace, cCF10, cOB1, cad, came, ebpA, ebpB, ebpC, efaAfs, fsrB, gelE, hylB, tpx*
NigeriaC242	Retailed chicken	STP-TOB-TET-ERY-CLI	*aac(6′)-aph(2″), ant(6)-Ia, lsa(A), erm(B), tet(M), tet(L)*	IS6	rep9c	ST477	*ElrA, SrtA, ace, cCF10, cOB1, cad, came, ebpA, ebpB, ebpC, efaAfs, fsrB, gelE, hylB, tpx*
NigeriaC243	Retailed chicken	STP-TOB-TET-ERY-CLI	*aac(6′)-aph(2″), ant(6)-Ia, lsa(A), erm(B), tet(M), tet(L)*	IS6	rep9c	ST477	*ElrA, SrtA, ace, cCF10, cOB1, cad, camE, ebpA, ebpB, ebpC, efaAfs, fsrB, gelE, hylB, tpx*
NigeriaT23	Retailed turkey	STP-TOB-TET-ERY-CLI	*aac(6′)-aph(2″), ant(6)-Ia, lsa(A), erm(B), tet(M), tet(L)*	IS6	rep9c	ST477	*ElrA, SrtA, ace, cCF10, cOB1, cad, camE, ebpA, ebpB, ebpC, efaAfs, fsrB, gelE, hylB, tpx*
NigeriaT44	Retailed turkey	STP-TOB-TET-ERY-CLI	*aac(6′)-aph(2″), ant(6)-Ia, lsa(A), erm(B), tet(M), tet(L)*	IS6	rep9c	ST477	*ElrA, SrtA, ace, cCF10, cOB1, cad, camE, ebpA, ebpB, ebpC, efaAfs, fsrB, gelE, hylB, tpx*

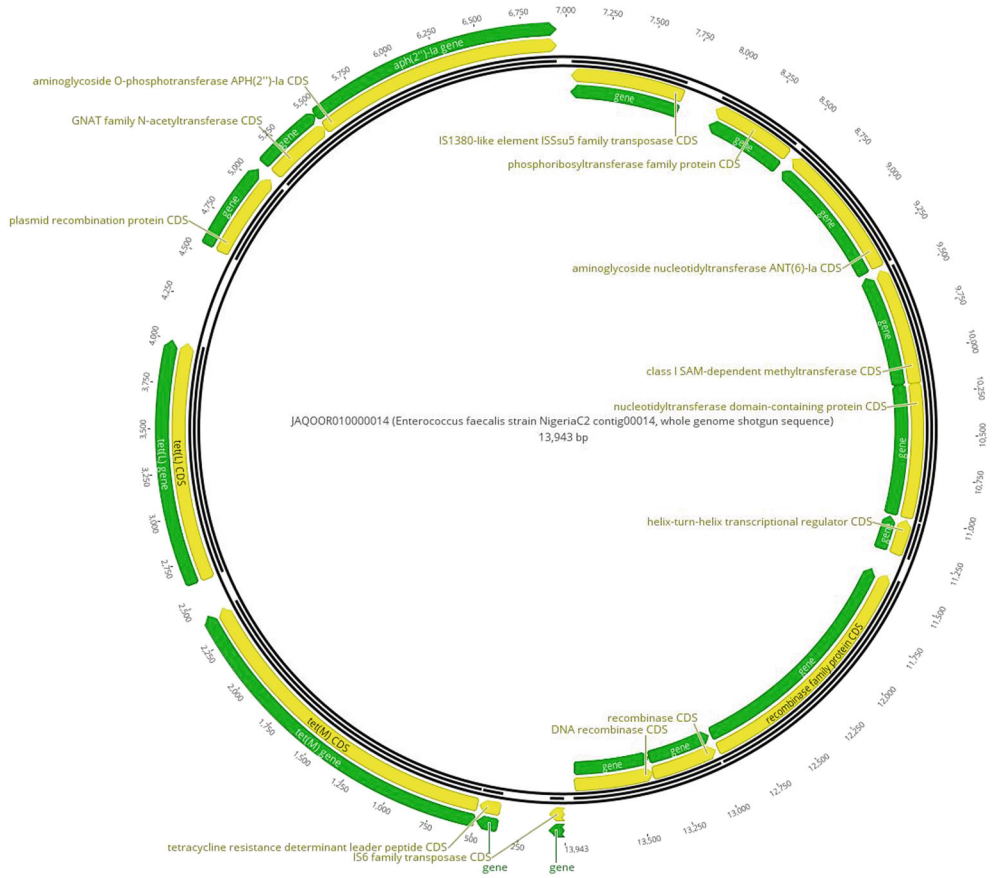


FIGURE 3: Circular genomic annotation of the genetic cassette carrying resistance genes in *Enterococcus faecalis* isolate NigeriaC2. This figure presents a visualization of the genomic region containing resistance determinants *aac(6')-aph(2")*, *ant(6)-Ia*, and *tet(M)* in isolate NigeriaC2 (Accession Number: JAQOOR010000014). The annotation highlights the relative positioning and orientation of these resistance genes alongside associated mobile genetic elements, including *IS1380* transposase, *IS6* family transposase, and plasmid recombinase family proteins. Genes are color-coded, with green representing resistance genes and regulatory elements, while yellow indicates protein-coding sequences (CDS). Arrows denote the transcriptional orientation of individual genes, providing insights into their synteny and potential functional interactions within the genomic context.

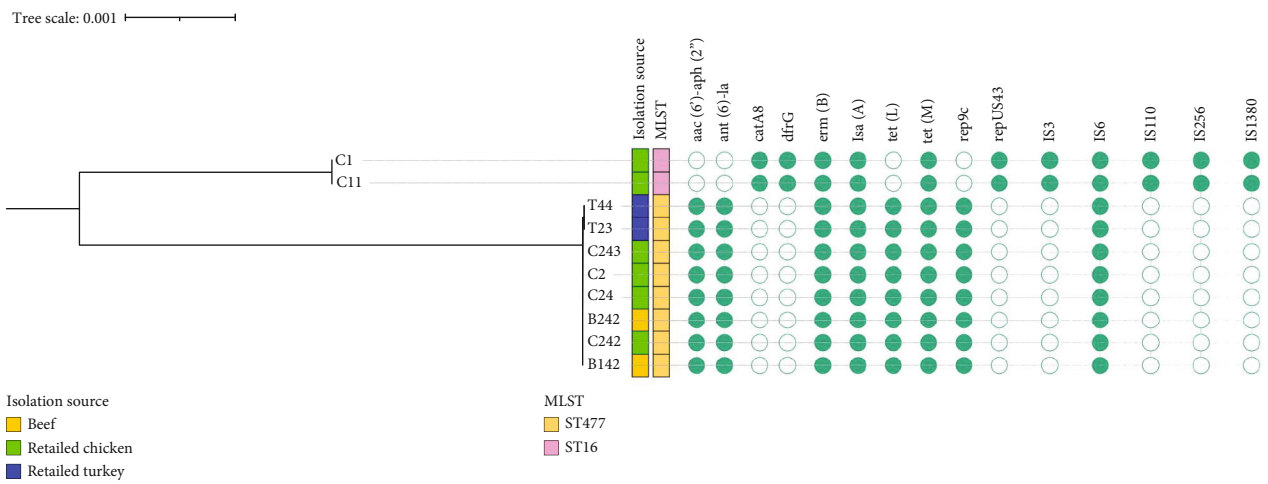


FIGURE 4: SNP-based maximum likelihood tree showing phylogenetic relationships among *E. faecium* isolates recovered from retail meat. The tree illustrates two main clades, each linked to specific sequence types (STs) and genomic characteristics. Annotations highlight key genomic features, including STs, isolation sources, resistance genes, plasmid replicons, and insertion sequences. Isolates are visually distinguished by color-coded boxes, indicating their distribution across different meat sources.

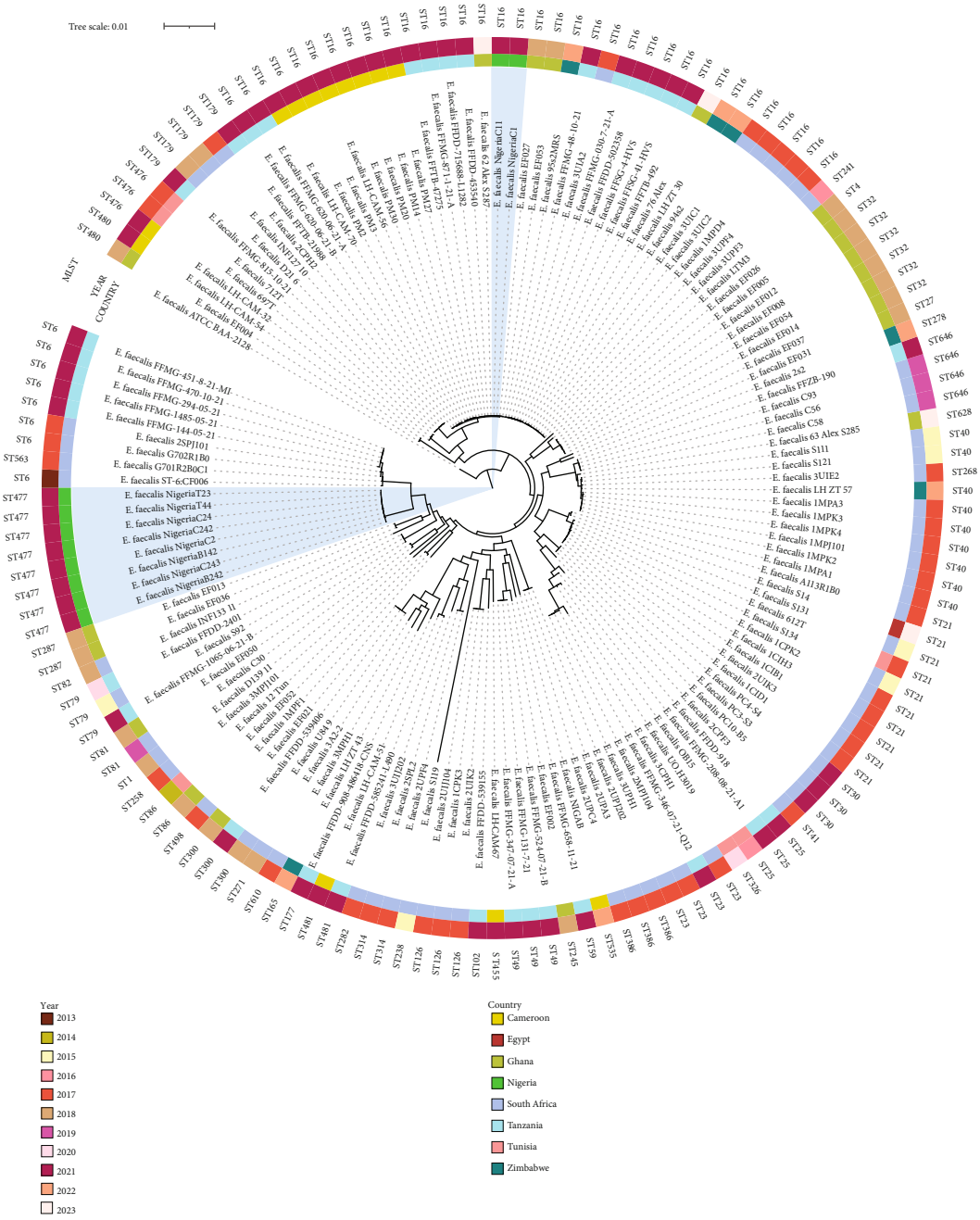
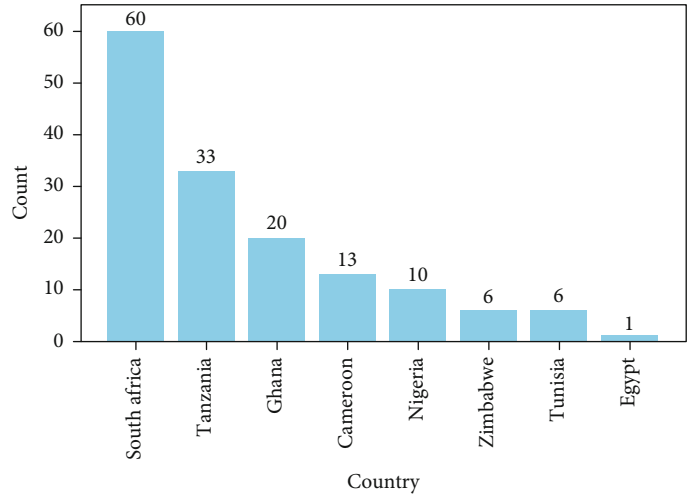


Figure 5: Maximum likelihood phylogenetic tree of *Enterococcus faecalis* isolates from African countries (2013–2023). A core-genome phylogenetic tree was constructed from 149 *E. faecalis* genomes retrieved from BV-BRC and annotated using iTOL. The tree is rooted using *E. faecalis* ATCC BAA-2128 (Accession: NAQY00000000.1) as the reference genome. The outer ring represents the sequence type (ST), the middle ring denotes the year of isolation, and the inner ring displays the country of origin. Isolates from this study are marked with a blue background.

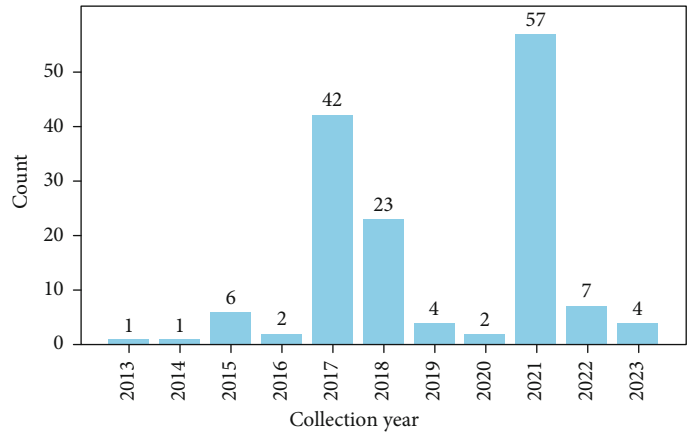
exclusively in the current Nigerian isolates, appeared geographically restricted. Other notable country-specific lineages included ST21 (Tunisia and Egypt) and ST646 (South Africa). A network analysis of the Top 10 STs and their country associations (Figure 6d) further illustrated these patterns. South Africa and Tanzania exhibited the highest ST diversity, with multiple connections to ST6, ST16, and ST646 (Figure 6d).

4. Discussion

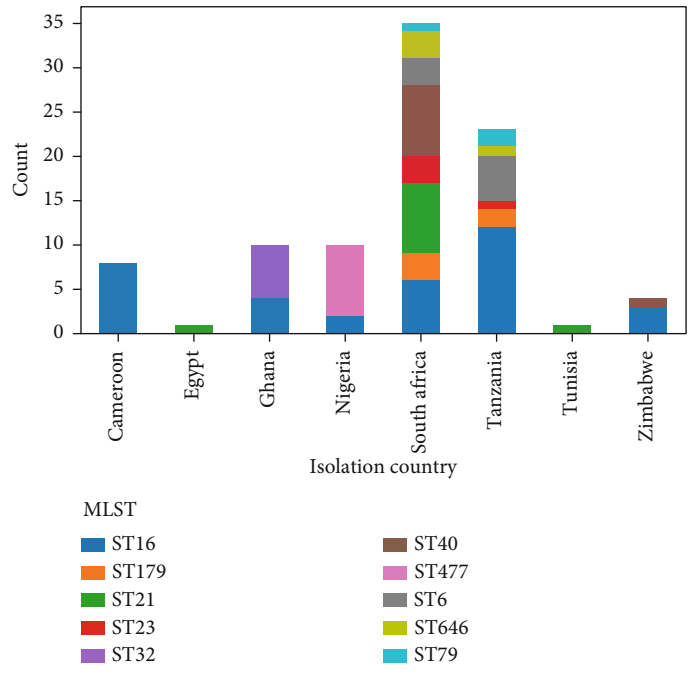
The presence of *E. faecalis* in retail meats highlights the potential role of foodborne transmission in the dissemination of AMR [13]. Consistent with previous reports, chicken meat yielded the highest recovery rate of *E. faecalis*, reinforcing its role as a major reservoir for enterococci in food systems [14, 15]. Although not a classical foodborne



(a) Geographic distribution

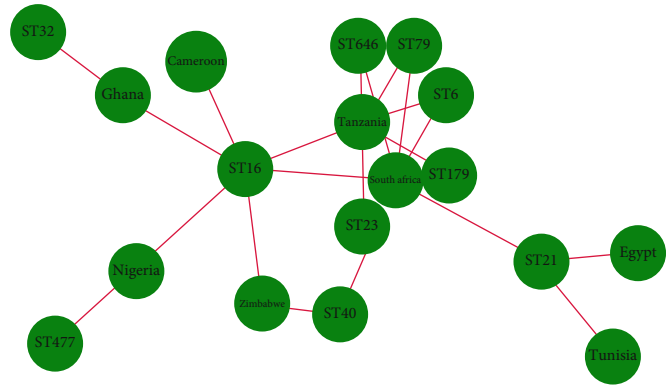


(b) Temporal trends



(c) MLST distribution by country

Figure 6: Continued.



(d) Network of countries and Top 10 MLSTs

Figure 6: (a) Geographic distribution of *E. faecalis* isolates across Africa. A bar chart illustrating the number of *E. faecalis* isolates obtained from different African countries. (b) Temporal trends in *E. faecalis* isolations across Africa. A bar chart depicting the number of *E. faecalis* isolates collected per year from 2013 to 2023. (c) Distribution of major sequence types (*STs*) among African *E. faecalis* isolates. A stacked bar chart showing the distribution of the most prevalent *STs* across different African countries. (d) A network graph visualizing relationships between African countries and their associated *E. faecalis STs*. Nodes represent countries and *STs*, with edges indicating connections based on isolate presence.

pathogen, the species' capacity to horizontally transfer resistance genes within the human gut microbiota elevates its public health significance [16]. These findings support calls for strengthened hygiene protocols and antimicrobial stewardship in poultry production, a sector characterized by intensive antimicrobial use [13].

All isolates exhibited resistance to clindamycin, erythromycin, and tetracycline, mirroring global resistance trends and suggesting sustained selection pressure from widespread use of these agents in animal husbandry [17–19]. The absence of vancomycin resistance is encouraging; however, the species' genomic flexibility raises concerns about future acquisition through horizontal gene transfer [20]. The detection of *catA8* among chicken isolates, despite the relatively low phenotypic resistance to chloramphenicol, signals the emergence of latent resistance and highlights the risk of resurgence [21]. These findings reinforce the critical need for integrated AMR surveillance across food and clinical sectors, especially in high-burden regions [22].

Genomic analysis revealed a consistent resistome dominated by *aac(6′)-aph(2″)*, *ant(6)-Ia*, *lsa(A)*, *erm(B)*, *tet(M)*, and *tet(L)* conferring resistance to aminoglycosides, macrolides, lincosamides, and tetracyclines. These genes, conserved across meat types and lineages, mirror globally recognized resistance profiles and likely reflect coselection pressures exerted by agricultural antibiotic use [3]. Of particular note, *dfrG* and *catA8* were confined to ST16 isolates from chicken, suggesting lineage-specific resistance acquisition possibly driven by poultry-associated selective environments [23]. These resistance determinants were not randomly distributed but instead embedded within complex mobile genetic architectures. A multidrug resistance gene cassette colocalizing *aac(6′)-aph(2″)*, *ant(6)-Ia*, and *tet(M)* was detected within a genomic region enriched with IS6 and IS1380 transposases and plasmid recombinase genes, forming a transferable module capable of *en* bloc dissemination of resistance traits. Such modularity enhances the potential for inter-species gene flow, especially within gut microbiota of exposed consumers [3].

Plasmid replicons further delineated lineage-specific resistance pathways. The widespread *rep9c* replicon, present in all ST477 isolates, was consistently colocated with the *fsrB–gelE–hylB* virulence cluster, suggesting clonal expansion and vertical maintenance of resistance–virulence hybrids [24]. In contrast, *repUS43* was exclusive to ST16 isolates, co-occurring with *catA8* and *dfrG*, reinforcing its role in ST-specific resistance dissemination [25]. The structural linkage between plasmid types, ISs, and ARGs underscores the dynamic interplay of vertical inheritance and horizontal transfer in shaping the resistome of *E. faecalis*. ISs were found at multiple resistance loci and appear to drive genomic fluidity by facilitating recombination and gene mobilization. Their lineage-specific distribution highlights ongoing adaptive evolution under antimicrobial pressure. These findings illustrate a highly structured yet flexible resistance landscape in foodborne *E. faecalis*, propelled by mobile elements and reinforced by selective agricultural practices.

The widespread detection of virulence genes among *E. faecalis* isolates from retail meats reveals a pathogenic potential far beyond commensal behavior. Conserved adhesion factors including *ace*, *efaAfs*, *ElrA*, *SrtA*, and the *ebp* operon (*ebpA*, *ebpB*, *ebpC*) were uniformly present, underscoring a baseline capacity for epithelial colonization, biofilm formation, and immune modulation [26, 27]. The concurrent presence of conjugative factors (*cCF10*, *cOB1*) suggests an additional role in promoting gene exchange within host or environmental niches, facilitating coselection and persistence of resistance–virulence traits.

Lineage-specific virulence signatures were particularly striking. ST477 isolates recovered from beef, chicken, and turkey harbored the *fsrB–gelE–hylB* cluster, a constellation of genes associated with quorum sensing, extracellular matrix degradation, and immune evasion, previously linked to device- and bloodstream-associated infections [28, 29].

This combination of biofilm-promoting and immunomodulatory functions positions ST477 as a high-risk foodborne lineage with potential for clinical crossover. In contrast, ST16 isolates exclusively from chicken exhibited an enhanced virulence profile characterized by cytolysin operon genes (*cylA*, *cylB*, *cylL*, and *cylM*) and *hylA*, features associated with epithelial disruption and proinflammatory host responses [30]. Notably, these isolates also carried *agg*, a plasmid-borne aggregation substance linked to increased virulence and conjugation efficiency. The co-occurrence of *repUS43* with this virulence suite suggests a plasmid-mediated mechanism facilitating the emergence of hypervirulent clones.

The convergence of resistance and virulence within specific plasmid backgrounds and STs, especially ST16 and ST477, raises substantial public health concerns. Mobile elements, including IS3, IS110, IS256, and IS1380, were frequently associated with virulence loci, further implicating horizontal gene transfer in the amplification of pathogenic potential. These findings parallel global reports of virulence-enriched *E. faecalis* STs and affirm their relevance in zoonotic transmission and foodborne disease [31, 32]. In this context, foodborne *E. faecalis* strains function not only as reservoirs of resistance but also as potential vectors for invasive disease, particularly in immunocompromised hosts. Their emergence in the food chain, paired with genomic signatures linked to hospital-adapted strains, underscores the urgency of a One Health surveillance framework integrating food safety, environmental monitoring, and clinical microbiology.

Comparative phylogenomics of *E. faecalis* isolates across Africa uncovered a geographically structured but genetically diverse population. South Africa contributed the highest number of isolates, an observation likely shaped by differences in surveillance capacity, sequencing infrastructure, and public health prioritization. The scarcity of isolates from earlier years (2013–2016) and the sharp rise in submissions post-2017, interrupted briefly by the COVID-19 pandemic, reflect both historical data gaps and the growing momentum of genomics-based AMR monitoring on the continent [33–35].

Temporal and geographic analyses revealed that ST ST16 was the most broadly distributed, identified in multiple countries and across diverse ecological contexts, suggesting a well-adapted and potentially mobile lineage [9, 36]. Its widespread detection aligns with prior observations of ST16's capacity for environmental persistence and inter-host transmission. In contrast, ST477 was restricted to Nigeria, while ST21 was confined to Tunisia and Egypt, implying localized evolutionary trajectories shaped by selective pressures such as antimicrobial usage patterns, ecological boundaries, and food production systems [37, 38]. Network-based analysis reinforced these findings by revealing distinct ST-country associations, with South Africa and Tanzania exhibiting the highest ST diversity. These clusters suggest transboundary transmission possibly facilitated by trade, food import/export routes, or shared agricultural practices [39, 40]. Conversely, the geographic confinement of ST477 to Nigeria raises concerns about the emergence of a potentially endemic, foodborne high-risk clone with a stable resistome signature.

These patterns support a dual model of *E. faecalis* evolution in Africa: one shaped by clonal expansion of regionally successful lineages and another driven by horizontal gene transfer across environmental and host reservoirs. This genomic duality complicates control efforts and underscores the need for longitudinal, cross-sectoral surveillance. The convergence of clinically relevant traits in isolates recovered from food reinforces the risk of zoonotic spill-over, particularly in settings with limited food safety regulation and AMR control. In light of these findings, a coordinated genomic surveillance strategy that integrates human, animal, and environmental health guided by the One Health framework is essential for tracking the emergence, evolution, and dissemination of high-risk *E. faecalis* clones in Africa. This study, while limited by its modest sample size, contributes a critical dataset to the continental AMR landscape and provides a foundation for future longitudinal studies on *E. faecalis* as a foodborne pathogen of increasing public health concern.

5. Conclusion

This study provides critical genomic insights into the antimicrobial resistance and virulence landscape of *Enterococcus faecalis* isolates recovered from retail meats in Nigeria, underscoring the role of food systems as reservoirs and potential amplifiers of clinically relevant pathogens. The identification of high-risk lineages such as ST477 and ST16 harboring multidrug resistance determinants, virulence genes, and MGEs highlights the convergence of resistance and pathogenicity within the food chain. The presence of plasmid-encoded resistance–virulence modules and the widespread occurrence of ISs suggest an active mobilome facilitating gene exchange and adaptation across ecological boundaries. Comparative phylogenomic analysis across Africa revealed geographically structured transmission dynamics, marked by the emergence of regionally dominant clones and country-specific evolutionary trajectories. These findings reflect broader challenges in AMR control, particularly in LMICs where food safety infrastructure and genomic surveillance remain limited.

To mitigate the growing threat of foodborne antimicrobial resistance, we advocate for enhanced genomic monitoring of foodborne pathogens, stringent regulation of antimicrobial use in agriculture, and integration of One Health strategies across the human–animal–environment interface. The insights presented here serve as a foundation for future regional surveillance initiatives and emphasize the need for proactive, genomics-informed interventions to safeguard public health.

Data Availability Statement

The sequence data supporting this study are available in GenBank under BioProject Accession Number PRJNA928459. All additional data generated or analyzed during this study are included within this manuscript and its supporting information.

Ethics Statement

Institutional approval for the study protocol and sampling approach was granted by the Department of Microbiology,

Adekunle Ajasin University (Approval Reference: DM: AAU/2021). All meat samples were obtained from open retail markets in Akungba-Akoko, Nigeria, and were purchased anonymously as part of routine food supply, without involving live animals or interventions. The objectives of the study were clearly explained to meat vendors to ensure transparency and voluntary participation in the sampling process.

Disclosure

The authors acknowledged that a preprint (10.1101/2025.04.15.648955v1) [41] has been published; a reference to this preprint has been included in the reference list accordingly.

Conflicts of Interest

The authors declare no conflicts of interest.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. *(Supporting Information)* Table S1: List of *E. faecalis* genomes from African countries used in comparative phylogenomic analysis, including metadata such as country of origin, year of isolation, and sequence type.

References

[1] M. Farman, M. Yasir, R. R. al-Hindi, et al., "Genomic Analysis of Multidrug-Resistant Clinical *Enterococcus faecalis* Isolates for Antimicrobial Resistance Genes and Virulence Factors From the Western Region of Saudi Arabia," *Antibiotic Resistance and Infection Control* 8, no. 1 (2019): 55, https://doi.org/10.1186/s13756-019-0508-4.

[2] M. de Mesquita Souza Saraiva, K. Lim, D. F. M. do Monte, et al., "Antimicrobial Resistance in the Globalized Food Chain: A One Health Perspective Applied to the Poultry Industry," *Brazilian Journal of Microbiology* 53, no. 1 (2022): 465–486, https://doi.org/10.1007/s42770-021-00635-8.

[3] K. Hegstad, T. Mikalsen, T. M. Coque, G. Werner, and A. Sundsfjord, "Mobile Genetic Elements and Their Contribution to the Emergence of Antimicrobial-Resistant *Enterococcus faecalis* and *Enterococcus faecium*," *Clinical Microbiology and Infection* 16, no. 6 (2010): 541–554, https://doi.org/10.1111/j.1469-0691.2010.03226.x.

[4] D. S. Daniel, S. M. Lee, H. M. Gan, G. A. Dykes, and S. Rahman, "Genetic Diversity of *Enterococcus faecalis* Isolated From Environmental, Animal and Clinical Sources in Malaysia," *Journal of Infection and Public Health* 10, no. 5 (2017): 617–623, https://doi.org/10.1016/j.jiph.2017.02.006.

[5] L. Guan, M. Beig, L. Wang, et al., "Global Status of Antimicrobial Resistance in Clinical *Enterococcus faecalis* Isolates: Systematic Review and Meta-Analysis," *Annals of Clinical Microbiology and Antimicrobials* 23, no. 1 (2024): 80, https://doi.org/10.1186/s12941-024-00728-w.

[6] I. N. Okeke, A. O. Aboderin, A. Egwuenu, et al., "Establishing a National Reference Laboratory for Antimicrobial Resistance Using a Whole-Genome Sequencing Framework: Nigeria's Experience," *Microbiology* 168, no. 8 (2022): 001208, https://doi.org/10.1099/mic.0.001208.

[7] M. D. Ndahi, R. Hendriksen, B. Helwigh, et al., "Determination of Antimicrobial Use in Commercial Poultry Farms in Plateau and Oyo States, Nigeria," *Antimicrobial Resistance and Infection Control* 12, no. 1 (2023): 30, https://doi.org/10.1186/s13756-023-01235-x.

[8] L. Schnirring, *Survey Reveals High Antibiotic Use in Nigerian Poultry Production* (CIDRAP, 2023), Retrieved from https://www.cidrap.umn.edu/antimicrobial-stewardship/survey-reveals-high-antibiotic-use-nigerian-poultry-production (accessed on April 5, 2025).

[9] J. Monteiro Marques, M. Coelho, A. R. Santana, D. Pinto, and T. Semedo-Lemsaddek, "Dissemination of Enterococcal Genetic Lineages: A One Health Perspective," *Antibiotics* 12, no. 7 (2023): 1140, https://doi.org/10.3390/antibiotics12071140.

[10] K. E. Raven, S. Reuter, T. Gouliouris, et al., "Genome-Based Characterization of Hospital-Adapted *Enterococcus faecalis* Lineages," *Nature Microbiology* 1, no. 3 (2016): 15033, https://doi.org/10.1038/nmicrobiol.2015.33.

[11] R. S. Alimi, "An Analysis of Meat Demand in Akungba-Akoko, Nigeria," *Nigerian Journal of Applied Behavioural Sciences* 1 (2013): 96–104.

[12] Clinical and Laboratory Standards Institute (CLSI), *Performance Standards for Antimicrobial Susceptibility Testing* (CLSI, 33rd edition, 2023), Retrieved from http://www.clsi.org.

[13] S. Conceição, M. C. Queiroga, and M. Laranjo, "Antimicrobial Resistance in Bacteria From Meat and Meat Products: A One Health Perspective," *Microorganisms* 11, no. 10 (2023): 2581, https://doi.org/10.3390/microorganisms11102581.

[14] M. Aslam, M. S. Diarra, S. Checkley, V. Bohaychuk, and L. Masson, "Characterization of Antimicrobial Resistance and Virulence Genes in *Enterococcus* spp. Isolated From Retail Meats in Alberta, Canada," *International Journal of Food Microbiology* 156, no. 3 (2012): 222–230, https://doi.org/10.1016/j.ijfoodmicro.2012.03.026.

[15] J. R. Hayes, L. L. English, P. J. Carter, et al., "Prevalence and Antimicrobial Resistance of *Enterococcus* Species Isolated From Retail Meats," *Applied and Environmental Microbiology* 69, no. 12 (2003): 7153–7160, https://doi.org/10.1128/AEM.69.12.7153-7160.2003.

[16] B. Krawczyk, P. Wityk, M. Gałęcka, and M. Michalik, "The Many Faces of *Enterococcus* spp.—Commensal, Probiotic and Opportunistic Pathogen," *Microorganisms* 9, no. 9 (2021): 1900, https://doi.org/10.3390/microorganisms9091900.

[17] C. A. Arias and B. E. Murray, "The rise of the *Enterococcus*: Beyond Vancomycin Resistance," *Nature Reviews Microbiology* 10, no. 4 (2012): 266–278, https://doi.org/10.1038/nrmicro2761.

[18] T. F. Landers, B. Cohen, T. E. Wittum, and E. L. Larson, "A Review of Antibiotic Use in Food Animals: Perspective, Policy, and Potential," *Public Health Reports* 127, no. 1 (2012): 4–22, https://doi.org/10.1177/003335491212700103.

[19] F. Lebreton, R. J. Willems, and M. S. Gilmore, "*Enterococcus* Diversity, Origins in Nature, and Gut Colonization," in *Enterococci: From Commensals to Leading Causes of Drug Resistant Infection*, eds. M. S. Gilmore, D. B. Clewell, Y. Ike, and N. Shankar (Massachusetts Eye and Ear Infirmary, 2014).

[20] S. J. van Hal, C. L. C. Ip, M. A. Ansari, et al., "Evolutionary Dynamics of *Enterococcus faecium* Reveals Complex Genomic Relationships Between Isolates With Independent Emergence of Vancomycin Resistance," *Microbial Genomics* 2, no. 1 (2016): e000048, https://doi.org/10.1099/mgen.0.000048.

[21] S. H. Bae, S. Yoon, K. Kim, Y. B. Kim, and Y. J. Lee, "Comparative Analysis of Chloramphenicol-Resistant *Enterococcus faecalis* Isolated From Dairy Companies in Korea," *Veterinary Sciences* 8, no. 8 (2021): 143, https://doi.org/10.3390/vetsci8080143.

[22] WHO, *No Time to Wait: Securing the Future From Drug-Resistant Infections* (Report to the Secretary-General of the United Nations, 2019), Retrieved from https://www.who.int/publications/i/item/no-time-to-wait-securing-the-future-from-drug-resistant-infections (accessed on April 1, 2025).

[23] C. J. Kristich, L. B. Rice, and C. A. Arias, "Enterococcal Infection—Treatment and Antibiotic Resistance," in *Enterococci: From Commensals to Leading Causes of Drug Resistant Infection*, eds. M. S. Gilmore, D. B. Clewell, Y. Ike, and N. Shankar (Massachusetts Eye and Ear Infirmary, 2014).

[24] R. J. Willems, J. Top, W. van Schaik, et al., "Restricted Gene Flow Among Hospital Subpopulations of *Enterococcus faecium*," *mBio* 3, no. 4 (2012): e00151, https://doi.org/10.1128/mBio.00151-12.

[25] W. van Schaik, J. Top, D. R. Riley, et al., "Pyrosequencing-Based Comparative Genome Analysis of the Nosocomial Pathogen *Enterococcus faecium* and Identification of a Large Transferable Pathogenicity Island," *BMC Genomics* 11, no. 1 (2010): 239, https://doi.org/10.1186/1471-2164-11-239.

[26] S. R. Nallapareddy, K. V. Singh, J. Sillanpaa, et al., "Endocarditis and Biofilm-Associated Pili of *Enterococcus faecalis*," *Journal of Clinical Investigation* 116, no. 10 (2006): 2799–2807, https://doi.org/10.1172/JCI29021.

[27] P. Șchiopu, D. A. Toc, I. A. Colosi, et al., "An Overview of the Factors Involved in Biofilm Production by the *Enterococcus* Genus," *International Journal of Molecular Sciences* 24, no. 14 (2023): 11577, https://doi.org/10.3390/ijms241411577.

[28] A. O. Johnson, B. M. Shipman, B. C. Hunt, et al., "Function and Contribution of Two Putative *Enterococcus faecalis* Glycosaminoglycan Degrading Enzymes to Bacteremia and Catheter-Associated Urinary Tract Infection," *Infection and Immunity* 92, no. 7 (2024): e0019924, https://doi.org/10.1128/iai.00199-24.

[29] D. Van Tyne, M. J. Martin, and M. S. Gilmore, "Structure, Function, and Biology of the Enterococcus *faecalis* Cytolysin," *Toxins* 5, no. 5 (2013): 895–911, https://doi.org/10.3390/toxins5050895.

[30] J. X. Zheng, Y. Wu, Z. W. Lin, et al., "Characteristics of and Virulence Factors Associated With Biofilm Formation in Clinical *Enterococcus faecalis* Isolates in China," *Frontiers in Microbiology* 8 (2017): 2338, https://doi.org/10.3389/fmicb.2017.02338.

[31] E. Fiore, D. Van Tyne, and M. S. Gilmore, "Pathogenicity of *Enterococci*," *Microbiology Spectrum* 7, no. 4 (2019): https://doi.org/10.1128/microbiolspec.GPP3-0053-2018.

[32] S. M. McBride, V. A. Fischetti, D. J. LeBlanc, R. C. Moellering, and M. S. Gilmore, "Genetic Diversity Among *Enterococcus faecalis*," *PLoS One* 2, no. 7 (2007): e582, https://doi.org/10.1371/journal.pone.0000582.

[33] K. S. Baker, E. Jauneikaite, K. L. Hopkins, et al., "Genomics for Public Health and International Surveillance of Antimicrobial Resistance," *Lancet Microbe* 4, no. 12 (2023): e1047–e1055, https://doi.org/10.1016/S2666-5247(23)00283-5.

[34] H. M. Kajumbula, D. G. Amoako, S. K. Tessema, et al., "Enhancing Clinical Microbiology for Genomic Surveillance of Antimicrobial Resistance Implementation in Africa," *Antimicrobial Resistance & Infection Control* 13, no. 1 (2024): 135, https://doi.org/10.1186/s13756-024-01472-8.

[35] H. Tegally, J. E. San, M. Cotten, et al., "The Evolving SARS-CoV-2 Epidemic in Africa: Insights From Rapidly Expanding Genomic Surveillance," *Science* 378, no. 6615 (2022): eabq5358, https://doi.org/10.1126/science.abq5358.

[36] R. Zaheer, S. R. Cook, R. Barbieri, et al., "Surveillance of *Enterococcus spp*. Reveals Distinct Species and Antimicrobial Resistance Diversity Across a One-Health Continuum," *Scientific Reports* 10, no. 1 (2020): 3937, https://doi.org/10.1038/s41598-020-61002-5.

[37] F. Baquero, J. L. Martínez, V. F. Lanza, et al., "Evolutionary Pathways and Trajectories in Antibiotic Resistance," *Clinical Microbiology Reviews* 34, no. 4 (2021): e00050, https://doi.org/10.1128/CMR.00050-19.

[38] M. J. Bottery, J. W. Pitchford, and V. P. Friman, "Ecology and Evolution of Antimicrobial Resistance in Bacterial Communities," *ISME Journal* 15, no. 4 (2021): 939–948, https://doi.org/10.1038/s41396-020-00832-7.

[39] M. A. Salam, M. Y. Al-Amin, M. T. Salam, et al., "Antimicrobial Resistance: A Growing Serious Threat for Global Public Health," *Healthcare* 11, no. 13 (2023): 1946, https://doi.org/10.3390/healthcare11131946.

[40] A. J. Tatem, D. J. Rogers, and S. I. Hay, "Global Transport Networks and Infectious Disease Spread," *Advances in Parasitology* 62 (2006): 293–343, https://doi.org/10.1016/S0065-308X(05)62009-X.

[41] C. A. Osunla, A. Akinbobola, A. Elshafea, et al., "Genomic and Bioinformatic Insights Into Enterococcus faecalis From Retail Meats in Nigeria," *bioRxiv* (2025): https://doi.org/10.1101/2025.04.15.648955v1.