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Resistome, mobilome, virulome analysis and phylogenomics of *Enterococcus faecalis* isolated from raw muscle foods of beef origin in Gauteng, South Africa

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ABSTRACT

Enterococcus faecalis is a ubiquitous bacterium found in various environments, including processed beef meat, and is known for its importance in both food safety and public health. This pivotal significance stems not solely from its virulence but also from its adeptness in eliciting multidrug-resistant infections in humans. The aim of this study was to investigate the population structure, resistome, mobilome, and virulome of *E. faecalis* obtained from processed beef meat sources in South Africa. A total of eight genomes sequenced in this study were examined, alongside 78 publicly available, high-quality genomes of *E. faecalis*, with a comprehensive analysis conducted to identify antimicrobial resistance (AMR) determinants, virulence factors, and mobile genetic elements (MGE). Six distinct sequence types (STs) (ST79, ST860, ST40, ST238, ST21, and ST700) and 41 core virulence factors were found across all the genomes. The virulence factors included genes encoding adherence (*ace, asa1, Ef0485, ebpA, ebpB, ebpC, srtC*); exoenzyme (*Ef3023, Ef0818, gelE, sprE*); immunomodulation (*cpsA, cpsB, cpsC, cpsD, cpsE, cpsF, cpsG, cpsH, cpsI, cpsK*), and biofilm formation (*bopD, fsrA, fsrB, fsrC*). In addition, AMR genes were identified across all genomes, which include aminoglycoside resistance (*ant(6)-Ia*), trimethoprim resistance (*dfrA*), drug and biocide resistance (*efrA* and *efrB*), multidrug efflux pump (*emeA*), clindamycin quinupristin-dalfopristin, dalfopristin resistance (*lsaA*), and tetracycline resistance (*tetM*). The genomes of *E. faecalis* sequenced here contained a variety of MGEs, including Insertion Sequences (ISs), transposons, prophages, and plasmids, which may have facilitated genetic exchange within and between these species. The results highlight that beef meat products act as a reservoir for virulent *E. faecalis* strains possessing antibiotic-resistance traits. This study provides insight into the genomic characteristics, antimicrobial resistance genes, virulence factors, and genetic mobile elements associated with eight *E. faecalis* isolates from processed beef meat in the Gauteng province of South Africa.

1. Introduction

Enterococci inhabit the gastrointestinal tract of diverse animals, humans, insects, and nematodes [1,2]. They are Gram-positive lactic acid bacteria, non-spore-forming facultative anaerobes that thrive in various environments like soil, water, food, and feed for extended periods [3]. Due to their preference for the intestines, widespread presence, durability, and ease of cultivation, enterococci serve as indicators of faecal contamination for ensuring water and food hygiene [4]. Enterococci are one of the key indicators for antimicrobial resistance (AMR) in human and veterinary surveillance systems [5]. Despite their use in starter cultures, food fermentation, preservation, and as

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probiotics, *E. faecium* and *E. faecalis* are pathogenic. They contribute significantly to hospital-acquired infections, particularly among immunocompromised individuals like the elderly, infants, and those with weak immune systems [6]. The infections associated with the bacteria species include bacteremia, endocarditis, urinary tract, intra-abdominal, meningitis, surgical site, and device-associated infections [2].

Although *E. faecalis* is a prime cause of hospital-acquired infections, it's also found in various foods like vegetables, dairy, fish, and meats, possibly contributing to human transmission [3,7,8]. Several studies have demonstrated the transmission of *E. faecalis* from animals to humans through the consumption of contaminated food [9–11]. Despite being a mild pathogen, *E. faecalis* forms biofilms and acquires mobile genetic elements (MGEs) carrying drug resistance, notably to drugs like vancomycin, posing treatment challenges [2,12]. Moreover, due to intrinsic resistance mechanisms, the therapeutic options for *Enterococcus* infections are limited [12].

Diverse kinds of virulence factors have been reported to enhance the pathogenicity of *E. faecalis* strains by enabling the colonization and invasion of host tissue, translocation through epithelial cells, and evasion from the host's immune response [13]. The major virulence factors associated with the pathogenicity of enterococci virulent strains include enterococcal surface proteins (*Esp*), hyaluronidase (*Hyl*) aggregation substance (AS), gelatinase (*gelE*), and cytolysin (*Cyl*) [14,15]. In addition to having a variety of virulence factors and resistance genes, *E. faecalis* is also highly proficient at exchanging and transmitting many of these genes through horizontal gene transfer [15,16]. It has been reported that over the past ten years, genes for antibiotic resistance have been transferred between different strains of *E. faecium* as well as vancomycin resistance from *E. faecalis* to *Staphylococcus aureus* [10,17,18]. Epidemiological typing of *E. faecalis* has mainly been performed by pulsed-field gel electrophoresis. However, the advent of whole genome sequencing (WGS) has facilitated a more comprehensive and intricate examination of enterococcal antimicrobial resistance genes (ARGs), phylogenetics, and virulence [19,20]. As WGS becomes increasingly accessible and cost-effective, numerous previously stored isolate collections are being re-evaluated and compared with new isolates [21]. Subsequent to WGS, the adoption of novel sequence typing techniques for enterococci, like core-genome multi-locus sequence typing (cgMLST), has enabled enhanced analysis of relatedness among isolates from diverse sample sources [22]. WGS also permits the detection of emerging strains, outbreak analysis, and the delineation of resistance and virulence genes, including their genomic context and positioning [23]. There is a dearth of information about the role of this pathogen in food safety in South Africa (SA). However, there are a few studies on the WGS of enterococci in SA limited to the characterization of human clinical strains [20,21,22]. Therefore, the proliferation of *E. faecalis* as well as the specific genetic traits that determine its virulence and ability to acquire AMR in meat and meat products, must be closely monitored [24–29]. Therefore, the aim of the study was to employ WGS for exploring the population structure, resistome, mobilome, and virulome of *E. faecalis* obtained from beef meat products in South Africa.

2. Materials and methods

2.1. Bacterial isolation

The *E. faecalis* strains sequenced in this study were isolated from raw processed beef meat samples submitted to the Agricultural Research Council-Onderstepoort Veterinary Research (ARC-OVR) General Bacteriology Laboratory Section for routine diagnostic services (Table 1). From each sample, 10 g (ratio 1:10) were homogenized into 90 mL buffered peptone water, and then aliquots of 0.1 mL were inoculated onto a plate of bile esculin agar and a KF *Streptococcus* agar (both Oxoid, ThermoFisher, Johannesburg) and incubated for 18–24 h at 37 °C. Presumptive enterococci colonies were streaked onto blood agar supplemented with 5% sheep blood (Oxoid, ThermoFisher, Johannesburg), incubated for another 18–24 h at 37 °C, and identified by phenotypic characteristics and biochemical tests including Gram staining, catalase test, Lancefield grouping, lactose, arabinose, sorbitol, and mannitol.

2.2. Whole-genome sequencing, quality control, and de novo assembly

WGS of the isolates was performed at the Agricultural Research Council-Biotechnology Platform (ARC-BTP), Onderstepoort, Pretoria, South Africa. The DNA libraries were prepared using TruSeq and Nextera DNA library preparation kits (Illumina, San Diego, CA, USA), followed by 2 × 300 bp reads sequencing on Illumina HiSeq 2500 instrument (Illumina, San Diego, CA, USA) with 100 coverage. Quality control, including adapter removal of the raw data, was done using BBDuk v.37.90 (https://jgi.doe.gov/data-and-tools/bbtools/bb-tools-user-guide/bbduk-guide/). SPAdes v.3.12, [30] was used to create a *de novo* assembly of each isolate. QUAST v5.0.2 [31] was used to evaluate the quality of each resulting assembled genome. The genomes created were annotated using Prokka v.1.14.0 [32].

2.3. Acquisition and quality control of publicly available E. faecalis genomes

A total of 78 publicly available *E. faecalis* genomes from the National Center for Biotechnology Information (NCBI) database were downloaded (accessed 15 November 2022), and quality was assessed using QUAST as described above.

2.4. Taxonomic assignment

The 78 publicly available *E. faecalis* genomes from various sources of isolation and the eight meat isolates sequenced here were assigned to species level using the Genome Taxonomy Database Toolkit (GTDB-Tk) v.2.1.0 “classify_wf” workflow (default settings) and version R207_v2 of GTDB [30,33]. Pairwise average nucleotide identity (ANI) values were calculated between all 86 *E. faecalis* genomes using the command-line implementation of OrthoANI v1.40 [34] with default settings.

2.5. Multi-locus sequence typing and population structure analysis

Multi-locus sequence type (MLST) scheme were obtained from the

Table 1
Number of *Enterococcus faecalis* genomes sequenced in this study ($n = 8$).

Strain	Year of Isolation	Province	Animal	Sample Type	Isolation Source	Establishment Category	GTDB Species	MLST analysis	ANI Values
S92	2015	Gauteng	Cattle	Mince	Processed meat	Butchery	*Enterococcus faecalis*	ST79	96.8
S109	2015	Gauteng	Cattle	Mince	Processed meat	Retail	*Enterococcus faecalis*	ST860	98.4
S111	2015	Gauteng	Cattle	Patties	Processed meat	Butchery	*Enterococcus faecalis*	ST40	97.2
S116	2015	Gauteng	Cattle	Mince	Processed meat	Butchery	*Enterococcus faecalis*	ST860	98.2
S119	2015	Gauteng	Cattle	Wors	Processed meat	Retail	*Enterococcus faecalis*	ST238	97.0
S121	2015	Gauteng	Cattle	Mince	Processed meat	Butchery	*Enterococcus faecalis*	ST40	97.6
S131	2015	Gauteng	Cattle	Wors	Processed meat	Butchery	*Enterococcus faecalis*	ST21	98.3
S134	2015	Gauteng	Cattle	Mince	Processed meat	Butchery	*Enterococcus faecalis*	ST700	98.1

Enterococcus database hosted by the Pasteur Institute, France (https://pubmlst.org/organisms/enterococcus-faecalis). The *Enterococcus* MLST database contains 7 loci which were used to determine the sequence types of the assembled isolates. MLST v.2.18.0 program [35] was used to align all *de novo* assembled isolates against the MLST scheme to determine the sequence types for each of the genomes. The pan-genome composition was extracted using Roary v.3.12.0 [36] and a core-genome phylogenetic tree constructed with IQ-TREE v.1.6.6 [37]. Pan-genome clusters were defined as follows: core genes present in all isolates; softcore genes present in at least 95% of isolates; shell genes present between 15% and 95% of isolates; cloud genes in <15% of isolates. The core-genome phylogenetic tree was visualized using ggtree v.1.16.6 [38].

2.6. Phylogenetic reconstruction

Whole genome sequences of the *E. faecalis* isolates from the current study were compared with isolates curated from the GenBank website from different countries, including SA. SNPs were identified among all 86 *E. faecalis* genomes used in the study with kSNP3 v.3.92 [36,37] using default setting. The resulting core SNP alignment was used as input to IQ-TREE v.1.5.4 [37], which was used to construct a maximum likelihood (ML) phylogeny. The generated phylogenetic trees were viewed, annotated, and edited using ggtree v.1.16.6 [38].

2.7. The presence of antimicrobial resistance and virulence genes, their plasmid, and genomic context in E. faecalis

The presence of acquired and intrinsic virulence factors and resistance genes was determined using Abricate v.1.0.1 [32] with Virulence Factor Database (VFDB) (http://www.mgc.ac.cn/VFs/), ResFinder, the Comprehensive Antibiotic Resistance Database (CARD), and megares databases as input [41]. The Abricate v.1.0.1 [32] was used with default parameters. The plasmids associated with *E. faecalis* were determined by PlasmidFinder v.2.1 with default parameters [42]. The assembled genomes were further analyzed for MGEs, including insertion sequences, using ISFinder [43] and intact prophages using MobileElementFinder [46] and PHASTER [44], respectively. Integrative Conjugative Elements (ICE) and integrative and mobilizable elements (IME) were identified using the MobileElementFinder [45]. The virulence factors, resistance genes, and MGEs were considered significant if the identify and coverage percentage were > 90%.

3. Results

3.1. Genomic features population structure and pan-genome analysis of E. faecalis from meat isolates

An overview of the *E. faecalis* genome characteristics of the isolates sequenced in this study is presented in Table S1. The *E. faecalis* genome size ranged from 2.89 to 3.2 Mbp. The GC content of these genomes ranged from 37.19 to 37.51. The pan-genome analyses of the *E. faecalis* genomes revealed 11,656 pan-genome genes and 1690 core genes. The partitioning of genes across the pan-genome was as follows: core, 1690; softcore, 346; shell, 1313; and cloud, 8307 genes.

Eight strains of *Enterococcus* that had been isolated from processed beef products collected from Gauteng province in 2015 and 2016 underwent WGS and were assigned to the *E. faecalis* genomospecies using the Genome Taxonomy Database (GTDB) (Table 1). The MLST analysis revealed that the eight isolates sequenced here were assigned to six different STs (Table 1). *E. faecalis* ST40 and ST860 were reported from two isolates recovered from patties and mince, respectively. The other identified STs were ST21 (processed wors meat), ST79 (processed mincemeat), ST238 (processed wors meat), and ST700 (processed mincemeat). The MLST of the 78 publicly available genomes varied across these isolates, with ST9 ($n = 6$), ST476 ($n = 6$), and ST40 ($n = 5$) being the most frequently observed STs (Fig. 1 and Table S1).

3.2. Reference-free single nucleotide polymorphism identification and core-SNP phylogenetic clustering of E. faecalis

The 86 *E. faecalis* genomes from various sources of isolation used in this study were compared by means of phylogenomic analysis (Fig. 1). A total of 11,419 core-SNPs was identified across all the genomes. The core-SNP phylogenetic clustering revealed that the two genomes (S116 and S109) from this study clustered together and separated from the rest of the genomes. These genomes belonged to ST860 and were isolated from processed minced meat (Fig. 1). The S92 genome, which was assigned to ST79, branched separately from the clade dominated by ST79 genomes of African origin. The genomes of S134 and S131 clustered together and were closely related to two genomes (S39–4 and DRD-158), both belonging to ST19 from Europe, which was isolated from faecal and cheese samples, respectively. A similar trend was observed with isolates S111 and S121 (Fig. 1). Sample S119 was observed in a clade comprising of genomes from Asia and Europe and closely related to BL25_10 isolated from milk (Fig. 1). The eight genomes sequenced here shared >95% ANI. The ANI values corroborated the core-genome phylogeny analysis and MLST prediction results (Table S1). Another observation from these results was that the isolates sequenced from this study did not cluster with any of the other genomes from South Africa or other African countries (Fig. 1).

3.3. Virulence factors associated with E. faecalis strains from meat isolates

Genes coding for known virulence factors were identified by comparing the *E. faecalis* genomes against the Virulence Factor Database (VFDB), along with manual searches for putative virulence gene sequences that have been previously described in *E. faecalis*. A total of 30 different virulence genes were identified in our sequenced isolates and a total of 41 different virulence genes were identified across all the isolates (Fig. 2 and Table S3). The major *E. faecalis* virulence genes identified were associated with adherence (*ace, asa1, Ef0485, ebpA, ebpB, ebpC, srtC*); exoenzyme (*Ef3023, Ef0818, gelE, sprE*); immunomodulation (*cpsA, cpsB, cpsC, cpsD, cpsE, cpsF, cpsG, cpsH, cpsI, cpsK*), and biofilm formation (*bopD, fsrA, fsrB, fsrC*) (Fig. 2). The virulence-associated genes that were present in all isolates sequenced in this study included biofilm formation-associated genes (*bopD*); undecaprenyl diphosphate synthase gene (*cpsA*), and phosphatidate cytidylyltransferase (*cpsB*); endocarditis and biofilm-associated pilus (*ebpA, ebpB, and ebpC*); endocarditis specific antigen (*efaA*), and sortase (*srtC*) (Fig. 2). The exotoxin operon, which comprises of eight genes, *i.e. cylA/B/I/L/M/R1/R2/S* was not detected in the isolates sequenced in this study but was observed in other publicly available isolates used in the current study (Fig. 2). Other virulence-associated genes identified were the fibrinogen binding protein (*fss1–3*), and aggregation substance (*PrgB/Asc10*).

3.4. South African E. faecalis strains from meat isolates display multidrug-resistant capabilities.

Several AMR genes were present in the *E. faecalis* isolates from this study and were associated with predicted resistance to different antimicrobials classes. The putative AMR phenotypes observed include aminoglycoside resistance (*ant(6)-Ia*), trimethoprim resistance (*dfrA*), drug and biocide resistance (*efrA* and *efrB*), multidrug efflux pump (*emeA*), clindamycin quinupristin-dalfopristin, dalfopristin resistance (*lsaA*), and tetracycline resistance (*tetM*) (Table S2). All our genomes carried these AMR genes, with the exception of the *IsaA*, *tetM*, and *ant(6)-Ia_2*, which were found in 75% ($n = 6$), 62% ($n = 5$), and 12% ($n = 1$) of our isolates, respectively. The AMR elements observed in our genomes were also present in the majority of the other publicly available genomes used in this study (Table S2). The quinolone resistance determinant

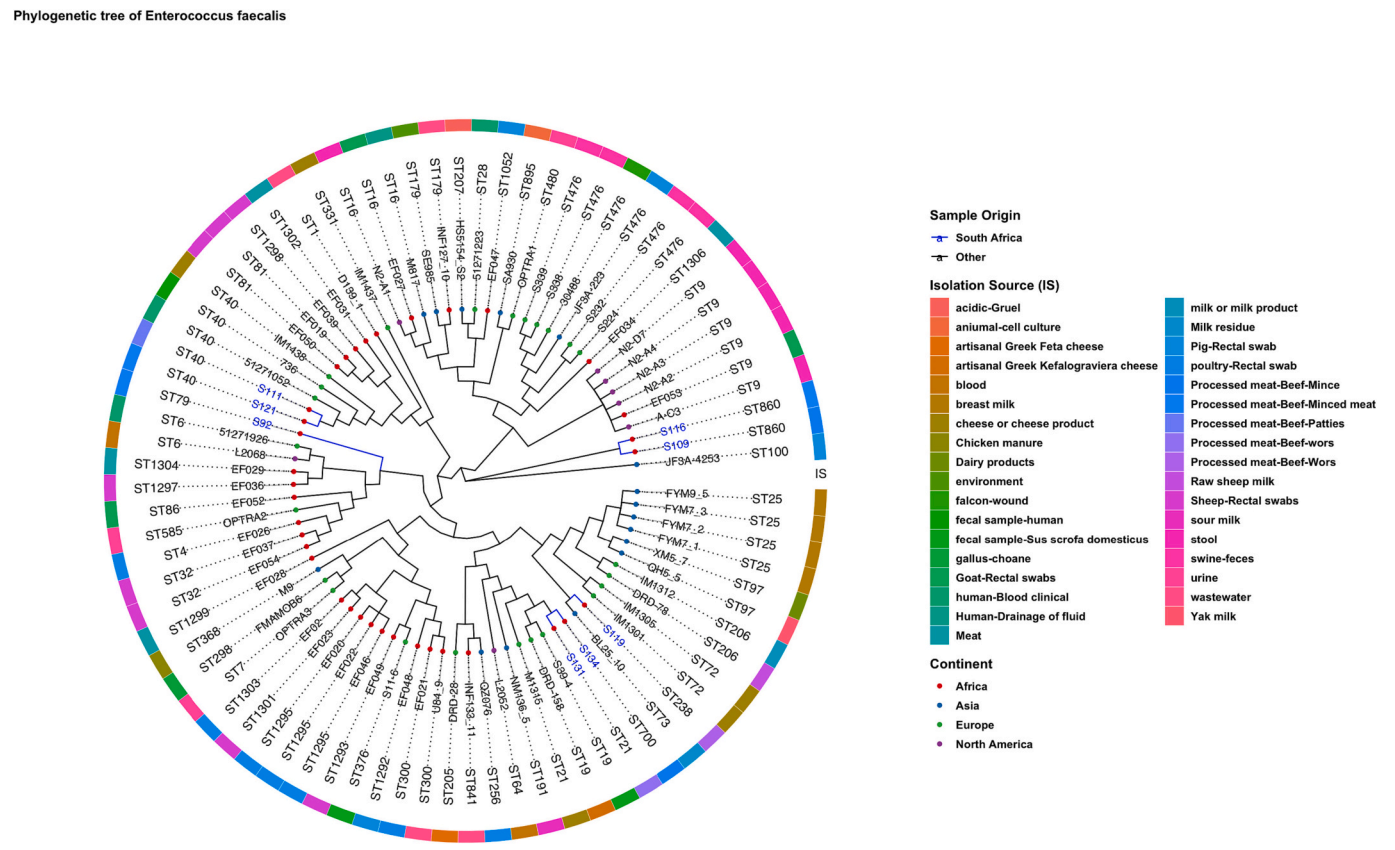

Fig. 1. Phylogenetic tree based on SNP differences in the core genomes of the *Enterococcus faecalis* strains isolated from meat isolates in South Africa in comparison to the publicly available datasets from humans, animals, environment, and food samples. Tip label colors denote the continent from which each strain was reportedly isolated. The branch colored in blue denote genomes sequenced in this study. The ring surrounding the phylogeny denotes the isolation source reported for each strain. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

regions (*QRDRs*) of the DNA gyrase (*gyrA*) and DNA topoisomerase IV genes (*parC*), were analyzed for point mutations in all isolates. The *parC* (*parC:p.E310K* and *parC:p.G250A*) showed putatively novel mutations that were not linked to any known phenotypic resistance in isolates S109 and S119, and the *gyrA* gene was found without point mutations (Table 2). Interestingly, the quaternary ammonium compound, a small multidrug resistance (SMR) family of drug efflux pump resistance genes *qacJ* and *qacH* were only detected in isolate S116 from minced meat collected in butchery. These genes provide resistance against the common detergents used in the food industry. Overall, these results shows that various AMR determinants are variably present within *E. faecalis* genomes may harbour AMR determinants predictive of an MDR phenotype. However, these results should be interpreted with caution, as AMR potential was not evaluated phenotypically in this study.

3.5. Characterization of MGEs found in South African E. faecalis genomes from meat isolates

PlasmidFinder detected a total of 10 different plasmids replicons at 95% identity, including pS86, rep7a_16_repC (Cassette), pAD1, pBEE99, EF62pC, pCF10, pPD1, pVEF3, DOp1, and pTEF1 in the 8 genomes sequenced in this study (Tables 3, 4). The most common plasmid replicons were DOp1 ($n = 4$; 36%) followed by pS86, rpAD1, EF62pC, pVEF3 which were all found in 18% ($n = 2$) of the genomes sequenced in this study. Isolate S119 had the highest number of plasmids replicons in comparison to the rest of the isolates. Intact prophages were found in all our genomes analyzed in this study. The intact prophages that were detected are Entero_phiFL2A_NC_013643 ($n = 3$; 37.5%), Entero_phiFL1ANC_013646 ($n = 2$; 25%), Entero_phiEf11_NC_013696 ($n = 2$; 25%), Entero_phiFL4A_NC_013644 ($n = 1$; 12.5%), Lactob_Lj928_NC_005354 ($n = 1$; 12.5%), and Lister_LP_101_NC_024387 ($n = 1$; 12.5%) (Table 4). A total of 3 IS families were detected in our *E. faecalis* genomes, including IS6, IS256, and IS982. The insertion sequences belonging to these families were ISEfm1, ISEfm2, ISEnfa4, ISLgar5, and ISS1N (Table 4). Five isolates harboured Tn6009 (Table 4). Transposons (cn_8629_ISS1N, cn_18965_ISS1N) were also detected in isolate S134 (Table 4). The comparison of the mobile genetic elements between the current genomes and publicly available genomes shows that these genomes harbour similar plasmids replicons and prophages.

3.6. The associations of gene-carrying MGEs and antimicrobial resistance in E. faecalis genomes from meat isolates

Mobile Element Finder, classifies AMR genes as either MGE-associated or carried by an MGE, depending on their relative location. In the current study, AMR genes were not carried by MGEs but instead were located in proximity to one or more MGEs (Fig. 3). The AMR genes *tetM* and *ClpL* were associated with transposons (Tn6009), an Integrative Conjugative Element (ICE) located on the same contig. The association of AMR genes with plasmid replicons was also observed (Table 3). Most associations were found on contigs with the same plasmid replicons (repUS43) except for isolate S116 which had two plasmid replicons (repUS43 and rep9a) (Table 3). Gene-carrying prophages in *E. faecalis* genomes were further detected. The results show that intact prophages from isolate S119 were found to harbour virulence genes, including *ebpA, ebpB, ebpC,* and *srtC*. None of these prophages harboured any AMR genes. The MGEs in this study were found to be associated with virulence genes *agg* and *cCF10* located on the same contig.

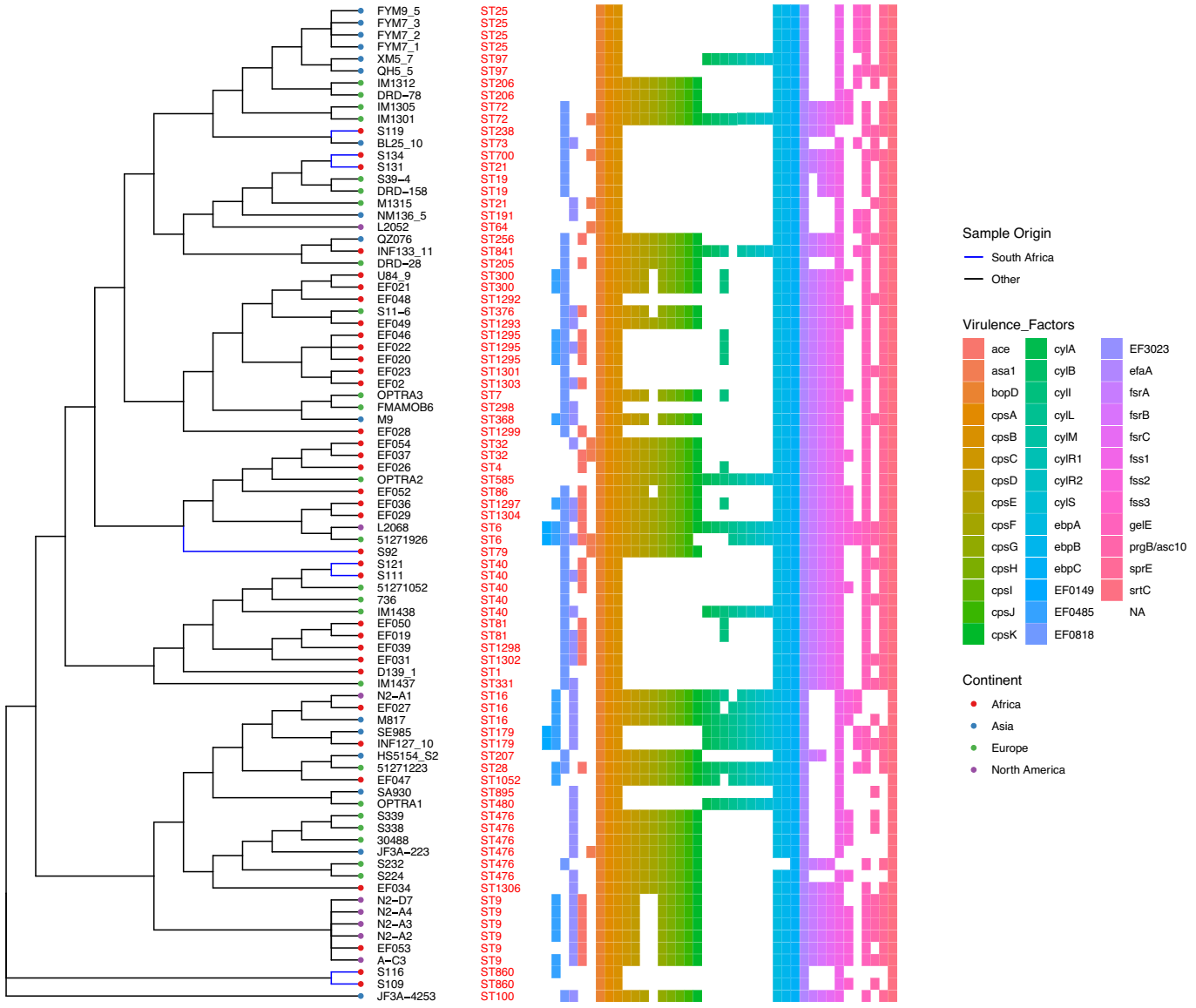

Fig. 2. Virulence factors associated with *E. faecalis* strains. The heatmap shows the presence (various colors) and absence (white colour) of the virulence genes from all the isolated, including the publicly available dataset used. Tip label colors denote the continent from which each strain was reportedly isolated. The branch colored in blue denote genomes sequenced in this study. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Point mutation in the *gyrA* and *parC* (quinolone resistance) genes in South African *E. faecalis* genomes from meat isolates.

Isolates	Mutation	*parC*		*GyrA*	
		Nucleotide change	**Amino acid change**	**Nucleotide change**	**Amino acid change**
S109	parC:p.E310K	gaa - > aaa	e - > k	No hits	No hits
S119	parC:p.G250A	ggc - > gcc	g - > a	No hits	No hits

Table 3
Mobile genetic elements associated with antimicrobial resistance genes in South African *E. faecalis* genomes from meat isolates.

Isolates	Sequence types (STs)	Contig number	Association of accessory genes plasmid replicons and MGEs		
			MGEs/ plasmid replicons	**ARGS**	**Virulence**
S119	ST238	8	repUS43, Tn6009	*tet(M), ClpL*	*Agg*
S116	ST860	9	repUS43, rep9a, Tn6009	*tet(M)*	*Agg*
S109	ST860	12	repUS43, Tn6009	*tet(M)*	
S134	ST700	2	repUS43, Tn6009	*tet(M)*	*cCF10*

4. Discussion

The MLST approach has widely been utilised in studying the population structure of *E. faecalis* [43,44]. In the current study, MLST identified six different sequence types (ST21, ST40, ST79, ST238, ST700, and ST860) that are associated with processed beef meat products in the

Table 4
Distribution of antimicrobial resistance genes and mobile genetic elements in *E. faecalis*.

Isolates	Sequence types (STs)	Isolation source	Antibiotic Resistance Genes (ARG)	Plasmid replicon Type	Mobile Genetic Elements (MGEs)			
					Intact prophages	**Insertion sequences (IS)**	**Integrative Conjugative Element (ICE)**	**Composite transposon (CN)**
S92	ST79	Processed meat-Beef-Minced meat	*ant(6)-Ia_2*, *dfrA*, *efrA*, *efrB*, *emeA*, *lsaA*, *tetM*	rep9b_2_prgW (EF62pC)	Entero_phiEf11, Entero_phiFL4A	ISEfm1	No hits	No hits
S109	ST860	Processed meat-Beef-Minced	*dfrA*, *efrA*, *efrB*, *emeA*, *lsaA*, *tetM*	rep9a_1_repA(pAD1), repUS43_1_CDS12738(DOp1), repUS1_2_rep(pVEF3)	Entero_phiFL2A, Lactob_Lj928	ISEnfa4, ISLgar5	Tn6009	No hits
S111	ST40	Processed meat-Beef-Patties	*dfrA*, *efrA*, *efrB*, *emeA*, *tetM*	No hits	Entero_phiFL1A	ISLgar5	Tn6009	No hits
S116	ST860	Processed meat-Beef-Minced meat	*dfrA*, *efrA*, *efrB*, *emeA*, *lsaA*	rep9a_1_repA(pAD1), repUS43_1_CDS12738(DOp1)	Entero_phiEf11	ISLgar5	Tn6009	No hits
S119	ST238	Processed meat-Beef-Wors	*dfrA*, *efrA*, *efrB*, *emeA*, *lsaA*, *tetM*	repUS43_1_CDS12738(DOp1), rep9b_2_prgW(EF62pC), rep6_1_repA(pS86), rep7a_16_repC(Cassette), repUS1_2_rep(pVEF3)	Entero_phiFL2A, Entero_phiFL3A	ISEfm1, ISEfm2, ISEnfa4	Tn6009	No hits
S121	ST40	Processed meat-Beef-Minced meat	*dfrA*, *efrA*, *efrB*, *emeA*, *tetM*	rep9c_2_prgW(pCF10), rep6_1_repA(pS86)	Entero_phiFL1A	ISEfm1, ISLgar5, ISS1N	No hits	No hits
S131	ST21	Processed meat-Beef-wors	*dfrA*, *efrA*, *efrB*, *emeA*, *lsaA*	repUS56_1_EFA0012(pTEF1), rep9b_1_prgW(pBEE99)	Entero_phiFL2A	No hits	No hits	No hits
S134	ST700	Processed meat-Beef-Minced	*dfrA*, *efrA*, *efrB*, *emeA*, *lsaA*	rep9c_3_repA(pPD1), repUS43_1_CDS12738(DOp1), rep6_1_repA(pS86)	Lister_LP_101	ISS1N, ISLgar5, ISEnfa4,	Tn6009	cn_8629_ISS1N, cn_18965_ISS1N

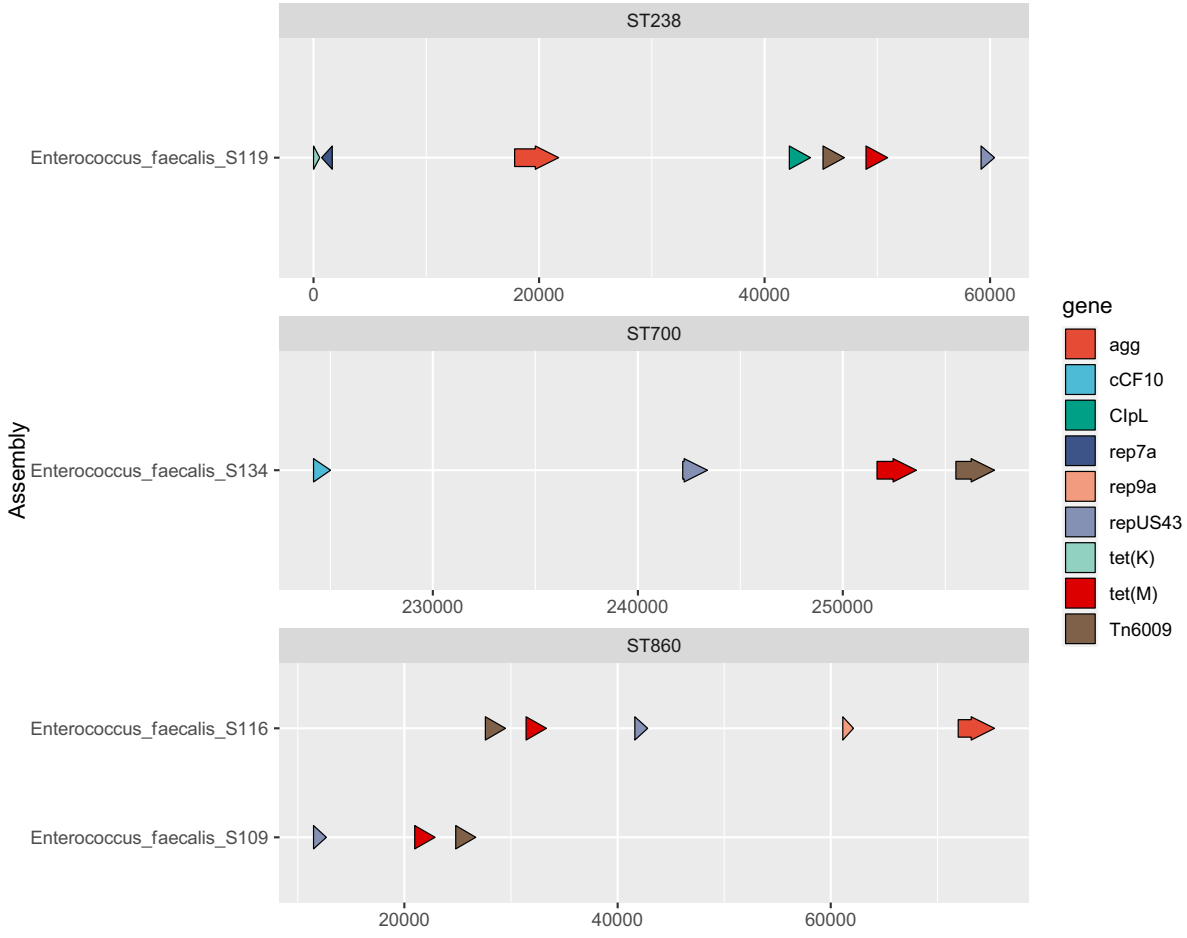

Fig. 3. Synteny of accessory genes and their associated MGEs. The colored arrows denote AMR and virulence genes located in proximity to one or more MGEs.

Gauteng province of South Africa. Although there are no prior accounts of these STs isolated in South Africa from meat products, ST21 and ST40 have been detected in South African public hospitals [27]. In many regions of the world, ST21 and ST40 are known to be a major causes of nosocomial infections [45–49]. Therefore, the presence of those STs in meat product pose a potential risk due to their association of human disease. It is important to note that other STs reported in the current study were also identified in hospital settings, food products, animal and human cases associated with *E. faecalis* globally [10,49–51]. *E. faecalis* possess several virulence factors that contribute to the severity of its infection [46]. A large group of genes conferring virulence factors (biofilm production or adherence to surfaces and capsular polysaccharide biosynthesis) were found in the genomes of *E. faecalis* strains in the present study. *E. faecalis* conserves many genetic factors that are associated with the production of biofilm and play an essential role in pathogenicity and infection as they promote virulence and antimicrobial resistance [53]. Several genes were identified in the present study which are involved in biofilm formation, including endocarditis and biofilm-associated pili genes (*ebpA, ebpB,* and *ebpC*), collagen adhesion precursor (*ace*), sortase (*srtE*), sugar-sensing transcriptional regulator (*bopD*), and the quorum-sensing mechanism (*fsrA, fsrB,* and *fsrC*). Similarly, these biofilm-conferring genes were isolated from *E. faecalis* from humans, food, and animals in other studies in different parts of the world [52,53].

Most of the isolates in this study carried the *fsrA, fsrB*, and *fsrC* genes, which are associated with the quorum-sensing mechanism and can control the expression of gelatinase (*gelE*) and serine protease (*sprE*) virulence genes. According to Bourgogne et al., [55] the *fsrB* gene is a quorum-sensing system signal molecule precursor that influences the expression of several interconnected genes, including *fsrA, fsrB, fsrC, gelE,* and *sprE*, which were also found in the majority of the strains in the current study. Extracellular metalloprotease gelatinase (*gelE*), can disintegrate haemoglobin, collagen, and gelatin as well as aid in the adhesion and the development of biofilms [56].

Many pathogenic bacteria maintain the genes that produce capsular polysaccharides to avoid phagocytosis and play a major part in pathogenesis by evading the immune system [57]. Most of the strains sequenced in the current study also contained genes (*cpsA, cpsB, cpsC, cpsD, cpsE, cpsF, cpsG, cpsH, cpsI,* and *cpsK*), that were capable of producing capsules. Similar results were reported from different strains of *E. faecalis*. The 8 enterococcal cytolytic toxin-encoding genes *cylA, cylB, cylI, cylL, cylM, cylR1, cylR2,* and *cylS* were not found in all the *E. faecalis* isolates from the current study. These cytolytic toxin production genes are commonly reported in *E. faecalis* isolated in human clinical settings but rarely from other sources [58]. Zaheer et al., [2020], suggest that high-virulent strains of *E. faecalis* harbour and express cytolytic toxin production genes, which promote virulence in enterococci pathogenicity by lysing both bacterial and eukaryotic cells in response to quorum signals. The most feasible explanation for the absence of enterococcal cytolytic toxin-encoding genes in our isolates is that these genes are mostly located on the pheromone-responsive rep9pAD1/pTEF2/pCF10 plasmid [59], which was not reported in the current study. Our findings indicate that *E. faecalis* isolates from beef meat products lacked virulence traits linked to the clinical isolates, which is encouraging and implies that there is likely a low risk of human-to-beef transmission of virulent *E. faecalis* in the food industry.

The virulence genes and virulence-associated genes for the aggregation substance (AS) *Ef0485*, *asaI*, and P*rgB/Asc10* were identified in the isolates sequenced in this study. Chajęcka-Wierzchowska et al., [60] indicated that AS frequently serves as a virulence factor and assists in transferring antimicrobial resistance genes. Similarly, AS contributes to the transmission of the plasmid, where the other virulence elements of *E. faecalis* such as cytolysin and antimicrobial resistance traits, are encoded [54]. The *asa1* gene coding for AS of the pheromone-responsive plasmid pADI has been well investigated, and the occurrence of AS genes in *E. faecalis* leads to fast conjugation [61]. The *prgB* gene, coding the surface protein, facilitates cell aggregation by conjugative transfer of the pheromone-responsive plasmid pCF10 in *E. faecalis,* which enables conjugation to distribute pathogenic material [62].

E. faecalis has the ability to resist a wide range of antimicrobial agents used in veterinary and clinical settings [46]. In the present study, genes conferring resistance to trimethoprim (*dfrA*), drug and biocide (*efrA* and *efrB*), multidrug efflux pump (*emeA*), and clindamycin quinupristin-dalfopristin (*lsaA*) were reported across all the isolates, with only one isolate having the aminoglycoside (*ant(6)-Ia*) resistant genes. This finding was consistent with the fact that *E. faecalis* has intrinsic resistance to clindamycin, quinupristin-dalfopristin, and trimethoprim-sulfamethoxazole, as well as exhibiting low-level resistance to aminoglycosides [61,62]. Holman et al., [64] reported multidrug-resistant *E. faecalis* isolated from a beef processing environment to harbour genes conferring resistance to a wide range of antibiotic compounds, which were consistent with the present study. Similarly, Zaheer et al., [58] reported multidrug-resistant *E. faecalis* harbouring resistant genes encoding for tetracycline (*tetM*, *tetS*, and *tetL*), quinupristin-dalfopristin (*lsaA* and *ermB*), and trimethoprim-sulfamethoxazole (*dfrG*) from a wide range of cattle samples. However, Future experimental efforts will thus be needed to investigate these differences further, as the study conducted here did not consider phenotypic data.

For years, mobile genetic elements (MGEs) have been implicated in the distribution of virulence factors and AMR between species and among pathogens through horizontal gene transfer [18,64]. Generally, enterococci are regarded as a core for MGEs, transferring AMR traits from one species to another, including both Gram-positive and Gram-negative [64,65]. The present study provides important information on MGEs such as plasmids, prophages, and ISs of *E. faecalis* isolated from beef meat products. Plasmids are commonly present in enterococci, and virulent and antimicrobial resistance genes are exhibited within these plasmids [54]. Research studies reported that plasmids serve as avenues for the transfer of antimicrobial resistance and virulence genes [18,64]. The detection of plasmids with virulent and antimicrobial resistance genes facilitates interest in understanding the evolutionary structure of plasmid distribution in regard to the pathogenesis [67]. Based on the WGS utilised in this study, 10 distinct plasmids replicons were identified including the detection of two or more unique replicons in an isolate. This reveals a higher occurrence of plasmids replicons in our *E. faecalis* isolates from beef meat products. The result obtained here agrees with previous reports stating that several kinds of plasmids replicons frequently occur in enterococci [22,67]. Plasmids (pPD1, pCF10, pBEE99, and pAD1) were identified in some of the present isolates of *E. faecalis*. This is congruent with the previous study by Wardal et al., [2013], who reported that pheromone-responsive plasmids, including pCF10, pBEE99, pPD1, pAD1, and pTW9 with their complex conjugation structure, mostly occur in *E. faecalis*. These plasmids are also responsible for carrying several resistance elements and genes coding bacteriocins and operons requiring virulence factors which include pili and cytolysin [60,68].

The current study detected these MGEs in close proximity to other MGEs which contain resistance genes (*tetM* and *ClpL)* and virulence genes (*agg* and *cCF10)* (Table 3 and Fig. 3). In addition, previous findings stated that pheromone-responsive plasmids promote the virulence of *E. faecalis* by supplying virulence factors such as cytolysin, pili, and aggregation substances [61]. Some of these genes were observed in or located in proximity to one or more MGEs (Table 3). Another study indicated that plasmids pAD1 and pCF10 encode characteristics that can promote virulence. For instance, pCF10 codes for tetracycline resistance and pAD1 for hemolysin and bacteriocin [46]. This indicates that the presence of pAD1 and pCF10 may increase the pathogenicity of these *E. faecalis* isolates and were detected in the present study. The repUS1 plasmid was detected in 2 of our isolates and this plasmid has been described as a likely carrier of the *optrA* gene which confers resistance to oxazolidinone and phenicol [70]. The pheromone-responsive plasmid

pCF10 was further detected in our study; it contributes majorly to the distribution of resistance genes and virulence factors within *Enterococcus* species [62]. A study by Tyson et al. 2018 [71] revealed that a major concern regarding the antimicrobial-resistant *E. faecalis* is the possibility of transferring resistance genes horizontally through plasmids to other pathogens.

Prophages play essential roles in *E. faecalis* genetic exchange, which results in many useful traits such as the acquisition of antimicrobial resistance genes, evolution, and adaptation [15,71]. Intact prophages were detected in all the *E. faecalis* genomes used in this study. The number of intact prophages per genome varied from 1 to 2. This shows that each isolate carried at least one prophage and that phages are ubiquitous among all the isolates. Of the 8 isolates, only 3 isolates (S92, S109, and S119) carried two different intact prophages each. The phi-FL2A prophage is the most widespread in all the isolates of *E. faecalis* from different beef meat products. The isolates sheltered the genes responsible for endocarditis and biofilm-associated pilus within the prophages. The genome of meat *E. faecalis* consists of a multiple of MGEs, including ISs, transposons, prophages, and plasmids that probably drive genetic exchange within and among these species [72].

5. Conclusion

In conclusion, our analysis revealed *E. faecalis* genomic characteristics, including virulence and resistance genes and mobile genetic elements. To the best of our knowledge, this is the first study to apply whole genome sequencing of *E. faecalis* strains isolated from processed beef meat in South Africa. Based on the virulence and resistance gene catalogues, all eight isolates in this study displayed potential pathogenic factors. The study's findings provide a glimpse of the pathogenic potential of *E. faecalis* and the water and meat safety risk posed by this pathogen.

Author contributions

IM performed all the microbiological analysis, source funding, and drafted the manuscript. RP supervised all the sequencing work and contributed to the drafting of the manuscript. TM performed all computational analyses and contributed to the drafting and editing of the manuscript. ACA, KM, and FT contributed to the drafting and editing of the manuscript.

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Author statement

IM performed all the microbiological analysis, source funding, and drafted the manuscript. RP supervised all the sequencing work and contributed to the drafting of the manuscript. TM performed all computational analyses and contributed to the drafting and editing of the manuscript. ACA, KM, and FT contributed to the drafting and editing of the manuscript. All authors agree to the publication of this work.

Declaration of Competing Interest

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.

Data availability

The whole Genome Shotgun project has been deposited at GenBank under the accession JARXOI000000000-JARXOP000000000 (Table S1)

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygeno.2023.110742.

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