Chapter

The Emergence and Spread of Antimicrobial Resistance in *Enterococcus* and Its Implications for One-health Approaches in Africa

Nathan Langat, Christine Inguyesi, Moses Olum, Peter Ndirangu, Ednah Masila, Ruth Onywera, Ascah Jesang, Esther Wachuka, Janet Koros, Peter Nyongesa, Edwin Kimathi and Monicah Maichomo

Abstract

Enterococcus bacteria, usually found in the gastrointestinal tracts of animals and humans, are used as an indicator of possible environmental contamination with enteropathogenic microorganisms. This group of bacteria is shed by healthy livestock and humans potentially contaminating the environment and water sources and may consequently cause public health problems in poor hygiene setups. Mitigation of the adverse effects arising from this requires a One-Health approach to reduce animal and human infections, and avail safe food of animal origin in a sustainable manner. Notably, *enterococcus* infections emerge as important nosocomial infections, aided by escalating antimicrobial resistance, increasing population of immunocompromised individuals and inadequate diagnostic techniques. This chapter will elucidate the intricate web of transmission and infection as pertains to *enterococcus* occurrence in food-producing animals. Prevalence, public health implications and mitigation strategy will be addressed.

Keywords: AMR, ARGs, environment, nosocomial, animal source food

1. Introduction

Enterococcus is a major genus of lactic acid bacteria in the Bacillota phylum, Bacilli class, *Lactobacillales* order and *Enterococcaceae* family. Its nomenclature has evolved over time. Based on Lancefield serologic typing, which characterizes the expression of beta-hemolysis on blood agar plates, it was designated as Group D Streptococci (GDS) in 1930 [1]. Sherman classified *streptococci* in 1937 into the viridans, pyogenic, enterococcal and lactic groups. Fast forward in 1984 *enterococcus* became a stand-alone

genus comprising *Enterococcus faecalis* and *Enterococcus faecium* (Efm) based on genomic DNA analysis by Schleifer and Kilpper-Balz [2].

Enterococci are catalase negative, Gram-positive, facultative anaerobic cocci that form short- to medium-length chains. They are typically regarded as commensals that are found in the gut microbiota of both humans and animals. They are also found in the female vaginal tract and, less frequently, the mouth cavity [3]. Once shed through feces, they are able to withstand the biotic and abiotic stressors to survive in the environment [4]. *Enterococci* are used as alternative indicators of possible water contamination by fecal waste [5]. Wastewater and slurry from animal holding facilities used for land application as manure as well as runoff from manure heaps onto pastures and plants are potential sources of *enterococci* that eventually find its way to the food chain.

In Africa, various *enterococcus* species have been identified, including *E. casseliflavus*, *E. faecalis*, *E. durans, E. gallinarum*, *E. thailandicus, E. devriesei*, *E. faecium*, *E. hirae*, *and E. mundtii* [6–9]. Since *enterococci* are part of the microbiota of many raw and non-sterilized food products, presence does not necessarily imply direct fecal contamination. Their ability to moderate the enteric environment against unwanted pathogens makes them relevant probiotics as is reported to control post-weaning diarrhea in piglets [10]. *Enterococcus durans* and *E. faecium* are actually recommended as co-starter cultures in yogurt and cheese production [11].

Most *enterococcus* species infections linked to healthcare facilities are caused by two of the most prevalent species in humans, *E. faecalis* and *E. faecium* [12]. They are opportunistic agents of clinical importance causing nosocomial infections in humans [13, 14]. From animal-based foods including meat, milk, and their derivatives, they appear to be the most often isolated *enterococcus* species [6, 7, 15]. Due to their enteric nature, they contaminate meat during slaughter. *Enterococci* are recognized as environmental pathogens contributing to mastitis, as they possess a capacity for prolonged survival in the environment and the ability to invade the mammary gland [16]. Its isolation from mastitic milk implies the likelihood of its transmission through milk to the consumers. This is particularly so in (*pastoral*) communities where milk hygiene is poor and the milk is consumed in different forms including raw and unpasteurised milk and their products.

High demand for poultry products for food and income source plays significant role as antimicrobial resistance (AMR) driver in Africa where indiscriminate use of antimicrobials arising from non-adherence to prescription by qualified animal health practitioners is still a challenge [17]. Poultry-driven AMR is further supported by the current practices addressing Sustainable Development Goals (SDGs) by the Vulnerable and Marginalized Groups (VMGs) with great focus on poultry farming as a way out of poverty for the rural folk. *Enterococci* from poultry products have shown AMR as reported in South Africa [18], Zambia [17] and Malaysia [19]. In Kenya, high AMR levels of 88.5% and multiple drug resistant (MDR) levels of 51% have been reported for *Enterobacteriaceae* from hospital clinical specimens [20]. Further, MDR *enterococci* to clinically relevant antibiotics, from beef and poultry carcasses, abattoir workers, cutting equipment in the slaughterhouses and the slaughterhouse environment have been reported in Kenya [8, 9].

Enterococci are of veterinary public health importance owing to their capacity for the development of resistance and horizontally transferring antibiotic resistance genes to additional bacteria through the food chain [16, 21–23]. Consequently, *enterococcus* species are seen as a strong indicator of antimicrobial resistance in the environment [24]. It is worth noting the role of pets such as cats and dogs as putative reservoirs of antimicrobial-resistant *enterococcus* for humans [25].

2. Epidemiology of *enterococcus*

2.1 Etiology and evolution

Enterococci are Gram-positive facultative anaerobic cocci in short and medium chains that were initially found in the human digestive system in 1899. Its nomenclature has evolved over time. In 1930, it was known as Group D Streptococci (GDS) based on Lancefield serologic typing that defines the expression of beta-hemolysis on blood agar plates [1]. In 1937, *streptococci* were divided into the pyogenic, viridans, lactic and enterococcal groups by Sherman and became a stand-alone genus comprising *Enterococcus faecalis* and *Enterococcus faecium* based on genomic DNA analysis [2]. In 1984, they were recognized as a different species from *streptococci* using DNA hybridization and 16S rRNA sequencing [26]. There are currently 58 species of *enterococci* known, with *E. faecium* and *E. faecalis* being the most significant and prevalent [27]. Other *enterococcus* species such as *E. avium*, *E. caccae*, *E. casseliflavus*, *E. dispar*, *E. durans*, *E. gallinarum*, *E. hirae* and *E. raffinosus* are increasingly being recognized as causes of human bloodstream and endovascular infections. These non-faecium and non-faecalis species of *enterococci* are becoming more prevalent in medical reports [28]. *Enterococci* exhibit remarkable resilience, enabling them to withstand challenging conditions such as common antiseptics and disinfectants. This robustness contributes to their widespread presence on typical hospital surfaces. Furthermore, the presence of *enterococci* on the hands of healthcare workers (HCWs) facilitates their effortless transmission [29]. In recent times, *enterococci* have garnered significant attention due to their escalating involvement in hospital-acquired (nosocomial) infections. A key factor fueling this trend is undoubtedly their inherent and acquired resistance to commonly prescribed antibiotics [30].

Colonization of the gastrointestinal tract by *enterococci* is the main factor that increases the risk of severe infections. These infections occur when *enterococci* move from the gut into the bloodstream. *Enterococci* have the ability to resist being killed by the body's immune system, even when they are phagocytosed. They can survive in a variety of harsh conditions, such as extreme temperatures, pH levels and high sodium chloride concentrations. This allows them to establish themselves in a wide range of environments. *Enterococci* have virulence factors like the Esp protein and aggregation substances that help them colonize their host. In recent years, *enterococci* have become a growing concern in healthcare settings due to their increasing resistance to certain antibiotics. Understanding the behavior, spread and virulence of *enterococcus* species is crucial to prevent and treat infections such as urinary tract infections, sepsis, endocarditis, wound infections and sepsis in newborns, and to slow down the development of antibiotic resistance.

2.2 Prevalence of *enterococcus* infections

Studies by Deshpande *et al*. [31], Wagenvoort *et al*. [32] and Noskin *et al.* [33] showed that *Enterococcus faecium* (Efm) is the predominant species among the hospital-related infections (HAI) because of its global distribution and propensity to endure in environments connected to healthcare. Other findings by Werner *et al*. [34] and Arias *et al*. [35] attributed its capacity for horizontal gene transfer and rapid rate of recombination enable it to quickly acquire resistant phenotypes. Compared to *E. faecalis*, acquired resistance to a number of antimicrobial agents is more commonly seen in *E. faecium*. Vancomycin-resistant *E. faecium* is regarded by the World Health

Organization (WHO) as a "high priority pathogen" that requires immediate attention and novel antibiotics for focused treatment [36]. Antimicrobial resistance (AMR)-exhibiting microorganisms pose a threat to the present global epidemiological shift in illness patterns, from communicable to non-communicable ones. By 2050, infectious diseases are predicted to reappear as the leading cause of death globally [37].

Enterococci have become significant healthcare-associated pathogens over the last few decades [38, 39]. The advancement of modern medical treatments toward more aggressive and invasive therapies for human diseases has certainly played a role in the rising prominence of these opportunistic pathogens. Additionally, the surge in antibiotic resistance among clinical strains of *enterococci* has been linked to this trend.

2.3 Public health (zoonotic) importance of *enterococcus*

Enterococcus species were considered inconsequential for an extended period of time in terms of medicine and safety for people. *Enterococcus* species have been employed extensively as starter cultures or probiotics in the food industry for the past 10 years due to their production of bacteriocins [40]. The presence of multi-antibiotic-resistant *enterococci* in animals, particularly poultry, is a growing public health concern globally due to their potential for human transmission [41]. *Enterococci* are indigenous members of the gastrointestinal microbiota in humans, as well as in a wide array of animal and insect species. *Enterococcus* spp. have been shown to propagate from animal reservoirs to humans through the food web. Given their commensal status in the intestinal tracts of humans and multiple animal species, including livestock and companion animals, *enterococci* possess the capacity to readily contaminate both food products and surrounding environments, thereby entering the food chain. Notably, during the evisceration stage at abattoirs, fecal *enterococci* are found to contaminate animal-derived food items, with contamination rates exceeding 90% as reported [42].

2.4 Sources and transmission of *enterococcus* infections

Less than 1% of the microbiota in the large intestine is made up of *enterococci*. In addition, they can be found in plants, sewage, food, water, soil, human skin, and the oral cavity [43].

2.4.1 Animal sources and mode of transmission

Enterococci naturally inhabit the intestinal tracts of various animals and humans and are able to contaminate food and the environment, thereby entering the food chain. Furthermore, some strains such as *E. faecalis* and *E. faecium* are significant pathogenic commensals that can cause a diverse array of infections [42].

2.4.2 Environmental sources

Enterococci are widely distributed in a diverse environmental habitat. They commonly occur in plant material, vegetables and foods, particularly food of animal origins [44]. Though earlier findings demonstrated the bacteria in flowers and buds, recent studies have expanded this to forage and crops [45] .Other extra enteric habitats are soil, water and sediments [46]. Freshwater and marine sediments as shown from other studies are significant reservoirs and sources of *enterococcus* [47]. High

enterococci densities in the soil are mainly attributed to the survival abilities as the Gram-negative bacteria compared to Gram-positive bacteria [48]. Investigation on *enterococcus* bacteria in soil environment that was carried out in watersheds particularly cattle grazing fields proved their survival and persistence [46].

2.4.3 Hospital sources and methods of transmission

Hospital setting plays a major role in disease transmission. Multidrug-resistant *enterococci* appear to be transmitted largely through the hospital environment [49]. Thermometer handles and thermometers have been identified as common surfaces associated with the transmission of vancomycin-resistant *Enterococci* (VRE) in healthcare settings. VRE is frequently present in these environments, with its highest concentrations observed on medical equipment such as blood pressure monitor, IV fluid pumps, stethoscopes as well as on items such as bed rails, gowns, bedside tables, bedpans, urinals and linens [49, 50]. The persistence of *enterococci* on environmental surfaces, as well as their subsequent transmission to the hands of healthcare professionals, has been underscored in many investigations.

3. Antimicrobial resistance and case studies for the genus *enterococcus*

3.1 Antimicrobial resistance

Enterococcus species belong to a category of pathogens referred to as indicator organisms. These microbes, including bacteria and viruses found in water bodies, serve as predictive markers to assess the existence of different pathogens within a particular environment. Typically nonpathogenic, *enterococcus* species exhibit limited or no growth in water and can be consistently detected even at low concentrati*ons* [51]. For a pathogen to qualify as a classical indicator organism, it must be present in higher proportions compared to the associated pathogen, while simultaneously exhibiting similar survival rates. This makes the *enterococcus* species ideal indicator bacteria for fecal contamination of water sources or water bodies by warm-blooded mammalian excreta [52].

Besides being an indicator of bacterium, this species contaminates food sources such as proteins of animal origin. A study by Holman et al. [53] isolated 10 different species in beef. Other studies have documented its contamination in milk and milk products and other processed foods [54]. Through this contamination, the pathogen has access to humans and other species where under different immunological competencies, it can cause infections. Infections caused by *enterococcus* species are common in hospital setups as nosocomial infections affecting the immunocompromised. Their resistance to environmental factors, along with a set of genetic determinants, allows *enterococcus* species to efficiently colonize their hosts. Additionally, they possess a remarkable capability to exchange genes with other bacteria [27].

Despite being a normal flora in most mammalian species, the genus has exhibited resistance over an extended time period against various antibiotics and possess the ability to acquire multiple antibiotic resistances. They can transfer genetic information among themselves and to non-pathogenic organisms through mechanisms such as plasmids and transposons [55–57]. The first proof of resistance to β-lactam antibiotics was detected before the bacteria were recognized as a genus. To get the effective treatment results, β-lactams were then combined with aminoglycosides [26].

This resistance has since advanced to novel advanced-generation Cephalosporin, Ceftaroline, complicating the treatment of *enterococcus* infections [54]. The emergence of vancomycin-resistant *Enterococci* (VRE) is a serious concern since vancomycin is typically used as a last resort when treating severe infections caused by Gram-positive bacteria that are resistant to other antibiotics [58]. In certain hospital settings, there has been a significant emergence of resistance to this antibiotic, with over 80% of *E. faecium* isolates demonstrating resistance to vancomycin [26]. Such establishments resorted to daptomycin against such VRE.

Genes that confer resistance to daptomycin and alter the charge and composition of cell membranes would eventually render the drug useless. These comprise the stress-sensing response component *liaF*, the glycerophosphoryl-diester-phosphodiesterase *gdpD*, and the cardiolipin synthase *cls* [59]. In the proceeding years, VRE developed resistance toward linezolid and tigecycline among other antibiotics through mutations in various efflux pumps [60] as shown in **Figure 1**.

Resistance by this genus is mediated by various mechanisms. Key among them is intrinsic resistance, which is exhibited against cephalosporins, aminoglycosides, lincosamides, and streptogramins [26]. This intrinsic resistance makes it easy for this genus of bacteria to acquire gene-mediated resistance through mobile genetic elements. This acquired resistance, demonstrated toward antibiotic classes like aminoglycosides, is the result of acquiring a plasmid-borne resistance factor. Over time, the genetic elements causing resistance against various antibiotics have been documented. Twelve genes have been discovered to provide resistance to various antibiotics in *enterococci*. These genes include erm(B) and erm(C) for resistance to erythromycin and tylosin, aph(3′)-IIIa for kanamycin, ant(6)-Ia for streptomycin, lnu(B) for lincomycin, vat(E) for Q/D, qnrE for ciprofloxacin, and tet(K), tet(L), tet(M), tet(O), and tet(S) for tetracycline [61]. This exhibits the multidrug resistance nature of *enterococci* leading to the stubborn nature of infections caused

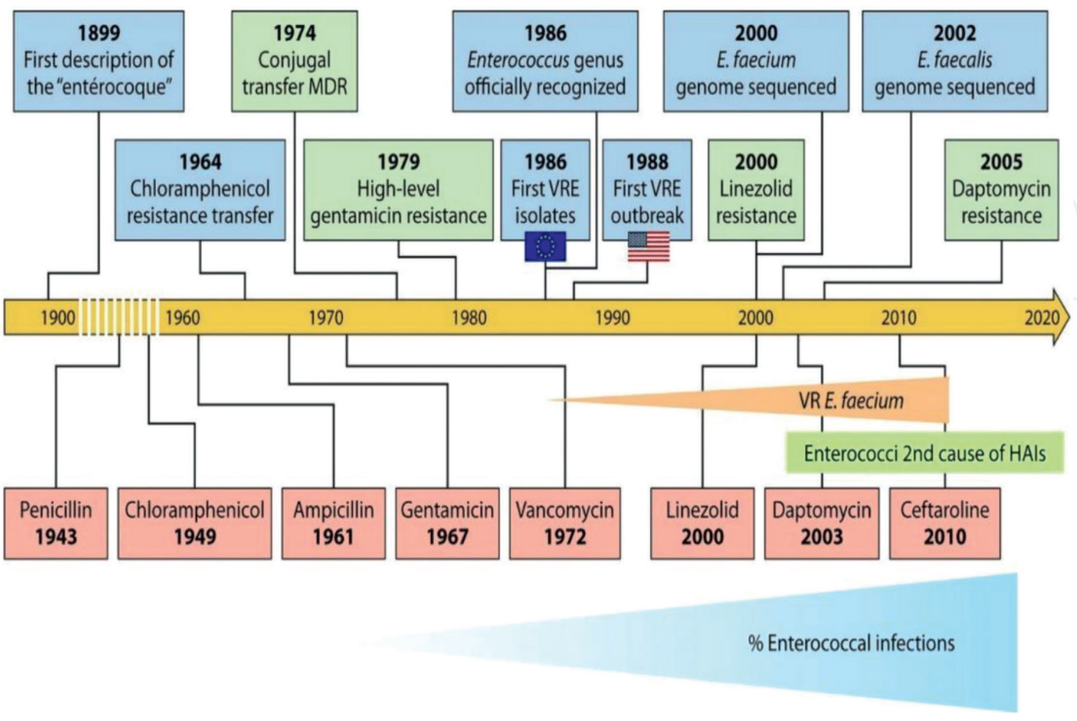

Figure 1.
History of enterococci antibiotic resistance in the genus. Adopted from Ref. [54].

by this pathogen. Furthermore, aminoglycoside-modifying enzymes, namely the bifunctional enzyme 6′-aminoglycoside acetyltransferase 2″-aminoglycoside phosphotransferase, are responsible for the high-level resistance that has been demonstrated against aminoglycosides [62]. The resistance mechanisms of *enterococcus* bacteria are captured in **Table 1**.

The most isolated species of the genus causing a myriad of infections are *E. faecalis* and *E. faecium*. They have shown multidrug resistance with the prevalence of the latter overtaking the former in hospital setups. This has partly been caused by increased colonal dissemination of the pathogen, inefficiencies of infection prevention and control measures and selective pressure by prolonged antibiotic use [54]. The efficiency with which both clinical and food isolates of the pathogen acquire genomic variation has been associated with lack of a functional Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system. This is also synergized by the efficient plasmid transfer system involving the production of pheromones. This leads to rapid acquisition of genetic resistance factors.

In animal studies, the occurrence of ampicillin or penicillin resistance in *enterococci* from livestock, pets, or wildlife, and animal food products has been shown to vary. This variance is majorly determined by geographical location and species of animal. This is particularly common for *E. faecium*. Other species associated with

Antimicrobial class (agents)	**Representative resistance gene(s)/operon(s)**	**Mechanism of resistance**
Aminoglycosides (gentamicin, kanamycin)	*aac-2′-aph-2″-le*, *aph-3′-IIIa*	Modification of the aminoglycoside
β-Lactams	*pbp4* (*E. faecalis*), *pbp5* (*E. faecium*)	Reduced affinity for the antibiotic
Chloramphenicol	*cat*	Acetylation of chloramphenicol
Clindamycin	*lsa*(A)	Putative efflux
Daptomycin	*liaFSR*	Alteration in membrane charge and fluidity
Erythromycin	*ermB*	Ribosomal methylation
Fluoroquinolones	*gyrA*, *parC*	Modifications in quinolone resistance-determining region
Glycopeptides	*vanA*, *vanB*, *vanD*, *vanM*	Modified peptidoglycan precursors terminating in D-lactate
	vanC, *vanE*, *vanG*, *vanL*, *vanN*	Modified peptidoglycan precursors terminating in D-serine
Oxazolidinones	rRNA genes	Mutations reducing affinity
	cfr	Methylation of 23S rRNA
Rifampin	*rpoB*	Point mutations reducing affinity
Streptomycin	*ant-6*	Modification of streptomycin
Tetracyclines	*tet*(L)	Efflux
	tet(M)	Ribosomal protection
Tigecycline	*tet*(L), *tet*(M)	Increased expression

Table 1.
Antimicrobial resistance mechanisms in enterococci [27].

animals include *E. faecalis*, *E. hirae*, *E. faecium*, and *E. durans*. The case studies below summarize the cases of *enterococci* in animals and animal products in Africa.

3.2 Case studies

3.2.1 Enterococcus isolated from camel meat and milk

Meat may be generally susceptible to microbial spoilage and can harbor a wide variety of zoonotic agents and foodborne including *Enterococcus* spp. that are common commensals of the gut of food-producing animals. These pathogens easily contaminate meat during its processing at the abattoir and, in turn, can result in human illnesses [57].

In a study on meat from markets in Tunisia, about 24.5% of the recovered *enterococci* displayed resistance to at least four antimicrobials. These isolates showed notable resistance to erythromycin and tetracycline. All isolates resistant to tetracycline carried the *tet*(L) and/or the *tet*(M) genes. About 78.5% of erythromycin-resistant isolates carried the *erm*(B) gene, the ant (6)-Ia gene was confirmed in 58.8% of the isolates resistant to streptomycin, and the *cat*(A) gene was found in one isolate resistant to chloramphenicol. Additionally, some isolates harbored the *gelE* gene and consequently demonstrated gelatinase activity. These findings suggest that meat may contribute to the transmission of *enterococci* with resistance characteristics and virulence factors through the food chain to humans raising concerns about their impact on human health [63].

Camels stand out for their ability to produce significant amounts of milk even in challenging feeding conditions, compared to other dairy species. The increasing awareness of the health and economic benefits of camel milk has made it a focal point, particularly in arid and semi-arid areas [64]. The informal and unregulated camel milk value chain in Africa, coupled with limited veterinary care, contributes to common mastitis issues among lactating females [65]. Despite being handled in unsanitary conditions, camel milk is often consumed raw, raising concerns about foodborne illnesses [66]. While camel milk contains natural antimicrobial factors, their protection against specific pathogens is brief [67]. The risk of contamination throughout the production chain, from producers to consumers, is a significant public health concern, with camel milk serving as a reservoir for antibiotic-resistant *Enterococcus* spp., including last resort antibiotics like vancomycin [68]. Contamination may arise from unhygienic conditions and various sources, such as human or animal feces, contaminated water, the animal's exterior, and milking equipment. The prevalence of antibiotic resistance in dairy isolates varies depending on strain, region, and isolation method [69]. A study by Chingwaru et al. [70] reported that *Enterococci* demonstrate growth at refrigeration temperatures (5°C) and at high temperatures (45°C), withstanding pasteurization allowing for their abundant detection in milk.

In a study by Naceur and BoudjemÃ¢a [69] in Algeria, milk samples from free-range camels were analyzed for the existence and antibiotic sensitivity testing of *Enterococcus* spp. The study revealed that 65% of the *enterococcus* isolates showed resistance to at least one antibiotic. Vancomycin resistance was observed in 13% of the isolates, while 26% displays resistance to erythromycin, tetracycline, or rifampin. Teuber et al. [71] reported 45, 64 and 32% resistance to tetracycline, chloramphenicol and erythromycin, respectively, emphasizing the concern regarding these antibiotics in dairy *E. faecalis* isolates. The prevalence of erythromycin resistance, representative

of macrolide antibiotics, is noteworthy and the frequent tetracycline resistance among dairy *enterococci* is possibly linked to its widespread use in veterinary practices.

Reports show that a substantial number of tetracycline-resistant isolates demonstrate co-resistance to chloramphenicol and/or erythromycin. This shows that the selection of tetracycline genotype could serve as a molecular foundation for further emergence of multidrug resistances [72].

Multiple studies report vancomycin-resistant *enterococci* (VRE) in animal-derived food, particularly in *E. faecium* and *E. faecalis*. The presence of VRE in camel milk is surprising due to the limited research on antibiotic resistance in these animals despite their habitat being mostly distant from urban areas [69]. Hammad et al. [73] also isolated VRE from cheese. One study reported *enterococci* levels at an average of 2.9×10^4 c.f.u./ml in camel milk indicating substantial fecal contamination during production and handling. Given their resistance to heat stress and adaptability in complex microflora environments, particularly in hot-camel rearing climates, *enterococci* appear to be more reliable fecal contamination indicator compared to coliforms [74].

3.2.2 Enterococcus in cattle value chain

Another study aimed to document the antibiotic resistance profiles and emphasize the existence of virulence genes in VREs obtained from feedlot cattle in the North-West Province of South Africa. From the *enterococcus* isolates, resistance genes were identified *(vanA, vanB* and *vanC)* in 176 *Enterococcus* spp. Multidrug resistances were confirmed in all the VRE isolates of this investigation. Virulence genes, namely *CylA*, *esp*, *gelE*, *hyl* and *asa1*, were also detected in eighty-six VRE isolates, with a majority displaying the virulence profile of *gelE-hyl*. These are potentially pathogenic multidrug-resistant VREs [75].

3.2.3 Enterococcus in pig value chain

In a South African farm-to-fork study carried out in an intensive pig production system, approximately 78% of the isolates showed multidrug resistance and were confirmed to have corresponding resistance genes. The results highlighted that intensive pig farming was a significant reservoir of antibiotic-resistant bacteria, raising concerns about the potential transmission to workers and consumers. The study underscores the necessity for more stringent guidelines on antibiotic use in intensive farming setups and advocates for inclusion of *Enterococcus* spp. in the antibiotic resistance monitoring for food animals [76].

Another study in the Eastern Cape Province of South Africa found high prevalence of multi-resistant VRE in the fecal samples of pigs in the studied farms. All isolates were found to be vancomycin, cloxacillin and streptomycin resistant and to a minimum of two distinct categories of antibiotics. About 93.8% isolates were found to be resistant to five or more antibiotics. Also, three virulence genes: adhesion of collagen, gelatinase and surface protein were detected in a majority of the isolates [77].

3.2.4 Enterococcus in poultry value chain

Enterococci in poultry can cause death of one-day-old chickens and pulmonary hypertension, bacteremia and amyloid arthropathy in adult poultry. Neurological disorders can be caused by fecal contamination of the embryo and young bird [78].

In Zambian cross-sectional study carried out in poultry farms, isolated *enterococcus* species displayed resistance to tetracycline, ampicillin, erythromycin, vancomycin, quinupristin/dalfopristin, linezolid, chloramphenicol, ciprofloxacin and nitrofurantoin. About 86% were multidrug resistant [17].

3.2.5 Enterococcus in the environment

Enterococci, entering the environment *via* feces, exhibit remarkable adaptability, readily colonizing soil, water and sewage [9, 57]. In sewage, *E. thailandicus* has been identified with multiple resistance genes [79]. Notably, the rate of multidrug resistance in *enterococci* is generally lower in environmental samples as compared to clinical ones, as per previous reports [80]. A study using bioinformatics tools and whole-genome sequencing to investigate *Enterococcus* spp. in a wastewater treatment plant and the associated water in South Africa detected genes conferring resistance including those encoding macrolides and tetracycline resistance. These were associated with transposons and insertion sequences. Evolutionary classification analysis showed that all *Enterococcus* spp. isolates were related more closely to environmental and animal isolates than to clinical isolates [81].

3.2.6 Enterococcus in slaughterhouses

The presence of antibiotic-resistant *enterococci* in animal environments, animal source foods and the handling equipment as well as in healthy persons, underscores the importance of evaluating *enterococci* found in slaughterhouse environments. In sub-Saharan countries, where animals for slaughter primarily come from pastoral areas with cases reported on self-medication and the misuse of antibiotics, the emergence of antibiotic resistance is a concern. Despite this, there is a scarcity of documented studies on antibiotic-resistant *enterococci* in foods of animal origin, thereby limiting the data on their prevalence and the antibiotic resistance in most African countries.

Different *Enterococci* species were isolated from samples taken from personnel, carcasses and the cutting equipment, during various slaughtering steps at five small and medium slaughterhouse enterprises in Kenya. About 56.7% of the isolates exhibited resistance to one or multiple antibiotic agents. The rise in *enterococci* resistance coincides with the introduction and extensive utilization of antibiotics. Notably, the widespread use of tetracycline, a commonly employed antibiotic in food-producing livestock in Kenya and Africa, aligns with the observed resistance. Differences in antibiotic resistance across countries may signify variations in veterinary antimicrobial use practices in these regions. Reports indicate common resistance of *enterococci* to rifampicin and erythromycin, especially in samples linked to animals [82]. Despite rifampicin being banned in livestock, the high resistance observed is possibly due to mutations or co-selection with fluoroquinolones [9, 82].

In a South African study, 15 *Enterococcus* spp. were isolated from food chain animals, emphasizing the importance of genomic surveillance to monitor antimicrobial resistance spread in these animals. Of the tested isolates, 30% exhibited resistance to at least two antibiotics and about 50% found resistant to multiple antibiotics. The study emphasizes that higher prevalence of VRE was found in environmental samples then followed by animal sources. In this study, a single *E. faecalis* isolate from a cow's water bucket underscores the importance of the One-Health approach to combat antibiotic resistance [83].

A study conducted in Gabon isolated *Enterococcus* spp. exhibiting a high resistance to rifampicin and tetracycline and closely related to clinical human isolates in the

Sample source	Sample type	Country	*Enterococcus* species	Prevalence of resistance
Camels	Fresh feces, raw meat, liver, hand swabs	Egypt	*E. faecalis, E. faecium, E. durans*	All isolates resistant to rifampicillin
Slaughterhouse	Meat	Tunisia	*E. fecalis*	About 24.5% resistant to at least four antibiotics
Camel	Milk	Algeria	*E. faecalis*, *E. faecium*, *E. avium*	65% were resistant to at least 1 antibiotic
Cattle, chicken	Milk, meat	Botswana	*E. faecalis* *E. faecium*	MDR against vancomycin, ampicillin, cephalothin
Cattle	Feces, water, soil	South Africa	*E. durans*	All VRE showed multiple resistance
Pigs	Farm, transport, abattoir, retail meat	South Africa	*E. faecalis*	78% had multidrug resistance
Pig	Feces	South Africa	*E. faecium*	All isolates found resistant to at least 2 different classes
Poultry	Cloacal swab samples	Zambia	Not specified	MDR against tetracycline, ampicillin, erythromycin, vancomycin, quinupristin/ dalfopristin, linezolid, chloramphenicol, ciprofloxacin, nitrofurantoin
Environment	Wastewater	South Africa		Tetracycline, macrolides
Slaughterhouse	Swabs of carcass, personnel, cutting equipment	Kenya	*E. fecalis*	57.6% of isolates resistant to at least 1 antibiotic
Chicken, cows, ducks, goats, horses, sheep, pigs, water, feedlot, soil	Rectal, oral fecal swabs	South Africa	*E fecalis, E. faecium, E. durans*	50% of isolates resistant to more than 2 antibiotics
Hens, swine, sheep cattle	Feces	Gabon	*E. faecium, E. hirae*	Resistance noted against tetracycline and rifampicin

Table 2.
Summary of the findings of enterococci *and their resistance in African studies.*

NCBI database. The *tet(M)* gene was identified in 65 isolates resistant to tetracycline with a large majority from being from hens (**Table 2**) [84].

4. Genomics of *enterococcus*

The emergence of next-generation sequencing (NGS) has significantly influenced bacterial genomics. Indeed, the study of enterococcal genomics has risen in the recent

years. Draft genomes and complete genomes have been done by researchers. These studies have given an insight into various characteristics of members of the genus *enterococcus* ranging from population structure to antimicrobial resistance mechanisms to virulence factors and possible vaccine candidates. This is especially important in current times when antimicrobial resistance is a global issue and strategies are being formulated to address this challenge.

Whole-genome sequencing (WGS) of several species of *enterococcus* isolated from mastitic milk from camels in Kenya has been carried out including the genomes of *E. faecalis* strain 1351 [7], *E. faecium* and *E. gallinarum* [6], *E raffinosus* CX012922 isolated from patient's fecal samples [85] and *E. casseliflavus* strain UFMG-H8 [86] isolated from the urine of healthy bovine heifers. Many more studies have carried out WGS on many species of *enterococcus*. *Enterococcus* species, including *E. raffinosus, E. faecalis, E. faecium*, *E. casseliflavus* and *E. gallinarum,* display variations in genome structure and size and the comprehension of these distinctions is crucial for unraveling their pathogenic potential. Typically, the *enterococcus* genome size ranges from 2.5 to 3.4 mega base pairs and possesses a single-circular chromosome as the primary genomic structure. Variations can, however, occur with the presence of plasmids or other mobile genetic elements, which can contribute to genetic diversity among strains. The general genomic characteristics of the *enterococcus* genus are summarized in **Table 3**.

Numerous genetic differences have been found across several *enterococcus* strains and species, according to comparative genomics research. These include variances in genes linked to antibiotic resistance, metabolism, virulence factors and other functional characteristics. Disparities in antimicrobial resistance profiles and pathogenic potential may be attributed to the presence of mobile genetic elements, pathogenic islands or strain-specific genes among distinct strains. A study carried out by Zhong et al. [87] revealed the significant influence of the environment on the genome of *E. faecium*, with human isolates exhibiting the largest average genome size, dairy isolates having the smallest average genome size and the lowest number of antibiotic resistance genes. Studying *enterococcus* species' pathogenicity requires an in-depth understanding of their genomic variants and structure. Certain genetic components, including genes for virulence factors or antibiotic resistance, can have an immediate effect on the capacity of an *enterococcus* species to spread illness and develop resistance. The detection and description of these genetic variants aid in the development of efficient treatment plans as well as the monitoring of the emergence and dissemination of antibiotic resistance. Moreover, by comprehending the structure of the genome, researchers can unravel the fundamental workings of how *enterococcus* species interact with their hosts and modify their environment to become more harmful.

	***E. faecalis* strain 1351**	***E. gallinarum***	***E. casseliflavus* strain UFMG-H8**
Size	2.91 Mbp	3.43 Mbp	3.6 Mbp
GC content	37.57%	40.83%	41.03%
Number of protein-coding genes	5342	2432	3347

Table 3.
General characteristics of the enterococcus *genus genome.*

4.1 Virulence factors

Studies have identified the virulence factors in different *enterococcus* species that enhance their ability to colonize susceptible hosts. These virulence factors include gelE, esp. and genes associated with cytolysin production such as cylA, cylR1, cylB, cylR2, cylLs, cylLl, asa1, cylM as well as cylI [75]. The *gelE* gene is responsible for producing the gelatinase enzyme, which aids the bacteria in breaking down proteins such as collagen and gelatin. The *esp* gene is responsible for encoding the Enterococcal Surface Protein (Esp), which plays a role in the formation of biofilms. The *cyl* genes encode for a hemolysin called cytolysin, which is a pore-forming toxin that has the ability to lyse cells, while the asa1 gene encodes for a surface protein that aids in aggregation.

The genomes of *enterococci* are recombinogenic which aids in acquisition of new genes or genetic elements that confer enhanced virulence or pathogenicity and antimicrobial resistance. Molecular epidemiological studies *of E. faecalis* using MLST (multi-locus sequence typing) have reported incongruent pairwise comparisons of the MLST loci sequences suggesting that *E. faecalis* undergoes frequent genetic recombination events, leading to genetic diversity within the population [88]. This recombinogenic nature of *E. faecalis* enables the organism to exchange genetic material with other bacteria, which promotes adaptability and evolution. Consequently, *enterococci* have acquired various antibiotic resistance traits, posing a significant challenge in healthcare settings.

Studies on *enterococcus* strains have revealed the existence of genomic islands that are specific region within the genome of a bacterium that contains a cluster of genes associated with enhanced virulence. Studies carried out by Tao et al. [89] revealed that *enterococcus* have a lower prevalence of CRISPR-Cas systems compared the typical occurrence among bacteria, suggesting that *enterococci* have a reduced capacity to defend against the integration of exogenous genetic material. This may contribute to an increased probability of the acquisition of mobile genetic elements such as plasmids and phages carrying antibiotic resistance genes, which may lead to increased antibiotic resistance of *enterococca*l strains [88].

Studies have also found out that various *enterococcus* species from bovine feces carry different antibiotic resistance genes. The study found genomes of several *Enterococcus* species containing genes that confer resistance to macrolides, likely due to tylosin phosphate use in cattle husbandry. Erm (B) was the predominant gene, whereas msrC was detected only in his *E. faecium*. *E. casseliflavus* and *E. gallinarum* genomes were found to contain the vanC operon, providing tolerance to low concentrations of vancomycin, while *E. faecium*_11 contained multiple ARGs, encompassing streptogramin A, aminoglycoside, streptothricin, MLSB, tetracycline and pleuromutilin resistance mechanisms [90]. In the process of reconstructing the evolutionary history of *E. faecium* through genomic analysis, the first genome-based study focused on sequencing seven isolates. By conducting a phylogenetic analysis of 649 protein sequences, it was demonstrated that the human commensal strain E980 exhibited a distant relationship from the remaining six *E. faecium* isolates, indicating a significant level of branching diversity within the species.

Variations in the genetic makeup of *E. faecium* bacterial genomes within the same species arise from changes in the sequences of common genes and the acquisition of new genes through horizontal gene transfer. An initial insight into the diversity in gene content among various *E. faecium* isolates was obtained through a research study that utilized comparative genomic hybridization to examine the additional gene pool

of the species. This analysis led to the discovery of 175 genes that were more prevalent in clinical isolates compared to non-clinical ones [88].

5. Management of *enterococcus* infections

5.1 Diagnosis

Diagnosis involves determination of the clinical disease (clinical or tentative diagnosis) as well as identification of the causative agent generally referred to as confirmatory diagnosis.

5.1.1 History and physical examination

A detailed patient history is essential, encompassing various aspects such as fever history, antibiotic usage along with duration, exposure to multidrug-resistant organisms, past hospitalizations or stays at healthcare facilities, cancer and HIV screening, surgical history, and underlying medical conditions such as diabetes or recent cardiac procedures. Additionally, examination of physical signs related to each organ system is crucial, particularly when the source of bacteremia is unclear. The most common clinical diagnostic outcomes include urinary tract infections (UTI), bacteremia and infective endocarditis (IE). Other less common clinical presentations include meningitis and may also manifest in diabetic and decubitus ulcers, surgical-site infections, prosthetic joint infections, dental issues, endophthalmitis and complications from root canal treatments [26].

5.1.2 Bacterial culture and identification

Culture and gram stain testing should be conducted on body fluids and blood before starting antibiotic treatment. Additional tests such as colonoscopy, a chest X-ray, CT scan of the abdomen and echocardiogram may also be required depending on the specific clinical situation of the infection.

- *Enterococci* are Gram-positive cocci that typically present as short chains, diplococci or single ovoid cells. They are facultative anaerobes that can grow on culture media, tolerating high salt concentrations of up to 6.5% and a variety of temperatures. While most *enterococci* are non-hemolytic, some may exhibit alpha or beta hemolysis.

- *Enterococci* can be identified in the laboratory as urease-negative, catalase-negative, able to hydrolyze esculin in 40% bile salts, positive for Lancefield group D antigen, and capable of hydrolyzing PYR, which helps to differentiate them from *Streptococcus gallolyticus*. Selective media and commercial testing kits utilize many of these characteristics for the identification of *enterococci*. To differentiate between different species within the *enterococci* group, factors such as carbohydrate fermentation, arginine hydrolysis, tolerance to tellurite, motility and pigmentation are considered.

- Traditional biochemical assays have been supplanted by modern genetic approaches in the detection of *enterococcus*. These cutting-edge techniques

include matrix-assisted laser desorption ionization-time of flight (MALDI-TOF), gene probes, polymerase chain reaction (PCR), nucleic acid amplification testing (NAAT), 16 s rRNA sequencing and other novel technologies. These instruments can quickly and precisely identify *enterococci* from other bacteria and even evaluate their profiles of antibiotic resistance by focusing on species-specific protein segments.

- Regularly evaluating *enterococcus* strains for high-level aminoglycoside resistance (HLAR), vancomycin resistance and penicillin resistance is advised. It is essential to do *in vitro* tests for linezolid and daptomycin susceptibility if resistance to beta-lactam or vancomycin is found.

- With enterococcal bacteremia, the DENOVA tool is used to predict endocarditis by accounting for variables such as the length of symptoms, the number of positive cultures, embolizations, origin, auscultation murmurs and valve disease. Certain specialists have recommended transthoracic echocardiograms for enterococcal bacteremia cases that are acquired in the community as well as nosocomial cases.

- As part of the assessment, recommendations have suggested considering routine colonoscopies for individuals with enterococcal bacteremia or infective endocarditis (IE) with unidentified sources, due to the increased incidence of detecting new colonic neoplasms in this group, aligning with the protocols for *Clostridium septicum* and *Streptococcus bovis.*

5.2 Treatment

Managing enterococcal infections can pose challenges. *Enterococcus* species naturally exhibit resistance to numerous antibiotics, such as clindamycin, cephalosporins, aminoglycosides and penicillinase-stable penicillins, and are also capable of acquiring resistance genes and mutations [38]. Furthermore, substances that hinder cell wall formation and are typically lethal to other Gram-positive cocci typically only have a bacteriostatic effect on *enterococci* [91]. This problem is significant in the treatment of endocarditis and other severe cases that necessitate the use of bactericidal drugs for successful treatment. For *enterococcal* infections, a combination of medications is typically required to achieve bactericidal effects synergistically. *In vitro* synergism refers to a 100-fold or more increase in bacterial killing within 24 hours when a combination of drugs is used compared to using each drug alone [38].

Therapeutic approaches differ based on various factors such as the sensitivity of microorganisms to β-lactams, aminoglycosides and glycopeptide or resistance to a combination of these antimicrobials, type of infection whether caused by single or multiple pathogens and whether the infection has affected heart valves or other internal vascular structures.

5.3 Prevention and control

Enterococcus is susceptible to a number of disinfectants that include 70% isopropyl alcohol, 0.041% sodium hypochlorite, 70% ethanol, phenolic and quaternary ammonia compounds [92, 93]. However, they are resistant to 3% hydrogen peroxide [93]. Killing or physical inactivation of the organism can be done using heat as

temperatures above 80°C kill the organism [94]. Doctors, nurses and pharmacists collaborate as an essential interprofessional team to manage and treat enterococcal infections.

- The Centers for Disease Control and Prevention (CDC) advises conducting immediate active screening using rectal and perirectal swabs or stool samples, followed by reporting of vancomycin-resistant *Enterococci* (VRE) in high-risk individuals such as those in intensive care units, transplant or oncology units, hemodialysis patients, or immunocompromised individuals, those with prolonged hospital stays, and those admitted from long-term care facilities. This approach has been found to be a cost-effective method for preventing colonization, infections and fatalities.

- Specialized cleaning methods like non-touch automated mobile ultraviolet units, along with regular chlorhexidine baths, are recommended for decreasing *enterococci* infections, including VRE, particularly in the intensive care unit (ICU).

- Training healthcare workers on hand hygiene has been linked to a 47% decrease in the acquisition of *enterococci* infections in hospital settings.

- Contact isolation is a common practice in hospitals for patients, but there is a lack of consistent data to fully support its effectiveness.

- It is crucial for antibiotic stewardship programs to restrict the use of cephalosporins, antibiotics targeting anaerobes, vancomycin and broad-spectrum antibiotics as they are instrumental in preventing the emergence and transmission of this pathogen.

- Zoonotic *enterococcus* infections can be prevented by practicing appropriate milk and meat hygiene practices. These include boiling milk before consumption, milk sterilization *via* ultra-heat treatment (UHT) or pasteurization as well as eating properly cooked meat.

6. Conclusion

Enterococcus bacteria, a normal enteropathogenic flora that is useful as environmental contamination indicator, possess probiotic potential against diarrhea and find application in food production. However, *Enterococcus faecalis* and *Enterococcus faecium* present significant healthcare-associated infection risks, contaminating animal source foods and contributing to antimicrobial resistance. Their prevalence in sub-Saharan Africa raises concerns for veterinary and public health, particularly in areas with poor hygiene leading to environmental contamination. The emergence of multiple drug resistance in *enterococcus* poses heightened risks for immunocompromised individuals, rendering nosocomial infections challenging to treat. Genomic analysis sheds light on recombination rates, antimicrobial resistance challenges and zoonotic transmission risks. Addressing the *enterococcus* challenge necessitates a comprehensive One-Health approach, emphasizing prevention and control of zoonotic transmission for effective management.

DOI: http://dx.doi.org/10.5772/intechopen.114340

Author details

Nathan Langat[1], Christine Inguyesi[1], Moses Olum[1], Peter Ndirangu[1], Ednah Masila[1], Ruth Onywera[1], Ascah Jesang[1], Esther Wachuka[1], Janet Koros[1], Peter Nyongesa[2], Edwin Kimathi[1] and Monicah Maichomo[1*]

1 Veterinary Research Institute, Kenya Agricultural and Livestock Research Organization, Nairobi, Kenya

2 Department of Medical Microbiology and Parasitology, Masinde Muliro University of Science and Technology, Kakamega, Kenya

*Address all correspondence to: monicah.maichomo@kalro.org

References

[1] Facklam R. What happened to the streptococci: Overview of taxonomic and nomenclature changes. Clinical Microbiology Reviews [Internet]. 2002;**15**(4):613-630. DOI: 10.1128/CMR.15.4.613-630.2002

[2] Schleifer KH, Kilpper-Balz R. Transfer of Streptococcus faecalis and streptococcus faecium to the genus enterococcus nom. Rev. as Enterococcus faecalis comb. nov. and Enterococcus faecium comb. nov. International Journal of Systematic Bacteriology [Internet]. 1984;**34**(1):31-34. DOI: 10.1099/00207713-34-1-31

[3] Carroll KC, Pfaller MA, Landry ML, McAdam AJ, Patel R, Richter SS, et al., editors. Manual of Clinical Microbiology. 12th ed. Washington, DC: ASM Press; 2019. 2 p

[4] Staley C, Dunny GM, Sadowsky MJ. Environmental and animal-associated enterococci. In: Advances in Applied Microbiology. 1 Jan 2014;**87**:147-186

[5] Bandh SA, Shafi S, Shameem N, editors. Freshwater Microbiology: Perspectives of Bacterial Dynamics in Lake Ecosystems. London, United Kingdom; San Diego, CA: Academic Press, an imprint of Elsevier; 2019. 433 p

[6] Maichomo M, Gachogo R, Masila E, Ogali I, Otieno L, Langat N, et al. Draft genome sequences of enterococcus faecium, enterococcus gallinarum, and Lactococcus lactis strains isolated from a mastitis-infected camel in Isiolo County, Kenya. Microbiology Resource Announcements. 24 Jan 2023;**12**(1)

[7] Ochieng J, Gicheru N, Indieka AS, Maichomo M, Wesonga OH, Murungi E. Draft genome sequence of enterococcus faecalis 1351, isolated from a mastitis-affected camel in Isiolo County, Kenya. Microbiology Resource Announcements. 15 Apr 2021;**10**(15):10.1128/mra.00143-21

[8] Oguttu JW, Qekwana DN, Odoi A. Prevalence and predictors of antimicrobial resistance among enterococcus spp. from dogs presented at a veterinary teaching hospital, South Africa. Frontiers in Veterinary Science [Internet]. 2021;**7**:589439. DOI: 10.3389/fvets.2020.589439/full

[9] Wambui J, Tasara T, Njage PMK, Stephan R. Species distribution and antimicrobial profiles of enterococcus spp. isolates from Kenyan small and medium Enterprise slaughterhouses. Journal of Food Protection [Internet]. 2018;**81**(9):1445-1449. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0362028X22086951

[10] Matsumoto H, Miyagawa M, Yin Y, Oosumi T. Effects of organic acid, Enterococcus faecalis strain EC-12 and sugar cane extract in feed against enterotoxigenic Escherichia coli-induced diarrhea in pigs. AMB Express [Internet]. 2021;**11**(1):68. DOI: 10.1186/s13568-021-01228-2

[11] Akpinar A, Saygili D, Yerlikaya O. Production of set-type yoghurt using *Enterococcus faecium* and *Enterococcus durans* strains with probiotic potential as starter adjuncts. International Journal of Dairy Technology [Internet]. 2020;**73**(4):726-736. DOI: 10.1111/1471-0307.12714

[12] Lebreton F, Willems RJL, Gilmore MS. Enterococcus diversity, origins in nature, and gut colonization. In: Gilmore MS, Clewell DB, Ike Y, Shankar N, editors. Enterococci: From

Commensals to Leading Causes of Drug Resistant Infection [Internet]. Boston: Massachusetts Eye and Ear Infirmary; 2014. Available from: http://www.ncbi.nlm.nih.gov/books/NBK190427/ [Accessed: Jan 30, 2024]

[13] Olawale K, Fadiora S, Taiwo S. Prevalence of hospital acquired enterococci infections in two primary-care hospitals in Osogbo, Southwestern Nigeria. African Journal of Infectious Diseases. 24 May 2011;**5**(2):40-46

[14] Ramos S, Silva V, Dapkevicius M, Igrejas G, Poeta P. Enterococci, from harmless bacteria to a pathogen. Microorganisms [Internet]. 2020;**8**(8):1118. Available from: https://www.mdpi.com/2076-2607/8/8/1118

[15] Sánchez Valenzuela A, Lavilla Lerma L, Benomar N, Gálvez A, Pérez Pulido R, Abriouel H. Phenotypic and molecular antibiotic resistance profile of enterococcus faecalis and enterococcus faecium isolated from different traditional fermented foods. Foodborne Pathogens and Disease. 2013;**10**(2):143-149

[16] Różańska H, Lewtak-Piłat A, Kubajka M, Weiner M. Occurrence of enterococci in Mastitic cow's milk and their antimicrobial resistance. Journal of Veterinary Research. 2019;**63**(1):93-97

[17] Mudenda S, Matafwali SK, Malama S, Munyeme M, Yamba K, Katemangwe P, et al. Prevalence and antimicrobial resistance patterns of *Enterococcus* species isolated from laying hens in Lusaka and Copperbelt provinces of Zambia: a call for AMR surveillance in the poultry sector. JAC-Antimicrobial Resistance [Internet]. 2022;**4**(6):dlac126. DOI: 10.1093/jacamr/dlac126/6955595

[18] Fatoba DO, Abia ALK, Amoako DG, Essack SY. Rethinking manure application: Increase in multidrug-resistant Enterococcus spp. in agricultural soil following chicken litter application. Microorganisms [Internet]. 2021;**9**(5):885. Available from: https://www.mdpi.com/2076-2607/9/5/885

[19] Wada Y, Irekeola AA, Shueb RH, Wada M, Afolabi HA, Yean CY, et al. Prevalence of vancomycin-resistant enterococcus (VRE) in poultry in Malaysia: The first meta-analysis and systematic review. Antibiotics [Internet]. 2022;**11**(2):171. Available from: https://www.mdpi.com/2079-6382/11/2/171

[20] Lord J, Gikonyo A, Miwa A, Odoi A. Antimicrobial resistance among *Enterobacteriaceae*, *Staphylococcus aureus*, and *Pseudomonas* spp. isolates from clinical specimens from a hospital in Nairobi, Kenya. PeerJ [Internet]. 2021;**9**:e11958. Available from: https://peerj.com/articles/11958

[21] Coburn PS, Baghdayan AS, Dolan G, Shankar N. Horizontal transfer of virulence genes encoded on the *Enterococcus faecalis* pathogenicity island. Molecular Microbiology [Internet]. 2007;**63**(2):530-544. DOI: 10.1111/j.1365-2958.2006.05520.x

[22] De Niederhäusern S, Bondi M, Messi P, Iseppi R, Sabia C, Manicardi G, et al. Vancomycin-resistance transferability from VanA Enterococci to Staphylococcus aureus. Current Microbiology [Internet]. 2011;**62**(5):1363-1367. DOI: 10.1007/s00284-011-9868-6

[23] Niederhausern S, Sabia C, Messi P, Guerrieri E, Manicardi G, Bondi M. Glycopeptide-resistance transferability from vancomycin-resistant enterococci of human and animal source to Listeria spp. Letters in Applied Microbiology [Internet]. 2004;**39**(6):483-489. Available from: https://academic.oup.com/lambio/article/39/6/483/6703023

[24] Hande G, Arzu F, Nilgün G, Serhat AS, Alper Ç, Ece K, et al. Investigation on the etiology of subclinical mastitis in Jersey and hybrid Jersey dairy cows. Acta Veterinaria [Internet]. 2015;**65**(3):358-370. DOI: 10.1515/acve-2015-0030

[25] Ghosh A, Dowd SE, Zurek L. Dogs leaving the ICU carry a very large multi-drug resistant Enterococcal population with capacity for biofilm formation and horizontal gene transfer. PLoS One. 19 Jul 2011;**6**(7):e22451

[26] Fiore E, Van Tyne D, Gilmore MS. Pathogenicity of enterococci. Microbiology Spectrum. 2019;**7**:4. DOI: 10.1128/microbiolspec.gpp3-0053-2018

[27] García-Solache M, Rice LB. The enterococcus: A model of adaptability to its environment. Clinical Microbiology Reviews. 2019;**32**(2):e00058-e00018

[28] Mastroianni A. Enterococcus raffinosus endocarditis. First case and literature review. Le Infezioni in Medicina. 2009;**17**(1):14-20

[29] Reyes K, Bardossy AC, Zervos M. Vancomycin-resistant enterococci: Epidemiology, infection prevention, and control. Infectious Disease Clinics. 2016;**30**(4):953-965

[30] Devriese L, Baele M, Butaye P. The Genus Enterococcus. In: The Prokaryotes. New York, NY: Springer US; 2006. pp. 163-174

[31] Deshpande LM, Fritsche TR, Moet GJ, Biedenbach DJ, Jones RN. Antimicrobial resistance and molecular epidemiology of vancomycin-resistant enterococci from North America and Europe: A report from the SENTRY antimicrobial surveillance program. Diagnostic Microbiology and Infectious Disease. 2007;**58**(2):163-170

[32] Wagenvoort JHT, De Brauwer EIGB, Penders RJR, van der Linden CJ, Willems RJ, Top J, et al. Environmental survival of vancomycin-sensitive ampicillin-resistant enterococcus faecium (AREfm). European Journal of Clinical Microbiology & Infectious Diseases. 2015;**34**(9):1901-1903

[33] Noskin GA, Stosor V, Cooper I, Peterson LR. Recovery of vancomycin-resistant enterococci on fingertips and environmental surfaces. Infection Control and Hospital Epidemiology : the Official Journal of the Society of Hospital Epidemiologists of America. 1995;**16**(10):577-581

[34] Werner G, Coque TM, Franz CMAP, Grohmann E, Hegstad K, Jensen L, et al. Antibiotic resistant enterococci—Tales of a drug resistance gene trafficker. International Journal of Medical Microbiology. 2013;**303**(6-7):360-379

[35] Arias CA, Panesso D, Singh KV, Rice LB, Murray BE. Cotransfer of antibiotic resistance genes and a hylEfm-containing virulence plasmid in enterococcus faecium. Antimicrobial Agents and Chemotherapy. 2009;**53**(10):4240-4246

[36] Contreras GA, Munita JM, Arias CA. Novel strategies for the management of vancomycin-resistant Enterococcal infections. Current Infectious Disease Reports. 2019;**21**(7):22

[37] Barreto ML, Teixeira MG, Carmo EH. Infectious diseases epidemiology. Journal of Epidemiology and Community Health. 2006;**60**(3):192-195

[38] Arias CA, Murray BE. The rise of the Enterococcus: Beyond vancomycin resistance. Nature Reviews. Microbiology. 2012;**10**(4):266-278

[39] Benenson S, Cohen MJ, Block C, Stern S, Weiss Y, Moses AE, et al. Vancomycin-resistant enterococci in long-term care facilities. Infection Control & Hospital Epidemiology. 2009;**30**(8):786-789

[40] Foulquié Moreno MR, Sarantinopoulos P, Tsakalidou E, De Vuyst L. The role and application of enterococci in food and health. International Journal of Food Microbiology. 2006;**106**(1):1-24

[41] Mwikuma G, Kainga H, Kallu SA, Nakajima C, Suzuki Y, Hang'ombe BM. Determination of the prevalence and antimicrobial resistance of enterococcus faecalis and enterococcus faecium associated with poultry in four districts in Zambia. Antibiotics. 2023;**12**(4):657

[42] Torres C, Alonso CA, Ruiz-Ripa L, León-Sampedro R, del Campo R, Coque TM. Antimicrobial resistance in Enterococcus spp. of animal origin. In: Antimicrobial Resistance in Bacteria from Livestock and Companion Animals. Washington, DC, USA: ASM Press; 2018. pp. 185-227

[43] Raza T, Ullah SR, Mehmood K, Andleeb S. Vancomycin resistant enterococci: A brief review. The Journal of the Pakistan Medical Association. 2018;**68**(5):768-772

[44] Giraffa G. Enterococci from foods. FEMS Microbiology Reviews. 2002;**26**(2):163-171

[45] Müller T, Ulrich A, Ott EM, Müller M. Identification of plant-associated enterococci. Journal of Applied Microbiology. 2001;**91**(2):268-278

[46] Byappanahalli MN, Nevers MB, Korajkic A, Staley ZR, HarwoodVJ.Enterococciintheenvironment. Microbiology and Molecular Biology Reviews. 2012;**76**(4):685-706

[47] Ferguson DM, Moore DF, Getrich MA, Zhowandai MH. Enumeration and speciation of enterococci found in marine and intertidal sediments and coastal water in southern California. Journal of Applied Microbiology. 2005;**99**(3):598-608

[48] Neely AN, Maley MP. Survival of enterococci and staphylococci on hospital fabrics and plastic. Journal of Clinical Microbiology. 2000;**38**(2):724-726

[49] Weinstein RA, Hota B. Contamination, disinfection, and cross-colonization: Are hospital surfaces reservoirs for nosocomial infection? Clinical Infectious Diseases. 2004;**39**(8):1182-1189

[50] Bonilla HF, Zervos MA, Lyons MJ, Bradley SF, Hedderwick SA, Ramsey MA, et al. Colonization with vancomycin-resistant enterococcus faecium comparison of a long-term–care unit with an acute-care hospital. Infection Control & Hospital Epidemiology. 1997;**18**(5):333-339

[51] Motlagh AM, Yang Z. Detection and occurrence of indicator organisms and pathogens. Water Environment Research. 21 Oct 2019;**91**(10):1402-1408

[52] Boehm AB, Sassoubre LM. Enterococci as indicators of environmental fecal contamination. In: Gilmore MS, Clewell DB, Ike Y, Shankar N, editors. Enterococci: From Commensals to Leading Causes of Drug Resistant Infection [Internet]. Boston: Massachusetts Eye and Ear Infirmary; 2014. Available from: http://www.ncbi.nlm.nih.gov/books/NBK190421/ [Accessed: Jan 29, 2024]

[53] Holman DB, Klima CL, Gzyl KE, Zaheer R, Service C, Jones TH, et al. Antimicrobial resistance in Enterococcus Spp. isolated from a beef processing plant and retail ground beef. Microbiology Spectrum. 2021;**9**(3):e0198021

[54] de Dapkevicius MLE, Sgardioli B, SPA C, Poeta P, Malcata FX. Current trends of enterococci in dairy products: A comprehensive review of their multiple roles. Food. 2021;**10**(4):821

[55] Kaçmaz B, Aksoy A. Antimicrobial resistance of enterococci in Turkey. International Journal of Antimicrobial Agents. 2005;**25**(6):535-538

[56] Klein G. Taxonomy, ecology and antibiotic resistance of enterococci from food and the gastro-intestinal tract. International Journal of Food Microbiology. 2003;**88**(2):123-131

[57] Gwida M, Zakaria A, El-Sherbiny H, Elkenany R, Elsayed M. Prevalence of campylobacter, enterococcus and Staphylococcus aureus in slaughtered camels. Veterinární Medicína. 2019;**64**:521-530

[58] Jamaly N, Benjouad A, Comunian R, Daga E, Bouksaim M. Characterization of enterococci isolated from Moroccan dairy products. African Journal of Microbiology Research. 2010;**4**:1768-1774

[59] Arias CA, Panesso D, McGrath DM, Qin X, Mojica MF, Miller C, et al. Genetic basis for in vivo daptomycin resistance in enterococci. The New England Journal of Medicine. 2011;**365**(10):892-900

[60] Fiedler S, Bender JK, Klare I, Halbedel S, Grohmann E, Szewzyk U, et al. Tigecycline resistance in clinical isolates of enterococcus faecium is mediated by an upregulation of plasmid-encoded tetracycline determinants tet(L) and tet(M). The Journal of Antimicrobial Chemotherapy. 2016;**71**(4):871-881

[61] Cho S, Barrett JB, Frye JG, Jackson CR. Antimicrobial resistance gene detection and plasmid typing among multidrug resistant enterococci isolated from freshwater environment. Microorganisms. 2020;**8**(9):1338

[62] Ferretti JJ, Gilmore KS, Courvalin P. Nucleotide sequence analysis of the gene specifying the bifunctional 6′-aminoglycoside acetyltransferase 2″-aminoglycoside phosphotransferase enzyme in Streptococcus faecalis and identification and cloning of gene regions specifying the two activities. Journal of Bacteriology. 1986;**167**(2):631-638

[63] Klibi N, Said LB, Jouini A, Slama KB, López M, Sallem RB, et al. Species distribution, antibiotic resistance and virulence traits in enterococci from meat in Tunisia. Meat Science. 2013;**93**(3):675-680

[64] Rahmeh R, Alomirah H, Akbar A, Sidhu J, Rahmeh R, Alomirah H, et al. Composition and properties of camel milk. In: Milk Production, Processing and Marketing. London, UK: IntechOpen; 2019. chapters/64764

[65] Muloi D, Alarcon P, Ombui J, Ngeiywa KJ, Abdullahi B, Muinde P, et al. Value chain analysis and sanitary risks of the camel milk system supplying Nairobi city, Kenya. Preventive Veterinary Medicine. 2018;**159**:203-210

[66] De Buyser ML, Dufour B, Maire M, Lafarge V. Implication of milk and milk products in food-borne diseases in France and in different industrialised countries. International Journal of Food Microbiology. 2001;**67**(1):1-17

[67] Obied AI, Bagadi HO, Mukhtar MM. Mastitis in Camelus dromedarius and

the somatic cell content of camels' milk. Research in Veterinary Science. 1996;**61**(1):55-58

[68] Abera T, Legesse Y, Mummed B, Urga B. Bacteriological quality of raw camel milk along the market value chain in Fafen zone, Ethiopian Somali regional state. BMC Research Notes. 2016;**9**(1):285

[69] Naceur B, BoudjemÃ¢a BM. Antibiotic resistance of enterococci isolated from raw camel milk in the south west of Algeria. AJMR. 7 Apr 2016;**10**(13):420-427

[70] Chingwaru W, Mpuchane SF, Gashe BA. Enterococcus faecalis and Enterococcus faecium isolates from milk, beef, and chicken and their antibiotic resistance. Journal of Food Protection. 2003;**66**(6):931-936

[71] Teuber M, Meile L, Schwarz F. Acquired antibiotic resistance in lactic acid bacteria from food. Antonie Van Leeuwenhoek. 1999;**76**:115-137

[72] Huys G, D'Haene K, Collard JM, Swings J. Prevalence and molecular characterization of tetracycline resistance in enterococcus isolates from food. Applied and Environmental Microbiology. 2004;**70**(3):1555-1562

[73] Hammad A, Hassan H, Shimamoto T. Prevalence, antibiotic resistance and virulence of Enterococcus spp. in Egyptian fresh raw milk cheese. Food Control. 2015;**50**:815-820

[74] Benkerroum N, Boughdadi A, Bennani N, Hidane K. Microbiological quality assessment of Moroccan camel's milk and identification of predominating lactic acid bacteria. World Journal of Microbiology and Biotechnology. 2003;**19**(6):645-648

[75] Tatsing Foka FE, Ateba CN. Detection of virulence genes in multidrug resistant enterococci isolated from feedlots dairy and beef cattle: Implications for human health and food safety. BioMed Research International. 2019;**2019**:e5921840

[76] Badul S, Abia ALK, Amoako DG, Perrett K, Bester LA, Essack SY. From the farms to the dining table: The distribution and molecular characteristics of antibiotic-resistant Enterococcus spp. in intensive pig farming in South Africa. Microorganisms. 2021;**9**(5):882

[77] Iweriebor BC, Obi LC, Okoh AI. Virulence and antimicrobial resistance factors of Enterococcus spp. Isolated from fecal samples from piggery farms in eastern cape, South Africa. BMC Microbiology. 2015;**15**(1):136

[78] Olsen RH, Frantzen C, Christensen H, Bisgaard M. An investigation on first-week mortality in layers. Avian Diseases. 2012;**56**(1):51-57

[79] Ybazeta G, Douglas L, Graham J, Fraleigh NL, Murad Y, Perez J, et al. Complete genome sequence of Enterococcus thailandicus strain a523 isolated from urban raw sewage. Genome Announcements. 2017;**5**(47):10-128

[80] Abriouel H, Omar NB, Molinos AC, López RL, Grande MJ, Martínez-Viedma P, et al. Comparative analysis of genetic diversity and incidence of virulence factors and antibiotic resistance among enterococcal populations from raw fruit and vegetable foods, water and soil, and clinical samples. International Journal of Food Microbiology. 2008;**123**(1):38-49

[81] Mbanga J, Amoako DG, Abia ALK, Allam M, Ismail A, Essack SY. Genomic analysis of Enterococcus spp. isolated

from a wastewater treatment plant and its associated waters in Umgungundlovu District, South Africa. Frontiers in Microbiology. 14 Jun 2021;**12**

[82] Li J, Zhou L, Zhang X, Xu C, Dong L, Yao M. Bioaerosol emissions and detection of airborne antibiotic resistance genes from a wastewater treatment plant. Atmospheric Environment. 2016;**124**:404-412

[83] El Zowalaty ME, Lamichhane B, Falgenhauer L, Mowlaboccus S, Zishiri OT, Forsythe S, et al. Antimicrobial resistance and whole genome sequencing of novel sequence types of Enterococcus faecalis, Enterococcus faecium, and Enterococcus durans isolated from livestock. Scientific Reports. 2023;**13**:18609

[84] Ekore DO, Onanga R, Nguema PPM, Lozano C, Kumulungui BS. The antibiotics used in livestock and their impact on resistance in enterococcus faecium and enterococcus hirae on farms in Gabon. Antibiotics. 2022;**11**(2):224

[85] Zhao H, Peng Y, Cai X, Zhou Y, Zhou Y, Huang H, et al. Genome insights of Enterococcus raffinosus CX012922, isolated from the feces of a Crohn's disease patient. Gut Pathogens. 2021;**13**(1):71

[86] Giannattasio-Ferraz S, Maskeri L, Oliveira AP, Barbosa-Stancioli EF, Putonti C. Draft genome sequences of three enterococcus casseliflavus strains isolated from the urine of healthy bovine heifers (Gyr breed). Microbiology Resource Announcements. 2020;**9**(21):e00386-e00320

[87] Zhong Z, Kwok LY, Hou Q, Sun Y, Li W, Zhang H, et al. Comparative genomic analysis revealed great plasticity and environmental adaptation of the genomes of Enterococcus faecium. BMC Genomics. 2019;**20**(1):602

[88] Guzman Prieto AM, van Schaik W, Rogers MRC, Coque TM, Baquero F, Corander J, et al. Global emergence and dissemination of enterococci as nosocomial pathogens: Attack of the clones? Frontiers in Microbiology. 2016;**7**:788

[89] Tao S, Zhou D, Chen H, Li N, Zheng L, Fang Y, et al. Analysis of genetic structure and function of clustered regularly interspaced short palindromic repeats loci in 110 Enterococcus strains. Frontiers in Microbiology. 2023;**14**:1177841

[90] Beukers AG, Zaheer R, Goji N, Amoako KK, Chaves AV, Ward MP, et al. Comparative genomics of Enterococcus spp. isolated from bovine feces. BMC Microbiology. 2017;**17**(1):52

[91] Krogstad DJ, Pargwette AR. Defective killing of enterococci: A common property of antimicrobial agents acting on the cell wall. Antimicrobial Agents and Chemotherapy. 1980;**17**(6):965-968

[92] Callahan KL, Beck NK, Duffield EA, Shin G, Meschke JS. Inactivation of methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococcus faecium (VRE) on various environmental surfaces by mist application of a stabilized chlorine dioxide and quaternary ammonium compound-based disinfectant. Journal of Occupational and Environmental Hygiene. 2010;**7**(9):529-534

[93] Saurina G, Landman D, Quale JM. Activity of disinfectants against vancomycin-resistant enterococcus faecium. Infection Control & Hospital Epidemiology. 1997;**18**(5):345-347

[94] Jabłońska-Trypuć A, Makuła M, Włodarczyk-Makuła M, Wołejko E, Wydro U, Serra-Majem L, et al. Inanimate surfaces as a source of hospital infections caused by fungi, bacteria and viruses with particular emphasis on SARS-CoV-2. International Journal of Environmental Research and Public Health. 2022;**19**(13):8121