

Enterococci in Meat: Understanding Consequences on Health and Food Safety

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Abstract

The focus of this review is the occurrence of Enterococci in meat and its consequence on health and food safety. Two species most commonly encountered in food as well as in hospital infections are *Enterococcus faecalis* and *Enterococcus faecium*. Members of the genus Enterococci are well known for their resistance to high temperatures, pH and salt concentrations. They are also known for their widespread resistance to a broad spectrum of antibiotics including some antibiotics of last resort such as vancomycin and linezolid. Antibiotic resistance is mostly mediated by genes borne on the chromosomes, plasmids or transposable elements. This also means they can acquire and transfer resistance easily. In addition, they possess a number of virulence determinants that aide invasion and colonization, as well as biofilm formation. Enterococci are found in different environments including soils, water, wastewater and slaughterhouses. Because of their widespread presence in different parts of the animal body, Enterococci easily find their way into foods of animal origin such as meat, poultry and milk. Strict personal and environmental hygiene must be observed while processing meat and other foods of animal origin to limit contamination with Enterococci and other foodborne pathogens. Prudent use of antibiotics both in healthcare and animal husbandry must be adopted to minimize the speed and scope of the development of resistance by Enterococci and other pathogens.

Introduction

Enterococci are a genus of Gram positive, non-spore forming, facultative anaerobic bacteria belonging to the phylum *Firmicutes*. Enterococcus was historically categorized as lactic acid bacteria of the Lancefield group D streptococcus (Klein, 2003). It is widely known for its ability to withstand the extremes of environmental conditions such as temperature (10°C to 45°C), high salinity (6.5% NaCl) and heating for up to 60°C for 30 minutes (Abouelnaga et al., 2016). Enterococci are also

widely distributed in the environment, occurring in such varied niches as soil, rivers, as well as fresh plant and animal products (Johnston & Jaykus, 2004). It is also an important commensal, found in the gastrointestinal tract of most animals including humans and it is found in the faeces of both humans and animals (Sustackova et al., 2004). Their resilience to environmental stress makes them hard to get rid of from food and from the environment (Krocko et al., 2008). Thus, enterococcus is among bacterial groups that serve as surrogates for the



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determination of faecal contamination of food or water (Ghosh & Zurek, 2015).

Enterococci are also known for their ability to produce bacteriocins that eliminate many types of harmful microbes, making them good candidates for biocontrol of food contamination (Sanlibaba et al., 2018). They are considered as agents of fermentation in the making of some traditional food products such as some artisanal cheeses (Franz et al., 2001). Enterococcus however, also serves as a source of contamination of food, especially foods of animal origin such as meat and milk products (Chajęcka-Wierzchowska et al., 2020). Although more than 50 genera of enterococcus have been isolated, the most important ones are *Enterococcus faecalis* and *Enterococcus faecium* (Ramos et al., 2020). Enterococcus has emerged in recent decades as an important nosocomial pathogen, ranked as number three among bacterial infections acquired in the hospital environment especially in immunocompromised individuals (Tansuphasiri et al., 2006; Fiore et al., 2019; Vyrostkova et al., 2021). The organism has been implicated in urinary tract infection, endocarditis and bacteremia (Ezeh et al., 2023). It possesses an array of virulence factors in addition to transmission and acquisition of antibiotic resistance often to multiple antibacterial agents (García-Solache & Rice, 2019). It is also capable of biofilm formation (Venkateswaran et al., 2022). Similarly, virulence and antibiotic resistance genes are capable of being transmitted between enterococci strains and species and to other species (Kayaoglu & Orstavik, 2004). This means that Enterococcus can vertically transfer various antibiotic resistance genes to unrelated species that hitherto do not carry such resistance genes (Klibi et al., 2015).

Resistance to important antibiotics such as vancomycin and linezolid is a serious challenge for the future of treatment of clinical infections (Goh et al., 2017; Chen et al., 2018). Thus, in spite of not being a primary pathogen, enterococci are bacteria of concern in both food and hospital environments. This review discusses the occurrence of enterococci in meat, with a focus on the virulence, antibiotic resistance, and resilience to environmental stress

that make these organisms a significant public health concern.

Occurrence of *Enterococci* in the Environment

As a testament to their hardiness, enterococci including *E. faecalis* and *E. faecium* have been isolated from different sources and environments such as soils, fresh and seawater, plants, foods of animal origin, feeds and in the hospital environment where they cause different opportunistic infections, in addition to their status as components of human and animal microbiota (Dapkevicius et al., 2021; Tuncay & Sancak, 2022). Due to the different operations involved in meat production from slaughter to the washing and cleaning of surfaces and equipment, slaughterhouses produce large volumes of wastewater (Igbinosa & Raje, 2019). Strains of *Enterococci* have been isolated from such wastewater coming from slaughterhouses and its different surfaces due to contact with slaughtered animals and their wastes, and from other environments including sewage treatment plants, farm animal faeces, poultry meat and manure from farm houses (Giraffa, 2002; Rabah, 2022).

Enterococci are commensals in the gastrointestinal tracts of many animals and are involved in the spoilage of some meat types (Anderson et al., 2016). In the intestinal environment, *E. faecalis* is the most abundant *Enterococcus* followed mainly by *E. faecium* and then *E. hirae* and *E. durans* (Braiek & Smaoui, 2019). This picture is similar to what obtains in the hospital, with additional occurrence of *E. avium*, *E. casseliflavus*, *E. gallinarum* and *E. raffinosus* from time to time (Giraffa, 2002). Antibiotic resistant strains of *E. faecium* have also been isolated from hospital patients (Hanchi et al., 2018). The natural resistance of *Enterococci* to environmental stress such as extremes in temperature, pH and salinity has been well described. They can grow at temperatures between 10 to 45°C and survive heating of up to 60°C for 30 minutes. Further, they can tolerate the presence of 6.5% NaCl and pH 9.6 with few exceptions by different species depending on the prevailing conditions (Foulquié Moreno et al., 2006). Their intrinsic ability to acquire and disseminate antibiotic resistance and virulence determinants

contribute to their selection for persistence in the environment (Araujo et al., 2021). Enterococci possess the ability to form biofilms, which is capable of worsening infection outcomes by making infections difficult to treat with antibiotics (Soni et al., 2022).

Presence of *Enterococci* in Meat

Because enterococci are normal flora of most farm and domestic animals, especially in the gut, they have been isolated in the meat of different farm animals such as cattle, goats, pigs as well as from poultry and other animal products such as milk and cheese (Krocko et al., 2008; Bortolaia et al., 2016). Foods of animal origin can easily become contaminated by faecal enterococci led by *E. faecalis* followed by *E. faecium*. The level of contamination with enterococci has been found to be as high as 90% in some foods of animal origin by many studies (Makarov et al., 2022). Studies suggest that enterococcal contamination of meat often occur when the meat come into contact with contents of the digestive tract during slaughter (Gokmen et al., 2017). The nature of meat contamination is such that the microbial continues to increase from the point of processing at the slaughterhouse until it reaches the final consumer (Nagarajan et al., 2018).

Although several studies used techniques such as the multilocus sequence typing (MLST) to demonstrate that enterococci can be highly host specific, for example the *E. faecium* CC17 that is confined to hospital infections and not found in healthy humans, some *E. faecalis* strains can share their colonizing ability equally between animals and humans (Kim et al., 2017). Studies have found the evidence of transfer of genetic elements such as *VanA* and *ErmB* between animals or their meat and humans, sometimes by colonizing human intestine after consumption of contaminated food and serving as donors of virulence factors, in spite of the host specificity that exists. But strict good hygiene is required when handling meat to minimize *Enterococcal* contamination since the organism is difficult to control due to their resistance to harsh environmental conditions (Hammerum et al., 2010). In a Polish study of vancomycin resistant *Enterococci* in slaughterhouses, 66% contamination with *E. faecium* and *E. faecalis* was detected from 140

samples of meat and previously decontaminated surfaces as well as mesophilic counts exceeding the acceptable limits of 10 cfu/cm² on the surfaces of the facilities and the hands of workers, as a result of poor hygiene (Lawniczek-Walczyk et al., 2022). Furthermore, some investigations on the spread of resistance from animals to humans found higher occurrence of resistant strains among individuals with close contact with animals such as veterinarians, farm workers and even restaurant workers (O'Dea et al., 2019).

Antibiotic Resistance in *Enterococcus*

Enterococci are known to be resistant to a broad range of antibiotics including aminoglycosides, cephalosporins, monobactams, lincosamides, macrolides, streptogramins and glycopeptides, some acquired and others intrinsic (Duckova et al., 2014). Although multidrug resistance used to be more widespread in hospital settings, now it is spreading to the community with negative consequences such as increase in mortality and cost of treatment (Naas et al., 2017). Introduction of antibiotics to animal feeds for growth promotion begun in the mid-20th century, a practice which increased risks of transfer of resistant bacterial strains from food animals to humans (Manson et al., 2019). The use of critically important antibiotics such as glycopeptides and aminoglycosides such as gentamicin for this purpose also becomes dangerous for making the treatment of infections caused by enterococci and other pathogens difficult due to resistance (Gwida et al., 2019; Sigirci et al., 2021). This widespread resistance makes vancomycin antibiotic of choice for the treatment of aminoglycoside resistant infections. However, resistance against vancomycin developed eventually as well (Onaran et al., 2019). Other antibiotics introduced to treat vancomycin resistance such as daptomycin and linezolid are becoming subject to resistance too (Mariam, 2021).

Animal commensal enterococci harboring resistance genes may come in contact with human pathogenic bacteria, after entering the environment by means of animal manure used as fertilizer or through discharges from wastewater treatment processes (Aun et al., 2021). When healthy people carry vancomycin resistant enterococci (VRE) it

becomes a latent source of serious infection when such individuals receive a medical device implant, become immunocompromised or hospitalized (Fang, 2020). Especially when prolonged admission periods occur (Graham et al., 2020). A study in Bangladesh and other places found the presence of resistant *E. faecium* and *E. faecalis* in poultry and meat. With high prevalence of VRE (44%; n=51) often with co-occurrence with linezolid resistance. Resistance often arises from the presence of resistance genes such as *vanA*, *vanC1* or *vanC2/3* carried either on the chromosome or on plasmid (Samad, 2021). In a study to assess the presence of enterococci and antimicrobial resistance (AMR) in beef processing facility and retail ground beef in Alberta, *E. faecalis* was more widespread in occurrence while *E. hirae* had higher isolation rate post hide removal partly because of higher faecal contact. *E. hirae* is the most common enterococci in livestock faeces (Zaheer et al., 2020).

Enterococci isolation rate in the carcasses postwashing and from the conveyor belt area was lower than in any other sample but 82.7% of ground beef from the plant was contaminated with enterococci nonetheless. Contamination may have come from equipment, cutting processes, workers and the environment. The study observed resistance of *E. faecalis* to tetracycline mediated by the *tetM* gene and inherently to lincomycin and quinupristin-dalfopristin mediated by the chromosomally carried *isaA* gene (Holman, 2021). Makarov et al. (2022) discovered AMR of *E. faecalis* and *E. faecium* from chickens, turkeys, and pigs to be higher than those from cattle, sheep, and ducks where resistance for bacitracin and virginiamycin occurred the most. The isolates from turkeys, chickens, and pigs were more resistant to tetracycline, erythromycin, ciprofloxacin, and the trimethoprim-sulfamethoxazole combination as well as rifampicin in all animal classes studied. *OptrA* gene often confers resistance to oxazolidinone classes of antibiotics such as linezolid in *E. faecalis* and *E. faecium* and it is mostly associated with food animals such as cattle as well as their products and processing environment (Zaheer, 2020). However, there exists a core

genome and pan-genome separation between animal and clinical *Enterococci* and genetic exchanges do not always readily occur between the two (O'dea, 2019).

Virulence Factors of *Enterococci*

The European Food Safety Authority (EFSA) issued a guide suggesting that any strain of *E. faecium* with ampicillin MIC > 2 µg mL⁻¹ and having the genetic elements IS16, *hylEfm* and *esp* in its genome are of clinical and epidemiological significance and should be regarded as unsafe (Mariam, 2021). Clinical *E. faecalis* isolates often carry more than five virulence genes simultaneously (Tuncay & Sancak, 2022). *Enterococci* often harbor virulence genes such as *ace* gene for collagen binding protein, *asa1* gene for aggregation substance, *cylA* gene for cytolysin, *efaA* gene for endocarditis antigen, *esp* gene for enterococcal surface protein, *gelE* for gelatinase and *hyl* gene for hyaluronidase (Anderson et al., 2016). These play roles in invasion, colonization, adhesion, manipulation of host defence mechanisms as well as biofilm formation (Giraffa, 2002; Anderson et al., 2016).

Biofilm formation is important in pathogenesis of enterococci, because of the important role it plays in colonization and drug resistance (Dapkevicius et al., 2021). It enables more resistance to environmental stress and persistence during infection or as continuous source of contamination in food processing environments (Barel et al., 2023). Strains residing in biofilms have been shown to exhibit much more resistance to antibiotics (Chajęcka-Wierzchowska et al., 2020). Bacterial biofilms can develop on tissue epithelia or on body implant devices, complicating the treatment process (Madeiros et al., 2014). Fahmy et al. (2021) found a fairly equal distribution of virulence markers between *E. faecalis* and *E. faecium* and an association between the *esp* & *asa1* genes and biofilm formation. Madeiros et al. (2014) also found strong positive correlation between the common presence of biofilm and the presence of *esp* and *ace* virulence factors, in a study in which all isolates of clinical and food sources formed biofilm *in vitro* using a technique of polyethylene adhesion.

Table 1. Antibiotic resistance profiles, virulence determinants, and occurrence of *Enterococcus* species isolated from meat and processing environments.

Enterococci Species	Antibiotic Resistance Phenotypes & Genotypes	Virulence Factors & Mechanisms	Sample Source	Citation
<i>Enterococcus faecalis</i>	Tetracycline (<i>tetM</i>)	Multi-virulence carrier (often >5 genes simultaneously):	Retail ground beef	Zaheer (2020)
	Lincomycin & Quinupristin-dalfopristin (<i>isaA</i>)		Beef processing plant (carcasses post-washing, conveyor belts)	Holman (2021)
	Oxazolidinones / Linezolid (<i>optrA</i>)	<i>ace</i> (Collagen binding protein)		Samad (2022)
	Vancomycin (VRE) (<i>vanA</i> , <i>vanC1</i> , <i>vanC2/3</i>)	<i>asa1</i> (Aggregation substance)	Farmed animals and poultry meat	Anderson et al. (2016)
	Co-resistance to linezolid and vancomycin	<i>cylA</i> (Cytolysin)	Cattle products	Tuncay & Sancak (2022)
		<i>efaA</i> (Endocarditis antigen)		Medeiros et al. (2014)
		<i>esp</i> (Surface protein)		
<i>Enterococcus faecalis</i>	Ampicillin (High-level: MIC > 2 µg mL ⁻¹)	<i>IS16</i> , <i>hylEfm</i> , and <i>esp</i> genetic elements (markers of clinical significance)	Chickens, turkeys, and pigs (higher AMR rates than cattle/sheep)	Mariam (2021)
	Vancomycin (VRE) (<i>vanA</i> , <i>vanC1</i> , <i>vanC2/3</i>)	Strong correlation between <i>esp</i> & <i>asa1</i> genes and in vitro biofilm production	Slaughterhouse environments and decontaminated surfaces	Makarov et al. (2022)
	Linezolid (<i>optrA</i>)			Ławniczek-Wałczyk et al. (2022)
	Multi-drug resistance (MDR) to tetracycline, erythromycin, ciprofloxacin, and trimethoprim-sulfamethoxazole		Pasteurized and fermented dairy products	Fahmy et al. (2021)
				Samad (2022)
<i>Enterococcus hirae</i>	Baseline livestock resistance profiles	Surface adherence elements facilitating initial attachment to hide and carcass tissue	Livestock feces, beef processing facilities, and hide-removal areas	Zaheer et al. (2020)
	Dominant species found post-hide removal due to direct fecal contact			Holman (2021)

Conclusion

Meat processing environments such as slaughterhouses are one of the commonest niches where contamination with enterococci and other foodborne bacterial pathogens occur. Strict hygiene must thus be observed and proper cleaning and disinfection done before and after a day's work to control contamination and foodborne illness. This also helps in preventing the formation of biofilms which make cleaning and prevention of contamination incredibly difficult. Antibiotic resistance and possession of an array of other virulence factors make enterococci potentially dangerous. Limiting antimicrobial use in animal husbandry and the establishment of a national AMR surveillance programme is crucial to curbing the rapid acquisition and spread of antibiotic resistance among farm animals. Although many studies suggest that there is no sufficient evidence to suggest that transfer of virulent enterococci or their genes occur between animals and humans, enterococci are serious opportunistic human pathogens in their own right with antibiotic resistance properties in the hospital environment. More investigations are needed to have deeper and more definitive understanding of the dynamics of pathogen and virulence exchanges between animals and humans as well as other entities in the ecosystem.

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Author contribution

AI drafted the manuscript, while AMM and UAD assisted with reviewing the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflicts of interest regarding this publication.

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