OCCURRENCE, ANTIMICROBIAL RESISTANCE AND PATHOGENIC FACTORS OF *ENTEROCOCCI*

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ABSTRACT

Enterococci are Gram-positive, non-spore-forming, catalase-negative, facultative anaerobic cocci and commensal flora of the gastrointestinal tract. Although believed initially to be of low clinical importance, they have been incriminated as nosocomial pathogens. With many of the species being considered drug resistant and resistance genes transfer culprits; they are reported as being responsible for many deaths. Public health concerns about the genus have made it necessary to further scrutinize the existing information on them. This work therefore reviewed studies on enterococci with emphasis on their occurrence, as well as their antimicrobial resistance and pathogenic factors. Available data showed that Enterococci were present in large quantities in the environment and that they are the chief source of food and water contamination, with the multi-drug resistant strains being especially worrisome. They were found resistant to physical and chemical agents; and proven to be extremely pathogenic as nosocomial infections. Their pathogenicity were linked to multidrug resistant strains having certain virulence determinants and the notable strains are the vancomycin-resistant enterococci (VRE). Good hygiene practices should be maintained during processing to reduce nosocomial exposure to and ingestion of these organisms in food and water.

Keywords: Enterococci, Multidrug resistant, Pathogenic, Nosocomial, Vancomycin-resistant enterococci, Virulence determinants

INTRODUCTION

In humans and livestock, enterococci are normal component of the commensal flora of the gastrointestinal tract (Hammerum, 2012). They are therefore found in great numbers in the faeces and having the ability to survive hostile conditions, are well-equipped to colonize several ecological niches (soil, water and food), thereby acting as markers of fecal contamination and sanitary quality (Anyanwu *et al.*, 2019; Igbinosa and Beshiru, 2019). The *Enterococcus* genus is the third most common lactic acid bacteria (LAB), next to *Streptococcus* and *Lactobacillus* (Ben Braiek and Smaoui, 2019). Although initially seen as an organism of low clinical impact, they are now considered common nosocomial pathogens, with patients dying at a rate as high as 61% (Fisher and Phillips, 2009). Some diseases caused by these organisms include bacterial endocarditis, bacteremia, urinary tract infection and meningitis (Economou *et al.*, 2016).

Enterococci are not just multidrug resistant; they are also characterized by the tendency to trade genetic materials (Giraffa,

2002). Mobile genetic components like plasmids and transposons, as well as chromosomal exchange and mutation, help to facilitate this special ability (Guerrero-Ramos *et al.*, 2016). The increasing antibiotic resistance of enterococci, as well as the presence of active gene transfer mechanisms, exacerbates their growing as nosocomial opportunists (Giraffa, 2002). Inherently, they are resistant to a number of antibiotics as well as the acquisition of resistance to the few antibiotics available for treatment (such as vancomycin) leading to therapeutic challenges. In the United States of America, there has been an upsurge in enterococcal infections, which now account for 10 to 14% of hospital-acquired illnesses (Rao *et al.*, 2020). Enterococcal infections usually originate from the intestinal microbiota of their victim and may be disseminated across individuals or contracted through the consumption of contaminated food and water (Brilliantova *et al.*, 2010). The overwhelming public health implications of enterococcal infections therefore necessitate a greater understanding of this 'unique' organism; its occurrence and pathogenicity.

MATERIALS AND METHODS

A systematic search of published articles in Google Scholar, PubMed and Science Direct databases from 2000 to 2019 was conducted using the following keywords: Enterococcus, Antimicrobial resistance in *Enterococcus*, virulence factors of *Enterococcus*, Pathogenicity of *Enterococcus* and *Enterococcus* in the environment. Excluded were studies classified as citation, dissertation, or thesis. The references in the identified articles were screened, yielding a total of 67 publications, 34 of which were excluded due to publication year. Between 2000 and 2019, 101 articles were found in the database using the keywords, of which 29 were duplicates and were excluded.

RESULTS AND DISCUSSION

Classification: According to Lee *et al.* (2019), Thiercelin defined enterococci as a Gram-positive diplococcus of intestinal origin in 1899. *Streptococcus faecium* and *Streptococcus faecalis* were then designated as enterococcal species within the genus *Streptococcus* (Sood *et al.*, 2008). Streptococci were eventually split into four classes by Sherman in 1937, including *Enterococcus*, pyogenic, viridians and lactic (Lee *et al.*, 2019). The term *Enterococcus* was therefore defined as organisms that grew between 10 and 45 °C, in 6.5% NaCl, at pH 9.6, survived at 60 °C for 30 minutes; and hydrolyzed esculin (Sood *et al.*, 2008). Their ability to survive for 30 minutes at 60°C, thrive in broth augmented with 40% bile salts and hydrolyze esculin distinguished them from *Streptococci* (Ben Braiek and Smaoui, 2019).

Molecular taxonomic approaches have made possible the recognition of *Streptococcus* and *Enterococcus* as distinct genera. Based on the 16S rRNA cataloguing in the 1980s, *Streptococcus* was divided into three genera: *Enterococcus*, *Lactococcus* and *Streptococcus* (Giraffa, 2002). Thus, the bacteria originally known as *Streptococcus faecium, Streptococcus faecalis, Streptococcus gallinarum* and *Streptococcus avium* were in 1984 renamed *Enterococcus faecium*, *E. faecalis*, *E. gallinarum* and *E. avium* respectively (Giraffa, 2002). Thirty-seven species were identified based on phylogenetic analysis using DNA-DNA hybridization and 16S rRNA sequencing; however, new species including *E. ureasiticus, E. caccae, E. pallens, E. thailandicus and E. cammelliae* were later discovered (Ben Braiek and Smaoui, 2019). In all, the most prevalent enterococcal species are *E. faecium* and *E. faecalis*.

Occurrence of Enterococci

Enterococci in animals: Most mammals, birds, insects and reptiles have enterococci as part of their natural intestinal flora (Oprea and Zervos, 2007). Some enterococci species are host-specific, while others are more widespread. The most common enterococcal species found in the intestines of livestock are *E. faecalis, E. hirae, E. faecium* and *E. durans*. In chicken, *E. faecalis* is found early in life and later *E. faecium*, which is then replaced by *E. cecorum*. Other species found in chickens include *E.*

cassseliflavus, E. gallinarum and *E. mundtii* (Oprea and Zervos, 2007).

It is worthy to note that enterococci of animal origin can colonize the human intestine (Bonten *et al.*, 2001) and that the presence of commensal enterococci in close proximity to resistant enterococci of animal origin can cause the transfer of genetic components associated with antimicrobial resistance (Sood *et al.*, 2008). Although, *E. faecium* of animal origin are usually not of significant threat themselves to humans, but they can serve as a reservoir of resistant genes to human pathogenic strains (Hammerum, 2012). The *E. faecalis* strains however, are almost identical to human pathogenic strains and are therefore of concern to humans (Larsen *et al.*, 2010).

Enterococci in animals are opportunistic pathogens, with infections largely associated with poor hygiene. Enterococci have been isolated from a variety of animal infections, including bovine mastitis. *Enterococcus faecium* and *E. faecalis* are the most commonly implicated species, while *E. durans*, *E. hirae, E. avium* and *E. pseudoavium* have also been associated with diseases in animals (Table 1).

Table 1: Members species of the *Enterococcus* groups

***Enterococcus* group**	**Member species**
***E. avium* group**	*E. avium, E. devriesei, E. gilvus, E. malodoratus, E. pseudoavium. E. raffinosus*
***E. cecorum* group**	*E. cecorum, E columbae*
***E. dispar* group**	*E. dispar, E. asini, E. canintestini, E. hermanniensis, E. pallens*
***E. faecalis* group**	*E. faecalis, E. caccae, E. haemoperoxidus, E. moraviensis, E. silesiacus, E. termitis, E. ureasiticus, E. quebecensis*
***E. faecium* group**	*E. faecium, E. canis, E. durans, E. hirae, E. mundtii, E. phoeniculicola, E. ratti, E. villorum, E. thailandicus*
***E. gallinarum* group**	*E. gallinarum, E. casseliflavus*
***E. saccharolyticus* group**	*E. saccharolyticus, E. acquimarinus, E. camelliae, E. italicus, E. sulfureus*

Culled from Economou et al. (2016)

Enterococcosis has been reported in a variety of avian species around the world, with the disease manifesting itself in acute, subacute and chronic forms (Economou *et al.*, 2016). Clinical signs of the disease include depression, lethargy, ruffled feathers, diarrhea, decrease in egg production, head tremors and in majority of the instances, death (Economou *et al.*, 2016).

Enterococci in humans: Enterococci are the most prevalent Gram-positive cocci in human faeces, with concentrations ranging from 105 to 107 CFU/g (Oprea and Zervos, 2007). The two species that are commonly associated with human clinical specimens are *E. faecium* and *E. faecalis*, but infections with *E. raffinosus* and *E. casseliflavus* have also been observed (Oprea and Zervos, 2007). *Enterococcus hirae, E. cecorum, E. dispar, E. durans, E. gilvus, E. gallinarum, E. avium, E. mundtii and E. pallens* are among few of the species that have occasionally been isolated from human sources (Oprea and Zervos, 2007). Hospitalized patients' alimentary canals, ulcers and soft tissue wounds are the main sites where enterococci colonize. However, the genitourinary tract, the epidermis and the perineum in particular, are occasionally colonized (Sood *et al.*, 2008).

Contrary to previous assumptions, enterococci strains have been identified as the second leading cause of wound and urinary tract infections (Pinto *et al.*, 2021), as well as the third leading cause of bacteremia in Europe (Uda *et al.*, 2021). As of 2005, there were 7066 cases of enterococci bacteremia reported in the United Kingdom (Table 2). Sixty three percent (63%) and 28% of those cases were attributed to *E. faecalis* and *E. faecium*, respectively, with significant antibiotic resistance rates (Fisher and Phillips, 2009). Typically, enterococci infections occur in elderly patients with major underlying illnesses and in other immunocompromised individuals who have spent a long time in the hospital. A patient's use of intrusive gadgets or receipt of broad-spectrum antibiotics is also attributed to it (Oprea and Zervos, 2007).

Enterococci in feedstuff: One of the lactic acid bacteria (LAB) that is crucial in foods is enterococci. Vegetables, plant matter and food,

Table 2: Intergroup comparison of demographic and clinical characteristics of patients with enterococcal bacteraemia and risk factors for the acquisition of *Enterococcus faecalis* and *E. faecium*

Variables	*E. faecalis* (n = 88)	*E. faecium* (n = 94)	*P-value*	Adjusted OR(95% CI)	*p-value*
Age (years), median (IQR)	73.5(66–80)	72(65–75)	0.073		
Male sex, n(%)	53(60)	54(58)	0.82		
Hospitalization ward, n(%)					
Medical ward	24(27)	30(32)	0.60		
Surgical ward	41(47)	33(35)	0.15		
Intensive Care Unit	23(26)	31(33)	0.40		
Comorbidities, n(%)					
Chronic renal failure	35(40)	32(34)	0.52		
Dialysis	8(9.1)	10(11)	0.9		
Diabetes mellitus	21(24)	18(19)	0.55		
Cardiovascular disease	23(26)	12(13)	0.058		
Previous cardiac valve replacement	11(13)	9(9.6)	0.69		
Coronary artery bypass grafting	3(3.4)	3(3.2)	0.9		
Hepatobiliary tumor	5(5.7)	20(21)	0.005	3.01(0.87-10.5)	0.083
Other solid tumors	15(17)	13(14)	0.69		
Hematologic tumor	3(3.4)	13(14)	0.027	7.85(1.96–31.4)	0.004
Solid organ transplant recipient	1(1.1)	1(1.1)	1		
Bone marrow transplant recipient	1(1.1)	2(2.1)	1		
Neutropenia	0(0.0)	9(9.6)	0.008		
Hepatobiliary disease	6(6.8)	3(3.2)	0.43		
Collagen disease	1(1.1)	10(11)	0.018	8.41(0.91–77.7)	0.061
Source of infections, n(%)					
Central venous catheter	18(21)	23(25)	0.64		
Cholecystocholangitis	8(9.1)	30(32)	<0.001	5.21 (1.89–14.3)	0.001
Urinary tract infection	14(16)	4(4.3)	0.017		
Intra-abdominal infection	3(3.4)	9(9.6)	0.17		
Febrile neutropenia	0(0.0)	8(8.5)	0.015		
Infectious endocarditis	4(4.6)	0(0.0)	0.11		
Wound infection	2(2.3)	1(1.1)	0.95		
Unknown	24(27)	14(15)	0.041		
Others	6(6.8)	2(2.1)	0.24		
Hospital stay before the onset of bacteremia(days), median (IQR)	23.5 (8–56.5)	31(13.3–75.8)	0.13		
qSOFA score ≥ 2, n(%)	27(31)	29(31)	1		
Recent surgery, n(%)	32(36)	31(33)	0.75		
Invasive devices, n(%)					
Central intravenous catheter	39(44)	50(53)	0.29		
Urinary catheter	43(49)	39(42)	0.40		
Immunosuppression (within 30 days), n(%)					
Immunosuppressive treatment	2(2.3)	9(9.6)	0.079		
Corticosteroid treatment	13(15)	26(28)	0.053		
Chemotherapy	5(5.7)	13(14)	0.11		
Previous antibiotic therapy(within 30 days)					
Non-antipseudomonal penicillins					
Number of patients (%)	16(18)	27(29)	0.13		
Duration of use, median (IQR)	6(3–9)	5(2.5–8.5)	0.68		
Antipseudomonal penicillins					
Number of patients (%)	14(16)	42(45)	<0.001	4.04(1.81–9.0)	<0.001
Duration of use, median (IQR)	7(4.3–9.5)	6(4–8)	0.89		
Cephalosporins					
Number of patients (%)	51(58)	53(56)	0.95		

Duration of use, median (IQR)	5(3–7)	5(2–10)	1		
Carbapenems					
Number of patients (%)	16(18)	42(45)	<0.001	3.33 (1.51–7.36)	0.003
Duration of use, median (IQR)	7(3.8–9)	6.5 (4.3–10.8)	0.24		
Quinolones					
Number of patients (%)	9(10)	19(20)	0.097		
Duration of use, median (IQR)	4(4–6)	7(4–9)	0.69		
Aminoglycosides					
Number of patients (%)	0(0.0)	3(3.2)	0.27		
Duration of use, median (IQR)	0(0–0)	3(2.5–3.5)	<0.001		
Anti-MRSA agents(VCM)					
Number of patients (%)	15(17)	28(30)	0.065		
Duration of use, median (IQR)	5(2.5–9.5)	4(2–5.3)	0.26		
Anti-MRSA agents(DAP, LZD)					
Number of patients (%)	6(6.8)	10(11)	0.52		

Culled from Uda et al., 2021

especially those with animal origin, like fermented sausages and cheeses, all contain LAB often in high concentrations (Giraffa, 2002). This occurrence is frequently linked to their ubiquity and resilience in harsh environments. Thus, they are prepared to colonize various ecological niches and spread throughout the environment via infected animals and contaminated foods.

Due to their critical role in the development of organoleptic characteristics during the ripening of cheeses and their inclusion in starter cultures for cheese, enterococci have significant effects in the dairy sector (Foulquié Moreno *et al.*, 2006). Their actions, however, are undesirable in processed meats since they result in deterioration. Enterococci are able to contaminate final products during food processing because they have the ability to survive high heat levels. As a result, enterococci are commonly found in fermented meat and dairy products like cheeses, among many other foods (Table 3). Enterococci have also been isolated from egg contents, a phenomenon that is not frequently reported (Economou *et al.*, 2016).

Some enterococci from dietary sources also have some advantageous biotechnological qualities, such as the ability to produce bacteriocin and probiotic properties. This illustrates why they are used in fermented food products. Bacteriocins (enterocins), also known to have anti-Listeria activity, inhibit or kill some enterococci strains, clostridia, bacilli and staphylococci (Oprea and Zervos, 2007).

Table 3: Origin of bacteriocinogenic *Enterococcus* strains

Source	Strain(s)
Dairy products	*E. faecium* DPC1146
	E. faecium 7C5
	E. faecium RZS C5
	E. faecalis INIA 4
	E. faecium CRL 35
	E. faecium WHE 81
	Screening for Bac$^+$ strains
	Screening for enterocin AS-48 producer strains
	E. faecalis TAB 28
	Screening for Bac$^+$ strains
	E. faecalis FAIR-E 309
	E. faecium FAIR E-198
	Screening for Bac$^+$ strains
Fermented sausages	*E. faecium* L1
	E. faecium CTC492
	E. faecalis EFS2
	E. faecium T136
	E. faecium P13
	E. faecium L50
	E. faecium AA13
	E. faecium G16
	E. faecium P21
	E. casseliflavus IM 416K1
Other fermented foods	*E. faecium* N15
	E. faecium B1
	E. faecium B2
	Screening for Bac$^+$ strains
	Screening for Bac$^+$ strains
Fish	Screening for Bac$^+$ strains
Vegetables	*E. faecalis* 226
	E. faecium BFE 900
	E. mundtii ATO6
	E. faecium 6T1a
Silage	Screening for Bac$^+$ *E. faecium* strains
	E. faecium RZS C13

	E. faecium NIAI 157
	E. faecalis K-4
Veterinary	*E. faecium* CCM 4231
	E. faecium BC25
	E. faecium J96
	E. faecalis BFE 1071
	Screening for Bac^+ strains
	E. gallinarum 012
Water	*E. faecalis* EJ97
Human origin	*E. faecalis* S-48
	E. faecium YI717
	Screening for Bac^+ strains
	E. faecium RC714

Culled from Foulquié Moreno et al. (2006)

Additionally, enterococci are used as probiotics to improve the intestinal flora's balance and treat both human and animal gastroenteritis (Eaton and Gasson, 2001). The safety of enterococci used in food processing or as probiotics, however, has been questioned due to their role in several diseases. This is frequently related to their interaction with humans in terms of their intestinal habitat; incorporation into the food chain; propensity for developing antibiotic resistance and potential participation in food-borne illnesses as a result of the presence of virulence factors.

There are a number of issues with enterococci in foods, despite the fact that they are not typically thought of as a foodborne pathogens. *Enterococci faecium* and *E. faecalis* strains are frequently present in clinical specimens from humans and also commonly found in foods and used as starter cultures (Giraffa, 2002). Enterococci are believed to produce biogenic amines that lead to food intoxication; however, this has not yet been proven (Giraffa, 2002). The first enterococci-related foodborne illness occurred in 1926, when there were two outbreaks of gastroenteritis linked to cheese. Enterococci were incriminated for the contamination due to its extensive distribution in the implicated foods and the absence of other pathogens like *S. aureus* or *Salmonella* spp. (Oprea and Zervos, 2007).

Enterococci in the environment: According to Hayes *et al.* (2003), enterococci are known to exist in extra-enteral habitats such soil, sewage, water bodies, plants, meat (Table 4) and dairy products in addition to the intestinal tracts of most animals. Therefore, given the large numbers of enterococci in faeces, their presence in recreational waters and foods are evidence of fecal pollutions.

Table 4: Prevalence of *Enterococcus* spp. among retail meat products from Iowa

Meat class	Number sampled	Number positive	% Positive
Turkey	227	226	99.6
Chicken	237	236	99.6
Pork	255	247	96.9
Beef	262	262	100.0
All meats	981	971	99.0

Culled from Hayes et al. (2003)

It is no longer a secret that the naturally occurring enterococci in poultry could serve as a source of antimicrobial-resistant organisms and resistance genes (Graham *et al.*, 2009). Following antibiotic usage, resistant organisms expelled in feces (litter) may add to the reservoir of resistance genes in the environment. Hence, animal waste disposal on land is a common practice that has been implicated in introducing germs resistant to antibiotics into the environment (Graham *et al.*, 2009). Enterococci thrive in a variety of ecological niches because they are extraordinarily resilient and adaptable to a wide range of environmental stimuli. While enterococci are primarily found in the gastrointestinal tracts of both humans and animals, they have also been found in other places, such as the oral cavity (Staley *et al.*, 2014). This has given rise to the belief that both human and animal digestive tracts provide passive environment for the conjugation of resistant genes and that resistance genes from animals may have been incorporated into human strains (Staley *et al.*, 2014). Animal sources are thought to be the source of extra-intestinal enterococci because of fecal contamination. The most commonly incriminated enterococcal species: *Enterococcus faecium* and *E. faecalis* are more frequently found in humans than in animals (Table 5); whereas others, such as *E. casseliflavus, E. mundtii* and *E. sulfureus* are usually found in plants. Other species, such as *E. cecorum*, *E. hirae* and *E. asini* are typically

Table 5: Commensal association of enterococci with various hosts

Animal host	*Enterococcus* species											Comments
Empty Cell	*E. faecalis*	*E. faecium*	*E. durans*	*E. hirae*	*E. mundtii*	*E. cecorum*	*E. columbae*	*E. casseliflavis*	*E. gallinarum*	*E. avium*	*E. raffinosus*	Empty Cell
Human	++	+								+		
	+*	++*										
Dog or cat	+	+	+			+			+	+	+	
Chicken	++	++	++	++	+	++		+	+		+	Succession of species from day 1 (*E. faecalis*) –day 10 (*E. facium* group) to adult (*E. cecorum*)
Pig	++	+										*E. faecalis* higher in intestine than feces. *E. faecium* higher in feces than intestine
Calf	++	++										
Cow	+	+				+				+		*S. bovis* tends to replace enterococci in adult cattle
Wild birds	++						++					*E. faecalis* predominant in ducks; *E. columbae* predominant in pigeons
Invert-ebrates	++	++						++				

++: predominant isolate; +: occasional isolate; ++: predominant following vancomycin treatment; +*: occasional following vancomycin treatment. Culled from Staley et al. (2014)*

associated with the animal's flora, including poultry, pigs and donkeys (Santagati *et al.*, 2012).

In both tropical and temperate climates, enterococci can be found in a range of soil types with a concentration of between 10 and 103 MPN g-1 of soil. It has been demonstrated that enterococci from sewage contamination can survive for long in a variety of soil types and are also a modest component of the native microbial assemblage of soils in tropical climates (Staley *et al.*, 2014). It is widely acknowledged that sediments from fresh and salt water are the most abundant sources of extra-enteric enterococci (Staley *et al.*, 2014). As a result, when sediments in both biomes are disturbed, the concentration of enterococci in these water bodies rises. The growth and persistence of enterococci in most extra-enteric habitat are influenced by the presence of biotic and abiotic factors (Table 6). In the same vein, their survival in secondary habitats is impacted by competition from nutrients and indigenous microbes, even though the majority of these habitats offer some degree of protection from various abiotic stressors like sunlight (Staley *et al.*, 2014).

Table 6: Distribution, growth and transport mechanisms of enterococci in extra-enteric habitats

Habitat	Distribution	Growth	Environmental transport
Soil	Tropical and temperate soils. Highest concentrations found in surface soils.	Possible if indigenous microbiota are inhibited.	Wind, gravity and precipitation.
Sediment	Ubiquitous in surface sediments. Undetectable at depth > 5 cm.	Suggested but not demonstrated.	Sediment re-suspension Precipitation.
Freshwater	Tropical and temperate waters.	Unlikely, but may occur sporadically in the presence of excess nutrients.	River flow, lake currents, and precipitation.
Marine water	Tropical and temperate waters. Higher concentration in near shore waters. Low to undetectable concentrations in offshore waters.	Unlikely.	Wave action and precipitation.
Beach sand	Tropical and temperate sands. Higher concentrations in surface sand and nearer to shoreline.	Demonstrated *in vitro.*	Tidal washing and precipitation.
Vegetation	Patchy distribution on terrestrial plants. High concentrations in submerged vegetation.	Unlikely on terrestrial vegetation. Possible on aquatic vegetation.	Wind, gravity, water current and wave action.

Culled from Staley et al. (2014)

Antimicrobial resistant enterococci in food producing animals: Drug-resistant enterococci strains (Table 7) are relatively common in livestock and their products (Zhou *et al.*, 2012). Contaminated foods, especially those of animal origin, as well as the environment therefore may serve as entry points for antibiotic-resistant enterococci into the human gut (Giraffa, 2002). Hence, enterococci associated with food may act as reservoirs for antibiotic resistance and when consumed may survive the stomach acid and multiply, leading to sustained intestinal carriage (Hooper *et al.*, 2002). Several researchers however have reported antimicrobial resistance among enterococci isolates from food animals and their environment as represented in Table 8.

Enterococcal species, *E. faecium* and *E. faecalis*, isolated from European cheeses were found to exhibit variable degrees of resistance to antibiotics such as penicillin, tetracycline, chloramphenicol, erythromycin, gentamicin, lincomycin, rifampicin, fusidic acid and vancomycin (Zhou *et al.*, 2012). Giraffa (2002) found strains with high levels of gentamicin and kanamycin resistance in hospitalized patients and French milk cheeses. Another study found that 73% of Swedish retailed chicken isolates were resistant to one or more antibiotics, such as tetracycline, erythromycin and vancomycin. On the other hand, 9, 55 and 14% isolates from Swedish pork, Danish chicken and Danish pork respectively, were resistant to same drugs (Giraffa, 2002). Enterococci species isolated from bovine mastitis (80%), pigs (57%), chickens (62 – 64%) and animal-based foods were recorded to show high levels of glycopeptide, gentamicin, streptomycin, kanamycin and tetracycline resistance (Ben Braiek and Smaoui, 2019).

In a study conducted in Abia State, Nigeria, the antibiogram of enterococcal species in poultry and pigs found nearly 50% of the strains to be multiple drug-resistant. While the pig-derived strains (20 – 30%) showed quinolone resistance, 30 – 50% of those from poultry showed resistance to erythromycin, floxapen and norfloxacin, respectively; but low levels (3%) of resistance to gentamycin (Amaechi and Nwankwo, 2015). In a different investigation on the antibiogram of generic enterococci in horses in Nigeria, isolates resistance prevalence to chloramphenicol, erythromycin, tetracycline, rifampicin and vancomycin were found to be 38, 80, 50 and 27% respectively (Anyanwu *et al.*, 2019). Again, a study on the prevalence of tetracycline resistance in Ogun State, Nigeria (Ayeni *et al.*, 2016) found 100%

Table 7: Distribution of general mechanisms of resistance exhibited by *Enterococcus* spp. to selected classes

Antibiotic class	Target modification[a]	Altered access of drug	Drug Inactivation
β-lactams	+	-	+
Aminoglycosides	+	+	+
Glycopeptides/ lipoglycopeptides	+	-	-
Lipopeptides	+	-	-
Fluoroquinolones	+	+	-
Oxazolidinones	+	-	-
Macrolides/lincosamides	+	+	+[b]
Streptogramins	+	+	+
Pleuromutilins	+	+	-
Tetracyclines/glycylcyclines	+	+	-
Rifamycins	+	-	-
Sulfonamides/trimethoprim	+	-	-

[a]Includes by-pass of drug activity by activation of alternative biochemical pathways. [b]Lincosamides only. Culled from Economou et al. (2016)

Table 8: Resistant-enterococci isolates from food animal and their environmental samples

S/N	Anti-Microbial Agent	Number of Resistant Isolates (%)	Sample Involved	Food Animal Involved	Country /Location	Reference
1	Tetracycline, Erythromycin and Vancomycin	73	Retailed chicken	Poultry	Sweden	Giraffa, 2002
2	Tetracycline, Erythromycin and Vancomycin	9	Pork	Swine	Sweden	Giraffa, 2002
3	Tetracycline, Erythromycin and Vancomycin	55	Chicken	Poultry	Denmark	Giraffa, 2002
4	Tetracycline, Erythromycin and Vancomycin	14	Pork	Swine	Denmark	Giraffa, 2002
5	Glycopeptide, Gentamycin, Streptomycin, Kanamycin and Tetracycline	80	Mastitis	Bovine	Not specified	Ben Braiek and Smaoui, 2019
6	Glycopeptide, Gentamycin, Streptomycin, Kanamycin and Tetracycline	57	Pigs	Swine	Not specified	Ben Braiek and Smaoui, 2019
7	Glycopeptide, Gentamycin, Streptomycin, Kanamycin and Tetracycline	62-64	Chicken	Poultry	Not specified	Ben Braiek and Smaoui, 2019
8	Lincomycin, Tetracycline, Penicillin and Ciprofloxacin	80.3, 65.3, 61.1 and 49.6 respectively	Poultry litter	Poultry	British Columbia, Canada (USA)	Furtula *et al.*, 2013
9	Quinupristin-Dalfopristin	51-78	Chicken environment	Poultry	Eastern Seaboard, (USA)	Hayes *et al.*, 2001
10	Chloramphenicol, Quinolones and Erythromycin	76.7, 30-50 and 71.7 respectively	Chicken faeces	Poultry	Nigeria	Amaechi and Nwankwo, 2015
11	Chloramphenicol, Quinolones and Erythromycin	85.6, 20-30 and 71.8	Pig dung	Swine	Nigeria	Amaechi and Nwankwo, 2015
12	Rifampicin, Erythromycin, Ciprofloxacin, Ampicilin, Streptomycin, Tetracycline and Chloramphenicol	15.1, 73.5, 11.3, 47.1, 9.4, 43.3 and 81.1 respectively	Chicken (cloacal swab)	Poultry	Nigeria	Ngbede *et al.*, 2016

13	Rifampicin, Erythromycin, Ciprofloxacin, Ampicillin, Streptomycin, Tetracycline and Chloramphenicol	39.2, 71.4, 14.3, 14.3, 7.1, 42.9 and 60.7 respectively	Chicken feces	Poultry	Nigeria	Ngbede *et al.*, 2016
14	Rifampicin, Erythromycin, Ciprofloxacin, Ampicillin, Streptomycin, Tetracycline and Chloramphenicol	35.7, 35, 5, 15, 7.1, 35.7 and 25	Rectal swabs	Swine	Nigeria	Ngbede *et al.*, 2016
15	Rifampicin, Erythromycin, Ampicillin, and Tetracycline	75, 25, 50 and 33.3	Cattle manure	Bovine	Nigeria	Ngbede *et al.*, 2016
16	Erythromycin, Tetracycline, Amoxicillin, Ofloxacin, Vancomycin and Gentamycin	100, 81, 73, 68, 65 and 20 respectively	Chicken feces	Poultry	Nigeria	Ayeni *et al.*, 2016

resistance to erythromycin, 81% to tetracycline, 73% to amoxicillin/clavulanic acid, 68% to ofloxacin, 65% to vancomycin and 20% to gentamycin.

Pathogenicity of Enterococci

Enterococci have both commensal and pathogenic characteristics. While exhibiting their pathogenic properties, they first cling to the specific host tissue before invading it (Banerjee and Anupurba, 2015). Upon entering the tissue, they encounter environment very different from that at the colonization site. This environment usually possesses higher redox potentials, fewer necessary nutrients, phagocytic leukocytes and other host defenses (Kishen *et al.*, 2008). However, it has been found that enterococci express genes that promote tissue invasion, immune regulation, adhesion to host cells cum extracellular matrix and toxin-mediated harm (Kishen *et al.*, 2008). Therefore, the pathogenicity of enterococci is greatly influenced by virulence factors as well as increase in the prevalence of antimicrobial-resistant strains (Ben Braiek and Smaoui, 2019). As a result, *Enterococcus* species continue to pose a significant challenge to healthcare workers when described as the principal causative agent, especially in immunocompromised individuals (Ben Braiek and Smaoui, 2019). However, while *Enterococcus faecium* is the best species for characterizing antimicrobial resistance, *E. faecalis* is best for pathogenicity traits (Banerjee and Anupurba, 2015).

Virulence factors of enterococci: Numerous genes encoding virulence factors are present in enterococci, enabling them to survive under harsh conditions as well as infect and cause disease in vulnerable individuals (Azizi *et al.*, 2017). Virulence factors are effector molecules that boost an organism's likelihood of causing infection; and medical isolates of the *Enterococcus* species were shown to exhibit highest virulence features, followed by food isolates and then starter strains (Fisher and Phillips, 2009). A correlation was found between the severity of infections and virulence factors like cytolysin (cylA, cylB and cylM), gelatinase (gel-E) and aggregation substances (asa1) (Table 9). These virulence elements are therefore essential for an organism to be pathogenic.

Aggregation substances are surface proteins of enterococci strains that facilitate the formation of aggregates during bacterial conjugation, swapping of plasmids and specific attachment to epithelial cells during colonization (Ben Braiek and Smaoui, 2019). Ben Braiek and Smaoui (2019) stated that aggregation substances are capable of binding with extracellular matrix proteins such as collagen type I, thrombospondin and fibronectin. Their presence is believed to increase the aquaphobicity of enterococci surfaces, resulting in localization of cholesterol to phagosomes and delay in the fusion of phagosomes with lysosomal vesicles (Eaton and Gasson, 2002). Because the aggregate determinant is exclusive to *E. faecalis* strains (Ben Braiek and Smaoui, 2019), it is usually encrypted in *E. faecalis* pheromone-responsive mobile genetic elements and produced in

Table 9: Frequency of virulence Genes[a]

Variables	*cylA*	*cylB*	*cylM*	*cylABM*	
E. faecalis	83(87)	77(81.1)	52(54.4)	46(59)	
E. faecium	9(32)	8(28)	5(17)	0	
Total(126)	92(73)	85(67)	57(45)	46(37)	
	Asa1	***cylABM, Asa1***	***gelE***	***Esp***	***gelE, Esp***
E. faecalis	26(27)	17(36)	64(67)	55(59)	52(41)
E. faecium	0	0	0	11(40)	0
Total (126)	26(21)	(14.38)	64(51)	66(53)	41(33)

[a]*Values are expressed as Number(%). Culled from Azizi et al. (2017)*

response to pheromone induction (Franz *et al.*, 2003). Another virulent characteristic of enterococci, cytolysin (hemolysin), is associated with haemolytic effect in humans and bactericidal action against other Gram-positive bacteria. This peptic toxin causes the death of target bacterial cells by creating holes in the cellular membranes (Leblanc, 2006). According to Ben Braiek and Smaoui (2019), infections caused by cytolysin producing enterococci are five times more likely to be fatal than infections caused by enterococci that do not produce cytolysin. The cytolysin is a primary virulence factor of *E. faecalis*, which is controlled by a quorum-sensing mechanism that comprises of a system of two-components (Fisher and Phillips, 2009; Azizi *et al.*, 2017).

Gelatinase, a virulent factor of enterococci, is an extracellular endopeptidase that hydrolyzes bioactive peptides such as collagen, gelatin, insulin, hemoglobin and casein (Del Papa *et al.*, 2007). Its ability to split fibrin and destroy host tissue, which promotes microbial movement and spread, contributes to enterococci pathogenicity, particularly *E. faecalis* (Ben Braiek and Smaoui, 2019). Gelatinase is a protease that is fundamental to the development of biofilm which is necessary for tissue colonization and persistence at the site of infection (Del Papa *et al.*, 2007).

Furthermore, a crucial virulent indicator of enterococcal species is extra-cellular surface protein (esp), which is primarily present in *E. faecium*. It is well known to promote immune system evasion, colonization, adhesion and resistance to antibiotics (Foulquié Moreno *et al.*, 2006). Extracellular surface protein is responsible for the biofilm development which is linked to enterococci's tolerance to environmental stress and attachment to eukaryotic cells like those in the urinary system (Fisher and Phillips, 2009). Additionally, it has been discovered that altering the esp gene reduces the ability of *E. faecalis* to form biofilms, but plasmid transfer of the esp gene to esp-negative *E. faecalis* strains results in biofilm formation (Latasa *et al.*, 2006). Once more, strains of *E. faecium* carrying the gene espfm had higher conjugation rates than strains lacking this gene (Fisher and Phillips, 2009).

Further, it was discovered that the former had higher levels of resistance to imipenem, ciprofloxacin and ampicillin than the latter (Billström *et al.*, 2008). Extra cellular surface proteins are also responsible for the increased risk of microbial colonization in hospitalized patients and chronic urethral tract infections (Azizi *et al.*, 2017).

The formation of biofilm in enterococci is associated with infection persistence, resilience and contamination of the environment and the food chain (Ch'ng *et al.*, 2019). Endocarditis, periodontitis and other device-related diseases, as well as drug resistance, are usually related to biofilm formation (Duggan and Sedgley, 2007). The gene cluster linked with the endocarditis and biofilm-associated pilus (ebp) and biofilm generations in enterococci are connected. The genes encoding sortase C, srtC and ebpA, ebpB and ebpC make up the ebp operon (Singh *et al.*, 2007). Consequently, a non-piliated mutant of *E. faecalis* was shown to lack the ability to generate biofilms (Budzik and Schneewind, 2006). However, *E. faecium* seem to form biofilms less frequently than *E. faecalis* (Fisher and Phillips, 2009).

Hyaluronidase is another Enterococcus virulent indicator. It operates on mucopolysaccharide and is implicated in tissue injury. Hyaluronidase is a degradative enzyme and its gene sequence is encoded by the chromosomal hyl gene. It is believed to depolymerize the mucopolysaccharide moiety of animal tissue, facilitating the passage of enterococci and their toxins into host tissues (Kayaoglu and Ørstavik , 2004). The inoculation of semisolid media with mucopolysaccharide has been used for the detection of hyaluronidase synthesis in bacteria (Liu *et al.*, 2011). In all, comparing *E. faecalis* strains to those of *E. faecium* strains, the virulence factors are generally far less common in the former. However, in the absence of genes conferring antibiotic resistance, virulent characteristics alone are insufficient to adequately explain the pathogenicity of enterococci (Oprea and Zervos, 2007).

Antibiotic resistance in enterococci: Enterococci have been described as the most common antibiotic-resistant bacterial pathogen (Santagati *et al.*, 2012). Being present in the guts, continuous exposure of livestock to antimicrobial agents often results in their development of resistance (Vanderhaeghen and Dewulf, 2017). This is because antimicrobial resistance (AMR) is a natural byproduct of selective pressure on microbe evolution (Antonova *et al.*, 2019). The species are effective opportunistic microorganisms in nosocomial infections because their pathogenicity is greatly enhanced by resistance to commonly and frequently used antibiotic agents (Landete *et al.*, 2018). Therefore, frequent use of antibiotics as preventative measures and growth promoters in human and veterinary medicine respectively, as well as genetic mutations, all contribute to the rise in multi-drug resistance which helps them survive, particularly in hospital settings (Ben Braiek and Smaoui, 2019). In addition, antibiotic misuse, such as the use of antibiotics to treat viral illnesses; underuse, such as the early termination of antibiotic treatment; and overuse in agriculture, such as in intensive poultry farming; encourage the disastrous spread of AMR among microorganisms surrounding human habitats (Singer *et al.*, 2016).

Antibiotic resistant enterococci constitute a serious public health issue. Spread of AMR enterococci have been linked to the pollution, inadequate public health infrastructure and poor waste disposal methods in low- and middle-income nations (Jia *et al.*, 2014). AMR bacteria such as enterococci can be transmitted to people through consuming contaminated food (Price *et al.*, 2005; Verraes *et al.*, 2013; Van Boeckel *et al.*, 2015), breathing or drinking polluted air or water (Graham *et al.*, 2009), or by having direct contact with livestock exposed to antimicrobials (Smith *et al.*, 2013). Therefore, people who work closely with animals receiving antibiotics, such as farm cum slaughterhouse employees and veterinarians, run the risk of being exposed to resistant enterococci (Alam *et al.*, 2019).

Target modification, which refers to changes that disrupt the drug's access to the target site or enzymatic drug inactivation, is the primary means of achieving antibiotic resistance in enterococci (Economou *et al.*, 2016). According to Ben Braiek and Smaoui (2019), enterococci have innate resistance to the following antibiotics: aminoglycosides, sulphonamides, lactams, cephalosporins and lincosamides. Acquisition of resistance in enterococci strains usually results from random mutations or ingestion of foreign genetic material. It has been reported that acquired resistance in enterococci from other pathogenic organisms via mobile genetic elements is common in the following antibiotics: glycopeptides, particularly vancomycin, chloramphenicol, aminoglycosides, erythromycin, ampicillin, fluoroquinolones, penicillin and tetracycline (Jahan and Holley, 2016). Resistance acquisition by horizontal gene transfer in enterococci can be achieved by pheromone-sensitive or broad host-range plasmid exchange, or transposon movement, as earlier reported (Hollenbeck and Rice, 2012). However, high levels of resistance to aminoglycosides, ampicillin resistance driven by beta-lactamase synthesis and glycopeptide resistance are the most significant kinds of resistance seen in enterococci (Sood *et al.*, 2008).

Mechanism of resistance to antibiotics B-Lactam's resistance in enterococci: Enterococci are notably resistant or relatively immune to beta-lactam antibiotics, due to their low affinity penicillin-binding proteins (Sood *et al.*, 2008). By attaching to the transpeptidase and transglycosidase enzymes, often known as penicillin binding proteins, lactam antibiotics essentially prevent the formation of peptidoglycan (Economou *et al.*, 2016). The low affinity penicillin binding proteins (PBPs) found in enterococci (PBP4 and PBP5 in *E. faecalis* and *E. faecium* respectively) bind poorly to β-lactams. Due to low affinity penicillin binding proteins (PBPs), enterococci are resistant to the majority of beta-lactam antibiotics but are still able to synthesize components of the cell membrane at low concentrations (Sood *et al.*, 2008).

Penicillin normally exhibits minimum inhibitory concentrations (MICs) of 8 – 16 and 2 – 8 mg/mL for *E. faecium* and *E. faecalis* respectively (Hollenbeck and Rice, 2012). Enterococci are often "resistant" to all or any beta-lactams in addition to high MICs; that is, they are not killed by concentrations many times above the MIC (Andersson and Hughes, 2012). The majority of *E. faecium* clinical isolates (83%) with high or very high levels of resistance produce, respectively, increased levels of a different penicillin-binding protein (PBP5) with particular amino acid substitutions that are thought to cause noticeably lower levels of affinity toward benzylpenicillin (Shepard and Gilmore, 2002). The intensity of resistance seen in *E. faecium* (MIC 16 – 64 g/mL) is correlated with the overproduction of low affinity PBP5, a protein that can replace the function of all PBPs. This resistance can be mitigated by the concentration of penicillin available in plasma (MIC 1 – 8 g/mL) (Sood *et al.*, 2008). High levels of penicillin resistance in *E. faecalis* are thought to be caused by accumulation of lactamases, which are often encoded on plasmid or transposon-borne BLA genes (Economou *et al.*, 2016). In fact, compared to streptococci, enterococci are approximately 100 times less sensitive to beta-lactams (Shepard and Gilmore, 2002). It has been posited that the ability of enterococci to exchange resistance determinants with other Gram-positive bacteria is underlined by the acquisition of the *Staphylococcus aureus* beta-lactamase operon that leads to beta-lactamase synthesis, as supported by some genetic evidence (Tendolkar *et al.*, 2003). However, the most effective lactams against enterococci are ampicillin and penicillin, which work by preventing the production of peptidoglycan, a crucial element required for bacterial survival and the fundamental component of the bacterial cell membrane (Miller *et al.*, 2014).

Cephalosporins resistance in enterococci: Although the molecular underlying mechanism of enterococci's intrinsic resistance to cephalosporins is well recognized, it is not entirely understood. However, this intrinsic resistance frequently coincides with cephalosporins' lower propensity for binding to enterococcal PBPs, particularly PBP5 (Rice *et al.*, 2009). The bacterial two component regulatory systems (TCS), which control a number of regulatory pathways, are also implicated in the intrinsic resistance of enterococci to cephalosporins (Miller *et al.*, 2014). One of them, cognate response regulator (CroR), has been demonstrated to be essential for cephalosporin resistance. The CroR is phosphorylated by the sensor (CroS), which has histidine kinase activity and CroR is believed to inhibit transcription via a DNA binding domain (Comenge *et al.*, 2003).

Serine/threonine kinase IreK and phosphatase IreP make up another TCS linked to cephalosporin resistance (Kristich *et al.*, 2007). Serine kinase IreK and phosphatase IreP usually targets another protein, IreB, whose negative effect on the expression of cephalosporin resistance has been reported (Hall *et al.*, 2013). Furthermore, the simultaneous removal of IreB and IreK, or ablation of the threonine residue is reported to result in the relaxation of inhibition of the pathway, giving rise to the restoration of resistance. Since MICs for ampicillin and other drugs that act on cell membranes were not impacted, this method appears to be particular to cephalosporin resistance (Miller *et al.*, 2014).

Aminoglycoside resistance in enterococci: Aminoglycoside resistance is innate in enterococci and both *E. faecalis* and *E. faecium* are reported to be inherently resistant to aminoglycosides at clinically relevant doses (Hollenbeck and Rice, 2012). This trait is believed to be linked to poor antibiotic absorption (which necessitates larger concentrations to permit intracellular penetration) and inactivation by endogenous enterococcal enzymes, which reduces the possibility of binding to the ribosomal target due to steric hindrance (Miller *et al.*, 2014). It has been noted that beta lactam exposure frequently results in enhanced aminoglycoside uptake in enterococci (which increases the intracellular uptake). Therefore, when enterococci are cultured in the presence of cell membrane synthesis inhibitors like penicillin or vancomycin, the absorption of a radiolabeled aminoglycoside is enhanced, as observed when this process was monitored (Hollenbeck and Rice, 2012). This discovery explains how aminoglycoside-penicillin combination therapy improves clinical outcomes (Miller *et al.*, 2014). While the acquisition of mobile genetic elements explains high-level aminoglycoside resistance in both *E. faecalis* and *E. faecium*, intrinsic mechanisms explain low-level aminoglycoside resistance (Hollenbeck and Rice, 2012).

Tobramycin, sisomicin, kanamycin and netilmicin can all be modified by the chromosomally encoded 6'-acetyltransferase enzyme present in *E. faecium* (Chow, 2000). Many clinical isolates also contain the enzymes APH (3')-IIIa, an aminoglycoside phosphotransferase, which confers resistance to kanamycin via its phosphotransferase capacity and Ant (4'')-Ia, a nucleotidyl transferase, which causes tobramycin, amikacin and kanamycin tolerance. Furthermore, enterococci are known to use the ribosomal RNA (rRNA) methyltransferase EfmM to alter the target ribosome (Galimand *et al.*, 2011). This enzyme recognizes the cytidine at position 1404 of *E. faecium* 16S rRNA and methylation of this residue results in kanamycin and tobramycin resistance. These enzymes are less significant clinically because they cannot confer gentamicin or streptomycin resistance.

Gentamicin and streptomycin are the two aminoglycosides consistently used in medical practice for synergism with b-lactams, due to their resistance to the intrinsic enzymes produced by enterococci. This synergistic action of the drugs, however was found negated by high-level aminoglycoside resistance, as evidenced by MICs of 500 and >2000 mg/mL for streptomycin and gentamicin respectively, using the agar dilution method (Miller *et al.*, 2014).

Enterococci are known to exhibit high levels of aminoglycoside resistance (MIC >2000 g/mL), which is either mediated by ribosomes or by the synthesis of inactivating enzymes (Sood *et al.*, 2008). These enzymes render gentamicin and other structurally related aminoglycosides inactive by phosphorylating it at the 2'hydroxy position, while simultaneously acetylating the other aminoglycosides' 6'hydroxy positions. Thus, because the antibiotic can no longer bind to its intended site on the 30S ribosomal subunit, it loses its antibacterial efficacy. A bifunctional gene that codes for APH (2")-Ia-AAC (6')-Ie is frequently required to achieve high levels of gentamicin resistance (Hollenbeck and Rice, 2012). Except for streptomycin, all aminoglycosides are therapeutically ineffective against strains containing aph (2") Ia-AAC (6')-Ie (Chow, 2000). High-level resistance to streptomycin typically results from enzymatic alteration or by the addition, subtraction or deletion of a single nucleotide base in the ribosome. Ant (6')-Ia and Ant (3")-Ia, two well-known adenylyl transferases, are capable of inactivating streptomycin and other structurally related aminoglycosides (Chow, 2000). Similarly, enterococci are able to undergo ribosomal alterations that lead to streptomycin resistance (Hollenbeck and Rice, 2012). Evaluating susceptibilities to both medications is crucial since the processes through which enterococcal resistance to gentamicin and streptomycin manifests differ.

Glycopeptide resistance in enterococci: In the past three decades, enterococci resistance to glycopeptide has been a problem for both epidemiology and antibiotic use. Since their initial description in 1988, glycopeptide-resistant

enterococci (GRE) have been linked to nosocomial infections. In the United States, it was discovered that 30% of clinical enterococcal isolates were resistant to glycopeptides (Hollenbeck and Rice, 2012).

The occurrence and spread of high-level resistance to lactams and aminoglycosides among enterococci made the usefulness of glycopeptide antibiotics (vancomycin in particular) in the treatment of enterococci and other serious Gram-positive infections evident (Shepard and Gilmore, 2002). The majority of GRE infections are caused by *E. faecium*, although it is occasionally seen in other enterococci species.

A study showed that glycopeptides hinders the formation of cell membranes by crosslinking with the peptidyl-D-alanyl-D-alanine (D-Ala-DAla) termini of peptidoglycan intermediates at the cell membrane. It was found that Glycopeptide-resistant organisms modify pentapeptide precursors to replace the terminal D-ala with D-lac or D-ser. These altered cell membrane precursors were found to have a 1,000-fold reduced affinity for binding glycopeptides than regular precursors (Hollenbeck and Rice, 2012). The production of substitute peptidoglycan precursors with decreased affinity for teicoplanin and vancomycin leads to resistance to glycopeptides. However, it is believed that resistant genes, which are typically acquired via exchange among enterococcal strains, are what cause resistance to vancomycin and teicoplanin, a related glycopeptide that is licensed for therapeutic use in Europe (Shepard and Gilmore, 2002).

The van operon encodes glycopeptide resistance, which can be categorized into a number of variants, with the VanA and VanB genotypes being the most prevalent (Shahraki and Mousavi, 2017). The majority of glycopeptide-resistant enterococci fall into one of five phenotypes, each with its own species specifics for teicoplanin and vancomycin resistance patterns (Shepard and Gilmore, 2002). It was shown that VanA, VanB, VanD and VanE phenotypes can be acquired by the interchange of transposable elements; however the non-transferable VanC phenotype was found to be chromosomally encoded. Vancomycin and teicoplanin resistance are present in enterococcal strains carrying the VanA phenotype, whereas VanB phenotype isolates were found only resistant to vancomycin but susceptible to teicoplanin (Shepard and Gilmore, 2002). The VanC resistance phenotype, on the other hand, was found innate in *E. gallinarum* and *E. casseliflavus* (Hollenbeck and Rice, 2012). The VanE phenotype, which is chromosomally defined in strains of *E. faecalis*, were recorded to be characterized by limited resistance to vancomycin but susceptibility to teicoplanin, while the VanD phenotype were found to consist of homologs of the VanA/VanB gene cluster (Leblanc, 2006). Vancomycin and teicoplanin, two glycopeptide antibiotics, were the "final therapeutic option" for treating nosocomial pathogens. As a result of the rise in vancomycin-resistant enterococci (VRE), alternative medications are being sourced for possible replacement.

Tetracyclines and glycylcycline resistance in enterococci: Tetracyclines interfere with aminoacyl-tRNA docking by attaching to the ribosome thereby killing the bacteria. This occurs as a result of the ribosomal protein S7's interaction with various loops of the 16S rRNA. This process is however, reversible and tetracyclines have bacteriostatic properties.

Several researchers have reported 60 to 80% tetracycline resistance in enterococci (Cetinkaya *et al.*, 2000). It is known that tetracycline resistance in enterococci is achieved via antibiotic efflux and ribosome protection; and multiple genes are reportedly involved in the resistance (Table 10). Tetracycline, resistance is conferred through the plasmid-borne determinants tetK and tetL (Chopra and Roberts, 2001). A report has it that the Tn916 transposon can transfer the chromosomal resistance determinants tet(M), tet(O) and tet(S), which are associated to doxycycline, minocycline and tetracycline resistance (Miller *et al.*, 2014). Similar to bacterial elongation factors (EFs), these genes are known to produce a protein that is ready to hydrolyze GTP in the presence of ribosome modifying the structure of the ribosome and releasing bound tetracycline (Chopra and Roberts, 2001).

Table 10: Distribution of tetracycline resistance genes among Gram-positive bacteria, *Mycobacterium, Mycoplasma, Nocarida, Streptomyces* and *Ureaplasma*

One determinant		Two determinants		Three or more determinants	
Genus	**Gene**	**Genus**	**Genes**	**Genus**	**Genes**
Abiotrophia	*tet*(M)	*Actinomyces*	*tet*(L), *tet*(M)	*Eubacterium*	*tet*(K),*tet*(M), *tet*(Q)
Bacterionema	*tet*(M)	*Aerococcus*	*tet*(M),*tet*(O)	*Bacillus*	*tet*(K),*tet*(L), *tet*(M)
Gemella	*tet*(M)	*Bifidobacterium*	*tet*(M), *tet*(W)	*Listeria*	*tet*(K), *tet*(L), *tet*(M), *tet*(S)
Mycoplasma	*tet*(M)	*Gardnerella*	*tet*(M),*tet*(Q)	*Staphylococcus*	*tet*(K),*tet*(L), *tet*(M), *tet*(O)
Ureaplasma	*tet*(M)	*Lactobacillus*	*tet*(O), *tet*(Q)	*Clostridium*	*tet*(K),*tet*(L), *tet*(M), *tet*(P), *tet*(Q)
Nocarida	*tet*(K)	*Mobiluncus*	*tet*(O),*tet*(Q)	*Peptostreptococcus*	*tet*(K), *tet*(L), *tet*(M), *tet*(O), *tet*(Q)
		Corynebacterium	*tet*(M),*tet*(Z)	*Enterococcus*	*tet*(K),*tet*(L), *tet*(M), *tet*(O), *tet*(S), *tet*(U)
				Streptococcus	*tet*(K),*tet*(L), *tet*(M), *tet*(O), *tet*(Q), *tet*(T)
				Mycobacterium	*tet*(K), *tet*(L), *tet*(V), *otr*(A), *otr*(B)
				Streptomyces	*tet*(K), *tet*(L), *otr*(A), *otr*(B), *otr*(C), *tcr3*, *tet*

Source: Chopra and Roberts (2001)

Tigecycline, a synthetic derivative of minocycline also known as glycylcycline, has broad antibacterial activity against both Gram-positive and Gram-negative bacteria, including VRE and methicillin-resistant *Staphylococcus aureus*. Like all tetracyclines, tigecycline binds to the 30S subunit of the ribosome's 16S rRNA, which prevents the aminoacyl-tRNA from binding (Bauer *et al.*, 2004). However, its MICs are unaffected by normal tetracycline resistance determinants, unlike other tetracyclines (Miller *et al.*, 2014). Although the cause of resistance is unknown, two cases of tigecycline tolerance in enterococci have been linked to intra-abdominal procedures (Werner *et al.*, 2008).

Fluoroquinolone's resistance in enterococci: Before cellular division, the transcription and replication of the genome depend on the introduction and relaxation of supercoils in the DNA. DNA gyrase and topoisomerase IV which are two of the enzymes in charge of these activities and quinolones are known to target them. DNA gyrase primes the strand for replication and relaxes it in anticipation of the approaching polymerase by incorporating negative supercoils into the DNA strand. Topoisomerase IV, further separates the freshly reproduced interlocking DNA helix, permitting segregation to occur prior to cellular division.

The aforementioned processes require double-stranded breaks in the DNA and because quinolones stabilize the enzyme/DNA complex, strand continuity is broken thereby halting replication (Hawkey, 2003).

The enterococci are resistant to quinolones at low levels intrinsically, but they can also acquire high degrees of resistance through a variety of methods. Target gene mutations, notably those in gyrA and parC, are one of them, while *E. casseliflavus* and *E. gallinarum* do not have them; *E. faecalis* and *E. faecium* do (López *et al.*, 2011). It is known that these modifications affect the "quinolone resistance determining areas," which probably alters the antibiotic's affinity for binding.

Secondarily, another established mechanism of quinolone resistance is the externalization of the antibiotic via efflux pumps. The third mode of resistance discovered in *E. faecalis* (Miller *et al.*, 2014) is controlled by QNR, which further encodes a protein with a sequence of pentapeptide reruns similar to the chromosomally quinolone resistance genes previously reported in enteric bacteria. This protein is believed to guard DNA gyrase by reducing the likelihood of DNA/quinolone interaction, resulting in the development of quinolone-gyrase complexes (Tran *et al.*, 2005).

Streptogramins/macrolides/lincosamides resistance in enterococci: Quinupristin dalfopristin is basically a combination of two pristinamycin derivatives: dalfopristin and quinupristin (streptogramin A and B respectively). Quinupristin dalfopristin (QD) is active against *E. faecium* but not *E. faecalis*. The mechanism of QD's action is based on the synergistic interaction of the two pristinamycin molecules. The ribosomal complex is irreversibly inhibited when dalfopristin binds to produce a conformational shift inside the ribosome that reveals a high-affinity binding site for quinupristin (Canu and Leclercq, 2001). Resistance to QD is attained in *E. faecium* through a number of ways. First, the acetyltransferases VatD and VatE modify dalfopristin, rendering it useless and eliminating its synergistic activity with quinupristin (Werner *et al.*, 2002). And secondly, via the lactonases VgbA and VgbB cleaving the ring structure of streptogramin B, as first observed in staphylococci (Miller *et al.*, 2014). The macrolide, lincosamide and streptogramin B (MLSB) phenotype, which is common in enterococci due to the presence of the erythromycin ribosome methylase (erm) genes which comprise of ermA, ermB and ermC, is responsible for enterococci resistance to macrolides, streptogramin B and lincosamides, hence altering the target for quinupristin (streptogramin B) whereas dalfopristin (streptogramin A), remains active. The presence of ermB on the other hand, may have an effect on the in vivo performance of QD.

Several methylase genes, most notably ermB, are linked with the alteration of the 23S rRNA target (A2508) in cross-resistance to macrolides, as compared to the alteration of A2503 by cfr in linezolid resistance (Portillo *et al.*, 2000). Recently it was discovered that a genetic variation in the genetic makeup of eatA gene, which is involved in the removal of QD from the cell, confers resistance to susceptible *E. faecium* strains. Miller *et al.* (2014) also suggest that efflux pumps, such as msr (Portillo *et al.*, 2000), may contribute to this process. All *E. faecalis* strains are endowed with a chromosomal gene Lsa (Singh *et al.*, 2002), whose exact molecular function and the mechanism of action are unknown, but its presence confers intrinsic resistance to streptogramin A and lincosamides.

Oxazolidinones resistance in enterococci: Linezolid has bacteriostatic properties with potent activity against Gram-positive bacteria. The most prevalent means of linezolid resistance is still the observed mutations in the genes that encode 23S rRNA, a critical component of the drug-binding site in the ribosome (Miller *et al.*, 2014). The 23S rRNA gene is highly duplicated in enterococci, as it is in many other bacteria and the resistance phenotype is correlated with the number of mutant alleles (Marshall *et al.*, 2002). When compared to a JH2-2 mutant without recombination, interaction among these alleles has been shown to boost the rise in MIC in *E. faecalis* JH2-2 (Miller *et al.*, 2014). Linezolid MIC higher levels are also linked to genetic variations in the ribosomal proteins L3 and L4, which surround the peptidyl transferase site where linezolid acts (Miller *et al.*, 2014).

These alterations were first discovered in linezolid-resistant staphylococci, but they have since been found in resistant enterococci too (Chen *et al.*, 2013; Miller *et al.*, 2014). Also described in enterococci is the methylation of an adenine at position 2503 by an enzyme that modifies the 23S rRNA (Toh *et al.*, 2007). The causative gene, cfr, a plasmid-borne resistance determinant, has been found in *E. faecalis* clinical strains as well as other Gram-positive microbes with clinical significance (Diaz *et al.*, 2012). This cfr gene has been linked to the mobile transposable element IS256, the sequence of which was discovered to; regulate antimicrobial resistance gene transfer; modify the promoter sequence of regulatory proteins; initiate the appearance of existing resistance determinants and is common in multi-drug resistant staphylococci and enterococci (Hennig and Ziebuhr, 2010). This phenomenon explains how cfr tends to span species boundaries and how it is likely to spread widely in a clinical context.

Vancomycin-resistant enterococci (VRE): First identified as a hazard to public health globally in 1988 in Europe, VRE are now found

everywhere (Bonten *et al.*, 2001). Prevalence of VRE in nosocomial infections in intensive-care patients in the USA increased between 1989 – 2000 (Figure 1).

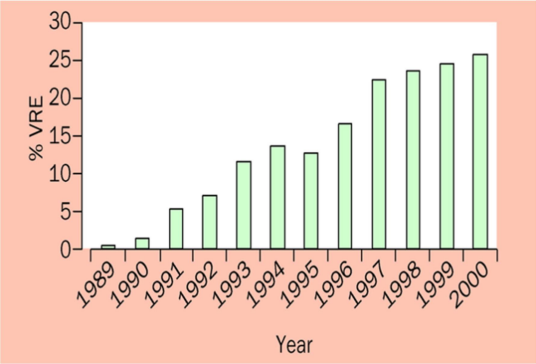

Figure 1: Vancomycin-resistant enterococci: Why are they here, and where do they come from? (*Bonten et al., 2001*)

Environmental sources of VRE include animal waste and human foods of animal origin (Torres *et al.*, 2018). There is abundant evidence of VRE transmission to people who come into contact with these sources, which have enlarged the human reservoir of VRE (Chastre, 2008). Numerous findings in Europe showed that VRE colonization typically occurs within the community (Shepard and Gilmore, 2002). Outside of the medical setting, sources of animal, environmental and food waste have been related to the colonization of VRE in the community (Giraffa, 2002). Once more, VRE could contaminate the environment via a variety of sources, including effluents from sludge treatment as well as livestock and poultry manure (Giraffa, 2002). According to Ford *et al.* (2015), VRE infection is frequently linked to an increase in mortality and there is currently no effective antibiotic therapy for many VRE infections (Forrest *et al.*, 2008). However, with the introduction and rapid spread of VRE, antimicrobials including linezolid, daptomycin and tigecycline are increasingly used in the treatment of VRE infection. In recent years though, it has unfortunately been reported that enterococci are becoming resistant to substances used to treat VRE including linezolid, tigecycline and daptomycin (Guzman Prieto *et al.*, 2016). Avoparcin, a vancomycin analog used as a growth promoter in Europe, was linked to the widespread use of VRE in livestock (Guzman Prieto *et al.*, 2016). Similar vancomycin resistance transposons in human and animal reservoirs offered a hint that enterococci originating from animal would be a source of antimicrobial resistance genes passed on to humans (Guzman Prieto *et al.*, 2016).

Before 1990, VRE were essentially nonexistent in hospitals in the USA. But at the moment, 87% of *E. faecium* and 14% of *E. faecalis* strains from nosocomial infections are now resistant to vancomycin (Guzman Prieto *et al.*, 2016). VRE have been reported to induce unusual infections which could not be handled with traditional antimicrobial agents; hence vancomycin resistance is an issue of major concern (Ben Braiek and Smaoui, 2019). VRE therefore offers a substantial challenge to physicians given that it was originally considered the "drug of last resort" for treating enterococcal infections and was commonly substituted for penicillin, ampicillin and aminoglycosides in individuals with allergies (Ben Braiek and Smaoui, 2019). Recently, it has been discovered that enterococci serve as donors of gene clusters encoding vancomycin resistance to more dangerous pathogens, such as Methicillin Resistant *S. aureus* (MRSA) (Miller *et al.*, 2014). This trend poses a serious challenge to public health.

Knowledge, Attitude and Practices in Farms that Predisposes to Antimicrobial Resistance: Antimicrobials are primarily used in modern food animal production worldwide to prevent and treat disease (Marshall and Levy, 2011). In animal production, antimicrobial drugs are widely used in four different contexts: treatment, metaphylaxis, prophylaxis and growth promotion. Although it generally seems legal to use antimicrobials for therapy, prophylaxis, or metaphylaxis, their use for growth promotion has been extremely contentious. Given its link to the development of VRE, the use of avoparcin as a growth booster in livestock has long been prohibited in many developed countries (Kühn *et al.*, 2005).

Avoparcin and virginiamycin are still used over the world despite being prohibited in many European nations (Hammerum, 2012).

Although the use of antibiotics for therapeutic, preventive and metaphylactic purposes seems reasonable and scientifically supported, the massive use of antibiotics in livestock husbandry has been linked to the emergence, spread and persistence of antibiotic-resistant bacteria (Marshall and Levy, 2011). The selection and mobilization of antimicrobial resistance genes within the microbiome of treated animals and the potential for subsequent spread into human pathogenic bacteria are issues of major concern, even though the occurrence of resistance in bacteria causing infections in animals is of primary concern. There is strong evidence that using antibiotics on animals favors the emergence of resistant commensals and zoonotic entero-pathogens (Halpern, 2009).

Poor biosecurity and hygienic practices in farms as illustrated in the Figure 2 have also been linked to the development of AMR, in addition to indiscriminate use of antibiotics (Davies and Wales, 2019).

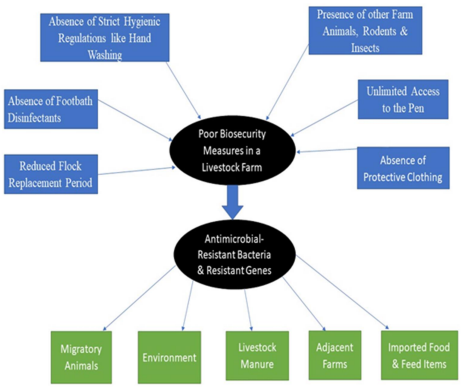

Figure 2: Components of poor biosecurity measures and channels of dissemination of antimicrobial-resistant bacteria/resistant genes in a livestock farm

Reduced flock replacement periods, presence of other animals, rodents and insects as well as unlimited access to the pens, absence of strict hygienic regulations like hand washing and sanitizing; changing into different boots and overalls before entering pens can give good results. The need for strict biosecurity and hygienic measures in different livestock farms is underscored by the possibility that antimicrobial-resistant bacteria and resistance genes could spread via a variety of routes, including between farms, through the environment, animal waste, migrating animals as well as imported food and feed products.

Management of Enterococcal Infections in Humans: *Enterococci faecalis* and, less frequently, *Enterococci faecium* are commonly blamed for enterococcal infections. *E. faecalis* is more likely to exhibit overt virulence traits and to be susceptible to at least one potent antibiotic. On the other hand, *Enterococcus faecium* is essentially devoid of overt pathogenic features and is more likely to be resistant to even antibiotics of last resort (Tendolkar *et al.*, 2003).

Better understanding of the interactions between enterococci, the hospital context and patients, judicious antibiotic use, maximum contact isolation in hospitals and improved surveillance are all required for effective management of multidrug-resistant enterococci (Vanderhaeghen and Dewulf, 2017). Despite stringent cleaning and sterilizing procedures, enterococci persist and spread in hospital environments due to widespread and ongoing environmental contamination with the bacteria on surfaces and medical equipment (Ellingson *et al.*, 2014).

The severity of the ailment, location, species and the resistance patterns seen in the clinical isolate are frequently used to guide treatment of enterococci infections (Hollenbeck and Rice, 2012). When handling clinical infections, it is important to differentiate enterococci to the species level and perform susceptibility tests on strains recovered from patients, due to the differences in resistance patterns between *E. faecium* and *E. faecalis*. Increased quest for additional drugs and alternative therapeutic techniques that are less susceptible to the cycle of drug introduction and resistance is crucial in management of drug-resistant enterococci.

A synergistic regimen is used to treat complicated or severe infections of enterococci, while uncomplicated infections can be successfully treated with monotherapy. The preferred treatment for a simple enterococcal infection is ampicillin, though it is preferable to combine it with a ß-lactamase inhibitor such as sulbactam for a better outcome.

The accepted practice when treating difficult infections like endocarditis in susceptible enterococcal infections is to combine an aminoglycoside with a cell membrane active drug for synergistic death (Hollenbeck and Rice, 2012). As previously established, only gentamicin and streptomycin are considered for synergistic therapeutic intervention. Animal models of high-level penicillin resistance have been effectively treated with a blend of aminoglycosides and other cell membrane active antibiotics such as vancomycin or daptomycin. Alternative therapies are employed for their synergistic effects in the treatment of difficult enterococcal infections that have high levels of resistance to gentamicin and streptomycin. Despite their resistance to both drugs, animals responded favorably to a combination of ceftriaxone and ampicillin (Gavaldà *et al.*, 2003).

Vancomycin resistant enterococcal strains pose a significant challenge in therapy due to the existence of VanA and VanB resistance determinants, which are often resistant to other classes of antibiotics. However, quinupristin-dalfopristin and linezolid have been demonstrated to be useful in the treatment of complex glycopeptide resistant enterococci infections. The new fifth generation cephalosporins, daptomycin, tetracyclines, tigecycline, quinolones and fosfomycin are also additional antimicrobial agents that have in vitro actions and are successfully used in specific situations (Hollenbeck and Rice, 2012).

Conclusion: Until recently, enterococci were considered to be of minor clinical significance. However, current investigations have noted their significant clinical impacts, especially in immunocompromised persons, given their capacity for antimicrobial resistance and possession of virulent components. At the moment, linezolid, daptomycin and tigecycline are drugs of choice for the treatment of VRE. Recent investigations have reported the emergence of resistance to this group of antimicrobials. This is of enormous public health and economic importance; therefore, there is a need to research for modifications or repurposing of the existing medications and discovery of novel drugs as potential replacements for them. Emergence of multi-drug resistant strains of enterococci can also be stalled significantly by responsible use antimicrobial agents in human and veterinary medicine as well as implementing efficient control measures to reduce the presence of enterococci in the environment and food sources.

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