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## Chapter

# 4

### **VANCOMYCIN-RESISTANT *ENTEROCOCCUS* COLONISATION: IS ISOLATION THE ONLY PRECAUTION?**

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## Contents

4.1. INTRODUCTION .....	79
4.2. GENUS DEFINITION .....	79
4.3. ENTEROCOCCUS IN THE ENVIRONMENT .....	79
4.4. DRUG RESISTANCE.....	80
4.5. GLYCOPEPTIDE RESISTANCE.....	81
4.6. DRUG-RESISTANT ENTEROCOCCI IN NONHUMAN RESERVOIRS.....	81
4.7. MICROBIAL COMMUNITIES AND ASSOCIATION WITH AMR .....	83
4.8. PREVENTING AND CONTROLLING VRE .....	84
4.9. SELECTIVE DECONTAMINATION OF THE DIGESTIVE TRACT (SDD) .....	85
4.10. PROBIOTICS (BACTERIOTHERAPY AGAINST VRE).....	85
4.11. <i>ENTEROCOCCUS</i> SPECIES AS PROBIOTICS IN FOODS.....	86
4.12. FAECAL TRANSPLANT (FAECAL MICROBIOTA TRANSPLANTATION) .....	87
REFERENCES .....	88

## 4.1. INTRODUCTION

The World Health Organization's World Health Day in 2011 highlighted the problems of antibiotic resistance under the title 'Antibiotic resistance: no action today, no cure tomorrow'. Every year 25,000 people in the European Union die because of serious resistant bacterial infections that are mostly acquired in healthcare settings.

## 4.2. GENUS DEFINITION

Thiercelin shared his observations of a saprophytic microbe that pullulated in the human gastrointestinal (GI) tract alongside the bacterium coli. He described this microbe as a Gram-positive diplococcus and proposed the name 'Enterocoque' to emphasise its morphology and its intestinal origin [1].

The core morphological and physiological features of enterococci are that they are Gram-positive spherical or ovoid cells that are arranged in pairs or chains [1]. They are nonspore-forming facultative anaerobes and obligatory fermentative chemoorganotrophs. They typically have an optimum growth temperature of 35 °C and a growth range between 10 and 45 °C [2,3]. They typically grow in broth containing 6.5 % NaCl and hydrolyse esculin in the presence of 40 % bile salts [4]. They are usually homofermentative and produce lactic acid as the end product of glucose fermentation without producing gas [5,6].

## 4.3. ENTEROCOCCUS IN THE ENVIRONMENT

The genus *Enterococcus* belongs to the *Enterococcaceae* family along with the *Atopobacter*, *Catelicoccus*, *Melissococcus*, *Pilibacter*, *Tetragenococcus* and *Vagococcus* genera [7]. The *Enterococcus* genus currently consists of 35 recognised species, with eight more species (*Enterococcus lactis*, *Enterococcus plantarum*, *Enterococcus quebecensis*, *Enterococcus rivorum*, *Enterococcus rotai*, *Enterococcus ureasiticus*, *Enterococcus ureilyticus*, and *Enterococcus viikiensis*) likely to be added [8-13].

Enterococci are common and commensal members of the gut community in mammals and birds. They also exist in various other extraenteric habitats, such as soil and sediment, beach sand, aquatic and terrestrial vegetation and ambient waters (*i.e.* rivers, streams and creeks). They may also be found in heterothermic habitats where temperatures are variable, which is in contrast

to the GI tract of warm-blooded animals where the temperature is relatively constant (*i.e.* the GI tracts of healthy individuals or hospitalised patients) [14]. Enterococci are found at high concentrations ( $10^4$ – $10^6$  bacteria/g wet weight) in human faeces [15].

A number of researchers have shown that enterococci are more prevalent in roof-harvested rainwater than *Escherichia coli*, and enterococci were suggested to be a better indicator in the assessment of faecal contamination [16]. Nevertheless, exposing hospitalised patients to antibiotics results in major modifications of the gut microbiota, which facilitates the colonisation of the GI tract by drug-resistant enterococci [17]. Among the enterococcal species, *E. faecalis* and *E. faecium* are the most commonly encountered species in human faeces [6,18].

Their low-acidification ability, proteolytic and lipolytic activities, carbohydrate metabolism and volatile compound and bacteriocin production ability allow enterococcal strains to be used in starter cultures in food fermentation or even as probiotics. Despite their use in traditionally-fermented products, some enterococcal strains show technological potential, antibiotic resistance and the presence of virulence factors, which hesitate the direct use of these strains in food technology [19].

#### 4.4. DRUG RESISTANCE

Antimicrobial resistance (AMR) in enterococci can be divided into two classes: intrinsic resistance and acquired resistance. Intrinsic resistance is caused by either a lack of target sites for the antimicrobial drug or insufficient penetration of the drug into the intracellular target site. In addition to being the leading multidrug-resistant pathogens in hospitals, they also serve as reservoirs and transmit resistance genes to other bacteria. *E. faecium* and *E. faecalis* are the enterococcal species that are most often associated with multidrug-resistant nosocomial infection, and approximately 30 years ago, both species acquired resistance to the important last-line bactericidal drug, vancomycin [20]. Many different transposons and plasmids have been identified in *E. faecalis* and *E. faecium* that confer resistance to a wide variety of antimicrobial drugs, including erythromycin, gentamicin, kanamycin, streptomycin, tetracycline and vancomycin [21].

## 4.5. GLYCOPEPTIDE RESISTANCE

Vancomycin, as well as teicoplanin, belong to a group of glycopeptide antimicrobials. These antimicrobials bind with high affinity to the D-alanyl-D-alanine (D-Ala-D-Ala) C-terminus of peptidoglycan pentapeptide precursors and block the addition of pentapeptide precursors by transglycosylation to the nascent peptidoglycan chain, thereby preventing subsequent cross-linking catalysed by transpeptidation [22,23].

The first clinical isolates of vancomycin-resistant enterococci (VRE), both *E. faecium* and *E. faecalis*) were detected in Europe in 1986 [24,25]. Since then, VRE have spread rapidly all over the world [26]. In 1988, French researchers discovered that glycopeptide resistance was plasmid-mediated [24]. A few years later, the same group identified that vancomycin resistance was located on a small mobile genetic element designated transposon Tn1546, encoding the vanA phenotype [27]). Tn1546 belongs to the Tn3 family of transposons that do not encode conjugative functions, therefore dissemination of the transposon can only occur after integration into transferable elements such as plasmids and conjugative transposons [21,27]. Furthermore, a second phenotype (vanB) was also identified on a different mobile element, designated transposon Tn1547 [28].

Molecular characterisation of DNA heterogeneity in the vanA gene cluster of Tn1546 in isolates from humans and animals revealed high levels of DNA polymorphisms due to point mutations, deletions and insertions of different sequences (*e.g.* IS1216V and IS1251). These polymorphisms can be used to study the epidemiology of Tn1546 [29,30]. Identical Tn1546 variants were identified among vancomycin-resistant *E. faecium* (VREF) from farm animals and humans, which could be the result of colonisation of animal-derived VREF in humans or the transfer of Tn1546 from animal VREF to human enterococcal isolates [31].

## 4.6. DRUG-RESISTANT ENTEROCOCCI IN NONHUMAN RESERVOIRS

**In the environment.** Sewage is an important reservoir of resistant enterococci, and tetracycline-resistant enterococci were isolated from sewage as early as the 1970s [32]. Glycopeptide resistance in *E. faecium* was first detected in 1986 in clinical isolates from hospitals in France and the United Kingdom [24,33]. The first indication of a reservoir outside hospitals was the detection of VRE in wastewater treatment plants in small German towns without a hospital and in sewage in the United Kingdom [34,35]. High-level aminoglycoside resistance has been observed globally among enterococci isolated from the environment [36]. More specifically, high-level kanamycin

resistance was observed among 34 % of 248 enterococcal isolates from sewage and water in the United States, streptomycin resistance was found in 5 % of sewage isolates in the United States and highly gentamicin-resistant enterococci were isolated from sewage and water in the United States and Germany [34,37].

**In animals.** Enterococcal infections in animals are rarely treated with antimicrobial agents; however, enterococci are exposed to antimicrobial selection in the GI tract during the treatment of other infections or when antibiotics are used as growth promoters or prophylaxis [38]. A multitude of studies have described the occurrence of VRE in nonhuman reservoirs, including cats, dogs, horses, birds, wood frogs, ostriches, pigs, pork, broilers, poultry meat, environmental samples and sewage. VRE were also reported in stool samples from farmers and nonhospitalised humans in the community, mainly in Europe [39]. In certain conditions, feeding animals low doses of antimicrobials can increase productivity by improving feed conversion and decreasing the morbidity and mortality caused by the infection [40]. The glycopeptide, avoparcin, was first introduced for growth promotion in 1975 and was mainly used for broilers, pigs, turkeys, veal calves and other animals [39-42]. As avoparcin confers cross-resistance to vancomycin, its use selected for the growth of VRE, and thus VRE were common in the intestinal flora of farm animals in Europe during the 1990s [43]. In contrast, VRE were not isolated from farm animals in the United States until 2008. Because of this connection, the use of avoparcin as a growth promoter was banned, and the prevalence of VRE in European farm animals rapidly declined but did not disappear [44]. A recent study modelling the persistence of VRE indicated that they will be present among farm animals for a long time, which is in agreement with today's view regarding the timeframe of AMR reversal [45,46].

**In food.** Antibiotic-resistant enterococci occur in meat products, dairy products and even within strains used as probiotics [47]. Al-Ahmad *et al.* demonstrated, that after the consumption of cheese, food-borne enterococci can integrate into the oral biofilm *in vivo* [48]. Thurnheer and Belibasakis confirmed that *E. faecalis* is able to colonize an *in vitro* established six-species oral biofilm in high numbers [49]. The finding that *E. faecalis* from food can incorporate into the oral biofilm and is prevalent in dental diseases raises the question as to whether the oral cavity serves as a reservoir for virulent and resistant strains of *E. faecalis* [48,50]. The prevalence of antimicrobial-resistant enterococci in food-producing animal is becoming a matter of concern, as these resistant bacteria may be transmitted to humans *via* the food chain [51]. Tetracycline resistance commonly appears as acquired antimicrobial resistance in *Enterococcus* [52]. Because tetracycline has been widely used to promote livestock growth and to treat human diseases, the widespread use of this antimicrobial has caused selective pressure and led to an increase in the number of acquired resistance genes among bacteria *Enterococci* resistant to multiple antimicrobial agents, including vancomycin.

## **4.7. MICROBIAL COMMUNITIES AND ASSOCIATION WITH AMR**

**Resistant Genes Exist In Nature.** Resistance to commonly-used antibiotics is present in the genes of bacteria everywhere, as researchers at the University of Lyon discovered. A global study of bacterial genomes found resistance across 71 environments, including oceans, soil and human faeces. They found that 30 % of all known antibiotic-resistance genes could be found in a single soil sample [53].

It is only in recent years that research regarding antibiotic resistance has focused on the environment from which the antibiotics were initially extracted (*i.e.* soil microorganisms and the soil ecosystem). With an ever-decreasing supply of novel antibiotics and increasing resistance, emphasis has turned to defining the natural antibiotic resistome and understanding the ecology and evolution of antibiotic resistance in the nonclinical environment [54].

There is evidence that antibiotics have played a role in microbial metabolism for millions of years [55]. Some work by destroying the bacterial cell wall, disorganising the peptidoglycan layer or interrupting enzyme synthesis or signal cascades [56]. In natural environments, certain bacterial species produce their own chemical compounds that are adept at killing other bacteria [57]. This is done to outcompete other species for habitat, nutrients and possible hosts. Antibiotics were seen to control and obliterate fungal and protozoal infections, control crop pests, maintain livestock health and help those with common physiological diseases [58]. However, the environment does not exist in a separate world to that of humans.

There is a constant flow to and from soil, especially in urban and agricultural environments. Human activities such as using antibiotics for the treatment of human and animal diseases and in agriculture, but also pollution and climate change, have altered the soil environment. If the soil is a reservoir of antibiotic-resistance mechanisms, it is important to identify how the actions of humans and climatic change will affect the soil resistome.

Antibiotic resistance in bacteria only leads to a loss of functional systems. Evolution requires a gain of functional systems for bacteria to evolve into man [59]. Therefore antibiotic resistance in bacteria is not an example of evolution in action but rather of variation within a bacterial kind. It is also a testimony to the wonderful design God gave bacteria.

**The Habitat.** It is estimated that the human body consists of approximately  $10^{13}$  cells and hosts  $10^{14}$ – $10^{15}$  individual microorganisms. These microorganisms can be divided into two groups: 1) those that usually remain constant in their normal habitat (indigenous flora) and 2) those that are accidentally acquired and have to compete with other microorganisms and host defences after their adherence to epithelial or mucosal surfaces.

It is accepted that infection is the result of interaction between the host, the microorganism and the environment. Pathogenicity is not only an intrinsic quality of microorganisms but the consequence of some properties of the microorganisms and the host [60].

Leonard *et al.* attempted to quantitatively estimate intrinsic pathogenicity in 40 infants admitted to a neonatal surgery unit for at least 5 days. The intrinsic pathogenicity index (IPI) for a species ( $y$ ) was defined as [61]:

The range of this index is 0–1. The highest IPI of 0.38 was found for *Pseudomonas* spp., while other isolated potential pathogens had IPI values of <0.1 (*i.e.* *Enterobacter* spp. = 0.08, *Staphylococcus aureus* = 0.06, *Klebsiella* spp. = 0.05, *E. coli* = 0.05, *Staphylococcus epidermidis* = 0.03 and *Enterococcus* spp. = 0). This index provides useful information about the relative pathogenicity of different microorganisms in a specific population and could be used to design antibiotic policies, both prophylactic and therapeutic, in groups of patients in which microbiological surveillance could be [62].

Interestingly, if the number of bacteria in the human body ( $3.9 \times 10^{13}$ ) is compared to the number of nucleated human cells ( $\sim 0.3 \times 10^{13}$ ), a ratio of approximately 1 : 10 is obtained [63]

## 4.8. PREVENTING AND CONTROLLING VRE

Risk factors for VRE carriage upon intensive care unit (ICU) admission include the duration of previous hospitalisations, glycopeptide administration, chronic heart failure, malignancy, insulin-dependent diabetes mellitus and previous enterococcal infection (VRE or vancomycin sensitive enterococci). Risk factors for VRE colonisation during an ICU stay include quinolone administration, chronic obstructive pulmonary disease, chronic renal failure and the number of VRE-positive patients in nearby beds [64]. Although the translocation mechanisms of enterococci across the GI tract are uncharacterised, it is known that the overgrowth of VRE in the gut microbiota is an initial condition in the development of VRE infections [17,65,66]. In November 1994, the Hospital Infection Control Practices Advisory Committee ratified a series of recommendations for preventing and controlling the spread of vancomycin resistance, with a special focus on VRE (isolation precautions to prevent patient-to-patient transmission of VRE). These recommendations included placing VRE-infected or colonised patients in private rooms, wearing gloves (clean and nonsterile gloves are adequate) [67].

Preventing colonisation of the upper and lower digestive tract is an approach to prevent ventilator-associated pneumonia (VAP) and other infections. This approach is built on the theory that the GI flora changes with acute illness. In particular, it assumes that the normal flora disappears and is replaced by an overgrowth of potentially-pathogenic microorganisms (PPM). This is followed



by aspiration of PPM, which could finally result in VAP. This review focuses on the decolonisation methods of VRE without using antibiotics.

#### **4.9. SELECTIVE DECONTAMINATION OF THE DIGESTIVE TRACT (SDD)**

Selective decontamination of the digestive tract (SDD) prevents severe infections and reduces mortality in critically ill patients. Historical arguments against its use, such as the development of bacterial resistance and the absence of influence on mortality, have not been confirmed. Recent clinical trials and meta-analyses that were designed to evaluate these variables showed remarkable reductions in the incidence of resistant bacteria and a significant beneficial effect on survival [68].

SDD consists of the oropharyngeal and gastric application of nonabsorbable antibiotics (often polymyxin, tobramycin and amphotericin) alongside a short course of intravenous antibiotics, often cefotaxime [69]. Sanchez-Garcia *et al.* demonstrated a reduction in the overall occurrence of nosocomial pneumonia following the use of SDD; however, the level of carriage of methicillin-resistant *S. aureus* and coagulase-negative staphylococci and enterococci was significantly higher in the SDD-treated patients [70]. SDD and selective oropharyngeal decontamination (SOD) are not active against resistant Gram-positive bacteria, therefore they may promote colonisation with bacteria such as *S. aureus* and *E. faecalis* [71]. As a result, SDD does not cover low-level pathogens, such as anaerobes, viridans streptococci, enterococci and coagulase-negative staphylococci, which rarely cause infections during an ICU stay [68].

#### **4.10. PROBIOTICS (BACTERIOTHERAPY AGAINST VRE)**

Another strategy uses topically-applied probiotics (live bacteria) that could alter the GI flora. Recent trials of different probiotic formulas suggest this strategy is also effective at preventing VAP.

Composed of nearly a thousand different types of microorganisms (some beneficial, others not), the human gut microbiota plays an important role in health and disease. The concept of selective decontamination with probiotics, with or without prebiotics, is at least partly based on colonisation resistance. Probiotics are live bacteria that could beneficially affect the host by altering the GI flora, while prebiotics are nondigestible sugars that selectively stimulate the growth of certain colonic bacteria. When administered in combination,

prebiotics can enhance the survival of probiotic strains as well as stimulate the activity of the endogenous flora. The combination of pre- and probiotics has been termed 'synbiotics'.

The administration of probiotics is not expected to eradicate PPM in the same way that antibiotics would, but it is expected to delay the time to colonisation while the patients are intubated and ventilated, which would be beneficial. Several probiotic and synbiotic formulas are known and used. They usually contain a combination of lactic acid bacteria (including *Lactobacillus* spp.) and prebiotics or a single-agent probiotic (*Lactobacillus* spp.) [71].

#### 4.11. ENTEROCOCCUS SPECIES AS PROBIOTICS IN FOODS

*Enterococcus* species are able to produce a wide variety of virulence factors, which highlights their relevance as safety indicators in foods; however, they are also able to produce bacteriocins, called enterocins, and promote specific food modifications during fermentation. Enterococci are present in a variety of ripened cheeses, especially from the Mediterranean region, and are responsible for the specific aromas and flavours that determine the characteristics of these artisan foods. Many *Enterococcus* species were characterised based on their probiotic potential and were included in commercial products to be consumed by humans and animals that aimed to promote health and well-being. Despite being known to possess virulence genes, many studies found the absent expression of such genes, especially in isolates obtained from food systems. This led to studies investigating their real relevance as pathogenic microorganisms [72].

One trial investigated *Lactobacillus rhamnosus* GG (LGG) in the form of commercially-available yoghurt for the treatment of VRE. Subjects were randomly assigned to either the treatment group (receiving 100 g day<sup>-1</sup> of LGG-containing yoghurt for 4 weeks) or control group (receiving standard pasteurised yoghurt). A total of three faecal samples were obtained at weekly intervals, and treated patients were tested for VRE at 8 weeks. Patients in the control group who failed to clear VRE after 4 weeks were given LGG-containing yoghurt for 4 weeks as an open continuation. Of the 27 enrolled patients, 23 completed the study. Two patients were lost to follow-up, one died and one withdrew. All 11 patients in the treatment group who completed the study were cleared of VRE. This was the first description of a probiotic therapy successfully treating the GI carriage of VRE in renal patients [73]. Szachta *et al.* used LGG to clear VRE colonisation in hospitalised paediatric patients. A statistically significant difference in the level of VRE-negative children was seen at the end of the third week of the trial, with 62.5 % in the probiotic group ( $n = 32$ ) and 24 % in the control group ( $n = 29$ ) [74]. Taken together, these studies show the potential of LGG to reduce the level of VRE in colonised

patients, and additional studies on larger cohorts of patients are needed to confirm the anti-VRE effect of LGG. In contrast, *L. rhamnosus* Lcr35, which is related to the GG strain, did not significantly decrease the VRE colonisation density in nine adult patients [75].

The identification of commensal species correlated with a VRE reduction or, for *Vibrio cholerae* and *Clostridium difficile*, supported the idea of using commensal single or mixed strains to stimulate protection against intestinal pathogens and pathobionts [76-78]. Ubeda *et al.* introduced the notion of commensal key species in VRE clearance. They demonstrated that the reintroduction of the normal intestinal microbiota eliminated VRE from the intestinal tract. VRE clearance was correlated with the presence of *Barnesiella intestihominis*. In addition, analyses of the gut microbiota in patients undergoing allogeneic hematopoietic stem cell transplantation showed that microbiota containing *Barnesiella* correlated with resistance to intestinal domination and infection with VRE. However, it was not proven whether the anti-VRE effect was mediated directly by *B. intestihominis*, indirectly through the host response or was consecutive with the restoration of the microbiota [76]. Therefore its role in colonisation resistance is still unknown.

The only publication reporting a preventive effect of probiotic administration on VRE initial overgrowth used the *Lactococcus lactis* MM19 strain. *L. lactis* MM19 reduced the number of mice harbouring detectable VRE in their microbiota 3 days after inoculation with a vanA-type VRE strain. The authors provided *in vitro* evidence to suggest that the production of the bacteriocin, nisin Z, was responsible for the probiotic effect. In the same study, another bacteriocin-producing bacterium, *Pediococcus acidilactis* MM33, decreased VRE persistence in the mice microbiota 6 days after inoculation, albeit to a lesser extent [79].

A reduction of microbiota diversity decreased the production of RegIII $\gamma$ , which promotes VRE overgrowth. Depletion of commensal GI microbiota can be compensated by oral administration of lipopolysaccharides or intraperitoneal injection of flagellin, which restore RegIII $\gamma$  expression and reduce VRE density [80,81]. In the future, the use of commensal bacteria will be a tool for the eradication of infective bacteria.

#### **4.12. FAECAL TRANSPLANT (FAECAL MICROBIOTA TRANSPLANTATION)**

A single course of antibiotics can destroy the good intestinal bacteria. If a person receives a course of antibiotics, there is a good chance that the microorganisms in their digestive tract will become out-of-balance or ruined. This also applies to the eyes, nose, throat, skin, vagina, bladder and other parts

of the body where a bacterial balance is essential for good health. Health professionals usually recommend that probiotics are used in this situation, and sometimes probiotic capsules and pills do help.

In a healthy person, bacteria, yeast, protozoa and other microorganisms are built up over a lifetime. A mother's milk contains important flora, and during the first few years of life when an infant is crawling around and putting virtually every available object in their mouth, they are building up an essential balance of gut flora that will protect and provide good health for the rest of their lives.

Gaita material was first given in China in the 4<sup>th</sup> century as 'yellow soup' to patients with food poisoning and severe diarrhoea. It was used in the 16<sup>th</sup> century to treat high fever, pain, vomiting and chronic diarrhoea or constipation, and in the 17<sup>th</sup> century it was used in veterinary medicine. In the 20<sup>th</sup> century, 'warm camel faeces' was recommended by Bedouins, and during the Second World War, it was used by German soldiers in Africa to verify the cause of the cure. In Anatolia, 'horse-donkey goat stools' were used to help recover wounded tissue. In 1958, doctors in Denver used an enema to administer faeces to their patients with fulminant and life-threatening pseudomembranous enterocolitis. The goal of this faecal microbiota transplantation (FMT) was to 're-establish the balance of nature' within the intestinal flora to correct the disruption caused by antibiotic treatment [82].

Faecal material for transplantation can be infused in various ways. The preferred route is colonoscopy; however, transplantation is possible using a nasogastric tube, the nasoduodenal pathway, upper gastrointestinal system (GIS) endoscopy and retention enema [83]. One study investigated the impact of FMT in a cohort of patients with digestive tract colonisation by carbapenem-resistant *Enterobacteriaceae* (CRE) or VRE. A total of eight patients were included: six carrying CRE and two colonised by VRE. Two patients were free from CRE after 1 month of FMT, and another patient was free from VRE after 3 months [84]. In an industry-sponsored trial using an experimental microbiota suspension, eight of 11 patients (73 %) became VRE negative at 1–6 months after enema-administered FMT; however, the patients may have experienced spontaneous eradication [85].

In conclusion, there has been a substantial increase in CRE and VRE carriage, and every new strategy to decolonise patients should be investigated.

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