NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

DEPARTMENT OF MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY

INVESTIGATION OF FECAL CARRIAGE RATES OF VANCOMYCIN-RESISTANT ENTEROCOCCI IN HOSPITAL AND COMMUNITY SETTINGS

M.Sc. THESIS

Ifedayo Seun OYEGOKE

Nicosia

February, 2022

NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

DEPARTMENT OF MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY

INVESTIGATION OF FECAL CARRIAGE RATES OF VANCOMYCIN-RESISTANT ENTEROCOCCI IN HOSPITAL AND COMMUNITY SETTINGS

M.Sc. THESIS

Ifedayo Seun OYEGOKE

Supervisor

Assoc. Prof. Dr. Emrah RUH

Nicosia

February, 2022

Approval

We certify that we have read the thesis submitted by Ifedayo Seun Oyegoke titled "**Investigation of Fecal Carriage Rates of Vancomycin-Resistant Enterococci in Hospital and Community settings**" and that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Educational Sciences.

Examining Committee	Name-Surname	Signature
Head of the Committee:	Prof. Dr. Nedim Çakır	……
Committee Member:	Assist. Prof. Dr. Ayşe Seyer	……
Supervisor:	Assoc. Prof. Dr. Emrah Ruh	……

Approved by the Head of the Department

…../…../2022

Prof. Dr. Nedim Çakır

Head of Department

Approved by the Institute of Graduate Studies

…../…../2022

Prof. Dr. Kemal Hüsnü Can Başer

Head of the Institute

Declaration

I hereby declare that all information, documents, analysis and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

Ifedayo Seun Oyegoke

10/02/2022

Acknowledgments

My most profound appreciation goes to God Almighty for his blessings and guidance all through my academic years and for granting me the grace, strength, determination, patients, and knowledge all through my research work. I would like to appreciate and express my deepest and sincere gratitude to my supervisor Assoc. Prof. Dr. Emrah Ruh for his support, guidance, mentoring, tutoring and encouragements. All his contributions and guidance made this work a success.

I will not forget to appreciate the effort of the Head of Department Prof. Dr. Nedim Çakır, Assist. Prof. Dr. Ayşe Seyer, Prof. Dr. Kaya Süer and B.Sc. Gülten Hastürk for all their efforts towards the success of this study.

I would also like to thank the entire Near East University Faculty of Medicine, Near East University Hospital and the Institute of Graduate Studies for the support given to this study.

I will not forget to appreciate the efforts of the technicians who guided, supported and supplied in time all what is needed for me all through my work in the laboratory.

Special thanks to my father, my adviser and my mentor for his contributions to my work, your statement “do things at dispatch” made sense to me when I started this program, he has always made sure I was provided with everything before it was needed, and to my mom for all her love, support and prayers throughout my time in school, I love you. To my pastors who kept calling and supporting me in prayers, thank you so much. I won’t fail to appreciate my friends Evelyn, Dino, Emmanuel and Patrick who believed in me. Thank you all for giving me the strength and support to fulfill my dreams and reach for stardom.

Özet

Vankomisine Dirençli Enterokokların Hastane ve Toplumdaki Fekal Taşıyıcılık Oranlarının Araştırılması

Oyegoke, Ifedayo Seun

Yüksek Lisans, Tıbbi Mikrobiyoloji ve Klinik Mikrobiyoloji Anabilim Dalı

Danışman: Doç. Dr. Emrah Ruh

Şubat 2022, 53 sayfa

Amaç: Bu çalışma, Kuzey Kıbrıs'ta hastanede yatan hastalarda ve toplumda vankomisine dirençli enterokokların (VRE) intestinal kolonizasyon oranının araştırılması amacıyla yapılmıştır.

Gereç ve Yöntem: Çalışmada toplam 110 dışkı örneği toplanmıştır. Örnekler Enterococcosel agara ekilmiş ve 37ºC'de inkübe edilmiştir. Kültür plaklarında üreyen koloniler steril %0,9 NaCl içerisinde süspanse edilmiş ve 0,5 McFarland standart bulanıklığa ayarlanmştır. Bunu takiben bakteri süspansiyonları, steril bir eküvyon kullanılarak Mueller-Hinton plakalarına ekilmiştir. Son olarak, plakların ortasına bir vankomisin diski (30 µg) yerleştirilmiştir. Kültür plakları 37°C'de inkübasyonunu tamamladıktan sonra inhibisyon zon çapları ölçülmüştür. VRE kolonizasyonunun sosyoekonomik ve epidemiyolojik faktörlere göre değerlendirilmesi için istatistiksel analiz yapılmıştır.

Bulgular: Çalışmada 110 katılımcının ikisinin (%1,8) VRE ile kolonize olduğu saptanmıştır. Her iki tür de *Enterococcus gallinarum* olarak tanımlanmıştır. Her iki katılımcı da kontrol grubuna aitti. İstatistiksel analiz sonucuna göre, VRE kolonizasyonu ile katılımcıların yaşı, cinsiyeti, eğitim düzeyi, medeni durumu ve sosyoekonomik durumu arasında bir ilişki olmadığı görülmüştür ($p>0.05$). Ayrıca, VRE kolonizasyonu ile örnek toplama sırasında katılımcılardaki gastrointestinal semptom varlığı, örnek toplanmasından önceki son altı ay içinde antibiyotik kullanımı, ishal, idrar yolu enfeksiyonu ve yurtdışına seyahat öyküsü arasında anlamlı bir ilişki olmadığı görülmüştür ($p>0.05$).

Sonuç: Bu çalışma, toplumda VRE fekal taşıyıcılığının mevcut olduğunu göstermektedir. Bu nedenle, VRE'nin Kuzey Kıbrıs'ta yayılmasının önlenmesi için kontrol önlemleri uygulanmalıdır.

Anahtar kelimeler: Vankomisine dirençli enterokoklar, antibiyotik direnci, *Enterococcus gallinarum,* fekal taşıyıcılık.

Abstract

Investigation of Fecal Carriage Rates of Vancomycin-Resistant Enterococci in Hospital and Community Settings

Oyegoke, Ifedayo Seun

M.Sc., Department of Medical Microbiology and Clinical Microbiology

Supervisor: Assoc. Prof. Dr. Emrah Ruh

February 2022, 53 pages

Aim: The aim of the study was to investigate the rate of intestinal colonization of vancomycin-resistant enterococci (VRE) in the hospitalized patients and the community in Northern Cyprus.

Materials and Methods: A total of 110 fecal samples were collected in the study. The samples were cultured on Enterococcosel agar and incubated overnight at 37ºC. The colonies that were grown on the culture plates were suspended in sterile 0.9% NaCl and adjusted to 0.5 McFarland standard turbidity. Following this, the bacterial suspensions were inoculated onto Mueller-Hinton plates by using a sterile swab. Finally, a vancomycin disc (30 µg) was placed at the center of the plates. After the culture plates were incubated overnight at 37ºC, the inhibition zone diameters were measured. In order to evaluate the intestinal colonization of VRE according to socioeconomic and epidemiological factors, statistical analysis was performed.

Results: In the study, two (1.8%) of 110 participants were colonized with VRE. Both of the species were identified to be *Enterococcus gallinarum*. Both participants belonged to the control group. The statistical analysis showed no correlation of VRE colonization with age, gender, level of education, marital status and socioeconomic status of the participants ($p>0.05$). Also, intestinal colonization of VRE was not significantly affected by the presence of gastrointestinal symptom at the time of sample collection; or history of antibiotic use, diarrhea, urinary tract infection, and travelling abroad within the last six months before the sample collection ($p>0.05$).

Conclusion: This study indicates that fecal carriage of VRE is present in the community. Therefore, control measures should be implemented to prevent further spread of VRE in Northern Cyprus.

Key words: Vancomycin-resistant enterococci, antibiotic resistance, *Enterococcus gallinarum*, fecal carriage.

Table of Contents

Approval……1
Declaration……2
Acknowledgements……...3
Özet……4
Abstract……..5
Table of Contents ……..6
List of Tables……9
List of Abbreviations……...10

CHAPTER I

Introduction……..11
Aim of the Study……..12

CHAPTER II

Literature Review...…..……..……...……...13
2.1. Important *Enterococcus* species……..…….……..14
2.1.1. *Enterococcos faecalis*…….....…….……...14
2.1.2. *Enterococcus faecium* ……14
2.1.3. *Enterococcus gallinarum* ……...……..……..…….14
2.1.4. *Enterococcus casseliflavus* ……..…….……..15
2.1.5. *Enterococcus avium* ……15
2.1.6. *Enterococcus durans* …….…….16
2.1.7. *Enterococcus hirae* ……...…….…….16
2.1.8. *Enterococcus malodoratus*……..16
2.2. Enterococcal infections……..……16
2.2.1. Urinary tract infections……17
2.2.2. Bacteremia……...17

2.2.3. Endocarditis ……..…….17
2.2.4. Cellulitis ……...…….……..17
2.2.5. Acute bacterial prostatitis..……...……..18
2.2.6. Wound infections……...……...……...…18
2.2.7. Osteomyelitis……...18
2.2.8. Abscesses of the abdomen……...……19
2.3. Virulence Factors of Enterococci…..…….19
2.4. Glycopeptides…....…….....20
2.4.1. Vancomycin…….…20
2.4.2. Vancomycin mechanism of action……..……...…..20
2.4.3. Mechanisms of vancomycin resistance……..…...…….....21
2.5. Resistant Enterococci…….....23
2.5.1. Intrinsic Resistance…….…….....24
2.5.2. Acquired Resistance……..……..24
2.6. Prevention of Vancomycin-Resistant Enterococci ……..……..25
2.7. Antibiotic Resistance in Enterococci…….....25
2.7.1. Beta-lactam resistance……..……...26
2.7.2. Aminoglycoside resistance……..……27
2.7.3. Glycopeptide resistance...……28
2.8. Epidemiology of Vancomycin-Resistant Enterococci……....28

CHAPTER III

Materials and Methods……..….31
3.1. Study Design and Participants…….……..…….31
3.2. Collection of Samples and Data……...……...……31
3.3. Initial Screening of Vancomycin-Resistant Enterococci……31
3.4. Confirmation and Identification of Vancomycin-Resistant Enterococci..…...31
3.5. Statistical Analysis…..…….....32

CHAPTER IV

Results ……..…….....33

4.1. General Characteristics of the Study Participants……...…33

4.2. Intestinal Colonization with VRE Isolates…….33

4.3. Characteristics of VRE Colonized Participants…….……34

4.4. Result of Statistical Analysis…….35

4.4.1. Correlation of intestinal colonization of VRE with demographic and socioeconomic factors……...……...…35

4.4.2. Correlation of intestinal colonization of VRE with epidemiological factors…….……...37

CHAPTER V

Discussion…….…….……..39

CHAPTER VI

Conclusion …….…….44

REFERENCES ……...45

List of Tables

Table 4.1. Distribution of VRE among Patient (n=46) and Control (n=64) Groups……..…..34

Table 4.2. Characteristics of the Participants who were Colonized with VRE……...35

Table 4.3. Correlation of Intestinal Colonization of VRE with Demographic and Socioeconomic Factors in the Study Group (n=110)…….….36

Table 4.4. Correlation of Intestinal Colonization of VRE with Epidemiological Factors in the Study Group (n=110).…..……..…38

List of Abbreviations

CDC: Centers for Disease Control and Prevention

CSLI: Clinical and Laboratory Standards Institute

DNA: Deoxyribonucleic Acid

ECDC: European Centre for Disease Prevention and Control

ESKAPE: *Enterococcus* spp., *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp.

GIS: Gastrointestinal Symptom

HAIs: Healthcare-Associated Infections

ICU: Intensive Care Unit

MIC: Minimum Inhibition Concentration

MRSA: Methicillin-Resistant *Staphylococcus aureus*

µg: Microgram

µl: Microliter

NaCl: Sodium Chloride

NCDO: National Collection of Dairy Organism

PFGE: Pulsed-Field Gel Electrophoresis

RNA: Ribonucleic Acid

rRNA: Ribosomal Ribonucleic Acid

TAVI: Transcatheter Aortic Valve Implantation

UTI: Urinary Tract Infection

VRE: Vancomycin Resistant *Enterococcus*

CHAPTER 1

Introduction

Antibiotic resistance is a major global concern, this is the major reason why it should not be used without prescription by a specialist. Antibiotic-resistant bacteria are on the rise, jeopardizing the effectiveness of antibiotics, which have changed medicine and saved millions of lives. Bacterial infections have returned decades after the first cases were treated with antibiotics. Antibiotic resistance has also been connected to antibiotic abuse and overuse, as well as the pharmaceutical industry's mortality of new medication research because to limited economic incentives and stringent regulatory restrictions (Ventola, 2015).

Nosocomial infections are a major contribution to antibiotics resistance as a result of continuous antibiotics usage. Centers for Disease Control and Prevention (CDC) published two publications about nosocomial infections (NIs) and the criteria for surveillance of certain types of NIs in 1988. Nosocomial infections are any systemic or localized diseases caused by an infectious agent or toxin reaction. *Enterococcus* spp. are a fast-rising antibiotic resistant microorganism. They are a broad, species-rich group of lactic acid bacteria gotten from different habitats, including humans, animals, and insect digestive systems, as well as natural biomes such as water, sewage, soil, and arable land. Enterococci have also been identified from plants such as olives and are found on wild plants. Some enterococci species are commensal, can stimulate the immune system, and have a substantial impact on intestinal homeostasis maintenance (Kouchak et al., 2012).

Enterococci, in the form of a probiotic, can be utilized to strengthen the immune system (diet supplement or therapeutic application). Similarly, enterococci have a function in food technology as the starting culture involved in meat and cheese fermentation and food preservation. Enterococci can act as pathogens (O'Driscoll & Crank, 2015). They are responsible for food contamination and they constitute an epidemic concern in the hospital environment due to their occasionally present virulence and multi-drug resistance (Guzman et al., 2016). According to research, they may also have a role in the development of colon cancer. They were categorized as the first of the ESKAPE organisms (*Enterococcus* spp., *Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas*

aeruginosa, *Enterobacter* spp.) as rising causes of nosocomial and antibiotic-resistant infections threatening public health (Santajit & Indrawattana, 2016).

Enterococcus spp. are gram-positive, facultative anaerobic cocci that were discovered in the human gastrointestinal tract for the first time in 1899, DNA hybridization and 16S rRNA sequencing identified them as a distinct species from streptococci. They are found in the intestine and gut, the vaginal tract and mouth on rare occasions. (Said, et al., 2021). Enterococci are opportunistic bacteria that become harmful when the immune system fails. (Santajit & Indrawattana, 2016).

Enterococcus species can cause diseases in the hospitals, particularly in the urinary system, surgical sites, and the bloodstream. Between hospitalized infections in the United States of America and the most prevalent isolated germs in the bloodstream, *Enterococcus* is the third most common isolated organism. After staphylococci and streptococci, enterococci exist as one of the most common causes of infected endocarditis. *E. faecalis* causes around 90% of enterococcal endocarditis, with *E. faecium* accounting for less than 5%. Enterococcal endocarditis has a significant morbidity and fatality rate. For the past 30 years, the numbers of patients requiring cardiac surgery is approximately 42%, as well as the 1-year mortality ratio (29%), have remained virtually unchanged, with recent data indicating that they may even be growing (AL Bloushy & Elbehiry, 2018).

Aim of the Study

The purpose of this study was to investigate VRE carriage rate in patients of Near East University Hospital and the community in Northern Cyprus.

CHAPTER II

Literature Review

Enterococcus was first categorized as D streptococci organisms, they contain the D-group specific substance (Said, et al., 2021). Enterococci are known by criteria other than immunologic interaction with group-specific antisera since the group D cell wall–specific antigen is a teichoic acid and so is not an antigenically good marker. They are part of the intestinal flora. (Donald et al., 2020). Occasionally, enterococci grow under unfavorable environmental circumstances like drying, extreme temperatures, and some antiseptic treatments (Cabral, 2010). This rate enables enterococci to infect surroundings and medical devices, allowing for epidemics to be transmitted to patients via health care personnel. Although enterococci were once thought to be low-virulent bacteria, they have become a significant cause of a range of illnesses that mostly affect immunocompromised people and are primarily healthcare infections in recent decades (Fiore et al., 2019).

They are typically non-hemolytic, but can be hemolytic on occasion. Resistance to antibacterial such as beta-lactams, glycopeptides and aminoglycosides has substantially increased in enterococci throughout the previous few decades, making many enterococcal infections difficult to treat (Semedo et al., 2003).Due to the shifting epidemiology of enterococcal infections, the microbiology laboratory must diagnose enterococci at the species level, as each *Enterococcus* spp. may have a unique epidemiology and pattern of antibiotic resistance. Recent studies have revealed the genomes of enterococci, most notably *E. faecalis* and *E. faecium* bringing fresh insight into enterococci biology. Comparative genomic investigation has demonstrated high intra species genomic variability especially within strains that are clonally linked which is mainly related to the varied existence of plasmids, phages, pathogenicity and conjugative elements strains (Byappanahalli et al., 2012).

Furthermore, animal experimental models were used to study the host immune response to enterococci, shedding information on the increased susceptibility of immunocompromised and critically unwell patients to enterococcal infections (Skendros & Mitroulis 2012). The bulk of *E. faecalis* (and, to a lesser extent, E. faecium) putative virulence determinants are involved in extracellular structure adhesion and biofilm formation, both of which seem to be crucial components in colonization and infection (Daniel & Magnus, 2013). Plasmids and transposons

are important for antibiotic resistance spread in enterococci as well as the transfer of resistance determinants to other bacteria like *Staphylococcus aureus* (Babakhani & Oloomi, 2018). Many more enterococcal genomes have been sequenced using next generation sequencing technologies for instance pyrosequencing, which has increased our comprehension of enterococcal virulence determinants and biology (Behjati & Tarpey, 2013).

Currently, little has been known about enterococci's adaptive response mechanisms to a variety of conditions. Advances in genomics, such as a better understanding of enterococcal virulence factors, adaptive response mechanisms to multiple conditions, and also a better understanding of the host immune response to these pathogens, will allow the development of novel strategies to decrease colonization and infection, as well as novel therapeutic options for complicated enterococcal infections (Mulani et al., 2019).

2.1. Important *Enterococcus* Species

2.1.1. *Enterococcus faecalis*

This is a gram-positive commensal bacteria found in the human gastrointestinal tract that is usually classified as a member of the group D streptococci. Like other *Enterococcus species*, *E. faecalis* can be seen in healthy humans and used as a probiotic (Ramos et al., 2020). A as an opportunistic pathogen,it can cause life-threatening infections, particularly in clinical settings, in which the naturally high levels of antibiotic resistance identified in *E. faecalis* add to its pathogenicity. *E. faecalis* was identified often in re-infected, root canal-treated dentition, with prevalence estimates varying between 30% to 90%. Re-infected root canal-treated tooth are about nine times more likely to host *E. faecalis* than initial infections. (Stuart et al., 2006).

2.1.2. *Enterococcus faecium*

They are gram-positive, non-hemolytic *Enterococcus* bacteria. It can be commensal in the GI tract, but it can also be harmful, causing disorders like neonatal meningitis or endocarditis (Khan et al., 2019).

2.1.3. *Enterococcus gallinarum*

This species possesses a low-rate intrinsic vancomycin resistance. Resistance is mediated by a chromosomal gene called *vanC*, which encodes for a terminal D-alanine-D-serine sequence

in the cell wall peptidoglycan precursor proteins, rather than the more common D-alanine-D-alanine sequence. This is a distinct mechanism from the resistance of vancomycin observed in *E. faecium* and *E. faecalis* isolates, which is transferred by *vanA or vanB.* Although considered extremely rare, this species is known to generate various types of illness. Apart from *E. faecalis and E. faecium* it is the only other enterococcal species known to cause outbreaks and spread in hospitals (Gholizadeh & Patrice Courvalin, 2000).

According to a 2018 study, these pathogenic gut bacteria can spread to other organs such as the lymph nodes, liver, and spleen, eliciting an inflammatory response in people and animals. *E. gallinarum* was discovered during liver biopsies of three patients with systemic lupus erythematosus and autoimmune liver disease. When an intramuscular vaccine or antibiotic was delivered, the autoimmune reaction was inhibited. Meningitis and sepsis are potentially possible complications of these bacteria. Antibiotics such as linezolid, daptomycin, gentamicin, levofloxacin, and penicillin G are all effective against this bacterium (Taylor et al., 2021).

2.1.4. *Enterococcus casseliflavus*

They are resistant to vancomycin because they belong to *E. gallinarum* family. They are mostly the causative agent for clinical-related diseases, specifically urinary tract, bloodstream and surgical wound infections. Infections caused by this bacteria is on the rise around the world, as they are known to infect people with hepatobiliary or oncohematological illnesses. With resistance of vancomycin caused by a chromosomally encoded *vanC* operon, the innate response to several antibiotics other than glycopeptides restricts therapy options (Monticelli et al., 2018). Furthermore, unlike the vancomycin resistance generated by transmissible plasmids in *Enterococcus faecalis* and *Enterococcus faecium,* their intrinsic vancomycin resistance poses different infection management concerns (Monticelli et al., 2018).

2.1.5. *Enterococcus avium*

This species is very frequent in birds. It is also a rare source of infection in people, and in such cases, it may be vancomycin-resistant, a condition known as VREA. Linezolid was used successfully to treat these situations in people (Said et al., 2021).

2.1.6. *Enterococcus durans*

These species were previously known as *Streptococcus durans*, it is found in the gastrointestinal tract and has been linked to Fouls, piglets, calves, and puppies can all have enteritis. E. durans was found to be the cause of diarrhea in 5 of 7 kittens who had diarrhea within the first 10 days of birth (Mahizan et al., 2019).

2.1.7. *Enterococcus hirae*

Its type strain is NCDO 1258 (National Collection of Dairy Organism). This specie is involved in growth depression in young chickens, and endocarditis and sepsis in humans (Lebreton et al., 2014).

2.1.8. *Enterococcus malodoratus*

This is a specie of the genus *Enterococcus*; it is a gram-positive bacterium capable of opportunistic pathogenic response. *Enterococcus* can be found in the alimentary canal of humans and other mammals. These microorganisms are covered with a dense polypeptide coating. *E. malodoratus* was discovered in the intestines and feces of swine during a study on the *Enterococcal* flora of the animals (Lebreton *et al*., 2014). *Enterococcus malodoratus* is a nonmotile, facultatively anaerobic and fermentative bacterium. The cells have a coccoid shape and are typically seen in pairs or short chains. Unlike many other *Enterococcus* species, *E. malodoratus* does not grow well at 45ºC (Fotadar et al., 2005).

2.2. Enterococcal infections

There are approximately 17 distinct enterococci. Numerous species naturally inhabit the digestive tract and do not typically cause illness. These bacteria, collectively referred to as resident flora, cause disease only in specific circumstances, such as when they invade other parts of the body. *Enterococcus faecium* and *Enterococcus faecalis* are the most common *Enterococcus* spp. that cause diseases in humans (cetinkaya et al., 2000).
Enterococci can affect immunocompromised people and cause a wide range of severe diseases (Radhouani et al., 2014). Though enterococci can cause community-acquired diseases, there has been an upsurge in the number of enterococcal healthcare-associated infections (HAIs) in the

past years. Enterococci are usually associated with mixed infections. Enterococcal HAIs are usually preventable and may cause poor outcomes and increased costs (Fiore et al., 2019). *Enterococci* are able to cause infections at numerous sites. The infections entail; urinary tract infections (UTIs), endocarditis, vascular catheter-related bloodstream infections, bacteremia, and cellulitis (soft tissue infections). Prostatitis, wound infections, osteomyelitis, abscesses of the abdomen (Natsis & Cohen, 2018).

2.2.1. Urinary tract infections

They are the major prevalent enterococci infection, and they mainly occur in chronically ill patients in the hospital, often in conjunction with blockage, catheterization, or instrumentation. They can also induce more serious UTIs such as pyelonephritis, perinephric abscesses, and chronic prostatitis, all of which can lead to bacteremia (Said et al., 2021).

2.2.2. Bacteremia

This occurs when bacteria escape the host's immune system or when the immune system's normally coordinated response fails to control bacterial spread due to inherent or acquired immunological weakness (Christaki & Giamarellos-Bourboulis, 2014).

2.2.3. Endocarditis

This is an infection of the endocardium, the inner lining of the heart. Up to 10% of these infections are caused by *E. faecalis* and other enterococci bacteria. This is often classified as acute or subacute disease based on the spontaneous clinical cause. Enterococcal infective endocarditis (IE) is becoming more common, occurring in 8% to 32% of enterococcal bacteremia episodes and usually lasting less than a week. Due to their advanced age and comorbidities, *Enterococci* was the most prevalent pathogen causing IE in participants who underwent trans catheter aortic valve implantation (TAVI) (Said et al., 2021).

2.2.4. Cellulitis

Cellulitis is a non-necrotizing disease of the skin and subcutaneous tissues that usually results from an acute infection. Although a portal of entrance may not be obvious, cellulitis

frequently occur when there is a breach in the skin; the breach may include tiny skin changes or the invasive abilities of some microbes (Swartz, 2004).

2.2.5. Acute bacterial prostatitis

This is a urinary tract infection that produces pelvic discomfort as well as urinary tract abnormalities like dysuria, frequent urination, and urine retention, as well as systemic symptoms like fevers, chills, vomiting, reflux, and malaise. Although the real incidence is unclear, acute bacterial prostatitis is thought to account for roughly 10% of all prostatitis cases. Although the majority of acute bacterial prostatitis infections occur in the community, some can occur as a result of transurethral manipulation techniques such as urethral catheterization and cystoscopy, as well as following transrectal prostate biopsy (Coker & Dierfeldt, 2016).

2.2.6. Wound infections

Wound infections develop when cuts, scrapes, animal bites, sutured wounds, and puncture wounds get infected, which usually occurs 24 to 72 hours after the incident. Pus, a red region or streak that is spreading, are all signs of a wound infection. A temperature, as well as increased pain and edema. A lymph node that has enlarged. *E. faecalis* wound infections are more difficult to treat than other forms of bacteria. A lengthier hospital stay, a repeated surgical operation, and prior antibiotic therapy are all connected with an increased risk of death, particularly in poor countries (AL Bloushy & Elbehiry, 2018).

2.2.7. Osteomyelitis

This is a painful bacterial infection of the cortical and cancellous bones. In the maxillofacial area, the jawbone is the most often impacted bone. It is a bone infection whose exact source is unknown, however it is thought to be caused by local invasion, bacteremia or trauma. Any bone may be affected; however, the long bones are the most usually affected (Pineda et al., 2009). It presents perniciously in children with fever, discomfort, impaired coordination or supporting bodyweight, making diagnosis challenging. Early on, laboratory and radiographic testing may be negative, thus they ought to be double-checked. Conventional radiograph abnormalities are typically not seen till 10-14 days after the beginning of the sickness. The following are the most prevalent causes of acute osteomyelitis: *Staphylococcus*

aureus, which includes a growing number of methicillin-resistant species *Streptococcus pyogenes* and *Streptococcus pneumoniae* (Pineda et al., 2009). For diagnosis, bone scans or magnetic resonance imaging are frequently employed. Enterococcus vertebral osteomyelitis is a rare infection that can be transmitted through the bloodstream following a urinary tract infection (AL Bloushy & Elbehiry, 2018).

2.2.8. Abscesses of the abdomen

The abscesses can be found anywhere in the abdomen, however, the majority is contained within the peritoneal cavity. To avoid the significant morbidity and fatality rates associated with this disease, it must be detected and treated early. Unless properly drained, abdominal abscesses pose a considerable risk of morbidity and mortality. The symptoms differ according on the location of the illness (Sarychev et al., 2018).

2.3. Virulence Factors of Enterococci

Enterococci are particularly opportunistic pathogens. Unlike other microbes, they lack well-defined virulence factors. Nevertheless, the followings are the most known:
Endocarditis, UTI, and bacteremia are all caused by *Enterococcus.* According to a survey of infectious organisms that cause hospital-acquired illnesses, enterococci is the top cause of hospital acquired infections in the U.S, with rates ranging from 20% to 30%, and the second greatest cause worldwide. According to a recent Chinese investigation, *E. faecium* was the most common bacterium (74%), second was *E. faecalis,* which contributed to 20% of bloodstream infections and a 24% death rate. These organisms cause diseases that are difficult to treat, endure a long time, and are typically bothersome. Several variables, consisting of but not restricted to cytolysin (CylLLLSM), have been implicated (Fiore et al., 2019).
Enterococcal pathogenicity is known to be provided by aggregation substance (AS), enterococcal surface protein (Esp), gelatinase (GelE), sex pheromones Cob*, E. faecium* cell wall adhesion factors, and Ccf. This same enterococcal membrane molecule is thought to have a role in immunological escape. The hemolytic and bactericidal actions of cytolysin against gram-positive bacteria contribute in the progression of enterococcal infection. A.S at the site of infection promotes bacterial breeding and recombination, resulting in bacterial proliferation. By hydrolyzing hemoglobin and other peptides, GelE promotes inflammation, and sex pheromones

can transmit plasmids encoding one or maybe more genes resistant to antibiotics (Cook et al., 2013).

2.4. Glycopeptides

Glycopeptides are a class of naturally occurring and chemically manufactured glycosylated peptides that prevent gram-positive bacteria from forming cell walls. This is performed by attaching to the bacterial cell wall's D-alanyl-D-alanine end and blocking peptidoglycan layer cross-linking. Vancomycin is a glycopeptide antibiotic that has shown clinical endurance and a consistent preference for the treatment of methicillin-resistant *Staphylococcus aureus* and *Enterococcus* spp. (Urbańczyk et al., 2017).

2.4.1. Vancomycin

Vancomycin is a glycopeptide antibiotic that is still in use and is part of the World Health Organization's list of important drugs. Previously, vancomycin was considered a "last resort" antibiotic for the therapy of numerous gram-positive bacteria resistant to other antibiotics (Zeng et al., 2016). It was used effectively to treat MRSA diseases for more than 30 years following its identification in 1952 by E.C. Kornfield. In 1988, the first isolates of VRE, *Enterococcus faecium* and *Enterococcus faecalis*, have been discovered in United Kingdom, followed shortly thereafter by other European countries and the United States (Walsh et al., 2021).

Vancomycin has antibacterial activity on Staphylococci, Streptococci, and other gram-positive bacteria. Bactericidal action of vancomycin results from inhibition of cell wall biosynthesis. Vancomycin and other antibiotics have no cross-resistance. Vancomycin does not have in vitro activity against gram-negative bacilli, mycobacteria, or fungi. (Cong et al., 2019)

2.4.2. Vancomycin mechanism of action

Vancomycin inhibits cell wall production by attaching to D-Ala-D-Ala pentapeptides, *van* operons, a two-component regulatory system, confers resistance to antibiotics (*vanS-vanR*). When stimuli are detected, the subsequent *vanH, vanA* or *vanB,* and *vanX* become active. *VanX* is a d,d-dipeptidase that cleaves D-Ala-D-Ala repeats, reducing the pool of D-Ala-D-Ala and providing free D-Ala to *van*(*A/B*). *VanH* is a d-hydroxyacid dehydrogenase which converts pyruvate into d-Lac in order to prepare the *van*(*A/B*) ligase. After that, *Van (A/B)* ligates D-Ala-

D-Lac to form D-Ala-D-Lac pentapeptides with poor vancomycin specificity. *VanY* is a D,D-carboxypeptidase that cleaves the D-Ala terminal peptide, resulting in the reduction of pools of pentapeptides with high vancomycin affinity (Mühlberg et al., 2020).

Lastly, *vanZ*, which really is found in *vanA*-carrying variants, imparts teicoplanin resistance via an independent mechanism. Resistance differences also are likely due to pentapeptide makeup. When pentapeptides are mostly constituted of low-affinity molecules, high-level resistance develops, whereas moderate-level resistance is composed of a more diversified mix of high- and low-affinity pentapeptides (Mühlberg et al., 2020).

Resistance-encoding genes are commonly shared on recombinant jumping genes, pheromone-responsive plasmids, and some other plasmids with a broad host range. Vancomycin interacts with the precursors of the cell wall D-Ala-D-Ala of the bacteria. By establishing H_2 bonds, vancomycin limits cell wall formation (dotted lines). In *van*A-mediated resistance, the peptidoglycan precursor's end of the D-alanine is substituted with D-lactate, leading to significant reduction of the peptidoglycan precursor's binding affinity to vancomycin (Sung & Lindsay 2007). D-Ala-D-Lac is represented by *vanA*, *vanB*, and *vanD*, whereas D-Ala-D-Ser is represented by *vanC* and *vanE*. The *vanA* and *vanB* gene clusters are linked to *E. faecalis* as well as *E. faecium* of which are responsible for the bulk of VRE outbreaks in humans (Moosavian et al., 2018).

2.4.3. Mechanism of vancomycin resistance

Vancomycin and lipoglycopeptides are inherently resistant to Gram-negative bacilli because the outer membrane blocks the huge chemicals crossing the cell and connecting to their specific locations. Resistance to vancomycin develops far more slowly in gram-positive bacteria than resistance to other non-glycopeptide antibiotics. Over 30 years after vancomycin was developed, the first clinical isolation of a vancomycin-resistant strain was reported in 1987 (Zeng D et al., 2016*)*.

The most frequent resistant mechanism of enterococci are *van-A* resistance (Depardieu et al., 2004). Tn1546 and adjacent elements express nine vancomycin-resistant polypeptides, which alter the carboxyl terminus peptidoglycan precursor away from the D-alanine to D-lactate. This enzymes comprise those required in the production of transformed peptidoglycan precursors (*vanH* and *vanA*), hydrolysis of normal precursors (*vanX* and *vanY*), resistance expression

regulation (*vanR* and *vanS*), transposition (products of ORF1 and ORF2), and a variety of others (*vanZ*). *VanH* is a dehydrogenase that catalyzes the conversion of pyruvate to D-Lac.while *vanA* is a ligase which promotes D-Ala-D-Lac synthesis, which substitutes D-Ala-D-Ala. Similarly, *vanX* and *vanY* lower indigenous D-Ala-D-Ala levels. *VanX* is a D,D-dipeptidase which hydrolysis of the D-Ala-D-Ala generated by a parent homologous D-Ala-D-Ala synthetase, whereas *van*Y is now a D,D-carboxypeptidase that catalyzes the hydrolysis of the terminal D-Ala of pentapeptide precursors if the D-Ala-D-Ala (Zeng et al., 2016).

The inducible expression of this resistance is regulated by the *vanR*/*vanS* two-component system.v*anS* is a surface sensor that detects glycopeptides and donates a phosphoryl group to *vanR*, the responsive promoter that triggers cotranscription of the *vanH*, *vanA*, *vanX*, and *vanY* genes by adhering to the PRES promoter and of the *vanR* and *vanS* genes by binding to the PREG marker (Zeng et al., 2016). (Dersch et al., 2017). This rearrangement provides greater resistance to vancomycin, teicoplanin & telavancin, albeit the MICs for telavancin remain 32- and 128-fold lower than those for vancomycin and teicoplanin, respectively (Sabharwal et al., 2015), *vanA*-type resistance, which may be transmitted from *Enterococcus* species to *S. aureus*, is the major mechanism for VRSA resistance (Depardieu et al., 2004).

The *vanB*-type resistance, similar to *vanA* resistance, is brought on by the existence of a tranposon (Tn1547 or Tn1549), which causes D-Ala-D-Lac to be synthesized rather than D-Ala-D-Ala, leading to lower antimicrobial adherence. Furthermore, the *vanB* cluster's architecture and functions are identical to those of *vanA*, producing a reductase, ligase, and dipeptidase, both of which are characterized by a high amount of structural similarity (67–76%) to the *vanA* operon's equivalent proteins. Nevertheless, the regulator's proteins in *vanB* kind of resistance are relatively remotely linked to *van*RS (34% and 24% respectively) and therefore are exclusively triggered by vancomycin and not teicoplanin; as a result, bacteria with all of this phenotype have high-level vancomycin resistance (Courvalin, 2006).

Enterococcus casseliflavus/*flavescens* and *Enterococcus gallinarum* exhibit *van*C-type resistance, which results in the synthesis of peptidoglycan components ending in D-Ala-D-Ser (Reynolds and Courvalin 2005). This clump comprises three critical resistance genes: *vanT* is a vancomycin-resistant *Enterococcus gallinarum* BM4174 bilayer serine racemase that synthesizes D-Ser; *vanC* is a ligase that speeds up D-Ala-D-Ser synthesis; and *van*XYC possesses D,D-dipeptidase and D,D-carboxypeptidase activity and hydrolyzes precursors terminating in D-Ala-

D-Ala. The *vanC* trait is shown on the chromosome either constitutively or inducibly and gives resistance to low amounts of vancomycin but not to other glycopeptides (Reynolds & Courvalin 2005).

Resistant rate of the *vanD* type produce the formation of peptidoglycan precursors with the sequence D-Ala-D-Lac, *vanD* genes are organized similarly like *vanA* and *vanB*; nevertheless, *vanD* genes are only found on the bacterial genome,their resistance are not transferred to other enterococci via conjugation (Depardieu et al., 2004). Furthermore, *vanD*-type strain lacks D,D-dipeptidase functionality, which is unnecessary since different point mutations prevent the D-Ala-D-Ala ligase from binding (Donald & Levine, 2006). Surprisingly, due to this deficiency of native D-Ala-D-Ala synthesis, *vanD* isolates need constant production of these resistance components to thrive, which they do by alterations in the *vanSD* sensor or *vanRD* regulator (Depardieu et al., 2004). *VanE*-type resistance is observed in intrinsically resistant *E. faecalis* and is structured similarly to *vanC* resistance (Courvalin, 2006).

The *vanG*- resistance is made up of genes derived by many *van* gene cluster: *vanG* is a D-Ala-D-Ser ligase (like *vanC*-type isolates); *vanXYG* is a potential polyfunctional D,D-peptidase and D,D-carboxypeptidase; *vanTG* is a serine racemase (like *vanE*-type strains); and *vanRG* and *vanSG* are ordering system sensor based enzymes (highest similarity to the *vanD*-type strains). Just like *vanC and vanE* genotypes, *vanG* bacteria generate D-Ala-D-Ser but are only moderately resistant to vancomycin (Depardieu et al., 2004).

2.5. Resistant Enterococci

Since the 1980s, antibiotic-resistant enterococci have been the primary cause of hospital-acquired infections. The emergence of multidrug-resistant enterococci is primarily due to specific antimicrobials like beta-lactams and aminoglycosides, as well as acquired resistance to quinolones, glycopeptides, macrolides, streptogramin and tetracyclines via mobile elements like transposons and plasmids (Michael et al., 2013). Enterococci have low-level resistance to antibiotics. Furthermore, in recent years, enterococci has established a rapid process of establishing high-level antibiotic resistance to glycopeptides, aminoglycosides and beta-lactams in recent years. (Miller et al., 2014).

High levels of streptomycin, gentamicin, resistance in both are documented in the United States and in Europe, and could affect both *E. faecalis* and *E. faecium* The primary clinical

implication of high rate of aminoglycoside resistance is a lack of synergistic action during attachment to vancomycin or ampicillin in endocarditis or other severe enterococcal diseases (Miller & Munita, 2014).

Resistance to beta-lactams and glycopeptides is particularly concerning, as both resistance types are primarily associated with *E. faecium.* Though the estimates of increased ampicillin resistance in the United States and Europe are very comparable, there are significant variances in the study of glycopeptide resistance in enterococci. Increased rate of glycopeptide resistance is typically related with the *vanA* gene cluster, it exhibits vancomycin resistance as well as cross-resistance to teicoplanin. Significant issue in medicine is that glycopeptide resistance can be transferred both intra and inter-species (Binda et al., 2014).

Finding the best therapeutic choice for enterococcal infections having high-level resistance to vancomycin and ampicillin is a difficult task that could necessitate the development of novel medicines (Said et al., 2021).

2.5.1. Intrinsic resistance

The common types of resistance seen in *Enterococcus* species are to glycopeptides, ampicillin, and aminoglycosides. A alteration or excess production of penicillin binding protein 5 causes high-level resistance to beta-lactam antibiotics (MIC 16-64 g/ml) (PBP5). The sluggish absorption system is the cause of low-level aminoglycoside resistance (MIC 62 to 500g/ml). *Enterococcus casseliflavus/flavescens* and *Enterococcus gallinarum* are two examples of species that display inherent resistance. They have the *vanC* operon, which confers low-level inherent resistance (Mühlberg et al., 2020).

2.5.2. Acquired resistance

Mutations of DNA, the addition of exogenous gene induce extrinsic resistance. A ribosomal mutation or a plasmid-based aminoglycoside modifying enzyme causes high-level aminoglycoside resistance (Coker & Dierfeldt, 2016). Chloramphenicol resistance is either plasmid-borne or enzymatic, whereas higher-level erythromycin resistance is produced by a transposon. Glycopeptide resistance could be plasmid-borne or chromosomal, and is caused by a group of genes that are absent among typical enterococci. *E. durans, E. faecalis* and *E. faecium, E. raffinosus* and *E. avium* are examples of species that have developed resistant traits. They

have the *vanA* or *vanB* operon, which is a sign of acquired resistance. Coker and Dierfeldt (2016).

2.6. Prevention of Vancomycin-Resistant Enterococci

The rise of VRE has raised increased concerns in many healthcare organizations, making infection and colonization with VRE prevention and control critical. The majority VRE prevention measures are equally relevant to other antibiotic-resistant Enterococci (Reyes et al., 2016). Because VRE colonization of the digestive system frequently precedes infection, it is critical to prevent it. Patients using antibiotics could get a higher density of VRE colonization and VRE in their faeces, this could promote and facilitate its spread. VRE can transmit from patient-patient via healthcare personnel (due to poor hygiene and lack of adherence to contact measures) and can contaminate medical equipment and surfaces (Kim et al., 2017). In summary, various risks for VRE colonization have been found. They include: admission to a critical care unit, intensity of sickness, exposure to other VRE patients, length of hospitalization, and antibiotic exposure (Kang et al., 2013).

Vancomycin, cephalosporins, quinolones, and anti-anaerobic drugs have been related to VRE acquisition in epidemiological studies. One critical concern for infection control program include preventing VRE spread in healthcare settings. Hand cleanliness, precautions against contact/barrier transmission, and source control are among these techniques. Isolation of sick healthcare personnel and patients like nurses; utilization of a separate space whenever it is required, colonization epidemiological studies, antibiotics should be used sparingly, environmental cleaning programs designed to maximize the cleaning of high-touch areas and medical equipment in hospitals (Levitus et al., 2021).

2.7. Antibiotic Resistance in Enterococci

Enterococci can be resistant to a variety of antibiotics, with beta-lactams, aminoglycosides, and glycopeptides being the most common. Enterococci have inherent resistance to various antibiotics. As a result, about all enterococcal species are beta-lactam or glycopeptide tolerant and resistant to aminoglycosides at low doses. Endocarditis and other severe enterococcal infections involve the integration of a cell-wall active molecule, beta-

lactam, glycopeptide, and an aminoglycoside to provide prolonged bactericidal effect (Yilema et al., 2017).

Enterococci can also gain resistance through a variety of ways, such as substitutions or the uptake of extra resistance genes carried by plasmids or transposons. Hence, new advances in bioinformatics have revealed new information about the spread and genome make up of transposons including moving genetic molecules in enterococci. These transposable elements are critical for the spread as well as durability of antibiotic resistance among enterococci, as well as the transfer of resistance determinants to other bacterial species (Miller et al., 2014).

Enterococcus spp. are a natural make-up of the intestinal flora. Use of antibiotic may exert some pressure on the gut flora, promoting the growth and release of resistant strains as well as the intra and inter transmission of resistance genome (Rudy et al., 2004). Some Enterococci, most notably *E. faecium*, now have antibiotic resistance at a high degree, such as vancomycin, ampicillin and aminoglycosides making treatment choices for severe enterococcal diseases problematic for physicians (Miller et al., 2014).

2.7.1. Beta-lactam resistance

Enterococcal spp. can have varying degrees of beta-lactam resistance. Thus, *E. faecium* typically exhibits more resistance to beta-lactam antibiotics than *E. faecalis* (Kristich et al., 2014)

Imipenem, ampicillin, piperacillin, and penicillin G are the most active beta-lactams, while ticarcillin, aztreonam, cephalosporins, methicillin, and ertapenem have little or no activity. Some novel cephalosporins like ceftaroline, ceftobiprole are very effective against *E. faecalis* species, nonetheless, their action against *E. faecium* is minimal (Kristich et al., 2014).

Previous research discovered that one of two UDP-GlcNAc 1-carboxyvinyltransferases encoded in *E. faecalis* (catalyzing the first committed step in peptidoglycan synthesis) necessary particularly for cephalosporin resistance (MurAA). MurAA's paralog (MurAB) cannot cause cephalosporin resistance. As a result, MurAA has a particular and specific ability to enhance cephalosporin resistance (Hollenbeck & Rice, 2012).

In some circumstances, enterococci, notably *E. faecalis* developed resistance through the ability to generate enzymes like beta-lactamases, however this resistance mechanism is seldom identified and is readily handled by employing a beta-lactamase inhibitor in conjunction with a

beta-lactam drug. Piperacillin-tazobactam, amoxicillin-clavulanic acid and ampicillin-sulbactam, are examples (Hollenbeck & Rice, 2012).

2.7.2. Aminoglycoside resistance

Having high gentamicin and streptomycin MICs, enterococci have an inherent intermediate to lower level of resistance to aminoglycosides. Gentamicin MICs vary from 8 to 64 g/mL, whereas streptomycin MICs range from 64 to 512 g/mL. The bacterial resistance contributes to a reduction in cell wall permeability, limiting aminoglycoside entry (Kristich et al., 2014). Notwithstanding this relatively low level of resistance, combining gentamicin or streptomycin with a cell wall active drug might result in a synergistic impact and a better therapeutic outcome (e.g., ampicillin, penicillin, or vancomycin). It is demonstrated that including a cell wall-strong chemical which inhibits peptidoglycan formation significantly enhances membrane fluidity and absorption of these aminoglycosides (Kristich et al., 2014).

In most situations, the synergistic effect with some aminoglycosides like netilmicin tobramycin and kanamycin cannot be obtained due to high levels of resistance created by multiple mechanisms of resistance (Krause et al., 2016). Several reports over the last four decades have shown increased resistant rate to gentamicin streptomycin; however, when gentamicin or streptomycin is joined with ampicillin, penicillin, or vancomycin, no impact is shown, and the antibacterial effect required for certain complicated bacteria is not improved, for example in meningitis or endocarditis. As a result, Clinical and Laboratory Standards Institute (CLSI) advised that enterococci be tested especially for high levels of gentamicin and streptomycin resistance (Schneider et al., 2014).

The European Centre for Disease Prevention and Control (ECDC) revealed that, the percentage of *E. faecalis* with increased resistance to aminoglycoside was greater than 50% in several countries, such as Hungary and Greece and within 30% and 50% in the most of other western countries. In 2009, *E. faecium* had a larger proportion of enterococcal bacteremia patients with high-level gentamicin resistance than *E. faecalis*.

Endocarditis caused by *E. faecalis* that is resistant to aminoglycosides has become a significant issue. In addition, combining cefotaxime or ceftriaxone in conjunction with ampicillin could be successful than ampicillin alone, and this good concept may be an alternate treatment

for persons with aminoglycoside-resistant *E. faecalis* endocarditis. However, no significant impact of these treatment combinations with *E. faecium* was identified (Kristich et al., 2014).

2.7.3. Glycopeptide resistance

High-level vancomycin resistance is a serious hospital and diagnostic issue because it commonly arises in enterococcal isolates, notably *E. faecium,* which has high-level ampicillin resistant rate (Gardete & Tomasz, 2014). Growing usage of vancomycin in the treatment of illnesses like methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant coagulase-negative staphylococci, and *Clostridium difficile* has been related to the rise of vancomycin resistant rate in enterococci in the United States. (Raza et al., 2018).

According to reports, resistant vancomycin can be transmitted within and between species, and the *vanA* cluster can spread from enterococci to many bacterial species including MRSA strains, posing significant challenge in clinical setup (Raza et al., 2018). According to a previous report, some countries reported resistance rates over 25% (Ireland, Greece, and Luxembourg), other nations documented resistant rates within 10% and 25% (UK, Portugal, Lithuania, Latvia, and South Cyprus) and most nation documented resistant rates between 1% and 10% (ECDC, 2009).

In enterococci, some antibiotics like macrolides, trimethoprim-sulfamethoxazole, fluoroquinolones and tetracyclines cause variable degrees of resistance. The existence of vancomycin-resistant *E. faecium* is relatively predominant in some U.S hospitals, but it vary widely between nations in Europe and are increasing in recent years in general (Kristich et al., 2014).

Modern antibiotics like daptomycin, linezolid, quinupristin-dalfopristin, lypoglycopeptides as well as tigecycline, show strong antibacterial efficacy against multidrug-resistant enterococci; nevertheless, tolerance to medications were just been discovered (Arias et al., 2010).

2.8. Epidemiology of Vancomycin-Resistant Enterococci

Antimicrobial resistance has emerged in enterococci to a range of antimicrobial medications (e.g., aminoglycosides, beta-lactams, glycopeptides), and as resistant rate rises, managing the appearance and spread of resistant enterococci becomes increasingly important

(Kristich et al., 2014). Controlling VRE in the hospitals has recently become a major challenge for epidemiologists and clinicians. The majority of VRE prevention techniques are applicable to other resistant bacteria as well. VRE can colonize the gastrointestinal tract, particularly the large bowel, and is a major cause of nosocomial infections (Miller et al., 2014). VRE bowel colonization can contaminate the skin due to fecal discharge. Patients infected with VRE serve as reservoirs, allowing the strains to spread to some patients via medical personnel and contaminated things (Levitus et al., 2021).

In patients admitted to hospitals, long-term care centers, and other healthcare facilities, VRE infections have proven difficult to cure. Several studies have discovered significant differences in VRE distribution between Europe and the United States, which is surprising. VRE infections in humans were initially detected in the late 1980s in Europe, and they were connected to the widespread usage avoparcin, a glycopeptide molecule, as a food component to stimulate animal growth (VRE was isolated from the gut flora of various species, including chicken, fowl, and pigs). However, there was a clear correlation between VRE transmission from an animal source and subsequent human transmission in European nations. (Cetinkaya et al., 2000). VRE outbreaks were later discovered in hospitals in the United States, where they were linked to an increase in the use of vancomycin in hospitalized patients. Furthermore, there was no real evidence of an animal source of VRE in the United States, where avoparcin was not used to enhance animal growth, as there was in Europe. A recent Michigan experiment confirmed the separation of VRE from pigs (Sundermann et al., 2020). A few *Enterococcus* species, like *E. gallinarum* and *E. casseliflavus/flavescens,* may take over the human digestive system, although enterococci with strong vancomycin resistance are unlikely to be colonists. Then, in humans, bowel colonization with high-level vancomycin-resistant enterococci containing the *vanA* or *vanB* gene cluster may occur from an animal source or via horizontal transfer within the hospital or other healthcare settings (Byappanahalli et al., 2012). Several publications have discovered VRE infections all across the world, however the prevalence of these disorders varies substantially between countries. Notably, the colonization of VRE infections in the U.S. has steadily increased over the preceding two decades, whereas in Europe, the frequency has stayed significantly lower than in the U.S. However, VRE have been documented in increasing numbers in some countries e.g the United Kingdom, Greece and Portugal, meanwhile their existence has remained comparatively low in others (Wang et al., 2019).

Many outbreaks of VRE have occurred in patients staying in the hospital, most notably in critically ill or immunocompromised persons in ICUs. In recent years, VRE has grown common in many clinics and health related settings. Methods employing techniques such as pulsed-field gel electrophoresis (PFGE) contributed a better understanding of the epidemiology of VRE in the hospital. As a result, a single VRE clone may spread across a hospital and cause epidemics, according to several examinations. VRE strains have the ability to horizontally transmit resistance to unrelated enterococcal strains (Cetinkaya, et al., 2000).

CHAPTER III

Materials and Methods

3.1. Study Design and Participants

During the period of March 2019 and July 2019, fecal samples were collected from 110 individuals. Forty-six participants were hospitalized for at least 72 hours at the Near East University Hospital, and 64 were community residents (control group) with no history of hospitalization six months before the research. Study inclusion requirements were that participants be over the age of 18 and live in Northern Cyprus. The Near East University Ethics Review Board granted ethical permission for this study (Project no: YDU/2019/65-717).

3.2. Collection of Samples and Data

In the investigation, fecal sample was taken from each participant, for a total of 110 samples. Participants completed a questionnaire form and their socioeconomic and epidemiological variables were recorded during the sample collection. Age, gender, level of education, and marital status were among the demographic and socioeconomic status factors. During the collection of samples, participants also supplied information on their gastrointestinal problems. More information was provided, including a history of antibiotic use, diarrhea, urinary tract infection (UTI), travel to foreign countries, and a hospital stay for at least 72 hours during the previous six months.

3.3. Initial Screening of Vancomycin-Resistant Enterococci

The fecal samples were initially inoculated on enterococcosel agar (including vancomycin at 1 mg/L concentration) for screening of the possible VRE isolates. The bacterial cultures that grew on the vancomycin-containing media (n=41) were stocked at -20^{o}C until use. Fifteen of the stocked bacteria were isolated from hospitalized patients, and 26 of them were recovered from the control group.

3.4. Confirmation and Identification of Vancomycin-Resistant Enterococci

In order to confirm the presence of VRE, the 41 stocked samples were analyzed. Firstly, the samples were cultured on enterococcosel agar and incubated overnight at 37^{o}C. The colonies

that grew on the culture plates were suspended in sterile 0.9% NaCl and adjusted to 0.5 McFarland standard turbidity. Following this, the bacterial suspensions were inoculated onto Mueller-Hinton media (Merck, Germany) by using a sterile swab. Finally, a vancomycin disc (30 μg) was placed at the center of the plates. The culture plates were incubated overnight at 37^{o}C. The inhibition zone diameters generated following the incubation period were measured. The zone diameters obtained in the disc diffusion test were evaluated (CLSI 2021). Thus, vancomycin resistance and the fecal carriage rate of VRE were determined among the study participants.

In the study, identification of the resistant isolates was done by using VITEK-2 automated system (bioMérieux, France).

3.5. Statistical Analysis

The demographic and epidemiological characteristics of the subjects were examined statistically in order to seek for probable factors associated with VRE fecal carriage. For categorical variables, frequency and percentage were provided, while arithmetic mean, standard deviation, and median were computed. Fisher's exact test was performed to assess the relationship between variables. The IBM SPSS statistics program for Macintosh was used for all statistical analysis (Demo version 22.0; Armonk, NY: IBM Corp.).

CHAPTER IV

Results

4.1. General Characteristics of the Study Participants

The total number of individuals in the investigation was 110; 46 were hospitalized for at least 72 hours, and 64 were from the community. The study group's mean and median ages were 44.57 (21.6) and 43.50, respectively (19-90). 46 (41.8 percent) of the participants were between the ages of 19 and 30. There were 64 (58.2 percent) participants in the 31-year-old and older age group. There were 77 men (70 percent) and 33 women (30 percent). Participants' education levels ranged from less than university to university and higher, with a total of 47 (51.7 percent) persons. Marital status was also analyzed and there was a total of 50 (45.5%) single and 60 (54.5%) married participants. Participants with low and middle income were 95 (86.4%) and total number of people with higher income were 15 (13.6%).

In the research individuals, 33 (30.0%) participants stated that they had gastrointestinal symptom (GIS) at the time of sample collection; while 77 (70%) had no GIS at the time of sampling. In the population, 57 (51.8%) of the participants had a history of antibiotic use in the last six months prior to the study, while 53 (48.2%) did not take any antibiotics in the last six months. A total of 31 (28.2%) participants had diarrhea while 79 (71.8%) of the participants never showed symptoms of diarrhea. Participants with UTI were 10 (9.1%) while 100 (90.9%) never presented any UTI symptoms. The travel history showed that 61 (55.5%) participants had travelled while 49 (44.5%) participants did not visit any other country within the last six months before the sample collection. A total of 33 (54.1%) participants had travelled to Turkey or Europe in the last six month while a total of 28 (45.9%) participants had no travel history to either Turkey or Europe. Furthermore, 28 (45.9%) had traveled to either Africa or Asia while 33 (54.1%) participants did not have any travel history to Africa or Asia in the last six months prior to the sample collection.

4.2. Intestinal Colonization with VRE Isolates

The percentage of VRE intestinal colonization was determined to be 1.8% (n=2/110) in the research. Both VRE strains were identified as *Enterococcus gallinarum* after being isolated

from the control group. In the patient and control groups, no notable change in VRE fecal carriage levels. (p=0.509) (Table 4.1)

Table 4.1.

Distribution of VRE among Patient (n=46) and Control (n=64) Groups.

Participants	VRE-positive n (%)	VRE-negative n (%)	Total n (%)
Patients	0 (0.0)	46 (100)	46 (100)
Controls	2 (3.1)	62 (97.4)	64 (100)
Total	2 (1.8)	108 (98.2)	110 (100)

4.3. Characteristics of VRE Colonized Participants

One of the VRE cases (no: 1) was a 27-year-old male participant, a postgraduate student, single and stayed in Cyprus for the past two years. He had a middle socioeconomic status; he had no GIS as at the period of sampling however there was diarrhea which occurred one month before the sampling in the last six months. He also stated he used antibiotics for one week within the previous six months before the study, also no UTI in the last six months, he also has no underlying diseases. He stated that he travelled to a European country within the last six months and had no history of hospitalization for at least 72 hours in the last six months. The other VRE case (no: 2) was a 33-year-old male participant, a postgraduate student, married and living in Cyprus for the past two years. He had a middle socioeconomic status; he had no GIS as at the period of sampling however there was no diarrhea in the last six months. He used antibiotics for one week within the last six months prior to the study, also no UTI in the last six months, he has no underlying diseases. He stated that he travelled within the last six months to an Asian country and had no history of hospitalization for at least 72 hours in the last six months before the sample collection (Table 4.2).

Table 4.2.

Characteristics of the Participants who were Colonized with VRE

Characteristic	Participant no: 1	Participant no: 2
Age	27	33
Gender	Male	Male
Education level	Postgraduate	Postgraduate
Marital status	Single	Married
Socioeconomic status	Middle	Middle
Gastrointestinal symptom	Yes	No
Antibiotic	Yes	Yes
Urinary tract infection	No	No
Travel history	Yes	Yes
Hospital stay	No	No
Species	*Enterococcus gallinarum*	*Enterococcus gallinarum*

4.4. Results of Statistical Analysis

4.4.1. Correlation of intestinal colonization of VRE with demographic and socioeconomic factors

According to the statistical analysis, no significant association was found between the age groups and intestinal colonization with VRE (p=1.000). Furthermore, there was no statistical association between gender of participants and the intestinal colonization (p= 1.000). No statistical correlation was detected between the education level of the participants and VRE colonization (p=0.506). In addition, marital status (p=1.000) and the socioeconomic status (p=1.000) of the participants did not significantly affect the fecal carriage of VRE (Table 4.3)

Table 4.3.

Correlation of Intestinal Colonization of VRE with Demographic and Socioeconomic Factors in the Study Group (n=110)

Risk Factors	Positive n/N (%)	*p* value
Age		
19-30	1/46 (2.2)	
31 and above	1/64 (1.6)	1.000
Total	2/110(1.8)	
Gender		
Male	2/77(2.6)	
Female	0/33 (0.00)	1.000
Total	2/110 (1.8)	
Education		
Lower than university	0/47(0.0)	0.506
University and higher	2/63 (3.2)	
Total	2/110 (1.8)	
Marital status		
Single	1/50 (2.0)	
Married	1/60 (1.7)	1.000
Total	2/110 (1.8)	
Socioeconomic status		
Low and middle	2/95 (2.1)	
High	0/15 (0.0)	1.000
Total	2/110(1.8)	

4.4.2. Correlation of intestinal colonization of VRE with epidemiological factors

This research also looked at VRE colonization in the gut based on epidemiological characteristics. The prevalence of GIS as at the period of collection of sample was not a significant predictor of intestinal VRE colonization (p=1.000). Furthermore, no statistical relationship was detected between recent antibiotic use and VRE colonization (p=0.496). There was no significant relationship between a history of diarrhea and VRE colonization in the intestine (p=0.486). There was no significant association between UTI history and VRE fecal carriage, according to the statistical studies (p=1.000). The study found no statistical link between recent international travel and VRE carriage (p=0.501). Also visiting Turkey or Europe (*p*=1.000) and Asia or Africa (*p*=1.000) were not significant determinants for VRE carriage (Table 4.4).

Table 4.4.

*Correlation of Intestinal Colonization of VRE with Epidemiological Factors in the Study Group (n=110). (*The period covers the last six months before the study.)*

Risk factors	Positive n/N (%)	*p* value
Presence of any gastrointestinal symptom at the time of sample collection		
Yes	2/64 (3.1)	1.000
No	0/46 (0.0)	
Total	2/110 (1.8)	
History of antibiotic use*		
Yes	2/57 (3.5)	
No	0/53 (0.0)	0.496
Total	2/110 (1.8)	
History of diarrhea*		
Yes	1/31 (3.2)	
No	1/79 (1.3)	0.486
Total	2/110 (1.8)	
History of urinary tract infection*		
Yes	0/10 (0.0)	
No	2/100 (2.0)	1.000
Total	2/110 (1.8)	
Travel history*		
Yes	2/61 (3.3)	
No	0/49 (0.0)	0.501
Total	2/110 (1.8)	
Travel to Turkey or Europe*		
Yes	1/33 (3.0)	
No	1/28 (3.6)	1.000
Total	2/61 (3.3)	
Travel to Asia or Africa*		
Yes	1/28 (3.6)	
No	1/33 (3.0)	1.000
Total	2/61 (3.3)	

CHAPTER V

Discussion

The existence of enterococci in the intestinal flora, the increased use of medical devices, length of hospital stay, and, most critically, illogical and incorrect antibiotic administration have all contributed to the growth of VRE. The prevalence of VRE is on the rise all over the globe, which has been reported. Bacteremia caused by VRE is causing increased deaths and morbidity all over the world (Guzman et al., 2016).

The fast and increasing rate of nosocomial infections is a global issue with serious effects on antibiotics use (Santajit & Indrawattana, 2016), and *Enterococcus* spp. were defined as one of the leading causes of HAI, therefore it should be closely monitored.

According to numerous writers, the cause of VRE is food contamination, which could act as a source where non-hospitalized patients may get VRE (Santajit & Indrawattana, 2016), in this study, we also found the VRE carriers in the community setting which may be as a result of contaminated food products.

Despite the fact that no major VRE outbreaks have been reported, this study is significant since the gastrointestinal system has been identified as a probable reservoir for VRE according to Banerjee et al (2015). We found no VRE in the hospitalized patients in this investigation, which could explain why no outbreaks occurred.

E. faecalis accounts for approximately 80% of enterococcal infections, with almost all the remaining infections caused by *E. faecium.* Only rarely are other spp. such as *E. gallinarum* /*E casseliflavus* associated with colonization infection, but this organism are notable for their intrinsic low-level vancomycin resistance. Whole genome sequencing suggests that the patient's indigenous flora is the source of enterococcal infection in most cases (Monticelli et al., 2018). However, *E. gallinarum* are only responsible for a substantial percentage of enterococcal infection.

Vancomycin resistance is largely exhibited by *E. faecium* and, to a minimal rate in *E. faecalis* (Eshaghi et al., 2015), however *Enterococcus gallinarum* is innately resistant to *van*comycin. *vanC* is a chromosomally mediated nontransferable gene mediates the low-level resistance.

The fecal carriage rate of VRE was studied in both hospital and community settings in this investigation. The study enlisted the participation of 110 people. Forty-six participants were hospitalized for at least 72 hours at the Near East University Hospital, and 64 were community residents (control group) with no hospitalization history in the six months leading up to the investigation. Participants' stools were collected and analyzed for VRE isolates.

E. gallinarum was identified as the *Enterococcus* species (Table 4.1). Padiglione et al., (2000) found VRE isolates in 2 (0.2%) of the 1,085 (95 percent confidence intervals 0% -0.4%) specimens in the community study. Both strains were identified in agar and broth cultures and were *vanB E. faecium*.

There was no significant link between age groups and VRE colonization in the gut, according to the data analysis (p=1.000). In addition, there was no significant link between participant gender and intestinal colonization (p=1.000). There was no meaningful link found between the participants' educational level and VRE colonization (p=0.506). Furthermore, the participants' marital status (p=1.000) and socioeconomic status (p=1.000) had no effect on VRE fecal carriage.

The presence of GIS at the period of collection of samples wasn't just a significant predictor of VRE colonization in the intestine (p=1.000). Furthermore, no statistical relationship was detected between recent antibiotic use and VRE colonization (p=0.496). There was no significant relationship between a history of diarrhea and VRE colonization in the intestine (p=0.486). There was no significant association between UTI history and VRE fecal carriage, according to the statistical analyses (p=1.000). The study found no statistical link between recent international travel and VRE carriage (p=0.501). Also visiting Turkey or Europe (p=1.000) and Asia or Africa (p=1.000) were not significant determinants for VRE carriage.

Data on the incidence of *E. gallinarum* group isolates in clinics are scarce, and are frequently restricted to epidemiological investigations in hospitals or specialized groups. Other *Enterococcus* spp. infections, which are often regarded to be low-virulence pathogens, are uncommon and frequently limited to immunocompromised hosts. Nevertheless, their inherent vancomycin resistance causes therapeutic concerns comparable to those seen during the treatment of VRE in *E. faecalis* and *E. faecium*, since resistance to vancomycin *in E. faecalis* has been independently linked to a higher risk of morbidity and death (Monticelli et al., 2018).

Enterococcus gallinarum is thought to be a rare occurrence in the gut microbiota of humans and birds. Its presence can also be detected in human food, and it has been discovered in fish/crustaceans, meat, cheese-meat combos, minced beef and pork on occasion, although not as a sign of fecal contamination (Monticelli et al., 2018).

Numerous investigations on both colonized and infected people have shown different results regarding the relevance of previous antibiotic therapy as a risk factor for nosocomial VRE. The emergence of VRE has been associated to the use of vancomycin, cephalosporins, and antimicrobial medications having an aerobic spectrum (Sakka et al., 2008), but in this study, one of the VRE positive participants stated that he had used antibiotics but could not remember what they were.

A survey was administered to research participants in trying to determine the potential factors linked with VRE fecal carriage. Several studies have employed age to investigate the relationship between antibiotic resistance and age. Age and marital status had no significant relationship with VRE intestinal colonization in this study ($p=1.000$ and $p=1.000$, respectively) (Table 4.3).

Contrary to our study, Vasilakopoulou et al. (2020) discovered that extended VRE carriage is influenced by both hospitalization and old age. VRE may survive for a long time on surfaces, and human-to-human contact has been identified as a risk factor for VRE transmission to healthcare workers' hands and gloves, and then to patients (Cetinkaya et al.,2000).

In this investigation, two (1.8%) male community dwellers were reported to have been colonized by VRE; nevertheless, Biswas et al., (2016) indicated that colonized individuals with VRE infections were males 16 (6.4%) and females (8.2%). The reason for the prevalence of male participants colonized with VRE infections could be attributed to the fact that guys are more likely to seek medical assistance and may have received it from contact with people than females, who do not really associate and prefer to neglect their symptoms.

In our study, socioeconomic level had no statistically significant relationship with antibiotic resistance. Boeing et al., 2021 discovered no significant relationship between socioeconomic level and VRE colonization in the intestine in another investigation.

VRE can survive in the environment for extended periods of time (up to a week), can contaminate practically any surface, and can be passed from person to person. The patient's health status determines whether VRE colonization progresses to infection. Immunocompetent

people colonized with VRE have a low risk of infection; immunocompromised hosts (patients with hematologic abnormalities, transplant recipients, or critically ill patients) have a higher risk of infection after colonization. In a study of VRE colonization and antibiotic use (McKinnel et al., 2016), it was reported that 1,454 patients were admitted to a tertiary care medical intensive care unit, he identified variations in antibiotic exposure between patients who acquired VRE invasion and individuals who were never occupied with VRE in 83 cases of incident VRE colonization. However, in this study, 46 people were admitted for at least 72 hours, and 64 of participants were community residents, and there was no significant link between participants' antibiotic use and VRE intestinal colonization.

According to a previous study (Cetinkaya et al., 2000), VRE colonization in healthy persons does not necessarily imply danger of infection with the organisms. A prevalent culture study done some years ago at one Belgian hospital found that 3.5% of patients' bowel VRE was found in the isolates, although no illnesses related with VRE had been documented at the time at that institution. Gastrointestinal cultures from 11 (28%) of 40 healthy individuals that were not clinicians but had not used antibiotics in the previous year generated a wide range of vancomycin-resistant *E. faecium* isolates. In previous investigations, the same researchers identified VRE in the feces of about to 64% of participants who previously taken glycopeptides orally, this is in line with our study as the participants are community dwellers and one of the participants colonized had history of antibiotics use in the last six month before the study.

In this investigation, there was no significant association between a history of UTI within the previous six months and VRE colonization of the participants (p=1.000) (Table 4.4). This is contrary to Biswas et al. (2016) which found that majority of the participants in his study were colonized by VRE from history of UTI.

A study revealed that travel history, particularly to endemic regions of the world, is a potential risk for contracting VRE (Gouliouris et al., 2021). However, there was no significant association between travel history and VRE fecal carriage in our investigation (p=0.501) (Table 4.4) as a result of low number of VRE positive participants.

According to another study (Forstner et al., 2015), overall use of vancomycin, fluoroquinolones, third-generation cephalosporins, have been linked to the spread of VRE inside the healthcare setting. However, another study (Peel et al., 2012) found no link among glycopeptide usage and VRE isolation, but did find a link between a high prevalence of VRE-

bloodstream infectious disease and previous hospitalization (McKinnell et al., 2012). According to one research, it was unable to establish indisputably a possible application for reducing vancomycin use in the treatment of VRE (De Bruin et al., 2007). However, in a systematic case–control research performed at a Korean university hospital, vancomycin treatment significantly increased the period of VRE carriage in healthcare settings who were still infected by vancomycin-resistant *E. faecium* (Yoon et al., 2011). However, owing to the persistence of VRE in the community, measures different from limiting glycopeptide consumption are necessary to minimize its spread, such as patient isolation and enforced environmental cleaning (Marcel et al., 2008). At the time of admission, individuals who are at risk should be screened and housed in separate rooms. Said et al. (2021) investigated enterococcal colonization of the gastrointestinal tract as the primary risk factor for severe infections caused by gut translocation. Enterococci are phagocytosed and moved across the gut wall, but they are immune to lymphatic death. Some microbes can now be known to cause dangerous invasive diseases such as endocarditis, bacteremia, and urinary tract infection, and pelvic infection in hospitalized patients or patients with weakened immune systems, and that they can be easily transmitted within the hospital through surfaces or person-to-person contact, potentially leading to an outbreak. Despite the fact that current clinical microbiology laboratory algorithms do not routinely test for such resistant genes in these species, this research highlights the importance of further exploration of any acquired enterococci such as *E. gallinarum* in medical settings.

CHAPTER VI

Conclusion

In this study, the fecal carriage rate of VRE was found to be 1.8% (n=2/110). *Enterococcus gallinarum* was identified as the VRE isolate. Statistical analysis revealed that there was no significant relation of fecal carriage of VRE with any of the socioeconomic and epidemiological factors ($p>0.05$).

For the isolation in human specimens, *E. gallinarum* have been a rare finding. However, avoiding long-term aggressive treatment, strengthening immunity as well as quality control of food products can help prevent the existence of *E. gallinarum* infections. The keys to achieve optimal efficacy are quick detection and diagnosis with rational antibiotic administration. Colonization and infection with *E. gallinarum* are less common than *E. faecalis* and *E. faecium*, but their prevalence may be growing globally.

In our study, statistical analysis showed no significant result, but this can be attributed to the low percentage of VRE colonization in the study group. Therefore, future studies that include more participants will be essential. Also, in order to understand the characteristics of vancomycin resistance in *Enterococcus* spp., *van* genes should be searched and identified in Northern Cyprus.

References

AL Bloushy AA, Elbehiry A. (2018). History, Virulence Genes, Identification and Antimicrobial Resistance of *Enterococcus faecalis* Isolated from Diabetic Foot Patients. *Int J Curr Microbiol Appl Sci.;7(1):*2136–54.

Arias, C. A., Contreras, G. A., & Murray, B. E. (2010). Management of multidrug-resistant enterococcal infections. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases*, *16*(6), 555–562. https://doi.org/10.1111/j.1469-0691.2010.03214.x.

Babakhani, S., & Oloomi, M. (2018). Transposons: the agents of antibiotic resistance in bacteria. *Journal of basic microbiology*, *58*(11), 905–917. https://doi.org/10.1002/jobm.201800204.

Banerjee, T., Anupurba, S., Filgona, J., & Singh, D. K. (2015). Vancomycin-resistance Enterococcal Colonization in Hospitalized Patients in Relation to Antibiotic Usage in a Tertiary Care Hospital of North India. *Journal of Laboratory Physicians*, *7*(02), 108–111. https://doi.org/10.4103/0974-2727.163123.

Behjati, S., & Tarpey, P. S. (2013). What is next generation sequencing?. *Archives of disease in childhood. Education and practice edition*, *98*(6), 236–238. https://doi.org/10.1136/archdischild-2013-304340 .

Binda E, Marinelli F, Marcone GL. (2014). Old and New Glycopeptide Antibiotics: *Action and. Resistance. Antibiotics (Basel);3(4):572-594. Published 2014 Nov 4. doi:*10.3390/antibiotics3040572.

Biswas PP, Dey S, Adhikari L, Sen A. (2016). Detection of vancomycin resistance in enterococcus species isolated from clinical samples and feces of colonized patients by phenotypic and genotypic methods. *Indian J Pathol Microbiol*;59(2):188-193. doi:10.4103/0377-4929.182015.

Boeing, C., Correa-Martinez, C. L., Schuler, F., Mellmann, A., Karch, A., & Kampmeier, S. (2021). Development and Validation of a Tool for the Prediction of Vancomycin-Resistant Enterococci Colonization Persistence-the PREVENT Score. *Microbiology spectrum*, *9*(2), e0035621. https://doi.org/10.1128/Spectrum.00356-21.

Byappanahalli MN, Nevers MB, Korajkic A, Staley ZR. (2012). Harwood VJ. Enterococci in the environment. *Microbiol Mol Biol Rev* 76(4):685-706. doi:10.1128/MMBR.00023-12.

Cabral J. P. (2010). Water microbiology. Bacterial pathogens and water. *International journal of environmental research and public health*, *7*(10), 3657–3703. https://doi.org/10.3390/ijerph7103657.

Cetinkaya, Y., Falk, P., & Mayhall, C. G. (2000). Vancomycin-resistant enterococci. *Clinical microbiology reviews*, *13*(4), 686–707. https://doi.org/10.1128/CMR.13.4.686.

Christaki, E., & Giamarellos-Bourboulis, E. J. (2014). The beginning of personalized medicine in sepsis: small steps to a bright future. *Clinical genetics*, *86*(1), 56–61. https://doi.org/10.1111/cge.12368.

Clinical and Laboratory Standards Institute (CLSI). (2021). Performance Standards for Antimicrobial Susceptibility Testing. 31st ed. CLSI supplement M100. USA.

Coker, T. J., & Dierfeldt, D. M. (2016). Acute Bacterial Prostatitis: Diagnosis and Management. *American family physician*, *93*(2), 114–120.

Cong Y, Yang S, Rao X. (2019). Vancomycin resistant *Staphylococcus aureus* infections: *A review of case updating and clinical features. J Adv Res;*21:169-176. Published 2019 Oct 12. doi:10.1016/j.jare.10.005.

Cook, L. C., LaSarre, B., & Federle, M. J. (2013). Interspecies communication among commensal and pathogenic streptococci. *mBio*, *4*(4), e00382-13. https://doi.org/10.1128/mBio.00382-13.

Courvalin, P. (2006). Vancomycin Resistance in Gram-Positive Cocci. *Clinical Infectious Diseases*, 42(Supplement_1), pp.S25-S34.

Daniel Johansson, & Rasmussen, M. (2013). Virulence factors in isolates of Enterococcus faecalis from infective endocarditis and from the normal flora. *Microbial pathogenesis*, *55*, 28–31. https://doi.org/10.1016/j.micpath.2012.09.009.

De Bruin MA, Riley LW. (2007). Does vancomycin prescribing intervention affect vancomycin-resistant enterococcus infection and colonization in hospitals*? A systematic review. BMC Infect Dis;7:24.*

Depardieu, F, Perichon, B, Courvalin, P. (2004). "Detection of the van alphabet and identification of enterococci and staphylococci at the species level by *multiplex PCR". J Clin Microbiol.* vol. 42. 2004. pp. 5857-60.

Dersch, Petra & Khan, Muna & Muehlen, Sabrina & Görke, Boris. (2017). Roles of Regulatory RNAs for Antibiotic Resistance in Bacteria and *Their Potential Value as Novel Drug Targets. Frontiers in Microbiology.* 8. 10.3389/fmicb.2017.00803.

Donald L. Reynolds and J. Dustin Loy. (2020). Avian Diseases, *Volume 64, Number 4 DOI: 10.1637/aviandiseases*-D20-00060.

Donald P. Levine. (2006). Vancomycin: A History, *Clinical Infectious Diseases*, Volume 42, Issue Supplement_1, January, Pages S5–S12, https://doi.org/10.1086/491709.

Eshaghi, A., Shahinas, D., Li, A., Kariyawasam, R., Banh, P., Desjardins, M., Melano, R. G., & Patel, S. N. (2015). Characterization of an Enterococcus gallinarum Isolate Carrying a Dual vanA and vanB Cassette. *Journal of clinical microbiology*, *53*(7), 2225–2229. https://doi.org/10.1128/JCM.03267-14.

European Centre for Disease Prevention and Control (2009) www.ecdc.europa.eu/en/publications/Publications/1011_SUR_annual_EARS_Net.pdf.

Fiore, E., Van Tyne, D., & Gilmore, M. S. (2019). Pathogenicity of Enterococci. *Microbiology spectrum*, *7*(4), 10.1128/microbiolspec.GPP3-0053-2018. https://doi.org/10.1128/microbiolspec.GPP3-0053-2018.

Forstner, C., Diab-Elschahawi, M., Kivaranovic, D. (2015). Non-linear significant relationship between use of glycopeptides and isolation of vancomycin-resistant *Enterococcus species* in a university hospital setting. *Antimicrob Resist Infect Control* **4,** 25 https://doi.org/10.1186/s13756-015-0064-5.

Fotadar U, Zaveloff P, Terracio L. (2005) Growth of Escherichia coli at elevated temperatures. *J Basic Microbiol*;45(5):403-404. doi:10.1002/jobm.200410542.

Gardete, S., & Tomasz, A. (2014). Mechanisms of vancomycin resistance in Staphylococcus aureus. *The Journal of clinical investigation*, *124*(7), 2836–2840. https://doi.org/10.1172/JCI68834.

Gouliouris, T., Coll, F., Ludden, C., Blane, B., Raven, K. E., Naydenova, P., Crawley, C., Török, M. E., Enoch, D. A., Brown, N. M., Harrison, E. M., Parkhill, J., & Peacock, S. J. (2021). Quantifying acquisition and transmission of Enterococcus faecium using genomic surveillance. *Nature microbiology*, *6*(1), 103–111. https://doi.org/10.1038/s41564-020-00806-7.

Guzman Prieto, A. M., van Schaik, W., Rogers, M. R., Coque, T. M., Baquero, F., Corander, J., & Willems, R. J. (2016). Global Emergence and Dissemination of Enterococci as Nosocomial Pathogens: Attack of the Clones? *Frontiers in microbiology*, *7*, 788. https://doi.org/10.3389/fmicb.2016.00788.

Hollenbeck, B. L., & Rice, L. B. (2012). Intrinsic and acquired resistance mechanisms in enterococcus. *Virulence*, *3*(5), 421–433. https://doi.org/10.4161/viru.21282.

Kang, Y., Vicente, M., Parsad, S., Brielmeier, B., Pisano, J., Landon, E., & Pettit, N. N. (2013). Evaluation of risk factors for vancomycin-resistant Enterococcus bacteremia among previously colonized hematopoietic stem cell transplant patients. *Transplant infectious disease : an official journal of the Transplantation Society*, *15*(5), 466–473. https://doi.org/10.1111/tid.12120.

Khan, R., Petersen, F. C., & Shekhar, S. (2019). Commensal Bacteria: An Emerging Player in Defense Against Respiratory Pathogens. *Frontiers in immunology*, *10*, 1203. https://doi.org/10.3389/fimmu.2019.01203. https://www.frontiersin.org/article/10.3389/fimmu.2019.01203.

Kim, S., Covington, A., & Pamer, E. G. (2017). The intestinal microbiota: Antibiotics, colonization resistance, and enteric pathogens. *Immunological reviews*, *279*(1), 90–105. https://doi.org/10.1111/imr.12563.

Kouchak, Farideh & Askarian, Mehrdad. (2012). Nosocomial Infections: The Definition Criteria. Iranian journal of medical sciences. 37. 72-3.

Krause KM, Serio AW, Kane TR. (2016). Connolly LE. Aminoglycosides: An Overview. *Cold Spring Harb Perspect Med*.6(6):a027029. Published 2016 Jun 1. doi:10.1101/cshperspect.a027029.

Kristich CJ, Rice LB, Arias CA. (2014) Enterococcal Infection—Treatment and Antibiotic Resistance. In: Gilmore MS, Clewell DB, Ike Y, Shankar N, eds. Enterococci: *From Commensals to Leading Causes of Drug Resistant Infection*. Boston: Massachusetts Eye and Ear Infirmary; February 6th.

Lebreton, F., Willems, R., & Gilmore, M. S. (2014). *Enterococcus* Diversity, Origins in Nature, and Gut Colonization. In M. S. Gilmore (Eds.) et. al., *Enterococci: From Commensals to Leading Causes of Drug Resistant Infection*. Massachusetts Eye and Ear Infirmary.

Levitus, M., Rewane, A., & Perera, T. B. (2021). Vancomycin-Resistant Enterococci. In *StatPearls*. StatPearls Publishing.

Mahizan, N. A., Yang, S. K., Moo, C. L., Song, A. A., Chong, C. M., Chong, C. W., Abushelaibi, A., Lim, S. E., & Lai, K. S. (2019). Terpene Derivatives as a Potential Agent against Antimicrobial Resistance (AMR) Pathogens. *Molecules (Basel, Switzerland)*, *24*(14), 2631. https://doi.org/10.3390/molecules24142631.

Marcel, J. P., Alfa, M., Baquero, F., Etienne, J., Goossens, H., Harbarth, S., Hryniewicz, W., Jarvis, W., Kaku, M., Leclercq, R., Levy, S., Mazel, D., Nercelles, P., Perl, T., Pittet, D., Vandenbroucke-Grauls, C., Woodford, N., & Jarlier, V. (2008). Healthcare-associated infections: think globally, act locally. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases*, *14*(10), 895–907. https://doi.org/10.1111/j.1469-0691.2008.02074.x.

McKinnell, J., Kunz, D., Chamot, E., Patel, M., Shirley, R., Moser, S., Miller, L. (2012). Association between Vancomycin-Resistant Enterococci Bacteremia and Ceftriaxone Usage. Infection Control & Hospital Epidemiology, 33(7), 718-724. doi:10.1086/666331.

Michael S Gilmore, Francois Lebreton, Willem Van Schaik,Genomic. (2013). Transition of enterococci from gut commensals to leading causes of multidrug-resistant hospital infection in the antibiotic era. *Current Opinion in Microbiology,Volume 16, Issue 1,* 2013,Pages 10-16,ISSN 1369-5274.

Miller, W. R., Munita, J. M., & Arias, C. A. (2014). Mechanisms of antibiotic resistance in enterococci. *Expert review of anti-infective therapy*, *12*(10), 1221–1236. https://doi.org/10.1586/14787210.2014.956092.

Monticelli, J., Knezevich, A., Luzzati, R., & Di Bella, S. (2018). Clinical management of non-faecium non-faecalis vancomycin-resistant enterococci infection. Focus on Enterococcus gallinarum and Enterococcus casseliflavus/flavescens. *Journal of infection and chemotherapy : official journal of the Japan Society of Chemotherapy*, *24*(4), 237–246. https://doi.org/10.1016/j.jiac.2018.01.001.

Moosavian, M., Ghadri, H., & Samli, Z. (2018). Molecular detection of *vanA* and *vanB* genes among vancomycin-resistant enterococci in ICU-hospitalized patients in Ahvaz in southwest of Iran. *Infection and drug resistance*, *11*, 2269–2275. https://doi.org/10.2147/IDR.S177886.

Mühlberg, E., Umstätter, F., Kleist, C., Domhan, C., Mier, W., & Uhl, P. (2020). Renaissance of vancomycin: approaches for breaking antibiotic resistance in multidrug-resistant bacteria. *Canadian journal of microbiology*, *66*(1), 11–16. https://doi.org/10.1139/cjm-2019-0309.

Mulani, M. S., Kamble, E. E., Kumkar, S. N., Tawre, M. S., & Pardesi, K. R. (2019). Emerging Strategies to Combat ESKAPE Pathogens in the Era of Antimicrobial Resistance: A Review. *Frontiers in microbiology*, *10*, 539. https://doi.org/10.3389/fmicb.2019.00539.

Natsis, N. E., & Cohen, P. R. (2018). Coagulase-Negative Staphylococcus Skin and Soft Tissue Infections. *American journal of clinical dermatology*, *19*(5), 671–677. https://doi.org/10.1007/s40257-018-0362-9.

O'Driscoll, T., & Crank, C. W. (2015). Vancomycin-resistant enterococcal infections: epidemiology, clinical manifestations, and optimal management. *Infection and drug resistance*, *8*, 217–230. https://doi.org/10.2147/IDR.S54125.

Peel, T., Cheng, A. C., Spelman, T., Huysmans, M., & Spelman, D. (2012). Differing risk factors for vancomycin-resistant and vancomycin-sensitive enterococcal bacteraemia. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases*, *18*(4), 388–394. https://doi.org/10.1111/j.1469-0691.2011.03591.x.

Pineda, C., Espinosa, R., & Pena, A. (2009). Radiographic imaging in osteomyelitis: the role of plain radiography, computed tomography, ultrasonography, magnetic resonance imaging, and scintigraphy. *Seminars in plastic surgery*, *23*(2), 80–89. https://doi.org/10.1055/s-0029-1214160.

Radhouani, H., Silva, N., Poeta, P., Torres, C., Correia, S., & Igrejas, G. (2014). Potential impact of antimicrobial resistance in wildlife, environment and human health. *Frontiers in microbiology*, *5*, 23. https://doi.org/10.3389/fmicb.2014.00023.

Ramos S, Silva V, Dapkevicius MdLE, Igrejas G, Poeta P.(2020) Enterococci, from Harmless Bacteria to a Pathogen. *Microorganisms*;8(8):1118. https://doi.org/10.3390/microorganisms8081118.

Raza, T., Ullah, S. R., Mehmood, K., & Andleeb, S. (2018). Vancomycin resistant Enterococci: A brief review. *JPMA. The Journal of the Pakistan Medical Association*, *68*(5), 768–772.

Reyes, K., Bardossy, A. C., & Zervos, M. (2016). Vancomycin-Resistant Enterococci: Epidemiology, Infection Prevention, and Control. *Infectious disease clinics of North America*, *30*(4), 953–965. https://doi.org/10.1016/j.idc.2016.07.009.

Reynolds, Peter & Courvalin, Patrice. (2005). Vancomycin Resistance in Enterococci Due to Synthesis of Precursors Terminating in D-Alanyl-D-Serine. *Antimicrobial agents and chemotherapy. 49. 21-5*. 10.1128/AAC.49.1.21-25.2005.

Rudy M Nowakowska, M., Rogala-Zawada, D., Wiechuła, B., Radosz-Komoniewska, H., & Zientara, M. (2004). Czynniki etiologiczne zakazeń układu moczowego u dzieci i ich wrazliwość na antybiotyki [Urinary tract infections in children--etiologic agents and susceptibility to antibiotics]. *Wiadomosci lekarskie (Warsaw, Poland : 1960)*, *57*(9-10), 438–443.

Sabharwal ER, Sharma R. (2015). Fosfomycin: An Alternative Therapy for the Treatment of UTI Amidst Escalating Antimicrobial Resistance. *J Clin Diagn Res*;9(12):DC06-DC9. doi:10.7860/JCDR/2015/15227.6951.

Said MS, Tirthani E, Lesho E. (2021). Enterococcus Infections. Sep 28. *In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; Jan–. PMID*: 33620836.

Sakka, V., Tsiodras, S., Galani, L., Antoniadou, A., Souli, M., Galani, I., Pantelaki, M., Siafakas, N., Zerva, L., & Giamarellou, H. (2008). Risk-factors and predictors of mortality in patients colonised with vancomycin-resistant enterococci. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases*, *14*(1), 14–21. https://doi.org/10.1111/j.1469-0691.2007.01840.x.

Santajit, S., & Indrawattana, N. (2016). Mechanisms of Antimicrobial Resistance in ESKAPE Pathogens. *BioMed research international*, *2016*, 2475067. https://doi.org/10.1155/2016/2475067.

Sarychev, L. P., Sarychev, Y. V., Pustovoyt, H. L., Sukhomlin, S. A., & Suprunenko, S. M. (2018). Management of the patients with blunt renal trauma: 20 years of clinical experience. *Wiadomosci lekarskie (Warsaw, Poland : 1960)*, *71*(3 pt 2), 719–722.

Schneider, A. P., 2nd, Zainer, C. M., Kubat, C. K., Mullen, N. K., & Windisch, A. K. (2014). The breast cancer epidemic: 10 facts. *The Linacre quarterly*, *81*(3), 244–277. https://doi.org/10.1179/2050854914Y.0000000027.

Semedo, T., Almeida Santos, M., Martins, P., Silva Lopes, M. F., Figueiredo Marques, J. J., Tenreiro, R., & Barreto Crespo, M. T. (2003). Comparative study using type strains and clinical and food isolates to examine hemolytic activity and occurrence of the cyl operon in enterococci. *Journal of clinical microbiology*, *41*(6), 2569–2576. https://doi.org/10.1128/JCM.41.6.2569-2576.2003.

Skendros, P., & Mitroulis, I. (2012). Host cell autophagy in immune response to zoonotic infections. *Clinical & developmental immunology*, *2012*, 910525. https://doi.org/10.1155/2012/910525.

Stuart, C. H., Schwartz, S. A., Beeson, T. J., & Owatz, C. B. (2006). Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. *Journal of endodontics*, *32*(2), 93–98. https://doi.org/10.1016/j.joen.2005.10.049.

Sundermann, A. J., Babiker, A., Marsh, J. W., Shutt, K. A., Mustapha, M. M., Pasculle, A. W., Ezeonwuka, C., Saul, M. I., Pacey, M. P., Van Tyne, D., Ayres, A. M., Cooper, V. S., Snyder, G. M., & Harrison, L. H. (2020). Outbreak of Vancomycin-resistant Enterococcus faecium in Interventional Radiology: Detection Through Whole-genome Sequencing-based Surveillance. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, *70*(11), 2336–2343. https://doi.org/10.1093/cid/ciz666.

Sung, J. M., & Lindsay, J. A. (2007). Staphylococcus aureus strains that are hypersusceptible to resistance gene transfer from enterococci. *Antimicrobial agents and chemotherapy*, *51*(6), 2189–2191. https://doi.org/10.1128/AAC.01442-06.

Taylor HL, Wellman LL, Conran RM. (2021). Educational Case: Neisseria Meningitis. *Acad Pathol*.;8:23742895211043244. Published 2021 Sep 26. doi:10.1177/23742895211043244.

Urbańczyk, M., Góra, J., Latajka, R., & Sewald, N. (2017). Antifreeze glycopeptides: from structure and activity studies to current approaches in chemical synthesis. *Amino acids*, *49*(2), 209–222. https://doi.org/10.1007/s00726-016-2368-z.

Vasilakopoulou, A., Karakosta, P., Vourli, S., Tarpatzi, A., Varda, P., Kostoula, M., Antoniadou, A., & Pournaras, S. (2020). Gastrointestinal Carriage of Vancomycin-Resistant Enterococci and Carbapenem-Resistant Gram-Negative Bacteria in an Endemic Setting:

Prevalence, Risk Factors, and Outcomes. *Frontiers in public health*, *8*, 55. https://doi.org/10.3389/fpubh.2020.00055.

Ventola C. L. (2015). The antibiotic resistance crisis: part 1: causes and threats. *P & T : a peer-reviewed journal for formulary management*, *40*(4), 277–283.

Walsh, L., Johnson, C. N., Hill, C., & Ross, R. P. (2021). Efficacy of Phage- and Bacteriocin-Based Therapies in Combatting Nosocomial MRSA Infections. *Frontiers in molecular biosciences*, *8*, 654038. https://doi.org/10.3389/fmolb.2021.654038.

Wang, T. Z., Kodiyanplakkal, R., & Calfee, D. P. (2019). Antimicrobial resistance in nephrology. *Nature reviews. Nephrology*, *15*(8), 463–481. https://doi.org/10.1038/s41581-019-0150-7.

Yilema, A., Moges, F., Tadele, S., Endris, M., Kassu, A., Abebe, W., & Ayalew, G. (2017). Isolation of enterococci, their antimicrobial susceptibility patterns and associated factors among patients attending at the University of Gondar Teaching Hospital. *BMC infectious diseases*, *17*(1), 276. https://doi.org/10.1186/s12879-017-2363-3.

Yoon, Y. K., Lee, S. E., Lee, J., Kim, H. J., Kim, J. Y., Park, D. W., Sohn, J. W., & Kim, M. J. (2011). Risk factors for prolonged carriage of vancomycin-resistant Enterococcus faecium among patients in intensive care units: a case-control study. *The Journal of antimicrobial chemotherapy*, *66*(8), 1831–1838. https://doi.org/10.1093/jac/dkr204.

Zeng, D., Debabov, D., Hartsell, T. L., Cano, R. J., Adams, S., Schuyler, J. A., McMillan, R., & Pace, J. L. (2016). Approved Glycopeptide Antibacterial Drugs: Mechanism of Action and Resistance. *Cold Spring Harbor perspectives in medicine*, *6*(12), a026989. https://doi.org/10.1101/cshperspect.a026989.