# ISOLATION AND ANTIBIOTIC SUSCEPTIBILITY OF *ENTEROCOCCUS* SPECIES FROM HUMAN STOOL AND ENVIRONMENTAL SAMPLES

**BY**

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**18/4894**

**A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES AND BIOTECHNOLOGY, COLLEGE OF PURE AND APPLIED SCIENCES, CALEB UNIVERSITY, IMOTA,**

**LAGOS, NIGERIA**

**IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.SC) IN MICROBIOLOGY AND INDUSTRIAL BIOTECHNOLOGY FROM THE DEPARTMENT OF BIOLOGICAL SCIENCES AND BIOTECHNOLOGY, CALEB UNIVERSITY, IMOTA, LAGOS**

**JUNE, 2022**

## DECLARATION

I, ALUKO PELUMI BUKUNMI hereby declare that this project is entirely my work and composition. The work in this project has not been submitted in candidature for any degree and is not concurrently being submitted for any other degree. All references made to works of other persons have been duly acknowledged.

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## CERTIFICATION

This is to certify that this project was carried out by me ALUKO PELUMI BUKUNMI, a student of the Department of Biological Sciences and Biotechnology (Microbiology and Industrial Programme), College of Pure and Applied Science, Caleb University, Imota, Lagos.

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## DEDICATION

This project report is dedicated to Almighty God and my parent MR and MRS ALUKO.

## ACKNOWLEDGEMENTS

I thank God for his grace, protection, provision, guidance and wisdom given to me and for seeing me through till this moment.

To my supervisor, Dr E. A. ADEMOLA, thank you for your time, efforts, advice and your moral and mental support for this to be possible, may the Almighty Lord bless you abundantly.

I am also grateful to my H. O. D, Dr C. C. Ezeanya-Bakpa, my lecturers Dr B.C. Kotun, Mr O. Onoja, Dr T.C. Bayo-Olajide, Dr O.A. Folorunsho, Dr O.S. Asaka, Dr Amos Olajide, Mr J.Ayedun for their great impact in my life.

My acknowledgement also goes to my parent Mr and Mrs Aluko, my wonderful siblings Olamide, Tomiwa and Emmanuel, my big aunts Mrs Ayanwola and aunt Temi for always being there financially, physically, spiritually and mentally. I also want to say a big thank you to Mr Gbenga, Aunt Taiwo, Mrs Pemi, Mr Peter, Aunt Lola, Mrs Olukoya, and Mrs Fransisca for their support and love.

Finally my appreciation goes to my ever supportive friends Onafeko Ibrahim, Moriamo, Oluwadamilola, Emmanuella, Jessica, Ebitariere, Misky, Ayomipo, Tolulope, Enoch, Gbolahan, Mojisola, Ibiayo, Bolatito, Kemi, Archie, Ademakinwa, Ruth, Olamide, Bolanle, Kanyinsola, Sylvia, Deborah and my course mates, thanks for always helping my lazy self-thanks for your endless love.

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## ABSTRACT

The genus *Enterococcus* comprises of a group of Gram-positive, non-spore forming cocci, facultative anaerobes bacteria that undergo lactic acid fermentation found in the gastrointestinal tract of vertebrate and invertebrate animals, soil, surface waters, oceans and fermented food products. They can cause health care- associated infections such as Urinary tract infection, bacteremia, endocarditis, and wound infection. This study is aimed at the isolation, identification and antibiotic susceptibility of *Enterococcus* species from faecal and environmental samples obtained from Caleb University. Isolation was carried out on blood agar using standard methods and identification was done using conventional morphological and biochemical test. Antibiotic susceptibility test was carried out using disc diffusion method. A total of 34.1% of the samples used in this study were positive for *Enterococcus* species with occurrence of 52.4%, 12.5% and 28.6% in stool, soil and waste water samples, respectively and fifteen isolates comprising of 10(66.7%) *Enterococcus faecalis* and 5(33.3%) *Enterococcus faecium* were obtained. The highest susceptibility of the Enterococcus species was recorded with Ciprofloxacin (100%), followed by Tetracycline (80%) and Vancomycin (53.3%) while 93.3%, 80%, 60%, 60% and 53.3% were resistant to Metronidazole, Ceftazidime, Ampicillin, Gentamicin and Erythromycin, respectively. The stool and environmental samples isolates had high level of resistance to antibiotics.

*Enterococci* species were identified in this study, the resistance pattern showed by *Enterococcus* specie isolated in the study could be due to the ability of *Enterococci* to acquire resistance.



**KEY WORD:** *Enterococci*, *Enterococcus*, Faecal, Environmental, *Enterococcus faecalis, Enterococcus faecium*, Antibiotic resistance .

# CHAPTER ONE

## INTRODUCTION

*Enterococcus* is a highly adaptable bacterium genus found primarily in the human and animal gastrointestinal tracts (Psoni *et al*., 2006). Due to their high heat tolerance and capacity to survive in difficult environmental circumstances, they can also thrive in the external environment and colonize a variety of habitats (Psoni *et al*., 2006). *Enterococci* are opportunistic microorganisms that are found in many animals' gut and are innocuous in healthy people but cause infections in patients in intensive care facilities, those who have serious underlying diseases and those who are immunocompromised (Koort *et al.,* 2004). One of the most prevalent species in nosocomial infections is *enterococci* and cause bacteremia, endocarditis, urinary tract infections, and other illnesses (Kuzucu *et al*., 2005). In the outpatient situation, the third most frequent cause of endocarditis is *enterococci*; while some of these infected persons may have an underlying heart valve problem, this is often unknown, and they appear "healthy" with no hospital exposure (Agudelo and Huycke, 2014).

The broad usage as methods for measuring recreational water quality worldwide is due to their abundance in faeces from humans and other animals, ease of culture, and connection to human health effects in freshwater and marine environments (Wade *et al*., 2008). In risk assessment and other modeling applicants, *enterococci* are frequently used as fecal indicator bacteria (FIB), or general indicators of fecal contamination. However, they can also be used as substitutes for pathogens and/or health effects (Tseng and Jiang, 2012). However, more than three decades of research have shown that these bacteria are widely dispersed in a variety of natural habitats, even

when there are not any human or animal excrement present. Extra-enteric habitats include soil and sediments, beach sand, aquatic and terrestrial vegetation, and ambient waters (rivers, streams, and creeks). They may also be referred to as heterothermic habitats because they experience varying temperatures as opposed to the relatively constant gastrointestinal tract of warm-blooded animals (Gilmore, 2002).

Antibiotic resistance is widespread among *Enterococci*. This trait permits the ability of *enterococci* to endure in an environment where antibiotics are often administered and promotes the spread of resistant microorganism (Lin *et al*., 2015). This resistance can either be acquired, mediated by genes on plasmids or transposons, or innate, mediated by genes on the chromosome, a property shared by practically all *enterococci* strains. Resistance to many β-lactams, low levels of aminoglycosides and resistance to cephalosporins, sulphonamides and lincosamides (Frederick and McGraw, 2007). The acquired genetic determinants provide resistance to all antimicrobials, including chloramphenicol, tetracycline, erythromycin, rifampicin, ampicillin, and glycopeptides. The main problem is that pheromone-mediated conjugative plasmids or transposons might be used to transfer the genes responsible for all of these antibiotic resistant traits to other *enterococci* or even more deadly pathogens (Schwarz *et al*., 2001).

## 1.1 STATEMENT OF PROBLEM

In the gastrointestinal consortia of a wide range of hosts, including humans and other mammals, birds, reptiles, and insects, *enterococci* have developed as well suited members for eons, but they became one of the major causes of hospital-acquired, drug-resistant illnesses in the 1970s for unknown reasons. Over the last ten years, the taxonomy of enterococci has evolved significantly, and the genus now contains over forty unique species with a wide range of habitats, tropisms,

metabolic, and behavioral properties. The ecosystems are supported by their animal hosts as well as by plants, soil, water and man-made foods including fermented foods and dairy products. In a few of these situations, antibiotic-resistant *Enterococci* strains have developed and novel resistance mechanisms in *enterococcus* are being discovered at an alarming rate.

From one health approach, the presence of *Enterococci* in various non-human ecosystems, as well as the increase in their resistant to antibiotics, is of great interest, most especially with the continuous interaction of human with the various components of the ecosystem.

## 1.2 JUSTIFICATION FOR STUDY

*Enterococcus* species can be spread by hand contact with open wounds harboring the bacteria or by contacting contaminated environmental surfaces, where the organisms can survive for weeks because of their propensity to cause potentially fatal infections such as endocarditis, bacteremia, intra-abdominal, and urinary tract infection (UTI), as well as their rising resistance to many antimicrobial treatments, *Enterococci* have obtained a lot of attention in recent years (Teixeira and Facklam, 2003).

The acquisition of bacteria put patients' health at risk due to antibiotics resistance. When *Enterococci* encounter drugs or pick up genetic resistance components from nearby species, they quickly develop resistance (Kristich *et al.*, 2014). As a result, human, environmental, and animal reservoirs can transmit Vancomycin Resistant *Enterococcus* across the community. Due to the multiple antibiotic resistance of VRE, therapeutic issues including patient longer hospital stays results in higher healthcare expenses and, in the worst-case scenario, patient death. The goal of this study is to see if *Enterococcus* species isolated from the environment, such as water, soil, and stool, are susceptible to antibiotics.

## 1.3 AIM AND OBJECTIVES

The aim of this study is to isolate, characterize and determine the antibiotic susceptibility of *Enterococcus* species from human and non-human samples.

The main objectives of the study are:

1. To isolate *Enterococcus* species from stool, soil and water samples.
2. To identify the *Enterococcus* species using their morphological and biochemical characteristics.
3. To determine the antibiotics susceptibility of the isolated *Enterococcus* species.
4. To compare the antibiotic resistance of the isolates from human and environmental samples.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 THE *ENTEROCOCCUS* GENUS

*Enterococci* are Gram-positive bacteria found in the gut microbiota of both vertebrates and invertebrates, in fermented food products, in relation with plants, in surface waters, the soil, the ocean, and as disease causing pathogens in humans have all been studied (Sedlácek *et al.*, 2013). Depending on the data collection and analytic requirements, they have a genus core genome with 605 to 1,037 genes, 2,154 to 5,107 predicted genes, a 2.3 to 5.4Mb genome size and a low-GC genome content of between 34 and 45 percent (Zhong *et al.,* 2017). The pan-genome of *Enterococci* is bigger, reflecting the highly flexible character based on their niche adaptations and genomes. Thiercelin invented the term "entérocoque" in 1899 to characterize commensal bacteria in the intestines that could turn pathogenic (Thiercelin, 1899). Up until the mid-1980s, the *Enterococci* were regarded as belonging to the genus *Streptococcus* (Albert *et al*., 2012) and classified as group D *Streptococci* due to morphological and biochemical similarities. Although the pyogenic lactic *Streptococci,* viridans *Streptococci*, pyogenic *Streptococci,* and *Enterococcus* were recognized as four distinct branches of Streptococci, in contrast to being a monophyletic genus, Gram-positive cocci isolated from the gut or faeces were more commonly called "*Enterococcus*". Kalina argued in favor of giving the so-called enteric *Streptococci* their own genus in 1970. *Enterococcus*, based on a careful investigation of biochemical and culture properties. The formal introduction of the genus *Enterococcus* did not take off until 1984, additionally, it was recognized as a separate genus from the *Streptococci* in an editorial update in Bergey's Manual of Systematic Bacteriology in 1986 (Mundt, 1986). The *Enterococcus'* genus

comprises 58 species with recognized names. (Parte, 2014). Ludwig *et al.,* (2009) suggested the *Enterococcaceae* family originally comprised *Enterococcus, Vagococcus, Tetragenococcus,* and *Melissococcus* based on 16S rRNA gene similarities. *Catellicoccus* and *Pilibacter* are two additional speculative genera belonging to the *Enterococcaceae* (Lawson *et al.,* 2006). Because of the low number of reported and sequenced species in each genus, *Tetragenococcus, Melissococcus, Catellicoccus*, and *Pilibacter* are not known to be in a specific phylogenetic position, and the possibility that at least one *Tetragenococcus* species and *Melissococcus* species may branch within *Enterococcus* (Lebreton *et al.,* 2017). The *Enterococcaceae* belong to the *Lactobacillales* order, which includes other important medicinal and economic families like as the *Lactobacillaceae* and *Streptococcaceae*, both of which belong to the *Firmicutes* phylum's class Bacilli.

## 2.2 *ENTEROCOCCUS* SPECIES AND LABORATORY DIFFERENTIATION

*Enterococci* are ovoid, non-spore-forming ovoid bacteria that can exist by themselves, in pairs, chains, or groups. Švec and Franz (2014) describe them as homo-fermentative chemo-organotrophic facultative anaerobes with lactic acid as the primary end product of carbohydrate fermentation. Various selective media have been used to isolate and identify *enterococci*; nevertheless, to identify *Enterococcus* from other catalase-negative gram-positive cocci, there are no conclusive biochemical techniques. The majority of *Enterococci* are esculin hydrolytic, salt tolerant (up to 6.5%), bile resistant (up to 40%), oxidase and catalase negative capable of growing in the presence of sodium azide (up to 0.4 percent). Glucoside are produced by the enzymes leucine arylamidase and glucosidase. Acid from the glycosides salicin, methyl β-D-

glucoside as well as the sugars D-fructose, galactose, -gentiobiose, glucose, lactose, maltose, D-mannose, ribose, trehalose, cellobiose, and N-acetylglucosamine. Due to the fact that *Enterococci* are urease negative and do not generate acid from D-arabinose, erythritol, D- and L-fructose, and L-xylose, methyl-D-xyloside these metabolic properties have been employed in the development of commercial testing kits. At temperatures between 10 and 45 degrees Celsius, growth takes place, with most species growing best at 35 to 37 degrees Celsius (Švec and Devriese, 2015).

Švec and Devriese (2015) found that dessication is remarkably ineffective against *Enterococci*. *Enterococcus casseliflavus, E. flavescens* and *Enterococcus gallinarum* are the only two enterococcal species known to be migratory (Stephen, 2012). As early as 1920, Orla-Jensen suggested separating *Streptococcus faecalis* and *Streptococcus faecium* into two species based on the former's capacity in order to produce black colonies and withstand potassium tellurite. To improve species identification, along the way, biochemical tests were added, such as the capacity to transform tetrazolium salts into the chromogenic formazan in the presence of glucose (Kurt and John, 2009).

Lancefield (1933) proposed a widely used method for classifying and differentiating *Enterococci* based on serological grouping in a seminal study. The method used to separate *enterococcus* from most *Streptococcus* species is currently used to categorize enteric *streptococci* as belonging to antigenic group D. *Enterococci* can be α, β, or non-hemolytic when grown on horse blood agar and produce 1- to 2-mm colonies with a moist look. A variety of selective culture mediums used to isolate *Enterococci* have been created based on their metabolic capacities; these selective media contains esculin or tetrazolium salts, sodium azide, antibiotics, and bile salts. Although the most therapeutically significant enterococcal species can grow in

these selective media, despite the fact that not all enterococcal species can. A bile esculin hydrolysis test, Catalase testing and pyrrolidonyl arylamidase/pyrrolidonyl-aminopeptidase (PYR) testing, are the most used clinical tests for enterococcal identification. In order to standardize and enhance the detection of *Enterococci* in the clinical setting, commercial kits have been developed, however they all require the organisms to be isolated and cultured first, which can delay diagnosis. Furthermore, precise species distinction among species groupings is not often achieved solely by phenotypic tests (Chantal *et* al., 2016). The clinical importance of species-level identification of *Enterococci* can be seen in the antibiotic resistance profiles of the numerous *Enterococci* that are pathogenic. Enhanced species identification and quicker testing options has been established with the integration of molecular methods into clinical microbiology laboratories; these methods are crucial for surveillance and epidemiology as well as the diagnosis of difficult cases. Techniques for molecular diagnosis are becoming more popular, but they are still not frequently used in clinical microbiology laboratories in resource-constrained areas. Molecular-based techniques have the potential to improve diagnostic precision, provide details on antibiotic resistance, and save time and money when compared to conventional cultivation and phenotypic testing.

Nucleic acid amplification tests (NAATs), peptide nucleic acid fluorescent in situ hybridization (PNA-FISH), Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDITOF MS) and multi-locus sequence typing (MLST) are some of the newer systems for classifying and identifying *Enterococci*. Clinical microbiology laboratories are starting to adopt MALDI-TOF MS-based identification, which is a potent, quick, and reliable method, for routine detection and species identification (Singhal *et al.,* 2015). The MALDI-TOF MS-based analysis in clinical practice allows for the quick detection of *Enterococci* directly from blood culture

bottles, possibly speeding up the commencement of antibiotic therapy (Lagace-Wiens *et al.,* 2012). Due to its high sensitivity, MALDITOF MS can distinguish between closely related species and identify roughly 94 percent of isolates down to the species level (Stepien'-Pysniak *et al.,* 2017). It may also be useful for profiling antibiotic resistance, such as identifying the presence of van genes, although it is not yet being used in clinical practice (Nakano *et al.,* 2014).

NAAT techniques are based on PCR amplification and subsequent sequencing, array/hybridization, or real-time PCR amplification of one or more genes that are helpful for identifying organisms to the genus level or species level, as well as detecting genes for antibiotic resistance (Ljungstrom *et al.,* 2015). For diagnostic and phylogenetic purposes, various genes have been utilized. The 16S rRNA gene is frequently used to identify bacterial species and allows differentiation of *Enterococci* down to the species level (Bosshard *et al.,* 2004); however, it can be challenging to distinguish between species that are members of the same species group, such as the *E. faecium* group (Bosshard *et al.,* 2004). Several other genes, groES and groEL, as well as ddl (D-alanine:D-alanine), atpA (ATP synthase), sod (superoxide dismutase), and tuf (elongation factor Tu), have all been proposed to aid in the differentiation of enterococcal species (Tan *et al.,* 2017).

Species and potential antibiotic resistance genes can be simultaneously determined using multiplexed real-time PCR and genus- and species-specific assays have been developed with the aim of speedy detection (Fang *et al.,* 2012). The use of PNA-FISH, enables for the fast detection of *Enterococci* in blood culture bottles by targeting species-specific rRNA. *E. faecalis*, *E. faecium*, and other less frequent enterococcal species can be distinguished using these assays (Deck *et al.,* 2014). MLST can identify strains and has been used to research molecular epidemiology and epidemics (Ruiz-Garbajosa *et al.,* 2006). Due to its higher reproducibility and

ease of use, pulsed-field gel electrophoresis (PFGE) analysis is often used. Whole-genome sequencing (WGS) and average nucleotide difference analysis are more expensive to adopt than Core genome MLST (cgMLST), which increases the number of genes from 7 or so housekeeping genes to up to 1,423 (de Been *et al.,* 2015), has recently been implemented to improve resolution (Lytsy *et al.,* 2017). In a study, 495 clinical *E. faecium* isolates and 11 reference genomes were used to compare MLST and WGS, and they largely attributed these disparities to MLST's lack of resilience due to a high level of recombination between isolates (Raven *et al*., 2016). Bayesian analysis of population structure (BASP) is a tool for better identifying deep-branching lineages and recombination compared to MLST-based research employing data on DNA sequence or molecular marker (Top *et al.,* 2007).

## 2.3 *ENTEROCOCCUS* SPECIES WITH SIGNIFICANCE CLINICALLY AND LESS-COMMON SPECIES

In the human gastrointestinal tract, *enterococci* are common commensal bacteria. However, they can also be pathogenic, leading to infections such as UTIs, bacteremia, endocarditis, burn and surgical site wound infections, abdominal and biliary tract infections, and infection of catheters and other medical devices. After *Staphylococcus aureus* and *Streptococci viridans*, the third most common cause of native valve endocarditis is *Enterococcus* (Slipczuk *et al.,* 2013). *E. faecium* and *E. faecalis* are the most prevalent strains in humans. In people who eat a westernized diet, *Lactobacillales* make up less than 1% of the gut microbiota. MacCallum and Hastings (1899) were the first to report a possible enterococcal infection, discussing an endocarditis case and identifying the isolated bacterium, which they named *Micrococcus zymogenes*. Thiercelin (1899)

described around communal enteric bacteria (an entérocoque) that can simultaneously cause septicemia and diarrhea. Endocarditis, puerperal fever, wound infections in First World War soldiers, fever and bacteremia were all attributed to *Streptococcus* or *Enterococcus faecalis* infection (Donald *et al*., 2013).

Since the late 1970s, the rate of enterococcal infections has been continuously rising (Arias and Murray, 2012). *Enterococci* being the second most typical pathogens associated with hospital-acquired Infection (HAI) in both Europe and the United States, with *E. faecalis* being the third most commonly isolated organism from central line-associated bloodstream infections (CLABSIs) and *E. faecium* being the eleventh and fifth most frequently isolated organism from catheter-associated urinary tract infections (CAUTIs) (Weiner *et al.,* 2016). The intrinsic resistance of *Enterococci* to a variety of antimicrobials and their capacity to develop novel resistance traits are two factors that contribute to their ability to become HAI agents. Since *E. faecalis* is more virulent than *E. faecium* yet exhibits less innate and acquired antibiotic resistance, it is the most frequent cause of HAIs. In the past, *E. faecalis* was discovered in about 50.3% of all enterococcal HAIs. However, *E. faecium*-related infections are becoming more common, owing to the emergence of β-lactam and vancomycin resistant *E. faecium* strains (Top *et al.,* 2007). Approximately 10% of *E. faecalis* isolates had vancomycin resistance, compared to 80% of *E. faecium* isolates (Weiner *et al.,* 2016). *E. faecium* and *E. faecalis* together account for around 75% of all enterococcal infections (Weiner *et al.,* 2016). All other non-faecalis *Enterococci* (OE), including non-typed *E. faecium* and *E. faecalis* make up about 24.6 percent of all enterococcal infections (Weiner *et al.,* 2016). However, the percentage of OE infections in nontyped presumptive *E. faecium* and *E. faecalis* infections is not reported separately. The number of Infections caused by OE has increased; from 2011 to 2014, cases of OE bacteremia in

U.S. hospitals ranked 10th among HAIs (Weiner *et al.,* 2016), up from 11th from 2009 to 2010. (Sievert *et al.,* 2013).

Human infection has been linked to *E. casseliflavus*, *E. raffinosus*, *E. gallinarum*, *E. durans*, *E. hirae*, *E. mundtii*, and *E. avium*, primarily in people with hematological malignancies, neutropenia, and past corticosteroid therapy. *E. durans*, *E. hirae*, and *E. mundtii* are members of the *E. faecium* species group suggests that their common ancestor had the capacity to become pathogenic (ŠVec and Franz, 2014). Vancomycin resistance is inherent in *E. casseliflavus* and *E. gallinarum*, which could provide a therapeutic dilemma if the number of illnesses caused by these organisms still increases (Navarro and Courvalin, 1994). A study by Manfredo Vieira *et al.,* (2018) links *E. gallinarum* to the induction of autoantibodies linked to autoimmune disease in mice with autoimmune susceptibility after translocation from the gut to the liver, raising the possibility that a similar mechanism may operate in people with autoimmune diseases like lupus erythematosus and indicating a new role for *Enterococci* in human health. *E. gilvus*, *E. raffinosus* and *E. pallens* all fall under the same species groupings (Švec and Franz, 2014). *E. pallens* has been isolated from ascites fluid and has been linked to spontaneous peritonitis in people with liver cirrhosis, with only four instances documented so far in the province of Quebec, Canada (Levesque *et al.,* 2016). This organism's significance as a human pathogen is still unknown. In the bile of a cholecystitis patient, *E. gilvus* was discovered to be a component of a mixed infection with *E. casseliflavus* and *E. faecium* (Tyrrell *et al.,* 2002). The majority of OE infections occur in critically ill patients with concurrent comorbidities, making it challenging to estimate the death rate of bacteremia caused by these organisms (Monticelli *et al.,* 2018).

## 2.4 *ENTEROCOCCUS* SPECIES VIRULENCE

The *Enterococci* do not have a high level of virulence, and the ability of *E. faecium* and *E. faecalis* to thrive in an environment that is antimicrobial-rich largely determines their pathogenicity in hospitals. Nonetheless, both species' pathogenic potential and capacity to cause disease have been linked to specific characteristics. These abilities include the capacity to avoid immune detection, the ability to attach to host cells, the extracellular matrix (EM), and inert objects like a variety of medical equipment, and the capacity to build biofilms that render them resistant to phagocytic and antibiotic attack (Mohamed and Huang, 2007).

*E. faecalis* has higher virulence factors, which could explain why it is still the most common cause of infections with *enterococci*. Numerous proteins have been recognized as components of pathogenic *Enterococci*'s pathogenicity repertoire. Surface elements called Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs) helps *Enterococci* adhere adhering to host tissues and cause infection. MSCRAMM genes are identified in nearly all *E. faecalis* strains and are expressed in vivo after human infection (Sillanpaa *et al.*, 2004). Ace, a protein that binds to collagen and promotes early heart valve colonization, may contribute to the formation of endocarditis, is one of the best-studied MSCRAMMs (Singh *et al.*, 2010). MSCRAMM genes are more frequent in endocarditis isolates, and genes from this family are concentrated in *E. faecium* clinical isolates (Sillanpaa *et al.*, 2009). The most researched MSCRAMM in *E. faecium* is a collagen-binding protein called Acm. Although one study indicated that a transposon damaged the acm gene in commensal isolates, rendering it nonfunctional, most health-care-associated isolates have the Acm gene (present in 99 percent of isolates studied) (Nallapareddy *et al.*, 2008).

### 2.4.1 Pilin gene clusters (PGCs)

They are present in *E. faecium* and *E. faecalis*, and they encode surface proteins with LPxTG-like motif that are in charge of producing long filamentous structures called pili that extend from the surface. Pili, like MSCRAMMs, function as adhesins. The (endocarditis- and biofilm-associated pilus) ebp PGC of *E. faecalis* has been linked to the etiology of endocarditis and urinary tract infections and is related to initial adherence and biofilm development (Nallapareddy *et al.,* 2006). The relevance of the pilum in *E. faecium* is unclear; nevertheless, there appears to be differences in PCG regulation and pilus protein assembly between clinical isolates and commensal strains (Hendrickx *et al.*, 2010).

### 2.4.2 Cytolysin (Cyl)

Cytolysin, also known as hemolysin is a virulence factor produced by E. faecalis infections and is encoded by the cylLL and cylLS genes. A two-peptide lytic protein called Cyl damages host cells and aids infection. It also acts like bacteriocin, which makes it harmful to Gram-positive bacteria (Cox et al., 2005). The cytolysin operon and aggregation substance genes are frequently found together on mobile elements like as conjugative plasmids or in the pathogenicity island (PAI) ( Joseph et al., 2000).

### 2.4.3 Aggregation substance

During conjugation, the pheromone-induced surface protein aggregate substance plays a dual role in the formation of mating pairs and pathogenicity. It helps produce the vegetation that causes infectious endocarditis, extracellular matrix adhesion, and phagocytosis protection, as well as potentiating Cyl's pathogenic action (Coburn and Gilmore, 2003).

### 2.4.4 Gelatinase (GelE)

A matrix metalloproteinase called gelatinase (GelE) hydrolyzes proteins like collagen and gelatin, it is an additional virulence factor that increases the possibility for disease caused by *E. faecalis.* GelE suppresses complement-mediated responses and has a part to play in the growth of endocarditis (Thurlow et al., 2010). gelE is co-transcribed with sprE, gene for a serine protease; the two genes combine to increase pathogenicity. The fsr locus, a master regulator involved in biofilm formation, surface protein expression, and metabolism, controls the expression of both genes (Bourgogne et al., 2006). The enterococcal surface protein (Esp) associated with the cell wall is involved in cell attachment in both *E. faecium* and *E. faecalis*, playing a role in urethral colonization and endocarditis, as well as fostering biofilm development (Heikens *et al*., 2011). Esp, on the other hand, is not present in all clinical isolates and neither is it sufficient for infection to spread.

### 2.4.5 Phosphotransferase system (PTS)

Phosphotransferase system genes code for transmembrane proteins involved in sugar absorption. Enterococci may use variety of sugars as sources of carbohydrates and the ability to adjust to changing conditions thanks to PTS diversification. The PTS can have a role in the overall stress response and *enterococci*-helping virulence factors and survival inside the host, as well as in the biofilm and endocarditis formation (Peng *et al*., 2017). A few of these virulence factors genes are frequently found together in mobile elements, such as PAIs making it easier for them to transfer between isolates. PAIs are huge components that bacterial pathogens can acquire by horizontal transfer and bestow pathogenicity (Gal-Mor and Finlay, 2006).

## 2.5 TREATMENT OF ENTEROCOCCAL INFECTIONS

Those β-lactams that display in vitro activity (predominantly ampicillin, but also penicillin and piperacillin) and vancomycin have been the pillars of antimicrobial therapy for enterococcal infections. For normal infections that do not require bactericidal therapy, a single such drug is usually sufficient (skin and soft tissue infections, urinary tract infections, surgically drained intra-abdominal infections, and intravenous line-associated bloodstream infections). Traditional treatment for infections that require bactericidal therapy (endocarditis, osteomyelitis, and meningitis) has comprised an active β-lactam or vancomycin in conjunction with gentamicin or streptomycin. Cure rates for enterococcal endocarditis with penicillin alone were around 40% prior to the adoption of combination therapy (Geraci and Martin, 1954). Geraci and Martin (1954) found that combining streptomycin and penicillin raised cure rates to 70% or more, and while there are no reliable randomized controlled studies to properly estimate cure rates, the majority of research utilizing combinations indicates cure rates exceeding 70% (Rice *et al.,* 1991).

A strain expressing aminoglycosides-modifying enzymes emerged after aminoglycosides were identified as a synergist with cell wall-active antibiotics in the treatment of endocarditis. This negated the in vitro synergism observed against strains that do not express these enzymes by causing high levels of resistance to gentamicin or streptomycin (Fong *et al*., 2007). Endocarditis induced by strains displaying significant levels of aminoglycoside resistance appears to cure at about the same rate as it did before synergistic therapy was utilized, necessitating other treatment techniques (Dharumadurai *et al*., 2016).

Mainardi *et al.,* (1995) were the first to describe cefotaxime and ampicillin's in vitro synergism against *E. faecalis,* proving that the presence of the other drug increased the

susceptibility to it in vitro. They theorized that the medications'combination was the cause of synergism more comprehensive suppression of all enterococcal PBPs. Gavaldà and colleagues (Gavaldà *et al.,* 1996) confirmed these findings in animal tests, finding comparable synergisms between ampicillin and ceftriaxone. Following human trials, whether or not the pathogenic strains demonstrated significant levels of resistance to aminoglycoside, the effectiveness of ceftriaxone and ampicillin in combination against ampicillin-resistant *E. faecalis* was confirmed (Gavaldà *et al.,* 2007). These findings have led to the recommendations of ampicillin-ceftriaxone combination therapy as a reasonable option for treating endocarditis brought on by strains that are high-level aminoglycoside-resistant and a reasonable substitute for strains that are not high-level of aminoglycoside. The best period of treatment is 4 to 6 weeks for enterococcal endocarditis, although there are no trials to help differentiate between the two. Lars *et al.,* (2002) conducted a study including 78-patients that revealed the combination regimen's aminoglycoside component could be stopped after 15 days without decreasing the cure rate but with beneficial effect on renal function. Retrospective data also suggests that cure rates comparable to those for native valve disease can be attained in enterococcal prosthetic valve endocarditis caused by susceptible strains (John *et al*., 2016). Recently, a noteworthy Danish study (Iversen *et al.,* 2018) found that a 17-day intravenous therapy for endocarditis due to one of four species *(S. aureus, E. faecalis*, *streptococci* or coagulase-negative *Staphylococci*), followed by approximately equal days of active oral therapy was non-inferior to an entire course of intravenous treatment. The study comprised 97 individuals with endocarditis due to *E. faecalis* (39 with prosthetic valve endocarditis), with results substantially identical to the final outcomes for each individual combined endpoint as well as the combined endpoints. At the time of randomization, the patients required to be in a clinically stable state, surgery to replace a valve was authorized before

switching to oral medicine, and it was not blind. This could have a significant influence on expenditures for endocarditis patients whose condition has improved after receiving intravenous therapy for two weeks or after surgical valve replacement for an infected valve. Some patients cannot tolerate β-lactam antibiotics, or may have strains of *E. faecium* that manufacture aminoglycoside-modifying enzymes and are resistant to vancomycin and all β-lactams. Unlike the 1990s, when there were no viable medicines for extremely resistant germs, we now have a number of options that exhibit in vitro effectiveness against resistant bacteria. The pristinamycin combination quinupristin-dalfopristin was the first to enter clinical trials. In opposition to *E. faecium* microorganisms deficient in the erm macrolide resistance gene, this combination proved synergistically effective (but not *E. faecalis*). Despite the fact that it was shown to be an effective treatment for vancomycin-resistant enterococcal infections, but it was also associated with severe myalgias and inflammation of the vein (Delgado *et al.,* 2000). The oxazolidinone antibiotic linezolid, which is accessible in both oral formulations and intravenous, was the next to receive approval, and in vitro testing has demonstrated that it routinely exerts against resistant *Enterococci*. When used for longer than 2 weeks, it can cause bone marrow suppression (Gerson *et al.,* 2002), additionally, individuals on selective serotonin reuptake inhibitors have a higher chance of developing serotonin syndrome (SSRIs) (Lawrence *et al.,* 2006). Despite being completely bacteriostatic, it has been shown to be just as effective in treating bacteremias as more bactericidal medications, and it's been used to treat enterococcal endocarditis in some cases (Chuang *et al.,* 2016). The Food and Drug Administration granted daptomycin's cyclic lipopeptide license in 2003. (Lauridsen *et al.,* 2012). Many enterococcal strains are susceptible to it. 4 mg/kg of body weight per day was the initial dose that was authorized, pharmacodynamic evaluations revealed that many strains would require greater doses (Foolad *et al.,* 2018).

Currently, Physicians are utilizing up to 8 to 10 mg/kg each day. Linezolid and daptomycin are contrasted while treating enterococcal bacteremia. A study (Chuang *et al.,* 2016) found that linezolid was superior than daptomycin at doses of 6 mg/kg/daily or below, the two regimens were comparable when daptomycin was administered at a dose of 6 mg/kg/daily. When exposed to β-lactams and a daptomycin antibiotic together, certain strains of *E. faecium* with higher daptomycin MICs had lower MICs (to which the strains are resistant). Although in certain cases, in the presence of ampicillin there was more daptomycin binding to the cell membrane, this apparent synergism's underlying mechanisms remain unknown (Sakoulas *et al.,* 2012). Other evidence suggests that strains with mutations in the liaFSR gene experience this synergism (Mishra *et al.,* 2012). The enhanced efficacy of these combinations against resistant microorganisms is not supported by strong clinical evidence. There are more uncertainties than answers about the treatment of severe multi-resistant enterococcal infections. A clinical trial consortium funded by the National Institutes of Health (NIH) known as the Gram-Positive Committee of the Antimicrobial Resistance Leadership Group recently identified a number of unmet needs in the treatment of enterococcal infections, including the function of combination β-lactams therapy against vancomycin-resistant *Enterococci,* the function of combination β-lactam therapy for enterococcal bloodstream infection and osteomyelitis, and the ideal length of therapy for vancomycin-resistant *Enterococci* (Doernberg *et al.,* 2017).

## 2.6 *ENTEROCOCCUS* SUSCEPTIBILITY TO ANTIMICROBIALS AND RESISTANCE MECHANISMS INHERENT

*E. faecium* and *E. faecalis* are distinguished by their low susceptibility to a variety of antistreptococcal and antistaphylococcal drugs. They have inherent resistance to almost all cephalosporins, antistaphylococcal penicillins, and aztreonam (with the probable exceptions of ceftaroline and ceftobiprole, which are effective against *E. faecalis* in vitro). Although strains of *E. faecalis* are responsive to carbapenems in vitro, their application in the management of human infections is supported by little clinical evidence. *Enterococcus* are naturally Vancomycin susceptible, however they are clinically significant resistance to trimethoprim-sulfamethoxazole, clindamycin and clinically attainable aminoglycoside doses. *Enterococcus* are innately susceptible to Erythromycin and Tetracycline, while these antibiotics often cause acquired resistance (regarding tigecycline) (Florescu *et al.,* 2008). *Enterococci* are resistant to daptomycin, televancin, oritavancin, linezolid and tedizolid, but *E. faecium* is solely resistant to the pristinamycin combination quinupristin-dalfopristin. Although ciprofloxacin's minimum inhibitory concentrations (MICs) are on the edge of being ineffective for non-urinary-tract infections, and clinical *E. faecium* strains frequently exhibit fluoroquinolone resistance (de Lastours *et al.*, 2017).

In the clinic, ampicillin is still the preferred treatment for strains that are susceptible in individuals who can handle it. Enterococcal resistance to β-lactams is linked with the production of low affinity penicillin-binding protein (PBPs) called PBP4 in *E. faecalis* and PBP5 in *E. faecium* (Infante *et al.,* 2016). Strains lacking these pbp genes exhibit decreased MICs for active

β-lactams and increases into the susceptible range for β-lactams with limited activity against wild-type strains (Arbeloa *et al.,* 2004). Many enterococcal strains are likewise resistant to the bactericidal activity of active β-lactams, with MICs far above bactericidal concentrations that are minimal. With cure rates of around 40% with only β-lactam antibiotics, this tolerance has therapeutic implications in the treatment of endocarditis.

In vitro, combining gentamicin or streptomycin with an active β-lactam brings about bactericidal synergism, with clinical cure rates of more than 70%. In experiments conducted by Moellering and Weinberg (1970), improved streptomycin entry into the cell was due to the presence of vancomycin or penicillin, this suggests that the aminoglycoside provided the killing activity once it had entered into the cell, which was facilitated by the cell wall-active agent. According to recent clinical data, the effectiveness of treating *E. faecalis* endocarditis with combinations of ampicillin, which is active against *E. faecalis*, and ceftriaxone, which is not, are comparable (Pericas *et al.,* 2014). It is considered that combining both antibiotics reduces all *E. faecalis* PBPs more effectively than either antibiotic does, despite the fact that the exact mechanism underlying this apparent clinical synergism is currently unknown. The limited penetration of aminoglycosides through the enterococcal cell envelope has been blamed for resistance to therapeutically feasible doses (John *et al*., 2016). The cause of this limited penetration is unknown, however it was suggested that the majority of enterococcal metabolism is anaerobic, inhibiting aminoglycoside transfer across the cytoplasmic membrane, which requires oxygen. The ABC superfamily of proteins that provides resistance to lincosamides, streptogramin and pleuromutilins is thought to be encoded by the ls(A) gene, a cell-derived antibiotic, is responsible for clindamycin resistance in *E. faecalis* (Singh *et al.,* 2002). Trimethoprim-sulfamethoxazole rsistance in *enterococci* is an invivo phenomena. In vitro testing

suggests that this combination is effective against wild-type *Enterococci*, however trimethoprimsulfamethoxazole is ineffective in animal models for treating enterococcal infections. This appears to be related to *Enterococci*'s ability to take in folate from the environment while avoiding the stages of folate synthesis that are hindered by the combination (Gilmore, 2002). There is no convincing clinical evidence that trimethoprim-sulfamethoxazole is effective in the treatment of human enterococcal infections.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 MATERIALS

### 3.1.1 Equipment Used

The major equipment used are:

Anaerobic jar, anaerogen, autoclave, petri-dishes, microscope, slides, refrigerator, weighing balance, measuring cylinder and antibiotic disk.

### 3.1.2 Reagents and Media Used

The media used in this study were; Clostridia agar (Titan Biotech Ltd India, December 2024), Nutrient agar (HiMedia Laboratories Pvt Ltd, February 2025), Mueller-Hinton agar (HiMedia Laboratories Pvt Ltd, September 2024), Mannitol salt agar (HiMedia Laboratories Pvt Ltd February 2024), Urease agar (HiMedia Laboratories Pvt Ltd December 2024), ethanol, peptone water, hydrogen peroxide, oil immersion, safranin, crystal violet, iodine, decoloriser, starch soluble, sodium chloride, phenol red, kovacs reagent. All the culture media were prepared as directed by the manufacturer.

## 3.2 METHODS

### 3.2.1 Sample Collection

A total of forty four (44) samples comprising of twenty one (21) stool samples, seven (7) waste water samples and sixteen (16) soil samples were collected for this study. The samples were

gathered in sterile bottles, given the proper labels, and sent to Caleb University, Imota Microbiology Laboratory of the Department of Biological Sciences and Biotechnology for analysis.

### 3.2.2 Isolation of *Enterococcus* spp.

The isolation of *Enterococcus* species was carried out using blood agar. Samples were directly inoculated on the agar plates by streaking with a wire loop then was incubated anaerobically using a anaerobic jar with anaerogen ($CO_2$ generating kit) at 37℃ for 48 hours. The plates were observed for smooth, creamy or whitish colonies appearance characteristics of *Enterococcus spp* then were selected and sub cultured until pure colonies were obtained.

### 3.2.3 Identification of *Enterococcus* spp.

*Enterococcus* spp colonies are identified using the morphological and biochemical characteristics.

#### 3.2.3.1 Morphological characteristics of *Enterococcus*

Morphological characteristics of *Enterococcus* includes; the shape, form, size, colour, elevation, edge, optical characteristics, texture surface, smell, Gram type.

#### 3.2.3.2 Biochemical Test of *Enterococcus*

The isolates were subjected biochemical characterization using;

##### 3.2.3.2.1 Catalase Test

Colonies were picked from the isolates using a sterile loop and placed on a clean grease-free glass slide; a drop of 3 percent Hydrogen Peroxide was added to the colony.

#### 3.2.3.2.2 Gram Staining

Colonies of the isolates were selected, put on a clean grease-free glass slide, smeared and let to dry. The smear were flooded for 30 seconds with crystal violet and rinsed with distilled water, they were flooded for 30 seconds with iodine and rinsed, decolorized with acetone and rinsed immediately. Finally, safranin was then added, allowed to sit for 30 seconds, washed off with distilled water and was air dried. Immersion oil was dropped on a glass slide and examined under the microscope using the objective lens x100.

#### 3.2.3.2.3 Sugar Fermentation Test

This was carried out in test tube containing fermentable sugars including glucose, mannitol, arabinose, raffinose and sucrose, prepared using peptone water with the addition of an indicator (phenol red). The isolates were inoculated into the sugar test tubes and incubated anaerobically using an anaerobic jar with anaerogen for 48hours.

#### 3.2.3.2.4 Indole Test

Prepared peptone water was dispensed into tubes sterilized at $121^0$C for 15 minutes. The isolates were inoculated into it and incubated at $37^0$C for 48 hours. A few drops of Kovac's reagent were added into the tubes after incubation.

#### 3.2.3.2.5 Urease Test

According to the manufacturer's instructions, Christensen's urea agar was prepared and dispensed into a sterile test tube, making it slant. Pure bacteria colonies were picked and inoculated in the slant and was incubated.

#### 3.2.3.2.6 Hemolysis Test

Supplemented clostridia agar with Blood agar was prepared, dispensed into a sterile petri-dish and allowed to solidify, after which the culture was streaked on the medium and then incubated anaerobically for 48hours.

#### 3.2.3.2.7 Starch Hydrolysis

One percent starch agar was prepared, autoclaved for 15minute and then dispensed into plates and allowed to set. The isolates were then spot inoculated into the plates and incubated anaerobically at $37^0$C for 48 hours, followed incubation. After incubation period, the plates were flooded with iodine solution and the result observed.

#### 3.2.3.2.8 Motility Test

Nutrient agar was prepared then dispensed into test tubes at half strength, making the bottles stand then colonies were stab in the middle of the bottles using a sterile needle. The test tubes were incubated anaerobically at $37^0$C for 48hours.

#### 3.2.3.2.9 Growth in 6.5% Sodium Chloride

Nutrient agar with sodium chloride was prepared and sterilized for 15 minute and poured in plates. The isolate were inoculated into the plates and was incubated for 48 hours anaerobically.

#### 3.2.3.2.10 Growth under aerobic condition

Clostridia agar supplemented with blood agar was prepared and isolates were inoculated on it and incubated for 24 hours aerobically.

### 3.2.3.2.11 Antimicrobial Susceptibility Test

The test was carried out using the Disk Diffusion Method on Mueller Hinton Agar. The antibiotic discs used were Ampicillin, Gentamicin, Vancomycin, Ciprofloxacin, Metronidazole, Tetracyclin, Erythromycin and Ceftazidime.

Normal saline was prepared, put into test tubes, then sterilized. Pure isolate cultures were inoculated into sterile normal saline and the cell suspension's turbidity was adjusted to 0.5 Macfarland standards. Sterile swab sticks were used to inoculate the isolates onto Mueller Hinton Agar plates. Using a sterile forcep, antibiotics disks were placed on the surface of the agar plates and the plates were then incubated aerobically at $37^0$C for 48 hours. Using a transparent ruler the zones of inhibition of the isolates by the antibiotics were observed, measured and recorded in millimeter (mm) and then results were interpreted.

# CHAPTER FOUR

## RESULTS

The occurrence of *Enterococcus* isolates in stool, soil and waste water samples used in this study was 34.1%(15/44) (Table 4.1). The highest occurrence was observed in stool samples which were 52.4%(11/21) while that of waste water and soil samples was 28.6%(2/7) and 12.5%(2/16), respectively.

The morphological characteristics of the isolates showed punctiformed cream colouration, with elevation ranging from flat to raised with entire edges, dry surface texture, opaque, with foul smell and they were all gram positive cocci. The Gram reaction was determined with purple colour indicating Gram positive while pink colour indicating gram negative organisms. The cell shape of the isolates was also determined (Table 4.2).

The biochemical characteristics of the isolates and the probable identity which are *Enterococcus faecium* and *Enterococcus faecalis* were identified. All isolates were catalase negative, the appearance of bubbles indicated a positive reaction for catalase production while the absence of bubbles indicates negative reaction. For the sugar fermentation test, colour change from red to yellow indicated positive result, no colour change indicated negative. A red or pink ring formed indicates a positive result for indole test. This test was carried out to identify bacteria that have the ability to degrade the amino acid tryptophan.

Urease production was indicated by a bright pink colour after incubation. The urease test identifies those organisms that are capable of hydrolyzing urea to produce ammonia and carbon dioxide. A clear zone around the colony indicated beta hemolysis, a partial clear zone indicates alpha hemolysis, and no clear zones indicated gamma hemolysis.

Starch Hydrolysis test carried out showed Positive test result which indicated a clear zone around the line of growth which means the organism has hydrolyzed starch while negative test result indicated a blue, purple, black colour on the medium.

Positive result for motility test was indicated by growths that spread throughout the medium making it slightly opaque while negative result was indicated by growth that is confined to the stabbed line leaving the surrounding medium transparent. The presence of growth in 6.5% Sodium Chloride indicated positive result while absence of growth indicated negative result. Growth on the plates after incubation aerobically indicated positive result while no growth indicated negative result (Table 4.3).

The frequency of occurrence of the *Enterococcus* isolates were expressed (Table 4.2). Majority of the isolates (66.7%) were *Enterococcus faecalis* while others *Enterococcus faecium* (33.3%).

The antibiotic susceptibility of all the *Enterococcus* isolates to antibiotics was shown (Table 4.5). In this study all isolates had 60% resistance to Gentamicin, 46.7% resistance to Vancomycin, 93.3% resistance to Metronidazole, 13.3% resistance to Tetracycline, 53.3% resistance to Ceftazidime.

The antibiotic susceptibility of *Enterococcus faecalis* isolates was shown (Table 4.6). In this study 40% was susceptible to Gentamicin, 70% was susceptible to Vancomycin, 100% was susceptible to Ciprofloxacin, 10% was susceptible to Metronidazole, 90% was susceptible to Tetracycline, 50% were susceptible to Erythromycin, and 30% was susceptible to Ampicillin.

The antibiotic susceptibility of *Enterococcus faecium* isolates was shown (Table 4.7). In this study all isolates had 60% resistance to Gentamicin, 80% resistance to Vancomycin, 100%

resistance to Metronidazole, 21% resistance to Tetracycline, 100% resistance to Erythromycin, 40% resistance to Ampicillin and Ceftazidime.

The antibiotic resistance of the *Enterococcus* isolates from different samples was shown (Table 4.8). Isolates from stool samples were 54.5% resistant to Gentamicin, 36.4% were resistant to vancomycin, 0% showed resistance to ciprofloxacin, 90.9% shows resistance to metronidazole, 9.1% shows resistance to Tetracyclin, 45.5% shows resistance to Erythromycin, 63.6% shows resistance to Ampicillin, 81.8% shows resistant to ceftazidime. Isolates from wastewater samples were 100% resistant to Gentamicin, metronidazole, Erythromycin and Vancomycin, 0% shows resistant to ciprofloxacin, 50% resistant to Tetracyclin, Ampicillin and Ceftazidime. 50% of isolates from soil sample were resistant to Gentamicin, erythromycin, ampicillin and Vancomycin, 0% was resistant to metronidazole and Tetracycline, 100% shows resistant to Ceftazidime.

**Table 4.1: The occurrence of *Enterococcus* spp in the sample**

| Sample | Number of Samples (%) | Occurrence of *Enterococcus* spp (%) |
|---|---|---|
| Stool | 21(47.7) | 11(52.4) |
| Waste Water | 7(15.9) | 2(28.6) |
| Soil | 16 (36.4) | 2(12.5) |
| Total | 44 (100) | 15(34.1) |

**Table 4.2: The morphological characteristics of the isolates**

| Isolate code | Form | Colour | Elevation | Edge | Optical characteristics | Texture surface | Smell | Gram type | Gram shape |
|---|---|---|---|---|---|---|---|---|---|
| S1a | Punctiform | Cream | Flat | Entire | Opaque | Dry | Foul | + | Cocci |
| S1b | Punctiform | Cream | Flat | Entire | Opaque | Dry | Foul | + | Cocci |
| S2a | Punctiform | Cream | Raised | Entire | Opaque | Creamy | Foul | + | Cocci |
| S3a | Punctiform | Cream | Flat | Entire | Opaque | Dry | Foul | + | Cocci |
| S3b | Punctiform | Cream | Flat | Entire | Opaque | Dry | Foul | + | Cocci |
| S4a | Punctiform | Cream | Raised | Undulate | Opaque | Creamy | Foul | + | Cocci |
| S5a | Punctiform | Cream | Flat | Entire | Opaque | Dry | Foul | + | Cocci |
| S6a | Punctiform | Cream | Raised | Undulate | Opaque | Creamy | Foul | + | Cocci |
| S7a | Punctiform | Cream | Flat | Entire | Opaque | Dry | Foul | + | Cocci |
| S8a | Punctiform | Cream | Flat | Entire | Opaque | Dry | Foul | + | Cocci |
| S9a | Punctiform | Cream | Flat | Entire | Opaque | Creamy | Foul | + | Cocci |
| WG1 | Punctiform | Cream | Flat | Entire | Opaque | Creamy | Foul | + | Cocci |
| WF1 | Punctiform | Cream | Flat | Entire | Opaque | Creamy | Foul | + | Cocci |
| SF1 | Punctiform | Cream | Raised | Entire | Opaque | Creamy | Foul | + | Cocci |
| SA1 | Punctiform | Cream | Flat | Entire | Opaque | Dry | Foul | + | Cocci |

**Key: + Positive**

**Table 4.3: Biochemical characteristics of the isolates and their probable identity**

| Isolate code | Catalase | Sucrose | Mannitol | Urease | Glucose | Starch hydrolysis | Growth in 6.5% sodium Chloride | Raffinose | Arabinose | Motility | Indole | Hemolysis | Growth under aerobic condition | Organism |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S1a | _ | + | + | _ | + | _ | _ | _ | _ | _ | _ | γ | + | *E.faecalis* |
| S1b | _ | + | + | _ | + | _ | + | _ | _ | _ | _ | γ | + | *E.faecalis* |
| S2a | _ | + | + | _ | + | + | + | _ | _ | _ | _ | γ | + | *E.faecalis* |
| S3a | _ | + | + | _ | + | _ | + | _ | _ | _ | _ | γ | + | *E.faecalis* |
| S3b | _ | + | + | _ | + | _ | + | _ | _ | _ | _ | γ | + | *E.faecalis* |
| S4a | _ | + | _ | _ | + | _ | + | _ | + | _ | _ | γ | + | *E.faecium* |
| S5a | _ | + | + | _ | + | _ | + | _ | _ | _ | _ | γ | + | *E.faecalis* |
| S6a | _ | + | _ | _ | + | _ | + | _ | + | _ | _ | γ | + | *E.faecium* |
| S7a | _ | + | + | _ | + | _ | + | _ | _ | _ | _ | γ | + | *E.faecalis* |
| S8a | _ | + | _ | _ | + | _ | + | _ | + | _ | _ | γ | + | *E.faecium* |
| S9a | _ | + | + | _ | + | _ | + | _ | _ | _ | _ | γ | + | *E.faecalis* |
| WG1 | _ | + | _ | _ | + | + | + | _ | + | _ | _ | γ | + | *E.faecium* |
| WF1 | _ | + | _ | _ | + | _ | + | _ | + | _ | _ | γ | + | *E.faecium* |
| SF1 | _ | + | + | _ | + | _ | + | _ | _ | _ | _ | γ | + | *E.faecalis* |
| SA1 | _ | + | + | _ | + | _ | + | _ | _ | _ | _ | γ | + | *E.faecalis* |

Key: - Negative, + Positive

**Table 4.4: Frequency of the occurrence of the *Enterococcus* isolates**

| Isolates | Number | % |
|---|---|---|
| *Enterococcus faecalis* | 10 | 66.7 |
| *Enterococcus faecium* | 5 | 33.3 |
| Total | 15 | 100 |

**Table 4.5: Antibiotic susceptibility of all the *Enterococcus* isolates**

| Antibiotics | Susceptible (%) | Intermediate (%) | Resistance (%) |
|---|---|---|---|
| **Gentamicin** | 6(40) | 0(0) | 9(60) |
| **Vancomycin** | 8(53.3) | 0(0) | 7(46.7) |
| **Ciprofloxacin** | 15(100) | 0(0) | 0(0) |
| **Metronidazole** | 1(6.7) | 0(0) | 14(93.3) |
| **Tetracycline** | 12(80) | 1(6.7) | 2(13.3) |
| **Erythromycin** | 5(33.3) | 2(13.3) | 8(53.3) |
| **Ampicillin** | 6(40) | 0(0) | 9(60) |
| **Ceftazidime** | 3(20) | 0(0) | 12(80) |

**Table 4.6: Antibiotic susceptibility of *Enterococcus faecalis* isolates**

| Antibiotics | Susceptible (%) | Intermediate (%) | Resistance (%) |
|---|---|---|---|
| **Gentamicin** | 4(40) | 0(0) | 6(60) |
| **Vancomycin** | 7(70) | 0(0) | 3(30) |
| **Ciprofloxacin** | 10(100) | 0(0) | 0(0) |
| **Metronidazole** | 1(10) | 0(0) | 9(90) |
| **Tetracycline** | 9(90) | 1(10) | 0(0) |
| **Erythromycin** | 5(50) | 2(20) | 3(30) |
| **Ampicillin** | 3(30) | 0(0) | 7(70) |
| **Ceftazidime** | 0(0) | 0(0) | 10(100) |

**Table 4.7: Antibiotic susceptibility of *Enterococcus faecium* isolates**

| Antibiotics | Susceptible (%) | Intermediate (%) | Resistance (%) |
|---|---|---|---|
| **Gentamicin** | 2(40) | 0(0) | 3(60) |
| **Vancomycin** | 1(20) | 0(0) | 4(80) |
| **Ciprofloxacin** | 5(100) | 0(0) | 0(0) |
| **Metronidazole** | 0(0) | 0(0) | 5(100) |
| **Tetracycline** | 4(80) | 0(0) | 1(21) |
| **Erythromycin** | 0(0) | 0(0) | 5(100) |
| **Ampicillin** | 3(60) | 0(0) | 2(40) |
| **Ceftazidime** | 3(60) | 0(0) | 2(40) |

**Table 4.8: Comparative antibiotic resistance of the *Enterococcus* isolates from the different samples**

| Samples | Stool (%) | Waste water (%) | Soil (%) |
|---|---|---|---|
| **GENTAMICIN** | 6(54.5) | 2(100) | 1(50) |
| **VANCOMYCIN** | 4(36.4) | 2(100) | 1(50) |
| **CIPROFLOXACIN** | 0 | 0 | 0 |
| **METRONIDAZOLE** | 10(90.9) | 2(100) | 2(100) |
| **TETRACYCLIN** | 1(9.1) | 1(50) | 0 |
| **ERYTHROMYCIN** | 5(45.5) | 2(100) | 1(50) |
| **AMPICILLIN** | 7(63.6) | 1(50) | 1(50) |
| **CEFTAZIDIME** | 9(81.8) | 1(50) | 2(100) |

**Key words:**

**CN- Gentamicin,** **VA- Vancomycin** **CIP- Ciprofloxacin**

**MTZ- Metronidazole** **TE- Tetracycline** **E- Erythromycin**

**AMP- Ampicillin** **CAZ- Ceftazidime**

# CHAPTER FIVE

## DISCUSSION AND CONCLUSION

### 5.1 Discussion

*Enterococci* are part of human intestinal flora and also found in soil and water. They have proven to be a major cause of community acquired pathogens and hospital acquired infections. In this study 52% occurrence of *Enterococcus* specie in stool samples is lower compared to what was reported in a study by Ismail and Sevil (2018) where 76% occurrence was recorded in stool samples. This variation can be as a result of size of the sample used in this study. The occurrence of *Enterococcus* species in environmental samples (wastewater and soil samples) in this study, 17.4%(4/23) is less than a study by Ndubuisi *et al*., (2017) who reported 25%(5/100) with sample size of 500 occurrence of *Enterococcus* species in environmental samples.

The prevailing *Enterococcus* specie in this study are *Enterococcus faecalis* and *Enterococcus faecium*, with *Enterococcus faecalis* having the highest prevalence of 66.7% and *Enterococcus faecium* had low prevalence of 33.3%. This result is comparable to a study by Ndubuisi *et al*., (2017) where *Enterococcus faecalis* was predominant with the percentage of 57.8% and *Enterococcus faecium* 23.5%.

In this study antibiotics susceptibility of the *Enterococcus* isolates in stool shows that 54.5% were resistant to gentamicin and this is lower compared to 80% which was reported by (Jabin *et* al., 2014) in a research on involvement of *Enterococcus* species in urinary tract infections. Similarly, 36.4% resistance to Vancomycin recorded in this study is lower compared to 34.61% reported by Ashagrie *et al*., (2021). 90.9% were resistant to metronidazole, 9.1% were resistant

to tetracycline, 45.5% were resistant to Erythromycin, 63.6% were resistant to ampicillin and 81.8% were resistant to ceftazidime.

In this study highest resistance to Gentamicin is 100%, Vancomycin (100%), Metronidazole (100%) and Erythromycin (100%) was recorded from wastewater while the lowest resistance to Gentamicin (50%), Vancomycin (50%), Erythromycin (50%) and Ampicillin was recorded in isolates from soil samples. There was no resistance to Ciprofloxacin in any of the isolates from the different samples.

### 5.2 Conclusion

The occurrence of *Enterococcus* specie in stool, water and soil sample. This prevalence could lead to occurrence of diseases caused by *Enterococcus.*

### 5.3 Recommendation

*Enterococcus* ability for infection and resistance has kept them ahead of efforts to limit the harm they cause to the health care system. There will be a need for methods to stop their spread and get rid of them from infected areas that are enterococcus prevalence in the environment.

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# APPENDIX

## APENDIX I

The antibiotic susceptibility of the isolates

| Sample code | CN | VA | CIP | MTZ | TE | E | AMP | CAZ |
|---|---|---|---|---|---|---|---|---|
| 1 | 21 | R | 31 | R | 20 | R | R | R |
| 2 | 15 | 21 | 22 | R | 19 | 12 | R | R |
| 3 | 13 | 22 | 30 | R | 24 | 23 | R | 16 |
| 4 | 23 | 23 | 31 | R | 30 | 25 | R | 12 |
| 5 | 28 | 30 | 30 | 24 | 18 | 33 | 30 | 16 |
| 6 | 21 | R | 32 | R | 23 | 10 | 10 | 33 |
| 7 | 19 | 10 | 22 | R | 33 | 25 | 27 | R |
| 8 | 21 | R | 30 | R | 12 | 10 | 20 | 30 |
| 9 | 19 | 23 | 30 | R | 34 | 16 | R | R |
| 10 | 19 | 25 | 32 | R | 34 | 12 | 31 | 21 |
| 11 | 19 | 22 | 33 | 18 | 30 | 32 | R | 20 |
| 12 | 11 | R | 25 | R | 22 | 12 | 24 | R |
| 13 | 15 | R | 25 | R | R | 11 | R | 35 |
| 14 | 11 | R | 25 | R | 23 | R | R | R |
| 15 | 20 | 20 | 30 | R | 24 | 22 | 28 | R |

**Key words:**

**CN- Gentamicin,** **VA- Vancomycin** **CIP- Ciprofloxacin**

**MTZ- Metronidazole** **TE- Tetracycline** **E- Erythromycin**

**APENDIX II**

**The antibiotic susceptibility of the isolates compared with CLSI standard**

| Isolate code | Gentamicin | Vancomycin | Ciprofloxacin | Metronidazole | Tetracycline | Erythromycin | Ampicillin | Ceftazidime |
|---|---|---|---|---|---|---|---|---|
| SF | S | R | S | R | S | R | R | R |
| SF | R | S | S | R | S | R | R | R |
| SF | R | S | S | R | S | S | R | R |
| SF | S | S | S | R | S | S | R | R |
| SF | S | S | S | S | R | S | S | R |
| SFC | S | R | S | R | S | R | R | S |
| SF | R | R | S | R | S | S | S | R |
| SFC | S | R | S | R | R | R | S | S |
| SF | R | S | S | R | S | R | R | R |
| SFC | R | S | S | R | S | R | S | R |
| SF | R | S | S | R | S | S | R | R |
| SFC | R | R | S | R | S | R | S | R |
| SFC | R | R | S | R | R | R | R | S |
| SF | R | R | S | R | S | R | R | R |
| SF | S | S | S | R | S | R | S | R |

**Key: S- Sensitive**

**R- Resistant**

**SF- Sample *faecalis***

**SFC- Sample *faecium***