

**INCIDENCE OF ANTIBIOTICS RESISTANT ENTEROCOCCI  
ISOLATED FROM HOSPITAL ENVIRONMENTS**

**BY**

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## CERTIFICATION

We certify that ARULEBA OLUWATOBI SUNDAY, with Matric. Number, MCB/11/0345, carried out this research work under the supervision of Dr. S.K.S. Ojo of the department of Microbiology, Faculty of Science; Federal University Oye-Ekiti.



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## **DEDICATION**

This research project is dedicated to God Almighty who preserves me all through the period of my learning as an undergraduate and also for giving me the opportunity to complete this research project.



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## ABSTRACT

Surface and equipment swabs were taken from two different Hospitals (Federal Medical center Ido-Ekiti, and Federal University Oye-Ekiti Health Center) to assay for the presence of antibiotic resistant enterococci. By using the disc diffusion method of antimicrobial susceptibility testing, the Emergency ward of FMC Ido-Ekiti was found to have the highest total viable count (TVC) of  $105 \times 10^3$  CFU while TVC of  $4 \times 10^2$  CFU was recorded in Male ward of FUYOYE Health Center. Biochemical tests revealed the presence of *Enterococcus faecalis* isolated from Emergency and Labour ward of FMC Ido-Ekiti and also from Female ward of FUYOYE Health Center. Antibiotics sensitivity tests were performed and results showed sensitivity to Gentamycin(25.2mm) and Ciprofloxacin(19.3mm); but resistance towards Cefazidime(0.00mm), Cefuroxime(0.00mm), and Amoxycillin(0.00mm). In order to avert the spread of nosocomial infection within health care centers; high levels of hygiene of the hospital environments as well as periodic monitoring of its microbial quality must be enforced. Isolated pathogens must be duly subjected to antimicrobial susceptibility testing to gain the knowledge of potent antibiotics capable of combating nosocomial pathogens.



## CHAPTER ONE

### 1.0 INTRODUCTION

Enterococci are part of normal flora of gastrointestinal tract of humans and animals, but they have also emerged as significant cause of serious infection such as endocarditis, urinary and blood stream infections, intra-abdominal and intra-pelvic abscesses ( Teixeira and Facklan, 2003). Among enterococci, *Enterococcus faecalis* and *Enterococcus faecium*, are responsible for the majority of infections in many cases of intra-hospital infections, and are becoming resistant to a wide range of antibiotics (Sievert *et al.*, 2013; Bereket, 2012; Hidron *et al.*, 2008; Chou, 2008; Low *et al.*, 2001).

Enterococci have both an intrinsic and acquired resistance (Murray, 2008). They are intrinsically resistant to penicillinase-susceptible penicillins, penicillinase-resistant penicillins, cephalosporins, sulphonamides, clindamycin and aminoglycosides. Acquired resistance to penicillins, chloramphenicol, tetracyclin, aminoglycosides and vancomycin evolves either by mutation or transfer of plasmids and transposons. Resistance of many beta-lactams is as a result of over-production and modification of penicilin-binding-proteins (PBPs), particularly PBP5, with low affinity for antibiotics (Brown *et al.*, 2006). Enterococci are among the most frequent causes of nosocomial infection, particularly in intensive care units (ICU). They are transmitted from person to person primarily on the hand of hospital personnel, some of whom may carry the organism in their gastrointestinal tracts. Occasionally, enterococci are transmitted on medical devices (Hidron *et al.*, 2008). In patients, the most common sites of enterococci infections are the urinary tract, wound and biliary tract along with other species of bacteria where it may be difficult to define the pathogenic role of the enterococci (Murray, 2008). In neonates, meningitis and bacteraemia may occur while endocarditis may occur in adults. Enterococci infection is



equally distributed between sexes (Gordon *et al*, 2009), although urinary tract infections are more common in healthy women than men and in elderly patients due to high incidence of urinary instrumentation.

In the routine microbiology laboratory, enterococci are distinguished from the non-group D streptococci by their ability to survive in the presence of 40% bile, ability to hydrolyze aesculin, growth in 6.5% NaCl and a positive pyrrolidonylarylamidase test (Facklam *et al*, 2002). The treatment of enterococci infection is usually problematic because they are usually resistant to  $\beta$ -lactam antibiotics and aminoglycosides, though synergistic action of a combination of these drugs may be effective. The glycopeptide, vancomycin was the drug of choice but resistance to this drug has emerged. As a result a combination of quinupristin and dalfopristin are employed to treat vancomycin resistant enterococci infection (Sievert *et al.*, 2013).

### **1.1. AIM AND OBJECTIVES OF STUDY**

The aim of this research is to isolate *Enterococci* species in different section of Federal Medical Center Ido-Ekiti and the Health Center of Federal University Oye-Ekiti. Specific objectives of this study are:

1. To determine the prevalence and distribution of *Enterococci* species in various sections of the hospital, (Emergency ward, Labour ward, Children ward, Male and Female ward).
2. To evaluate the susceptibility patterns of the isolated *Enterococci* species to certain antibiotics (antibacterial).



## CHAPTER TWO

### 2.0

### LITERATURE REVIEW

#### 2.1. DESCRIPTION OF ENTEROCOCCI

*Enterococcus* is a large genus of lactic acid bacteria of the phylum *Firmicutes*. *Enterococci* are Gram-positive cocci that often occur in pairs (diplococci) or short chains, and are difficult to distinguish from streptococci on physical characteristics alone (Gilmore *et al.*, 2002). Two species are common commensal organisms in the intestines of humans: *E. faecalis* (90-95%) and *E. faecium* (5-10%). Rare clusters of infections occur with other species, including *E. casseliflavus*, *E. gallinarum*, and *E. raffinosus*. Enterococci are facultative anaerobic organisms, that is, they are capable of cellular respiration in both oxygen-rich and oxygen-poor environments. (Fischetti *et al.*, 2000). Though they are not capable of forming spores, enterococci are tolerant of a wide range of environmental conditions: extreme temperature (10-45°C), pH (4.5-10.0) and high sodium chloride concentrations (Fisher *et al.*, 2009). Enterococci typically exhibit gamma-hemolysis on sheep's blood agar (Ryan *et al.*, 2004). Members of the genus *Enterococcus* (from Greek, *éntero*, "intestine" and, *coccus*, "granule") were classified as Group D *Streptococcus* until 1984, when genomic DNA analysis indicated that a separate genus classification would be appropriate (Schleifer *et al.*, 1984). In the routine microbiology laboratory, enterococci are distinguished from the non-group D streptococci by their ability to survive in the presence of 40% bile, ability to hydrolyze aesculin, growth in 6.5% NaCl and a positive pyrrolidonylarylamidase test (Facklam *et al.*, 2002). The treatment of enterococci infection is usually problematic because they are usually resistant to  $\beta$ -lactam antibiotics and aminoglycosides, though synergistic action of a combination of these drugs may be effective.



The glycopeptide, vancomycin was the drug of choice but resistance to this drug is now on the increase. Newer antibiotics, such as the combination of quinupristin and dalfopristin are currently used to treat vancomycin resistant enterococci infection (Arias & Murray, 2008)

## 2.2 ENTEROCOCCI AS PATHOGENS

Important clinical infections caused by *Enterococcus* include urinary tract infections, bacteremia, bacterial endocarditis, diverticulitis, and meningitis. Sensitive strains of these bacteria can be treated with ampicillin, penicillin and vancomycin. Urinary tract infections can be treated specifically with nitrofurantoin, even in cases of vancomycin resistance. Some enterococci are intrinsically resistant to  $\beta$ -lactam-based antibiotics (penicillins, cephalosporins, carbapenems), as well as many aminoglycosides. In the last two decades, particularly virulent strains of *Enterococcus* that are resistant to vancomycin (vancomycin-resistant *Enterococcus*, or VRE) have emerged in nosocomial infections of hospitalized patients, especially in the US (Mutnick *et al.*, 2003). Other developed countries, such as the UK, have been spared this epidemic, and, in 2005, Singapore managed to halt an epidemic of VRE. VRE may be treated with quinupristin/dalfopristin (Synercid) with response rates of approximately 70%. Tigecycline has also been shown to have anti-enterococcal activity as rifampicin (Gilmore, 2002).

Enterococcal meningitis is a rare complication of neurosurgery. It often requires treatment with intravenous or intrathecal vancomycin, yet it is debatable as to whether its use has any impact on outcome: the removal of any neurological devices is a crucial part of the management of these infections. New epidemiological evidence has shown that enterococci are major infectious agent in chronic bacterial prostatitis. Enterococci are able to form biofilm in the prostate gland making



their eradication difficult. In Europe, infection with *Enterococcus* species was considered harmless to humans for a long time. However in the last decade enterococci have been reported as the second most common cause of wound and urinary tract infection and the third most common cause of bacteraemia (De Fátima *et al.*, 2005). In 2005 in the UK there were 7066 reported cases of *Enterococcus* bacteraemia, 63% of these cases being due to *E. faecalis* and 28% to *E. faecium*, both of which have increasing antibiotic resistance (Health Protection Agency, 2007). In the USA approximately 12% of the hospital-acquired infections are *Enterococcus* species. *E. faecalis* is the most common species associated with clinical infection while *E. faecium* poses the higher antibiotic resistance threat (Giraffa, 2002).

### **2.3. CLINICAL EPIDEMIOLOGY**

*Enterococcus* specie is the third most frequent cause of hospital-acquired bacteremia, accounting for 8% of all nosocomial bacteremias in the United States (Karlowsky *et al.*,2004). High-level gentamicin resistance (HLGR) has emerged among *Enterococcus* spp over the last two decades, and is associated with the loss of synergy between cell-active antibiotics (e.g., vancomycin and penicillin, particularly ampicillin) and most aminoglycosides (e.g., gentamicin, tobramycin, amikacin, and kanamycin), thus posing additional treatment challenges (Moellenrig *et al.*,1992). Enterococcal bacteremia is associated with high mortality, which is even further increased when caused by HLGR strains compared with those caused by non HLGR strains. In Brazil, clinical and epidemiological studies of enterococcal infections have been less comprehensive, with most studies focusing on vancomycin-resistant *Enterococcus* (VRE), which is still restricted to a few Brazilian hospitals. Since *Enterococcus* specie is the second most common cause of Gram-positive blood stream infections in Brazil and that approximately 76% of all enterococcal



infections are due to *Enterococcus faecalis*. Clinical isolates of enterococci show a lower diversity than those obtained from the environment and other human sources, with *E. faecalis* being the dominant species (Kuhn *et al.*, 2003). The reason for this lack of diversity may be linked with the virulence factors associated with this species. The fact that *Enterococcus* species are opportunistic pathogens was highlighted by a study in Denmark which showed that hospitalized patients have a 57% isolation rate of *E. faecalis* whereas healthy individuals show only a 39–40% occurrence (Mutnick *et al.*, 2003). Hospitalized patients may have a greater incidence of enterococcal infection not only because of virulence, but because the hospital itself is a hub. This is illustrated by a report for the Department of Health in the UK, which highlighted the fact that enterococci may contaminate and survive around the patient for several days (Brown *et al.*, 2006). Enterococci also play a role in endodontic failure and are often isolated from the root canal system. The results of one study showed that out of 100 root-filled teeth with apical periodontitis, 69% of the isolated bacteria were facultative and 50% of those were enterococci (Dahlen *et al.*, 2006). *E. faecalis* is responsible for 80–90% of human enterococcal endodontic infection and is usually the only *Enterococcus* species isolated from the obturated root canal (Love, 2001; Peciuliene *et al.*, 2001).

## **2.4 FACTORS THAT INCREASE VIRULENCE AND GENETIC EXCHANGE MECHANISM IN *ENTEROCOCCI*.**

*Enterococcus* species with the highest virulence are medical isolates, followed by food isolates and then starter strains (Busani *et al.*, 2007; Omar *et al.*, 2004). Many factors determine the virulence of *Enterococcus* species, for example: ability to colonize the gastrointestinal tract,



which is the normal habitat; Ability to adhere to a range of extracellular matrix proteins, including thrombospondin, lactoferrin and vitronectin; and Ability to adhere to urinary tract epithelia, oral cavity epithelia and human embryo kidney cells.

Most infection is thought to be endogenous, by translocation of the bacteria through the epithelial cells of the intestine, which then cause infection via lymph nodes and thus spread to other cells within the body (Franz *et al.*, 2005). The aggregation substance (Agg) on the surface of *E. faecalis*, has been shown *in vivo* to form large aggregates and hence may contribute to pathogenesis. The presence of Agg increases the hydrophobicity of the enterococcal cell surface and induces localization of cholesterol to the phagosomes and is thought to delay or prevent fusion with lysosomal vesicles (Eaton & Gasson, 2002). Agg is a pheromone-inducible surface glycoprotein and mediates aggregate formation during conjugation, thus aiding in plasmid transfer as well as adhesion to an array of eukaryotic surfaces (Koch *et al.*, 2004). Pulsed-field gel electrophoretic analysis of clinical isolates of *E. faecalis* showed that the gene encoding Agg was not present in *E. faecium* isolates (Hällgren *et al.*, 2008). Another cell-surface protein present in *E. faecalis* is Ace (adhesion of collagen from *E. faecalis*). This is a collagen-binding protein, belonging to the microbial surface components recognizing adhesive matrix molecules (MSCRAMM) family. Ace may play a role in the pathogenesis of endocarditis (Koch *et al.*, 2004).

Extracellular surface protein (Esp) is a cell-wall-associated protein first described in *Enterococcus* species by (Shankar *et al.*, 1999). The *esp* gene consists of 5622 bp and is found at high frequency in infection-derived isolates. It is thought to promote adhesion, colonization and evasion of the immune system, and to play some role in antibiotic resistance (Foulquie *et al.*,



2006). Esp also contributes to enterococcal biofilm formation, which could lead to resistance to environmental stresses, and adhesion to eukaryotic cells such as those of the urinary tract (Borgmann *et al.*, 2004). Studies have shown that disruption of the *esp* gene impairs the ability of *E. faecalis* to form biofilms. Esp-negative *E. faecalis* strains, after receiving plasmid transfer of the *esp* gene, were able to produce biofilms (Latasa *et al.*, 2006). *E. faecium* strains that carry the gene *esp<sub>fm</sub>* have higher conjugation rates than strains that do not possess this gene. They also demonstrate higher resistance to ampicillin, ciprofloxacin and imipenem (Billström *et al.*, 2008).

The ability of enterococci to produce biofilms is fundamental in causing endodontic and urinary tract infections, as well as endocarditis. The formation of pili by enterococci is necessary for biofilm formation, the gene cluster associated with this being *ebp* (endocarditis- and biofilm-associated pili). The *ebp* operon consists of *ebpA*, *ebpB* *ebpC* and an associated *srtC* (encoding sortase C) gene (Singh *et al.*, 2007). A non-piliated mutant of *E. faecalis* was unable to produce a biofilm (Budzik & Schneewind, 2006). Enterococcal pili are heterotrimetric and the pilus shaft contains two minor pilins. A feature of Gram-positive pili is that a specific sortase is dedicated to their assembly (Mandlik *et al.*, 2008). The pili are constructed by cross-linking of multiple classes of precursor proteins that are assigned by sortases, which covalently anchor proteins with a C-terminal pilin-associated motif to the peptidoglycan (Nallapareddy *et al.*, 2006). *E. faecalis* contains two classes of sortase: sortase A links most proteins with a C-terminal sortase motif to



cell wall peptidoglycan, while sortase C is designated Bps (biofilm and pilus-associated sortase) and links the pilin subunits. Secreted virulence factors of *Enterococcus* species also have a function in pathogenesis. Cytolysin (also called haemolysin) is a bacterial toxin, the genes for the production of which are located on pheromone-responsive plasmids (Koch *et al.*, 2004). Cytolysin has  $\beta$ -haemolytic properties in humans and is bactericidal against other Gram-positive bacteria. The *cyll<sub>s</sub>* group of genes are the non-regulatory genes of the cytolysin operons (Hällgren *et al.*, 2008), and higher incidences of these genes occur in clinical isolates (33%, compared to 6% in food isolates) (Semedo *et al.*, 2003). Cytolysin is regulated by a quorum-sensing mechanism involving a two-component system. A group of hydrolytic enzymes including hyaluronidases, gelatinase and serine protease are involved in the virulence of *Enterococcus* species, although their precise roles are yet to be clearly understood (Semedo *et al.*, 2003). Hyaluronidase acts on hyaluronic acid and is a degradative enzyme which is associated with tissue damage. Hyaluronidase depolymerizes the mucopolysaccharide moiety of connective tissue, thus facilitating spread of enterococci as well as their toxins through host tissue (Kayaoglu & Orstavik, 2004). Hyaluronidase is encoded by the chromosomal *hyl* gene. One study showed that, out of 26 vancomycin-resistant *E. faecium* clinical isolates, seven (27%) carried the *hyl* gene, but it was found in only 14% of faecal isolates (Vankerckhoven *et al.*, 2004).



The main role of both gelatinase and serine protease in enterococcal pathogenesis is thought to be in providing nutrients to the bacteria by degrading host tissue, although they also have some function in biofilm formation (Mohamed & Huang, 2007; Gilmore, 2002). Gelatinase (GelE) is an extracellular zinc metallo-endopeptidase secreted by *E. faecalis* (Koch *et al.*, 2004). It is able to hydrolyse gelatin, casein, haemoglobin and other bioactive peptides. The gene (*gelE*) encoding GelE is located on the chromosome and is regulated in a cell-density-dependent manner. Another gene *sprE*, coding for a serine protease, is located directly downstream from and is cotranscribed with *gelE* (De Fátima *et al.*, 2006). Transcription of *gelE* and *sprE* is regulated in a growth-phase-dependent fashion by the quorum-sensing system encoded by the *fsr* (faecal streptococci regulator) locus (Sifri *et al.*, 2002). Quorum sensing occurs when a bacterial population produces a signal via an autoinducing peptide (AIP), regulated by a two-component system. AIP then accumulates in the environment by increased expression of the communication signal, or by increased numbers of cells producing the signal. Once the AIP reaches a threshold concentration, it interacts with a cell-surface receptor or re-enters the cell and causes a cascade of transcriptional regulation (Gobbetti *et al.*, 2007; Alksne & Projan, 2000). The *fsr* locus contains the *fsrA*, *fsrB* and *fsrC* genes. The *fsrA* gene is monocistronically transcribed into a response regulator, and *fsrB* and *fsrC*, encoding a processing enzyme and a sensor kinase respectively, are co-transcribed (Podbielski & Kreikemeyer, 2004). FsrB liberates gelatinase biosynthesis activating pheromone (GBAP) peptide, and with the accumulation of GBAP a transition from



exponential to stationary phase occurs and *gelE* and *sprE* are induced. It has been shown that in *E. faecalis* when mutations in *fsrA*, *fsrB* and *fsrC* are present, a reduction in biofilm formation of 28–32% occurs (Mohamed & Huang, 2007). 12 *E. faecalis* endocarditis strains has been shown to possess the *fsr* locus; and in a study by Podbielski and Kreikemeyer (2004) *fsr* locus was detected in 10 out of 19 stool strains, indicating the importance of *fsr* in virulence and disease.

## 2.5. ANTIBIOTIC RESISTANCE

The antibiotic resistance of *Enterococcus* is well documented, including resistance to glycopeptides such as vancomycin and teicoplanin, and to aminoglycosides (Kacmaz & Aksoy, 2005). Antibiotic resistance has been of growing concern for a number of years. Vancomycin was first used for treatment in 1972 and the first vancomycin-resistant enterococci were recognized only 15 years later. NNIS reported an increase of 7.6% in VRE between 1989 and 1993 (Metan *et al.*, 2005). It has been reported that if glycopeptide-resistant enterococci (GRE) are present in an infected patient rather than an antibiotic-susceptible strain, clinical treatment failure is increased by 20% and mortality is increased from 27% to 52% (Brown *et al.*, 2006). In both the Surveillance and Control of Pathogens of Epidemiological Importance (Antimicrobial Resistance Surveillance Program) databases, figures show that, of enterococcal isolates from the bloodstream, 2% of *E. faecalis* and 60% of *E. faecium* isolates are resistant to vancomycin



(Bearman & Wenzel, 2005). Resistance rates of *Enterococcus* species have reached endemic or epidemic proportions in North America, with Europe having lower, but increasing, levels (Mutnick *et al.*, 2003). Enterococcal antibiotic resistance is not exclusive to the clinical arena but is also prevalent in the food industry. The presence of VRE in individuals who have been hospitalized, when they have not previously been in hospital or taken antibiotics, suggests that VRE may have been contracted through the food chain. GRE may emerge in the food chain through use of avoparcin in animal feed (Manu *et al.*, 2003).

Glycopeptide resistance in enterococci involves a two-component system where the cell wall composition is altered from the peptidoglycan precursor d-Ala-d-Ala (vancomycin-susceptible) to d-Ala-d-lactate (d-Lac). The latter has 1000 times less affinity for vancomycin, while d-Ala-d-Ser has a sevenfold decrease in affinity for vancomycin, thus removing the susceptible target (Gilmore, 2002). The genes involved in this two-component system are *vanS/vanR*. The VanS sensor kinase is activated in response to vancomycin, resulting in the activation of d-Lac or d-Ser peptidoglycan precursor and the repression of d-Ala-d-Ala (Stephenson & Hoch, 2002). Six gene clusters associated with glycopeptide resistance have been identified in *Enterococcus* species: *vanA* to *vanG*. The three main types of resistance are those encoded by the *vanC*, *vanA* and *vanB* clusters. Intrinsic *vanC* resistance is specific to *E. gallinarum*, *E. casseliflavus* and *E. flavescens*, and the *vanC* operon is chromosomally located and is not transferable. The *vanA* resistance



operon comprises seven genes (*vanH*, *vanA*, *vanX*, *vanR*, *vanS*, *vanY* and *vanZ*) and is acquired through the Tn1546 transposon (Gilmore, 2002). Over 100 enterococcal isolates from humans, animals and food have shown *vanA* resistance residing on Tn1546 (Williams & Hergenrother, 2008). The transfer of *vanB* (acquired) resistance occurs through the exchange of transposon Tn1547 and/or Tn5382. Both *vanA* and *vanB* are present on the chromosome but can also be carried on a plasmid (Klare *et al.*, 2003; Gilmore, 2002).

*Enterococcus* species do not possess cytochrome enzymes and thus cannot produce the energy required to take up antibiotics into the cell. This means they show resistance to aminoglycosides at low levels (Klare *et al.*, 2003). Antibiotic resistance in *Enterococcus* species can be transferred by pheromone-mediated conjugative plasmids or transposons. The resistance genes may be passed on not only to antibiotic-susceptible enterococci, but also to other pathogens (Giraffa, 2002).

## **2.6. ANTIBIOTICS RESISTANCE OF ENTEROCOCCI IN HOSPITAL ENVIRONMENT**

During the last decade, enterococci have become important nosocomial pathogens, representing the third leading cause of bacteremia and the second leading cause of urinary tract infections in the USA. This increasing prevalence has been paralleled by the occurrence of vancomycin-resistant strains (VRE), which were first reported in 1988. Since then, strains resistant to



teicoplanin and/or vancomycin have appeared throughout the world. VRE frequently express additional resistance to multiple antibiotics, including ampicillin and aminoglycosides (including high-level resistance), thereby causing therapeutical problems (Mohamed & Huang, 2007). Between 1989 and 1993, the incidence of VRE colonization among patients in U.S. hospitals increased 26-fold, from 0.3 to 7.9% (Metan *et al.*, 2005). Higher incidences (13.6%) were reported among patients admitted to intensive care units (ICUs). Whereas the rapid emergence of VRE in the USA is has been attributed to the excessive use of vancomycin and/or cephalosporins, the occurrence of VRE in Europe either was “boosted” by the (former) use of glycopeptide analogs (e.g. avoparcin) as growth promoters in bioindustry and the consequent transmission of VRE via the food chain (Jones *et al.*, 2007).

As a hospital acquired pathogen, Enterococci have been associated with infections of the urinary tract, postsurgical wounds, septicemia, endocarditis and meningitis (Jones *et al.*, 2007; Emory and Gaynes 2005). They have a remarkable ability to adapt to exposure to antibiotics maintaining intrinsic resistance to penicillins and low- level resistance to aminoglycosides. In addition, they have demonstrated the capacity to acquire resistance to other antibiotics including high-level resistance to aminoglycosides and glycopeptides (Woodford *et al.*, 1993 and Jones *et al.*, 1995). The increasing resistance to antibiotics among enterococcal isolates has reduced the



choice of antibiotics available for treatment of infections caused by them (Eliopoulos 1993 and Mollering 1992).



## CHAPTER THREE

### 3.0.

### MATERIALS AND METHODS

#### 3.1. COLLECTION OF SAMPLES

Seventy –three (73) clinical specimens from beddings, clothing and surface equipment were collected using sterile cotton swabs from two different hospitals, which are Federal Medical Center Ido-Ekiti and Health Center of Federal University Oye-Ekiti. They were transported to the laboratory for processing, isolation on Sheep Blood agar and other appropriate culture media. Biochemical identification of enterococci was also performed.

#### 3.2. ISOLATION AND IDENTIFICATION OF ENTEROCOCCI

Enterococci were identified on Sheep Blood agar plate as non-haemolytic 0.5-1mm size streptococci-like colonies; on MacConkey agar as small dark-red magenta colonies. The colonies were confirmed as enterococci with Gram stain positivity, negative catalase test, growth in 6.5% NaCl broth and as *Enterococcus* species by specific sugar (glucose, lactose, mannitol, ribose and arabinose) fermentation reactions.

#### 3.3. BIOCHEMICAL CHARACTERISTICS OF ISOLATED ORGANISM.

Biochemical tests were performed to identify the isolates. The biochemical tests carried out on the organisms included:

- ❖ **Gram staining:** A thin smear of cell suspension was made on a clean greese free slides and then heat fixed by gently passing the slide over the blue flame. The smear was flooded with crystal violet for 1 minute and washed with water and then flooded with some drops of Grams iodine for 1 minute, the iodine decreases the solubility of the purple dye forming dye-iodine complexes, which was then washed with water. The purple dye-



iodine complexes was then decolorized with 95% ethanol for 15 seconds and rinsed with water. The smear was then counter stained with saffranin for 30 seconds and the excess stain was washed with water. The smear slide was then allowed to air dry. A drop of oil immersion was dropped on the smear and examined under the microscope using oil immersion objective (x100).

❖ **Catalase test**

A colony of the organism was picked and placed in a drop of 3% hydrogen peroxide on a clean glass slide. Effervescence caused by the liberation of oxygen as gas bubbles indicated the production of catalase by the bacterium while an absence of gas bubbles indicated a negative result.

❖ **Oxidase test**

The underlying principle of the 'oxidase reaction' is exclusively by virtue of an enzyme known as cytochrome oxidase that particularly catalyzes oxidation of reduced cytochrome by oxygen. A solution of tetramethyl p-phenylene diamine dihydrochloride [concentration 1.0 to 1.5 (w/v)] was poured gently as well as carefully over the colonies. If the area of inoculation turns dark-blue to maroon to almost black, then the result is positive, if a color change does not occur within three minutes, the result is negative.

❖ **Sugar Fermentation Test**

Sugar fermentation tests were carried out with the test organisms. The sugars used include: glucose, lactose, arabinose, ribose and mannitol. 1g of each sugar was weighed into different conical flask and labeled accordingly; into each flask, peptone (1g) was added and made up to 100ml with distilled water. 0.01g phenol red was added as an indicator. A 5ml each of the 100ml sugar solution was dispensed into different test tubes



with Durham's tubes inserted in inverted format. The tubes were labeled appropriately and covered tightly with cotton wool and aluminum foil paper and sterilized in an autoclave for 10 minutes at 121<sup>0</sup>C. These tubes were cooled to 40-45<sup>0</sup>C prior inoculation. After cooling to 40-45<sup>0</sup>C, the sugar solution in the test tubes were inoculated with the test organism and incubated at 37<sup>0</sup>C for 72 hours. Acid production was observed by a change in colour from red to yellow. Appearance of bubble in the Durham tubes indicates gas production. If otherwise, gas was not produced.

#### **3.4. ANTIBIOTIC SUSCEPTIBILITY TEST (Disk Diffusion method)**

The isolates were then subjected to antibiotic susceptibility testing by the disc diffusion method on Mueller-Hinton agar according to the National Committee for Clinical Laboratory Standards and Manual of Antimicrobial Susceptibility Testing guidelines (CLSI, 2013; Nkang *et al.*, 2009; Coyle, 2005). Commercially available antimicrobial discs were used in the study and included: Cefatadime (30µg), Cefuroxime (30µg), Gentamicin (25µg), Ciprofloxacin (10µg), Ofloxacin (5µg), Amoxycillin/Clavulanate (5µg), Nitrofurantoin (300µg) and Ampicillin (10µg). Plates were incubated at 37<sup>0</sup>C. Zones of inhibition were measured to the nearest millimeters (mm) and interpreted as resistant or sensitive using the interpretative charts as described by CLSI (2013). Zones of inhibition of ≥18mm were considered sensitive, 15-17 mm intermediate and < 14 mm resistant (CLSI, 2013; Nkang *et al.*, 2009; Coyle, 2005).



## CHAPTER FOUR

### 4.0. RESULTS

#### 4.1. ISOLATION RATE OF *Enterococcus faecalis* FROM DIFFERENT WARDS.

Table 1 shows the isolation rate of *Enterococcus faecalis* from sections of the two hospitals, FMC Ido-Ekiti (Emergency ward, Children Ward, Labour ward, and Laboratory), Health Center of FUYOYE (Male ward and Female Ward). Emergency ward has the highest isolation of fourteen (14) while Female ward has the lowest isolation rate of six (6).

#### 4.2. VIABLE COUNTS OF BACTERIA ISOLATED FROM HOSPITAL WARDS

Table 2 shows the result obtained from isolation of microorganisms from sections of the two hospital, FMC Ido-Ekiti (Emergency ward has , Children Ward, Labour ward, and Laboratory), Health Center of FUYOYE (Male ward and Female Ward).

#### 4.3. DISTRIBUTION OF *Enterococcus species* IN DIFFERENT HOSPITAL WARDS.

Table 3 shows the distribution of *Enterococcus spp* isolate in the different wards, *enterococci* specie were present in the emergency and labour wards of FMC Ido-Ekiti, while this organism was found only in the female ward of Health center of FUYOYE.

#### 4.4. MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF

##### *Enterococcus faecalis*.

The morphological and biochemical characteristics of the organisms used in this study are presented in Table 4. This shows the cultural, morphological and biochemical characteristics of these isolates.



#### **4.5. ANTIBIOTICS SUSCEPTIBILITY PATTERN OF *Enterococcus faecalis*.**

Table 5 shows the antibiotics susceptibility pattern of *Enterococcus faecalis*. Plates 1-3 also show the results of the antibiotics sensitivity test against *Enterococcus faecalis* from the wards.

#### **4.6. SUMMARY OF ANTIBIOTICS RESISTANT OF *E. faecalis***

Table 6 shows the summary of antibiotics resistant of *E. faecalis* isolated from Emergency, Labour and Female ward of FMC Ido-ekiti and Health Center of FUOYE respectively.



**Table 1: Isolation rate of *Enterococcus faecalis* from different wards.**

<b>Ward</b>	<b>FMC Ido-Ekiti</b>	<b>Health Centre FUOYE</b>
<b>Emergency</b>	<b>14</b>	<b>Nil</b>
<b>Labour</b>	<b>10</b>	<b>Nil</b>
<b>Children</b>	<b>Nil</b>	<b>Nil</b>
<b>Male</b>	<b>Nil</b>	<b>Nil</b>
<b>Female</b>	<b>Nil</b>	<b>6</b>

**Key: Nil= absent**



**Table 2: Viable counts of bacteria from each hospital wards**

Growth media	FMC Ido-Ekiti			FUOYE Health Center	
	Emergency ward	Children ward	Labour ward	Male ward	Female ward
<b>Nutrient Agar</b>	105x10 <sup>3</sup> CFU	6x10 <sup>2</sup> CFU	75x10 <sup>2</sup> CFU	45x10 <sup>3</sup> CFU	65x10 <sup>2</sup> CFU
<b>MacConkey Agar</b>	75x10 <sup>2</sup> CFU	54x10 <sup>3</sup> CFU	35x10 <sup>3</sup> CFU	22x10 <sup>2</sup> CFU	45x10 <sup>2</sup> CFU
<b>Sheep Blood Agar</b>	15x10 <sup>2</sup> CFU	5x10 <sup>2</sup> CFU	10x10 <sup>2</sup> CFU	4x10 <sup>2</sup> CFU	10x10 <sup>3</sup> CFU



**Table 3: Distribution of Enterococci isolates in different sections of the hospitals.**

ISOLATES	FMC Ido-Ekiti			FUOYE Health Center	
	Emergency ward (n)	Children ward (n)	Labour ward (n)	Male ward (n)	Female ward (n)
<i>Enterococcus faecalis</i>	+ (14)	- (0)	+ (10)	- (0)	+ (6)
<i>Enterococcus faecium</i>	- (0)	- (0)	- (0)	- (0)	- (0)

**Key: + = Present; - = Absent; n = number of isolates**



**Table 4: Morphological and biochemical characteristics of *Enterococcus faecalis*.**

Parameters	<i>Enterococcus faecalis</i>
Grams staining	+
Cellular morphology	Cocci
Catalase test	-
Oxidase test	-
Growth in 6.5% NaCl	+
Sugar Fermentation test	
Glucose	*
Lactose	*
Arabinose	×
Ribose	*
Mannitol	*

Keys: - = No growth, + = Growth, \* = sugar utilization, × = no sugar utilization



**TABLE 5. ANTIBIOTICS SENSITIVITY PATTERN OF *E. faecalis* ISOLATED FROM EMERGENCY, LABOUR AND FEMALE WARD OF FEDERAL MEDICAL CENTER IDO EKITI AND FUOYE HEALTH CENTER.**

Sample ID	(CAZ) 30µg(mm)	(CRX) 30µg(mm)	(GEN) 10µg(mm)	(CPR) 5µg (mm)	(NIT) 300µg(mm)	(OFL) 5µg(mm)	(AUG) 30µg(mm)	(AMP) 10µg(mm)
FMCE1	0.00	0.00	17.00	15.3	19.00	15.3	0.00	0.00
	R	R	I	I	S	I	R	R
FMCE2	0.00	0.00	18.00	15.3	20.00	11.3	0.00	0.00
	R	R	S	I	S	R	R	R
FMCE3	0.00	0.00	17.00	0.00	20.3	17.00	5.00	0.00
	R	R	I	R	S	I	R	R
FMCE4	13.00	10.2	22.4	15.2	23.2	18.3	2.32	0.00
	R	R	S	I	S	S	R	R
FMCE5	0.00	0.00	21.4	10.3	18.3	15.00	0.00	0.00
	R	R	S	R	S	I	R	R
FMCE6	0.00	0.00	17.00	18.3	15.00	21.3	0.00	0.00
	R	R	I	S	I	S	R	R
FMCE7	14.2	10.00	20.3	19.2	20.3	17.2	14.2	10.3
	R	R	S	S	S	I	R	R
FMCE8	0.00	0.00	18.2	19.3	15.00	20.00	5.00	0.00
	R	R	S	S	I	S	R	R
FMCE9	12.3	14.00	22.4	18.2	22.4	21.2	18.3	11.00
	R	R	S	S	S	S	S	R
FMCE10	10.2	15.3	23.2	16.5	0.00	22.3	10.3	12.2
	R	I	S	I	R	S	R	R
FMCE11	10.00	25.00	24.00	23.3	0.00	25.4	7.2	20.3



	R	S	S	S	R	S	R	S
FMCE12	0.00	0.00	13.2	16.4	20.3	0.00	0.00	0.00
	R	R	R	I	S	R	R	R
FMCE13	0.00	13.2	20.3	7.4	17.2	20.02	10.3	0.00
	R	R	S	R	I	S	R	R
FMCE14	14.2	14.00	25.2	15.3	15.2	23.4	0.00	5.3
	R	R	S	I	I	S	R	R
FMCL1	0.00	0.00	18.3	16.2	19.3	15.3	0.00	0.00
	R	R	S	I	S	I	R	R
FMCL2	0.00	0.00	23.00	17.2	12.3	11.3	0.00	0.00
	R	R	S	I	R	R	R	R
FMCL3	0.00	0.00	15.00	12.3	18.3	16.4	0.00	10.00
	R	R	I	R	S	I	R	R
FMCL4	11.00	12.2	23.4	16.4	12.3	14.3	0.00	0.00
	R	R	S	I	R	R	R	R
FMCL5	0.00	0.00	14.4	12.4	12.00	19.3	0.00	0.00
	R	R	R	R	R	S	R	R
FMCL6	0.00	0.00	19.2	18.3	15.3	16.3	16.00	0.00
	R	R	S	S	I	I	I	R
FMCL7	17.2	14.00	20.3	24.2	11.3	16.3	14.2	10.3
	I	R	S	S	R	I	R	R
FMCL8	0.00	0.00	16.2	19.3	15.2	19.2	8.00	0.00
	R	R	I	S	I	S	R	R
FMCL9	15.3	14.3	21.2	19.3	14.4	14.2	15.3	11.00
	I	R	S	S	R	R	I	R
FMCL10	12.2	13.3	21.2	15.1	0.00	22.3	12.3	14.2
	R	R	S	I	R	S	R	R



FHCF1	0.00	0.00	18.3	16.3	20.00	17.3	0.00	0.00
	R	R	S	I	S	I	R	R
FHCF2	0.00	0.00	13.3	17.3	23.2	11.3	0.00	0.00
	R	R	R	I	S	R	R	R
FHCF3	8.00	10.3	17.4	0.00	21.3	17.10	11.00	12.2
	R	R	I	R	S	I	R	R
FHCF4	14.00	11.2	22.4	17.4	20.2	18.3	3.20	0.00
	R	R	S	I	S	S	R	R
FHCF5	0.00	0.00	12.4	10.3	18.3	15.00	0.00	0.00
	R	R	R	R	S	I	R	R
FHCF6	0.00	0.00	12.3	12.3	14.3	21.3	0.00	15.2
	R	R	R	R	R	S	R	I

Zones of inhibition of:  $\geq 18$ mm(sensitive), 15-17mm(intermediate),  $< 15$ mm(resistant).

KEY1: CAZ= Ceftazidime, CRX= Cefuroxime, GEN= Gentamycin, CPR= Ciprofloxacin, NIT= Nitrofurantoin, AMP= Ampicillin, OFL= Ofloxacin, AUG= Amoxicillin/Clavulanate.

KEY2: FMCE= Federal Medical Center Ido Emergency, FMCL= Federal Medical Center Ido Labour, FHCF= FUOYE Health Center Female.



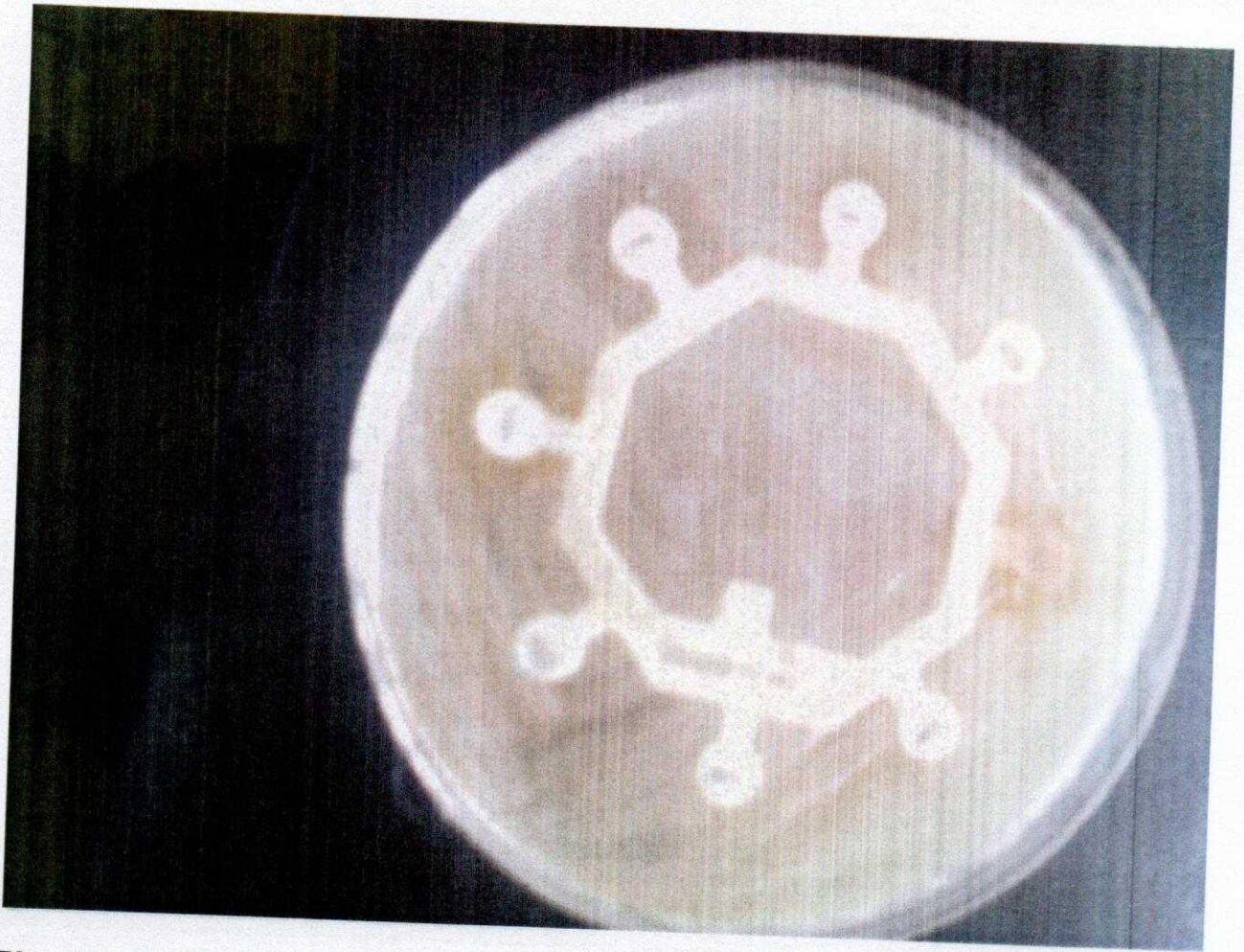
**TABLE 6: SUMMARY OF ANTIBIOTICS RESISTANT OF *E. faecalis* ISOLATED FROM EMERGENCY, LABOUR AND FEMALE WARD OF FMC IDO-EKITI AND HEALTH CENTER OF FUOYE RESPECTIVELY.**

ANTIBIOTICS	FMCE	FMCL	FHCF	PERCENTAGE (%) 100		
	TOTAL(n=14)	TOTAL(10)	TOTAL(6)	FMCE	FMCL	FHCF
DISCS						
CAZ	14	10	6	100	100	100
CRX	12	10	6	85.7	100	100
GEN	1	1	3	7.1	10	50
CPR	2	2	3	14.3	20	50
NIT	2	6	1	14.3	60	16.7
AMP	2	10	5	14.3	100	83.3
OFL	13	3	1	92.9	30	16.7
AUG	13	8	6	92.9	80	100
AVERAGE	7.38	6.25	3.88	52.68	62.5	64.59
RESISTANCE						

KEY1: CAZ= Ceftazidime, CRX= Cefuroxime, GEN= Gentamycin, CPR= Ciprofloxacin, NIT= Nitrofurantoin, AMP= Ampicillin, OFL= Ofloxacin, AUG= Amoxycillin/Clavulanate.

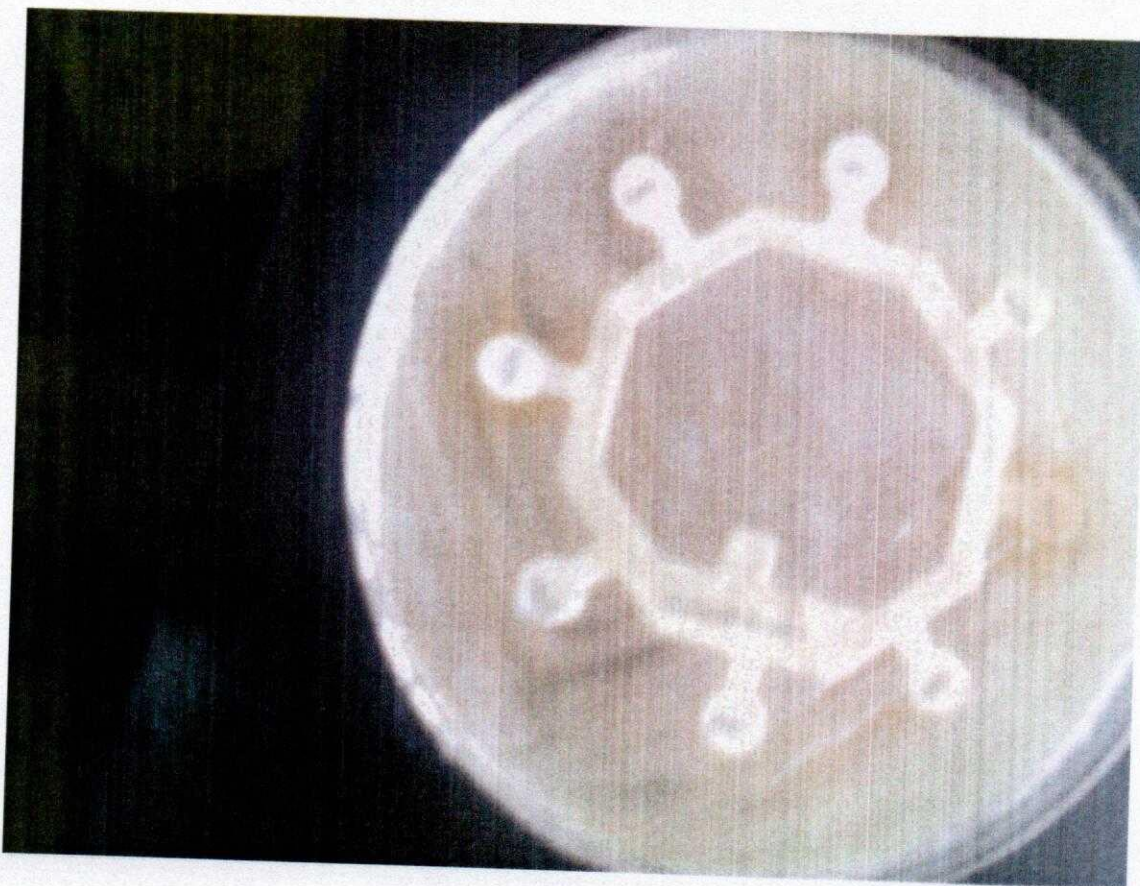
KEY2: FMCE= Federal Medical Center Ido Emergency, FMCL= Federal Medical Center Ido Labour, FHCF= FUOYE Health Center Female.





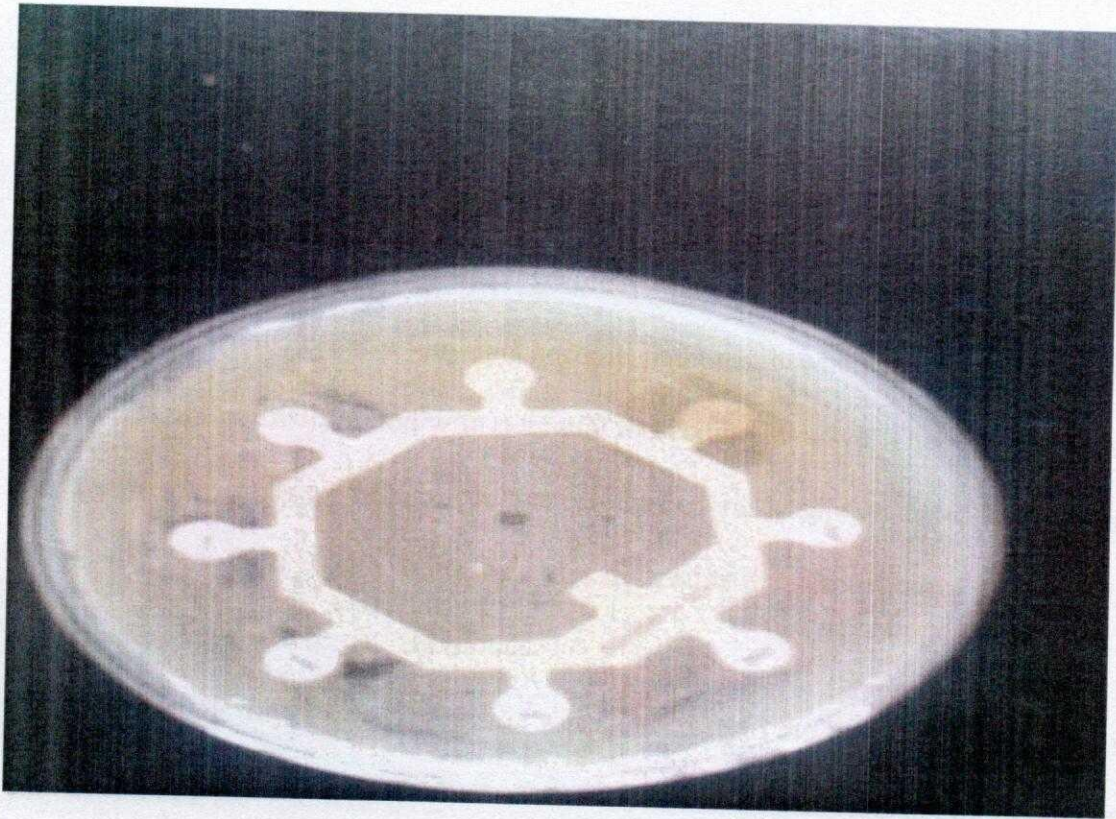
**Plate 1: Antibiotics sensitivity test against *Enterococcus faecalis* isolated from Emergency ward of Federal Medical Center Ido-Ekiti.**





**Plate 2: Antibiotics sensitivity test against *Enterococcus faecalis* isolated from Labour ward of Federal Medical Center Ido-Ekiti.**





**Plate 3: Antibiotics sensitivity test against *Enterococcus faecalis* isolated from Female ward of Federal University Oye-Ekiti Health Center.**



## CHAPTER FIVE

### 5.0. DISCUSSION

*Enterococci* have been largely regarded as commensal flora and generally disregarded when isolated from clinical specimens such as wound and urine. However, there are increasing reports that this opportunistic bacterium can become pathogenic when it colonizes ecological niche where it is not normally found with potential to become invasive. The aim of this study was to determine the presence of *Enterococci* specie in Federal Medical Center Ido-Ekiti and Federal University Oye-Ekiti Health Center and also determine the susceptibility pattern of the isolated *Enterococci* Specie to certain antibiotics.

From this study *Enterococcus faecalis* was isolated from the Emergency and Labour wards of FMC Ido-Ekiti and from the Female ward of FUOYE Health Center. The Emergency ward of FMC Ido-Ekiti was found to have the highest total viable count (TVC) of  $105 \times 10^3$  cfu/plate. This could be as a result of huge population of patients and relatives coming for visitation, while TVC of  $4 \times 10^2$  cfu/plate that was recorded in Male ward of FUOYE Health center could also be associated with the low rate of admission of patient into this ward. The antibiotics sensitivity pattern of *Enterococcus faecalis* from the sections show variation in resistance and sensitivity to the antibiotics used. In this research, *Enterococcus faecalis* was susceptible to Gentamycin and Ciprofloxacin with the percentage of 77.63% and 71.9% respectively. However *Enterococcus faecalis* was resistant to a number of antibiotics including Amoxycillin, Ceftazidime, Cefuroxime, and Ampicillin. In the work of Olawale *et al.*,(2011), *Enterococcus faecalis* was resistant to Ampicillin, Oxacillin, Ceftazidime and Gentamicin, unlike in this study which shows *E. faecalis* to be susceptible to Gentamycin.



In this research, the test organism was susceptible to Gentamycin with the zone of inhibition of (25.2mm), Ciprofloxacin with zones of inhibition of (19.3mm) while in the work of Olawale *et al.* (2011), Gentamycin was resistant with zone size of (8.00mm) and Ciprofloxacin with zone size of (0.00mm). However, in the work of Kacmaz and Aksoy (2005), *E. faecalis* was susceptible Gentamycin with zone size of (18.2mm), and Ciprofloxacin with zone size of (21.00mm), while Ceftazidime, Cefuroxime, and Amoxycillin were resistant with zone sizes of (0.00mm), (0.00mm), (0.00mm) respectively. Olawale *et al.*, (2011), reported resistance to Ceftazidime, Cefuroxime, and Amoxycillin with zone sizes of (12.00mm), (5.00mm), (0.00mm) respectively. Similarly, in the work of Kacmaz and Aksoy (2005), *E. faecalis* was also resistant towards Ceftazidime, Cefuroxime, and Amoxycillin were resistant with zone sizes of (0.00mm), (0.00mm), (12.00mm) respectively.

The spread of antibiotic-resistant bacteria has prompted greater attention to the need for potent control measures. Resistance could theoretically be reduced by controlling the spread of bacteria, using specific types of antibiotics to which bacteria are susceptible, performing sensitivity test for any infection before prescribing drugs for treatment. Also, health care workers should be screened for antibiotic-resistant nosocomial pathogens to avert transmission to patients (Olawale *et al.*, 2011).



## CHAPTER SIX

### 6.0 CONCLUSION AND RECOMMENDATION

#### 6.1 CONCLUSION

At the end of this research work, *Enterococcus faecalis* was isolated from emergency and labour ward of Federal Medical Center Ido-Ekiti and also from the Female ward of Federal University Oye-Ekiti Health center. The antibiotic sensitivity test shows that Gentamycin, Ciprofloxacin, were the most effective antibiotics against *Enterococcus faecalis*, while Ceftazidime, Cefuroxime, and Amoxycillin were found to have the highest percentage of resistance.

#### 6.2 RECOMMENDATION

It is recommended that appropriate antimicrobial susceptibility testing should be performed prior prescription of drugs for treatment of infectious diseases. In addition, the hospital management is advised to perform periodic disinfection of labour ward, emergency ward, Children ward, Male ward and Female ward to avoid transmission of nosocomial infections.



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