MOLECULAR CHARACTERIZATION OF VANCOMYCIN-RESISTANT ENTEROCOCCUS SPP ISOLATED FROM PATIENTS AND HOSPITAL ENVIRONMENTS IN SOME HOSPITALS IN ABUJA, NIGERIA

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BY

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN MICROBIOLOGY

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ZARIA, NIGERIA

NOVEMBER, 2017

DECLARATION

I declare that the work in this thesis entitled "**MOLECULAR CHARACTERIZATION OF VANCOMYCIN-RESISTANT ENTEROCOCCUS SPP ISOLATED FROM PATIENTS AND HOSPITAL ENVIRONMENT IN SOME HOSPITALS IN ABUJA, NIGERIA**" has been carried out by me in the Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria. The information obtained from the literature has been acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

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Name of Student	Signature	Date

CERTIFICATION

This thesis entitled "**MOLECULAR CHARACTERIZATION OF VANCOMYCIN-RESISTANT ENTEROCOCCUS SPP ISOLATED FROM PATIENTS AND HOSPITAL ENVIRONMENT IN SOME HOSPITALS IN ABUJA, NIGERIA**" by JOHN CHIMA NDUBUISI meets the regulations governing the award of the degree of Doctor of Philosophy in Microbiology of the Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

To my beloved mother Mrs Emilia Charles Ndubuisi, my twin brother James Ndubuisi, my brother Ben Charley Ndubuisi, my sister Maria Mbah and her husband Mr T.C. Mbah

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I return all the Glory to Almighty God for giving me the opportunity for this additional degree. I will praise and worship him for ever and ever for making a way for me where there was no way. I'm greatly indebted to my Supervisors, Professor O. S. Olonitola, Prof. A.T. Olayinka and Prof. E .D. Jatau, for their supervision, concern for the progress of the work and advice that made me not to derail. May God bless you all in Jesus Name. Amen.

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ABSTRACT

This study investigated *Enterococcus* spp isolated from patients and hospital environment in University of Abuja Teaching Hospital, National Hospital, Kubwa and Kuje General Hospitals Abuja, Nigeria for vancomycin resistant enterococci. A total of 500 samples comprising stool, urine, wound swabs and environmental samples were analysed. The clinical samples yielded 97 enterococcal isolates while environmental samples yielded 5 enterococcal isolates giving a total of 102 isolates. The breakdown of the frequency of occurrence of the isolates indicate *Enterococcus faecalis* (n= 59; 57.8%), *Enterococcus faecium* (n=24;23.5%), *Enterococcus casseliflavus* (n=1;1.0%), *Enterococcus gallinarum* (n=3;2.9%), *Enterococcus mundtii* (n=11;10.8%), *Enterococcus avium* (n=1;1.0%), *Enterococcus dispar* 2(2.0%) and *Enterococcus hirae* (n=1;1.0%). They were identified by conventional, Microgen test kit and 16S rRNA sequencing methods. Antimicrobial susceptibility testing of the isolates was done using disk diffusion method. The strains that showed resistance to vancomycin were confirmed by Minimum Inhibitory Concentration (MIC) with E-test strips based on Clinical and Laboratory Standards Institute (CLSI) guidelines. Phenotypically, thirty four (34) of the isolates were vancomycin resistant and showed multi-resistance to teicoplanin, ampicillin, chloramphenicol, ciprofloxacin, rifampicin, erythromycin, doxycycline, linezolid, nitrofurantoin, streptomycin and gentimicin. Twelve strains were confirmed to be intermediate Vancomycin Resistant *Enterococcus* and possessed *van* genes detected by Polymarase Chain Reaction (PCR). *Enterococcus mundtii, Enterococcus faecium and Enterococcus faecalis* were less susceptible to antibiotics used than other isolated species. The results indicated that a significant percentage of isolates from clinical samples were resistant to different antibiotics used suggesting the need for improved control strategies to avoid dissemination of resistant isolates and emphasizing the importance of surveillance for the detection of emerging resistance traits.

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LIST OF ABBREVIATIONS, DEFINITIONS, GLOSSARY AND SYMBOLS

50S	subunit
AGG	aggregation substance
AMAC	Abuja municipal Area Council
Ann	annealing
AWP	antibiotics with pattern
BA	blood agar
BEA	bile esculin azide agar
BHI	brain heart infusion
BLAST	basic local alignment search tool
BP	blood pressure
CHL	chloramphenicol
CIP	ciprofloxacin,
CLSI	Clinical and Laboratory Standard Institute
D-ALA	alanine
Den	denaturation
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTPs	Deoxy-nucleoside triphosphate
DOX	doxycycline
ERY	erythromycin
ESP	extracellular surface protein
FCT	Federal Capital Territory
FDA	Food and Drug administration
G+C	guanine+cytosine
GEN	gentamicin
GIT	gastro intestinal tract

GTP	guanosine triphosphate
HLAR	aminoglycoside resistance
I	intermediate
ICU	Intensive Care Unit
KW	Kubwa
LB	luria broth
LIN	linezolid
MAR	multiple antibiotic resistant
MDR	multidrug-resistant
MHA	Mueller-Hinton Agar
MIC	Minimum Inhibitory Concentration
mRNA	messenger ribonucleic acid
MRSA	Methicillin Resistant *Staphylococcus* aureus
NAG	N-acetylglucosamine
NAM	N-acetylmuramic acid
NCBI	National Center for Biotechnology Center
NH	National Hospital
NHA	National Hospital, Abuja
NIT	Nitrofurantoin
NNIS	National Nosocomial Infections Surveillance
PBS	penicillin-binding proteins
PCR	Polymerase Chain Reaction.
PDR	pandrug resistance
PFGE	Pulse Field Gel Electrophoresis
PYR	pyrrolidonyl aminopeptidase
R	resistance
RIF	rifampicin
rRNA	ribosomal ribonucleic acid

S	susceptibility
Spp.	species
STR	streptomycin
TEC	teicoplanin
Tn	transposons
UATH	University of Abuja Teaching Hospital
UTI	Urinary Tract Infection
UV	ultra violet
VAN	vancomycin
VP	Voges Proskauer
VRE	Vancomycin-Resistant Enterococci
VSE	Vancomycin-Sensitive *Enterococcus*
XDR	extremely drug-resistant
ZR	Zymo research

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Enterococci are facultative anaerobic Gram-positive cocci that share their morphology and Lancefield antigenicity with group D streptococci. The genus *Enterococcus* includes at least 17 species, distinguished on the basis of pigment production, motility, and ability to produce acids from various carbohydrates. They live as commensals in the gastrointestinal tract (GIT) of a variety of organisms including humans (Miller *et al*., 2014). Selective pressure of their competitive environment, have made these bacteria to develop different responses and genetic flexibility allowing them to thrive in the modern healthcare environment (Miller *et al*., 2014). Widespread use of antibiotic for patient care has increased in modern medicine and created opportunity for enterococci to use its antibiotic resistance determinants to thrive in the GIT from where multidrug-resistant (MDR) strains disseminate rapidly in the hospital environment. These coccoid-shaped bacteria are common in environments contaminated by animal and human faecal material.

After Staphylococcal infection, *Enterococcus* spp. are major causes of Gram-positive nosocomial infections. There is increased interest in enterococci not just because of their ability to cause serious infections such as endocarditis, bacteremia, intra-abdominal and urinary tract infection (UTI), but also because of their increasing resistance to many antimicrobial agents (Teixeira and Facklam, 2003). *Enterococci* are frequent causative agents of both nosocomial and community acquired infections (Maraha *et al*., 2001). Concern has especially been focused on enterococci that show high-level resistance to the glycopeptide antibiotic vancomycin which until recently has been the drug of last resort against multi-drug

resistant enterococci and against methicillin-resistant *Staphylococcus aureus* (Boneca and Chiosis, 2003).

French (1998) also noted that increased use of antibiotics in class such as aminoglycosides, cephalosporins, quinolones and glycopeptides contributed to resistance in enterococci. Collignon (1999) demonstrated the correlation between avoparcin usage as animal feed supplement and glycopeptides resistance of the human isolate. Transmission of vancomycin resistant enterococci (VRE) follows contact with faeces, urine, or blood of a patient infected or colonized with the organism (Salgado and Farr, 2007). It can also be spread via hand contact with open wounds containing the bacteria, or by touching contaminated environmental surfaces, where the organisms can survive for weeks. Bedrails, bed-side tables, Blood Pressure cuffs, and television remote control devices are among the many items that can harbor VRE (Martinez and Ruthazer, 2003).

Evidence strongly suggests that nurses and other healthcare providers carry VRE on their hands allowing cross contamination from one patient to another (Siegel and Rhinehart, 2006). Such transmission is more likely to occur when healthcare workers don't adhere to Center for Disease Control (CDC) recommendations for hand hygiene and glove use (Siegel and Rhinehart, 2006). One study found that during the care of patients, with VRE, contamination of gloves, gowns, or stethoscopes occurred two–thirds of the time (Salgado and Farr, 2007). Portals of entry for VRE typically include the urinary tract, intra-abdominal (gastrointestinal tract, biliary tract) or pelvic sources, wounds (surgical wounds, decubitus ulcers) and intravascular catheters (Bonten *et al*., 2001). The bacteria complicate and prolong patients' hospital stays and in some cases, cause fatal disease (National Institute of Allergy and Infectious Diseases, 2007). Patients at risk for VRE infection include those on antibiotics for long periods; immuno-compromised people or those who have undergone abdominal or

chest surgery; and those with urinary catheters or central intravenous lines (CDC, 2004). Vancomycin resistant enterococci (VRE) species have enhanced ability to transfer resistance genes to other bacteria (CDC, 2002). There are two types of vancomycin resistance in enterococci. The first type is intrinsic resistance; Isolates of *Enterococcus gallinarum* and *E. casseliflavus/E. flavescens* demonstrate an inherent, low-level resistance to vancomycin.

The second type of vancomycin resistance in enterococci is acquired resistance. Enterococci can become resistant to vancomycin by acquisition of genetic information from another organism. Most commonly, this resistance is seen in *E. faecium* and *E. faecalis*, but also has been recognized in *E. raffinosus*, *E. avium*, *E. durans*, and several other enterococcal species.

Several genes, including *van*A, *van*B, *van*C, *van*D, and *van*E, contribute to resistance to vancomycin in enterococci. Glycopeptide resistance in enterococci is encoded by *van* genes. *vanA* and *vanB* genes are the most significant ones. The *vanA* confers high-level inducible resistance to both vancomycin and teicoplanin and is found in *E.faecium, E.faecalis* and sometimes in other species (Woodford, 1998) while the phenotype *vanB* gene was characterized by moderate to low- level vancomycin resistance.

1.2 Statement of the Problem

Around the world, the rate of emergence of VRE are at their highest in North America with 35.5% between 2009 to 2010 ranking as the second most common cause of nosocomial infection in the US; in Canada, 6.0% was reported between 2007 and 2011; in Europe, 4% reported in 2013; in Asia-pacific, 11.9 was reported between 2007 and 2008; in Latin America, 12.9% was reported between 2007 to 2008 while 10.3% was reported worldwide between 2007 and 2012 (O'Driscoll and Crank, 2015).

Vancomycin-resistant infections such as bacteraemia increases the length of patient hospital stay which is attributable to mortality rate of up to 37%. Mortality rates associated with enterococcal infections may exceed 50% in critically ill patients, those with solid tumours, and some transplant patients (DiazGranados *et al*., 2005).

VRE infections are more difficult to treat than other infections with enterococci, because fewer antibiotics can kill the bacteria. Controlling transmission of VRE in the health care environment can be challenging. Vancomycin-resistant enterococci are capable of prolonged (>1 week) survival in the environment and can be transmitted from environmental sites to staff hands. Vancomycin-resistant enterococci have been isolated from virtually every site and every object in health care facilities (Duckro *et al*., 2005).

1.3 Justification

Acquisition of microorganisms resistant to multiple antibiotics represents a threat to patients' health. Enterococci easily acquire resistance when exposed to antibiotics or when they acquire genetic resistance factors from other organisms. Therefore, VRE can spread through the population via human, environmental and animal reservoirs.

The treatment problem such as prolonged hospital stay by patients translates to increased healthcare bills and eventual death of the patients due to multi-resistant nature of VRE to antibiotics. There is limited information on molecular detection of *van* genes in VRE in Nigeria which necessitated this study in order to provide the profile of VRE in the study population which will serve as a resource for healthcare policy makers or formulators.

1.4 Aim

The study was aimed at isolating, characterizing and molecularly determining vancomycin-resistant *Enterococcus* spp. from patients and hospital environments in some hospitals in Abuja, Nigeria.

1.5 Specific Objectives:

The objectives of this study were to:

1. isolate and characterize *Enterococcus* spp. obtained from UATH, NHA, Kubwa and Kuje General Hospitals, Abuja, Nigeria.
2. determine the susceptibility pattern of the isolates to commonly used antimicrobial drugs
3. determine multiple antibiotic resistance patterns among the isolates
4. determine vancomycin resistance among the isolated enterococci by E-test minimum inhibitory concentration (MIC).
5. screen the isolates for the possession of *van* genes from the phenotypically resistant *Enterococcus* spp. by Polymarase Chain Reaction (PCR).
6. confirm some of the isolates with *16S rRNA* sequencing.
7. determine the relatedness of the sequenced isolates by phylogeny

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Genus *Enterococcus*

The name "enterocoque" was first used by Thiercelin in 1899. The name was used to emphasize the intestinal origin of this new gram-positive diplococcus (Murray, 1990). The term ‘enterococcus’ originates from the Greek *enteron* meaning ‘the gut or intestine’ and *kokkos* meaning ‘a berry or kernel (Patel, 2003).

The normal intestinal flora of humans and animals include enterococci but are also important pathogens responsible for serious infections. The *Enterococcus* genus includes more than 17 species, but only *Enterooccus faecalis, E. faecium, E. avium, E. gallinarium, E. casseliflavus, E. durans, E. raffinosus* and *E mundtii* cause clinical infections in humans. *Enterococcus faecalis* and *E. faecium* account for over 90% of clinical isolates cultured from humans (de Perio *et al*., 2006).The enterococci have the group D group-specific substance and were previously classified as group D streptococci. It is because the group D cell wall specific antigen is a teichoic acid, it is not an antigenically good marker; enterococci are usually identified by characteristics other than immunologic reaction with group-specific antisera.They are usually non-haemolytic, but occasionally hemolytic, PYR-positive, grow in the presence of bile and hydrolyze esculin, grow in 6.5% NaCl and grow well at between 10°C and 45°C (dePerio *et al*., 2006).

With increasing antibiotic resistance, enterococci are recognized as nosocomial pathogens that can be challenging to treat with *E. faecium* representing the most vancomycin resistant *Enterococcus* specie (Marcus *et al.*, 1997; Nelson *et al.*, 2000).

2.2 Physiology of Enterococci

Enterococci grow at a range of temperatures from 5 to 50 °C. The minimum, optimum and maximum temperatures are 6.5, 42.7 and 47.8°C respectively on brain heart infusion (BHI) agar in aerobic conditions (Van den Berghe *et al.*, 2006). Both *E. faecalis* and *E. faecium* can survive heating at 60 °C for 30 minutes making *Enterococcus* species distinguishable from other closely related genera such as *Streptococcus* (Foulquie *et al.*, 2006). They tolerate hypotonic, hypertonic, acidic, or alkaline environments, sodium azide and concentrated bile salts, which inhibit or kill most microorganisms and are used as selective agents in agar-based media. As facultative organisms, enterococci grow under reduced or oxygenated conditions. Enterococci are usually considered strict fermenters because they lack a Kreb's cycle respiratory chain (Huycke, 1998).

During the lag phase, temperature is the most important factor influencing growth of enterococci, with stationary-phase cells being the most resistant to heat (Martinez *et al.*, 2003). The resistance of *E. faecalis* to a range of pH values according to Nakajo *et al.* (2005) is associated with its membrane durability and impermeability to acid and alkali or due to its membrane-bound H^+-ATPase activity. Heat resistance is also associated with membrane structure and has been related to lipid and fatty acid content.The heat resistance of enterococci is dependent on the temperature but also the phase of growth (Martinez *et al.*, 2003).

2.3 Taxonomy of Enterococcus

The genus *Enterococcus* are Gram-positive cocci, catalase-negative, non-sporing, facultative anaerobic bacteria that occur in singles, pairs and in chains. *Enterococcus* has been recognized since 1899, when it was identified as an intestinal organism. In 1937

Sherman classified it as a subgroup of *Streptococcus* species which belong to Lancefield group D (Klein, 2003). Traditional methods such as biotyping, serotyping and phage typing left questions as to which of the *Streptococcus* species actually belonged to the genus *Enterococcus* (Saeedi *et al.*, 2002).

Foulquie *et al* (2006) established that through the use of DNA hybridization and 16S rRNA sequencing, *Streptococcus faecium* and *Streptococcus faecalis* were distinct from the other *Streptococcus* spp. Nine species were transferred from the *Streptococcus* groups and now *Enterococcus* includes 28 species (Foulquie *et al.*, 2006).The *Enterococcus faecalis* complex includes *E. faecalis*, *E.haemoperoxidus* and *E.moraviens* whilst the *Enterococcus faecium* complex includes *E.faecium*, *E.durans*, *E.hirae*, *E.mundtii*, *E.porcinus* and *E.villorum* (Klein,2003).Fermentation test, enzyme activities, growth at defined temperatures and physiological characteristics are essential in the identification of *Enterococcus* species (Shanks *et al.*, 2006).

Shanks *et al.* (2006) noted the differences in the genomes of *E. faecalis* and *E. faecium* using DNA hybridization. Overall 6.4 % of the *Enterococcus* genome is associated with cell-surface proteins and 22.6 % of the differences between the two species are found in these genes. This variation in surface proteins is thought to have implications in the species avoiding different host immune responses (Shanks *et al.* 2006).

2.4 Virulence

According to Busani *et al.* (2004), *Enterococcus* species with the highest virulence are medical isolates, followed by food isolates and then starter strains. The factors that determine the virulence of *Enterococcus* species include: ability to colonize the gastrointestinal tract as a normal flora, ability to adhere to a range of extracellular

matrixproteins, including thrombospondin, lactoferrin and vitronectin; and ability to adhere to urinary tract epithelia, oral cavity epithelia and human embryo kidney cells. *E.faecalis* possesses aggregation substance (Agg) and cell-surface protein which contribute to pathogenesis. The presence of Agg increases the hydrophobicity of the enterococcal cell surface. This Agg is a pheromone-inducible surfaceglycoprotein and mediates aggregate formation during conjugation, thereby helping in plasmid transfer as well as adhesion to an array of eukaryotic surfaces (Koch *et al.*, 2004)

Extracellular surface protein (Esp) possessed by *Entero*coccus spp. is believed to promote adhesion, colonization and evasion of the immune system, and to play some role in antibiotic resistance (Foulquie *et al.*, 2006). Enterococcal biofilm formation according to Borgmann *et al.* (2004) is aided by Esp thus making the organism to resist environmental stresses, and adhesion to eukaryotic cells such as those of the urinary tract. Also pili formation in enterococci is important for biofilm production which is important in causing endodontic and UTI including endocarditis (Singh *et al.*, 2007).

Other virulence factors that contribute to the pathogenicity of *Enterococcus* species include haemolysin, a bacterial toxin; hyaluronidases, gelatinase and serine protease which are hydrolytic enzymes (Koch *et al.*, 2004).

2.5 Vancomycin

Vancomycin which is traditionally regarded as a drug of last resort is a glycopeptide antibiotic used in the prophylaxis and treatment of infections caused by Gram-positive bacteria. The organism that produced it is named *Amycolatopsis orientali*s and was first isolated from a soil sample in 1953 by Edmund Kornfeld (Shnayerson and Plotkin,2003).The compound was initially called compound 05865, but was eventually given the generic name

vancomycin derived from the term"vanquish"(Levine,2006).The original indication for vancomycin was for the treatment of methicilin-resistant *Staphylococcus aureus* (Levine,2006)

2.5.1 Pharmacology and Chemistry of Vancomycin

Vancomycin is a branched tricyclicglycosylated non-ribosomal peptide produced by the fermentation of the Actinobacteria species *Amycolatopsis orientalis*. Vancomycin inhibits cell wall synthesis in Gram-positive bacteria but is not active against Gram-negative bacteria except some non-gonococcal species of *Neisseria*. Vancomycin prevents incorporation of N-acetylmuramic acid (NaM) and N-acetylglucosamine (NaG) peptide subunits into the peptidoglycan matrix; which forms the major structural component of Gram-positive cell walls.The large hydrophilic molecule is able to form hydrogen bond interactions with the terminal D-alanyl-D-alanine moieties of the NaM/NaG-peptides.

Vancomycin is used for the treatment of serious, life-threatening infections caused by Gram-positive bacteria that are not responding to other less-toxic antibiotics.The increasing emergence of vancomycin-resistant enterococci resulted in the development of guidelines for use by the Centres for Disease Control and prevention (CDC) Hospital Infection Control Practices Advisory Committee (Rossi,2006).

2.5.2 The Origin of Vancomycin Resistance genes in Enterococci

The origin of the genes encoding high-level resistance to glycopeptides in enterococci is not known. It is postulated that enterococci may have acquired vancomycin resistance genes present in other microorganisms of the environment due to the pressure of increased glycopeptide usage both in agriculture and in clinical practice (Bates *et al.,* 1993; Aarestrup *et al.,* 1996).Intrinsically vancomycin-resistant bacteria such as *Leuconostoc,*

Pediococcus, and *Lactobacillus* with the glycopeptide-producing microorganisms are potential reservoirs. Alternatively, the genes may have originated through mutations in homologous genes within enterococci or other bacteria.

Three genes encoding homologues of *vanH*, *vanA*, v*anB* and v*anX* have been cloned from glycopeptide producing *Streptomyces toyocaensis* and *Amycolatopsis orientalis* (Marshall *et al.*,1998). Also, the arrangement of the *vanH*, *vanA* (*vanB*), and *vanX* genes in these organisms is identical to the arrangement found in VRE indicating that glycopeptide-producing organisms use a resistance mechanism that is similar to that found in VRE, which means that they have been the source of resistance genes in VRE. Marshall *et al*. (1998) also noted that the G+C (guanine+cytosine) contents of the VRE *vanH*, *vanA* (*vanB*) and *vanXgenes* are 5–10% higher than those of the adjacent *vanR*, *vanS*, *vanY*, and *vanZ* (or *vanW*) implying that the glycopeptide-producing organisms with different G+Ccontents than *S. toyocaensis* and *A. orientalis* genes may have served as donors of the *vanHA*(B)X gene cluster. Alternatively, bacteria that do not produce glycopeptide may have acquired the glycopeptide resistance genes as a defence mechanism, and eventually they may have passed them to enterococci.

2.6 Mechanisms of Resistance:

2.6.1. Mechanism of resistance to cell-wall active agents

2.6.1.1 ***Resistance to β-Lactam antibiotics***

Resistance to β-lactams is a characteristic feature of the genus *Enterococcus. E. faecalis* is typically10 to 100 times less susceptible to penicillin while *E. faecium* is at least 4 to 16 times less susceptible than *E. faecalis* (Murray, 1997). While most isolates of *E. faecalis* are inhibited by concentrations of penicillin or ampicillin (1 to 8 µg/ml) easily

achievable in humans, isolates of *E. faecium* usually require an average of 16 to 64 µg/ml to inhibit growth, although some isolates are even more resistant (Murray,1997). An additional problem with enterococci is that they are typically tolerant to β-lactams due to the production of low-affinity PBP (Fontana *et al*., 1985).Penicillin resistance is directly proportional to the amount of PBP5(penicillin-binding proteins) produced.*E. faecium* are less susceptible to ß-lactam antibiotics than *E. faecalis* because they produce low affinity penicillin-binding proteins for antibiotics. Loss of the ability of a strain of *E. faecium* to produce PBP5 caused this highly penicillin-resistant strain to become hyper-susceptible to penicillin (Fontana *et al*.,1985).

2.6.1.2 ***Resistance to vancomycin***

***Phenotypic description*.** There are five recognized phenotypes of vancomycin resistance, *vanA, vanB, vanC, vanD,* and *vanE* (Fines *et al*., 1999). Two of these (*vanA* and *vanB*) are mediated by newly acquired gene clusters not previously found in enterococci. The *vanA* and *vanB* resistance phenotypes were described primarily in *E. faecalis* and *E. faecium*. The *vanA*-resistant strains possess inducible, high-level resistance to vancomycin and teicoplanin (Arthur and Courvalin, 1993, Cetinkaya *et al*., 2000). The levels of vancomycin resistance among *vanB* isolates may range from 4 to ≥1,000 µg/ml but susceptible to teicoplanin (Cetinkaya *et al*., 2000). The *vanB* resistance determinants also reside on large mobile elements that can be transferred from one strain of *Enterococcus* to another (Quintiliani and Courvalin, 1994, Cetinkaya *et al*., 2000). The *vanC* resistance phenotype was described in *E. casseliflavus* and *E. gallinarum*, which demonstrate intrinsic, low-level resistance to vancomycin and are susceptible to teicoplanin (Moura *et al*., 2013).

Genotypic classification and resistance mechanisms. During peptidoglycan synthesis in enterococci, two molecules of D-alanine are joined by a ligase enzyme to form D-Ala-D-Ala, which is then added to UDP-*N*-acetylmuramyl-tripeptide to form the UDP-*N*-acetylmuramyl-pentapeptide. The UDP-N-acetylmuramyl-pentapeptide, when incorporated into the emerging peptidoglycan, permits the formation of cross-bridges and contributes to the strength of the peptidoglycan layer (Eliopoulos, 1997).Vancomycin binds with high affinity to the D-Ala-D-Ala termini of the pentapeptide precursor units, blocking their addition to the growing peptidoglycan chain and preventing subsequent cross-linking (Zaman *et al*., 1996).

VanA glycopeptide resistance. The *vanA* gene and other genes such as *vanR*, *vanS*, *vanH*, *vanX* and *vanZ* involved in the regulation and expression of vancomycin resistance are located on a 10,581-bp transposon (Tn*1546*) of *E. faecium*, which often resides on a plasmid (Arthur *et al*.1993). Bugg *et al*. (1991) noted that expression of these genes results in the synthesis of abnormal peptidoglycan precursors terminating in D-Ala-D-lactate instead of D-Ala-D-Ala. Vancomycin binds to D-Ala-D-Lac with markedly lower affinity than it does to the normal dipeptide product. The core protein functions favouring synthesis of pentapeptide terminating in D-Ala-D-Lac include vanA protein which is a ligase of altered substrate specificity that produces D-Ala-D-Lac in preference to D-Ala-D-Ala; *VanH* protein which is a D-hydroxy acid dehydrogenase that creates a pool of D-lactate for use in the above reaction and vanX protein is a D,D-dipeptidase lacking activity against D-Ala-D-Lac (Bugg *et*

al.,1991). This enzyme reduces pools of D-Ala-D-Ala produced by the native enterococcal ligase, thereby minimizing the competing synthesis of normal pentapeptide (Wu *et al*., 1995).

Resistance to vancomycin cannot be conferred alone by *vanA*, probably because D-hydroxy acids are neither natural products present in the environment of enterococci nor normally produced by enterococci (Leclercq and Couvalin, 1997). Thus, to synthesize D-lactate, enterococci must acquire the gene(s) within the vanA operon required to produce the substrate for vanA. The *vanH* is responsible for the synthesis of D-lactate.

Arthur *et al* (1997) noted that *vanR* and *vanS* proteins constitute a two-component regulatory system that regulates the transcription of the *vanHAX* gene cluster*vanS* detect the presence of vancomycin (Arthur and Courvalin, 1993) or, more likely, some early effect of vancomycin on cell wall synthesis. The *vanS* then signal *vanR*, the response regulator, which results in activation of the synthesis of some other proteins such as *vanH*, *vanA*, and *vanX* involved in resistance.

There is organizational heterogeneity of the *vanA* gene cluster. The Tn*1546* were present in some strains but had insertion-like sequences between *vanS* and *vanH* in others. These vancomycin resistance gene clusters may be incorporated into even larger mobile elements containing additional insertion-like elements (Handwerger *et al*., 1995).

Cross-linking of the precursors to the growing peptidoglycan is processed in bacteria by the PBPs, with PBP5 being used in enterococci (Fontana *et al*., 1985). The replacement of D-Ala by D-Lac does not impair cross-linking of the modified precursors to the growing peptidoglycan chain. However, PBPs other than PBP5, which are so far not known to play a role in cell wall synthesis, are probably required for processing of the altered precursors (Al-Obeid *et al*., 1992). These high-molecular-weight PBPs display a higher affinity for β-lactams.Since *VanA* resistance is inducible, a shift in the PBPs occurs only in the presence of

vancomycin and results in β-lactam hyper-susceptibility. This effect explains the synergy displayed by the combination of the two classes of drugs against vancomycin-resistant strains.

VanB glycopeptide resistanc. The *vanB* protein helps in the production of the pentadepsipeptide terminating in D-Ala-D-Lac**.** Enterococci resistance due to *vanB* glycopeptides is mediated by an abnormal ligase that is structurally related to *vanA* ligase (Evers and Couvalin, 1996). Baptista *et al*. (1996) reported that genes that are analogous to their class A resistance counterparts are designated $vanH_B$, $vanX_B$, $vanY_B$, $vanR_B$, and $vanS_B$. The activity of D, D-dipeptidase levels ($vanX_B$) correlate with levels of vancomycin resistance (Arthur *et al*., 1997). There is less homology between the RS and Y protein of *vanA* and *vanB* VRE but a high degree of sequence identity between *vanHAX* and $vanH_BBX_B$ (Evers and Courvalin, 1996).The $vanY_B$ is not found in all strains, and its position in the gene clusters differs fromthat of *vanY* in Tn*1546* (Evers and Courvalin,1996). DNA sequence heterogeneity among *vanB*-1, *vanB*-2, and *vanB*-3 *which are* three subtypes of the *vanB* ligase gene have been reported (Patel *et al.,* 1998*).* The *vanA* proteins are induced by Teicoplanin but not the production of *vanB*-related proteins. Vancomycin induces the synthesis of the resistance proteins of both *vanA* and *vanB.* That is to say that if a teicoplanin-susceptible *Enterococcus* with the *vanB* gene cluster is pre-exposed to vancomycin, the strain will become teicoplanin resistant as well.

Clark *et al* (1993) observed that the difference between *vanA*- and *vanB*-type resistances is that *vanA* is more widely distributed and predominates in Europe while *vanB* strains are fairly common in the United States. The v*anA* ligase gene has also been found not only in enterococcal species but in *Corynebacterium* spp., *Arcanobacterium haemolyticum*, and *Lactococcus* spp., while *vanB* has been found primarily in *E. faecium* and *E. faecalis*. The

difference in the dissemination of this resistance trait is observed to be due the location of v*anA* gene cluster on a transposon similar to Tn*1546,* which, in turn, can be a part of a conjugative plasmid (Handwerger *et al*., 1995).The *vanB* cluster which was thought not to be transferrable to other bacteria is often located on the host chromosome and can be transferable as part of large mobile elements.

VanC glycopeptide resistance. *Enterococcus gallinarum, E. casseliflavus,* and *E. flavescens* have low-level resistance to vancomycin. Clark *et al.* (1993) reported the nucleotide sequences of the *vanC*-1 gene in *E. gallinarum,* the *vanC*-2 gene in *E. casseliflavus,* and the *vanC*-3 gene in *E. flavescens*.The *vanC* ligase of *E. gallinarum* favours the production of a pentapeptide terminating in D-Ala-D-Ser. Substitution of D-Ser for D-Ala is thought to weaken the binding of vancomycin to the novel pentapeptide. Insertional inactivation of vanC1unmasks the associated production of the D-Ala-D-Ala pentapeptide in *E. gallinarum* (Reynolds *et al*., 1994). The presence of variable amounts of D-Ala-D-Ala relative to D-Ala-D-Ser could account for the variable levels of vancomycin resistance observed among isolates of VRE carrying the *vanC* phenotype (Murray, 1997).The *vanC-2* gene of *E. casseliflavus* demonstrates nucleotide sequence similarity to *vanC-1 and possess additional native ligase*. There is extensive homology between the gene sequences of *vanC2* and *vanC3* (Murray,1997). The *vanA* genes have been identified in strains of *E. gallinarum* and *E. casseliflavus* that confer high level resistance to glycopeptide in them (Dutka-Malen *et al*., 1994).

VanD glycopeptides resistance. *Enterococcus faecium* carrying vancomycin resistance gene designated *vanD* was first described in a New York Hospital in 1991 (Perichon *et al*., 1997). Partial sequencing of the ligase gene showed that it was distinct from but similar to the *vanA*

and *vanB* ligase genes. The *vanD* appears to be located on the chromosome and is not transferable to other enterococci.

VanE glycopeptide resistance. Fines *et al*. (1999) described *vanE* vancomycin resistance gene in *E. faecalis* BM4405, which is resistant to low levels of vancomycin and susceptible to teicoplanin.This new resistance phenotype has similarities to the intrinsic *vanC* type of resistance. The deduced amino acid sequence has a greater identity to *vanC* than to *vanA*, *vanB*, or *vanD* (Fines *et al*.,1999).

Other resistance classes and organisms. It has been noted that *Lactobacillus casei, Pediococcus pentosaceus*, and *Leuconostoc mesenteroides* are naturally resistant to glycopeptides even though they have D-Ala-D-Lac terminus as found in VRE with *vanA* or *vanB* phenotypes yet DNA from these organisms does not hybridize with resistance gene probes prepared from VRE (Handwerger *et al*., 1995).

Conjugal transfer of vanA-type vancomycin resistance genes from enterococci to other gram-positive cocci such as group A and viridans group of streptococci, *Listeria monocytogenes* and *Staphylococcus* has been done in the laboratory (Navarro,1994).Vancomycin resistance genes have already been found in human isolates of non-enterococcal organisms. A vanB-related gene sequence designated as vanB3 has been found in *Streptococcus bovis* (Poyart *et al*., 1997).

Vancomycin-dependent enterococci. Some strains of *vanA*- and *vanB*-type VRE require vancomycin for growth (Woodford *et al*., 1995). Vancomycin-dependent enterococci have been recovered from vancomycin-containing agar. The explanation for vancomycin dependence is that these enterococci turn off their normal production of D-Ala-D-Ala and then grow only if a substitute dipeptide-like structure is made. With most *vanA*- and *vanB*-

type enterococci, this occurs only in the presence of vancomycin, which induces the synthesis of associated dehydrogenase (*VanH*) and ligase (*vanA* or *vanB*) that make D-Ala-D-Lac. D-Ala-D-Ala synthesis is turned off in the presence of vancomycin since it is not necessary for cell wall synthesis by VRE (Murray, 1997). Once the vancomycin is removed, D-Ala-D-Lac is no longer synthesized, and without either D-Ala-D-Ala or D-Ala-D-Lac, the cell cannot continue to grow or replicate. Reversion to vancomycin independence has been observed; it probably occurs by either a mutation that leads to constitutive production of D-Ala-D-Lac or one that restores the synthesis of D-Ala-D-Ala.

2.6.2 Mechanisms of resistance to agents interfering with nucleic acid replication, transcription and synthesis

2.6.2.1 ***Rifampicin Resistance***

Rifampicin prevent the initiation of transcription by binding to the beta subunit of RNA polymerase thus inhibiting bacterial growth. The drug is used for the treatment of *Mycobacterium tuberculosis* and of recent has been used for the treatment of infections caused by *Staphylococcus* spp. Resistance to rifampicin results from mutations of specific sites in the genes encoding the beta subunit of the RNA polymerase which reduces the affinity of rifampicin for the polymerase. Also, enzymatic inactivation of rifampicin has been observed in some cases. Rifampicin is not commonly used for the treatment of enterococcal infections but acquired resistance to it is common. This could be due to the exposure of commensal enterococci to rifampicin during treatment for non-enterococcal infections including other unknown factors (Miller *et al*., 2014).

2.6.2.2 ***Quinolone Resistance***

Enterococcus spp exhibit moderate susceptibility to quinolones. Bacterial growth is inhibited by interfering with DNA replication by binding to the type II topoisomerase that control DNA supercoiling (DNA gyrase and DNA topoisomerase IV) and inhibiting their function that leads to lethal double-strand breaks in the DNA. Bacterial resistance to quinolones occurs via mutations in the "quinolone resistance determining regions" of the genes that encode gyrase and topoisomerase IV. These mutations prevent efficient binding of the antibiotic to the enzyme, which enables DNA replication to continue despite the presence of the antibiotic. Such mutations have been observed in clinical and lab-derived quinolone-resistant isolates of enterococci and presumably act to confer enhanced quinolone resistance through a similar mechanism (Miller *et al*., 2014).

Efflux of the antibiotic out of the bacterial cell is another mechanism of quinolone resistance in other species of bacteria. Such efflux is often a function of pumps with relatively broad or nonspecific substrate specificities, which are sometimes referred to as multidrug-resistance efflux pumps (MDRs). Genes encoding MDRs are usually found on the bacterial chromosome (Miller *et al*., 2014).

2.6.3 Mechanisms of resistance to antibiotics that interfere with protein synthesis

2.6.3.1 ***Aminoglycoside resistance***

Moderate and high-level streptomycin resistance has been demonstrated in enterococci.Moderate-level resistance is said to be due to low permeability and can be overcome by penicillin that increases the cellular uptake of the aminoglycoside.High-level resistance is ribosomally mediated or as a result of aminoglycoside- inactivating enzyme production (Moellering and Weinberg, 1971). Enterococcal resistance to gentamicin and streptomycin occur by different mechanisms. Gentamicin resistance is predominantly the result of the presence of the inactivating enzyme 2"-phosphotransferase-6'-acetyltransferase

conferring resistance to gentamicin, tobramycin, netilmicin, amikacin, and kanamycin. Hence, gentamicin resistance is a good predictor of resistance to other aminoglycosides except streptomycin. Streptomycin resistance is encountered mainly in enterococcal strains that produce streptomycin adenyltransferase; these strains remain susceptible to gentamicin (Herman and Gerding, 1991).

2.6.3.2 ***Linezolid resistance***

Linezolid is a member of the oxazolidinone family of antibiotics used against multidrug-resistant Gram-positive bacteria. Bacterial growth is interfered by linzolid as it inhibits protein synthesis through interaction with the translational initiation complex. Mutations within the central loop of domain V of 23S rRNA, including a G2576U mutation, are associated with resistance to linezolid, and are believed to prevent or reduce binding of the antibiotic to the ribosomal subunit. Analysis of linezolid-resistant *E. faecalis* and *E. faecium* isolates selected during therapy, or obtained from patients following linezolid treatment failure, revealed a direct correlation between the percentage of rRNA genes that carry a G2576U mutation (each genome encodes several copies of the rRNA genes—four in *E. faecalis* and six in *E. faecium*) and the phenotypic level of linezolid resistance, which suggests that the percentage of ribosomes that carry rRNA with the G2576U substitution is the primary determinant for the level of linezolid resistance.

2.6.3.3 ***Macrolide Antibiotics***

Erythromycin which belong to the class macrolides inhibit protein synthesis by binding to the 50S subunit of the ribosome. Macrolides are not used to treat enterococcal infections, but resistance to them is common. The production of an enzyme that methylates a

specific adenine in the 23S rRNA of the 50S ribosomal subunit which reduces the binding affinity of the macrolide for the ribosome is the most common form of acquired resistance to macrolides. The responsible enzyme is typically encoded by the *ermB* gene, and the phenotype is often referred to as MLS_B. An efflux pump, encoded by the transferrable *mefA* gene, is also known to pump macrolides out of the cell, but confers a lower level of resistance than *ermB* (Miller *et al*., 2014).

2.6.3.4 **Tetracyclines Resistance**

Tetracyclines are bacteriostatic agents and exert their antibacterial effect by binding to the ribosome and interfering with the docking of aminoacyl-tRNA which occurs through association with several loops of the *16SrRNA* and the ribosomal protein S7. Resistance is mediated by multiple genes but through the strategies of efflux of the antibiotic and ribosomal protection. Efflux pumps encoded by ***tetK*** and ***tetL*** are plasmid-borne determinants that encode proteins with 14 α-helices that make up the transmembrane domains and confer resistance to tetracycline. Expression of resistance is regulated by translational attenuation in the absence of the antibiotic due to the formation of a stem loop structure in the mRNA, which masks the second of two ribosomal binding sites In the presence of tetracycline, the ribosome complex is unable to synthesize the normal leader peptide, an alternate loop structure forms in the mRNA and the second binding site becomes accessible, allowing for synthesis of the efflux pump. The genes ***tetM***, ***tetO*** and ***tetS*** are chromosomal resistance determinants, which confer resistance to doxycycline and minocycline as well as tetracycline and can be transferred via the Tn***916*** transposon. These genes code for a protein with a significant homology to bacterial elongation factors (EFs), and like EFs they are able to hydrolyze GTP in the presence of the ribosome, which alters ribosomal conformation and displaces bound tetracycline (Miller *et al*., 2014).

2.6.3.5 ***Resistance to Chloramphenicol***

Chloramphenicol is a bacteriostatic agent that act by inhibiting protein synthesis. It inhibits protein chain elongation by inhibiting the peptidyl transferase activity of the bacterial ribosome. Chloramphenicol resistance is most often due to acetyltranferase which inactivate it. Chloramphenicol resistance may be due to efflux of chloramphenicol through specific membrane-associated transporters. The genes coding for chloramphenicol acetyltransferase and specific exporters, are often associated with plasmids, transposons or gene cassettes. Chloramphenicol resistance may also occur from mutations which reduce the expression of outer membrane proteins, mutations in the *23SrRNA*, inactivation of 3-*0*-phosphotransferases or target modification by a *23SrRNA* methylase (Mayers, 2009).

2.6.3.6 ***Resistance to Nitrofurantoin***

Nitrofurantoin is an antimicrobial of empiric therapy for community-acquired and nosocomial lower UTIs. A bacteriostatic or bactericidal agent depending on the concentration and susceptibility of the infecting organism. Nitrofurantoin is active against most common uropathogens such as *Escherichia coli* and *Enterococci*, the most frequent causes of nosocomial lower UTIs such as catheter-associated bacteriuria. Nitrofurantoin is also active against vancomycin-sensitive enterococci and vancomycin-resistant enterococci. Nitrofurantoin is activated by bacterial flavoproteins (nitrofuran reductase) to active reduced reactive intermediates that are thought to modulate and damage ribosomal proteins or other macromolecules, especially DNA, causing inhibition of DNA, RNA, protein, and cell wall synthesis. The overall effect is inhibition of bacterial growth or cell death. Nitrofurantoin is highly stable to the development of bacterial resistance, a property thought to be due to its multiplicity of mechanisms of action (Onoh *et al*., 2013).

2.7 Sources and Transmission of Resistant Organisms

2.7.1 Human Reservoir

Resistance emerges when native microorganisms are exposed to antibiotics or when they acquire genetic resistance factors from neighbouring organisms. Once resistant, microorganisms can spread through the population via human, environmental, or animal reservoirs (Miller *et al*., 2014).

There is an enduring consensus that antibiotic exposure lies at the root of the complex mechanisms through which resistance emerges. Most healthy human non-carriers administered a glycopeptide antibiotic (vancomycin or teicoplanin) orally will become colonized with VRE (Donskey and Rice, 1999). Antibiotic exposure can cause the emergence of VRE by inducing the expression of resistance genes and by selecting strains already expressing these genes. By altering the competing microbial flora in the GI tract, thereby increasing VRE concentration in the stools, antibiotic exposure can also facilitate the transmission of VRE (Donskey and Rice, 1999).

2.7.2 Environmental Reservoir

Hospital areas most likely to harbour VRE-colonized patients include ICUs, organ transplant, oncology, and dialysis and AIDS units. During outbreaks, 7 to 30% of environmental surface cultures in such areas may be positive for VRE. The highest contamination rates occur on bed rails, bedside Tables, objects near the patient and on multiple hospital fabrics (Neely and Maley, 2000).VRE is an environmental contaminant because it survives for long periods of time on dry surfaces: 60 min on gloves, telephones, and stethoscopes; 24 h on bedrails; and up to 7 days on countertops. Contaminated fomites

linked with VRE outbreaks include electronic thermometers, ear oxymeters and bronchoscopes (Noskin *et al*., 2000)

2.7.3 Animal Reservoir

VRE were first found outside health-care settings in 1993, when *E. faecium* was isolated in sewage treatment plant waste water in England and Germany. Since then, VRE have been isolated from pig and poultry faeces and from uncooked chicken in Europe (McDonald *et al*., 1997) European livestock feeds in Europe were supplemented with avoparcin antibiotic and therefore was thought to select for VRE (McDonald *et al*., 1997). This hypothesis has been supported by epidemiologic studies documenting an association between avoparcin-containing feeds and VRE recovery from animals (McDonald *et al*., 1997). The genetic similarity of the strains isolated from animals and workers indicates that VRE can be transmitted from animals to humans (Woodford *et al*., 1998). Based on this, the use of avoparcin was banned yet, VRE prevalence in some farm animals has remained high (Borgen *et al*., 2000).

2.8 Vancomycin Resistant Enterococcal (VRE) Colonisation and Risk Factors

Vancomycin Resistant Enterococci intestinal colonization does not result in symptoms but may serve as a reservoir for the transmission of VRE to other patients. VRE colonization is a precursor and a risk factor for VRE infection. According to Patel (2003), VRE was first identified in liver transplant patient at the Mayo clinic in 1995. In most cases, colonizing VRE were cultured from rectal swabs. Out of 53 colonized patients, six (11.3%) were documented with VRE infection (bacteraemia, wound infection, and/or intra-abdominal infection).

Exposure and susceptibility of a host to VRE is a risk of being colonized with VRE .It has been reported that proximity to other patients who are colonized with VRE especially those with diarrhoea (Sandoe *et al*., 2002) and those carrying the highest number due to antimicrobial therapy and length of hospital stay (Baden *et al*., 2001) are risk factors.

Severely ill patients and those on prolonged antimicrobial therapy are at most risk of becoming colonized with VRE when the proportions of patients with VRE are low (Beltrami *et al*., 2000). Baran *et al*. (2002) also noted that healthcare workers and their household are at risk of becoming colonized with VRE.

Stool incontinency which contaminates the environment with VRE has been associated with high-level stool colonization (Donskey *et al*., 2000). Patients may shed VRE for at least 5 years (Baden *et al*., 2001) which contributes to risk factors.

Elizaga *et al*. (2002) in their study observed that patients in long-term care facilities in urban referral hospitals in the USA have been shown to be a major reservoir of VRE, which can be transmitted to other inpatients in the urban referral hospital, in the long-term care facility and in smaller community hospitals. Ostrowsky *et al*. (1999) and Tokars *et al*. (2001) in their separate studies stated that organ transplant recipients and haemodialysis patients are also at increased risk of VRE colonization.

2.9 Vancomycin Resistant Enterococci Infections and Risk Factors

Vancomycin Resistant Enterococcal infections do arise in VRE-colonized patients, depending on the patient's population. Patel *et al*. (2003) reported clinical VRE colonisation in 53 (40 liver and 13 kidney transplant recipients) which often results to infection such as bacteraemia, wound infection and intra-abdominal infection. Patel *et al*. (2003) also reported that of seven liver transplant patients at the Mayo Clinic who were colonized with VRE at the

time of transplant, only one developed VRE infection post-transplant. On the other hand, Johnston *et al*. (2000) noted that colonization with VRE correlates with poor outcome in patients undergoing allogeneic blood and marrow transplants. Of seven patients undergoing allogeneic blood and marrow transplant that were colonized with VRE at the time of transplant, six developed VRE infection following transplantation and five died.

The ratio of VRE-infected to VRE-colonized patients depends on the specific patient population and would be substantially lower in a healthier population. Large numbers of VRE have been isolated from patients' stool whose blood, urine and wound specimen grew VRE on culture (Donskey *et al*., 2000). Vergis *et al*. (2001) and Husni *et al*.(2002) in their separate studies noted that risk factors for bacteraemia with VRE include haemodialysis, receipt of corticosteroids, anti-neoplastic agents or total parenteral nutrition, surgery, severity of illness, antimicrobial administration, indwelling bladder catheters, neutropenia and mucositis. According to Scagnelli *et al*. (2001), most cases of VRE bacteraemia in Northern Italy, occurred in neutropenic patients with haematological malignancies.

2.10 Mechanisms of Vancomycin Resistant Enterococcal infection

The entry points for VRE include the urinary tract, intra-abdominal tract, wounds and intravascular catheters. Isolation of VRE from the urine samples alone has limited clinical significance (Wong *et al*., 2000).Urinary tract infections caused by VRE are nosocomial and are associated with urinary instrumentation. Patients colonized with VRE have a high prevalence of skin colonization. In liver transplant recipients, the most common source of VRE bacteraemia is the abdomen.

2.11 Epidemiology of Enterococcal Infection

The origins of *Enterococcus* species vary from environmental to animal and human sources. Enterococci are essential part of the normal flora of both humans and animals. The

distribution of *E. faecium* and *E. faecalis* are the most common in the human gastrointestinal tract, *E.faecium* in production animals and *E. mundtii* and *E. casseliflavus* in plant sources (Klein, 2003). *E. faecalis* inhuman faeces range from 10^5 to 10^7 per gram, while *E. faecium ranges* from 10^4 to 10^5 per gram. *E. faecium* and *E. faecalis* are isolated more from human faeces than that of livestock's (Franz *et al.*, 1999).

The ecological and epidemiological studies of *Enterococcus* reported that *E. faecalis* and *E. faecium* are regularly isolated from fish, cheese, sausages, minced beef and pork (Foulquie *et al.*, 2006).

Kuhn *et al*. (2003) noted that 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. This could be associated to lack of diversity with the virulence factors of this species. Mutnick *et al*. (2003) observed that *Enterococcus* species are opportunistic pathogens as was shown in a study that hospitalized patient may have a 57 % isolation rate of *E. faecalis* whereas healthy individuals show only a 39–40 % occurrence. Hospitalized patients may have a greater incidence of enterococcal infection not only because of virulence, but because the hospital itself is a reservoir as the organism can contaminate and remain around the patient for days (Brown *et al*., 2006).

VRE have been found in many countries especially in Europe and The United States (Deshpande *et al*., 2007).

According to reports made available to centres for Disease Control and Prevention's National Nosocomial Infections Surveillance system (NNIS) from 1989 through 1993, the percentage of nosocomial enterococcal infections due to VRE increased from 0.3 to 7.9% (Fraser *et al*., 2010). This increase was because of a 34-fold rise (from 0.4 to 13.6%) of VRE

infections in intensive care unit (ICU) patients ,and non-ICU patients (Fraser *et al*., 2010).In 2003, the percentage of nosocomial enterococcal isolates exhibiting vancomycin resistance in ICU patients increased to more than 28%—an increase of 12% compared with 1998-2002.NNIS data reveal the pooled mean for vancomycin-resistant *Enterococcus* species from all ICUs, non-ICU inpatient areas, and outpatient areas were 13.9%, 12%, and 4.6%, respectively, from 1998 through June 2004 (NNIS, 2004). Vancomycin resistant enterococci (VRE) was initially isolated mainly in large university hospitals, but subsequent reports demonstrate the presence of significant VRE epidemics in community hospitals and chronic care facilities, whereby a single clone can easily spread.

The nosocomial pathogenicity of enterococci has emerged in recent years, as well as increasing resistance to glycopeptide antibiotics. Vancomycin resistance occurs when a susceptible enterococci is exposed to a resistant strain. Colonisation with VRE precedes infection; therefore, knowledge of the epidemiology of VRE is essential to prevent it from spreading. According to Boyce (1997), monoclonal outbreak of *vanB E. faecium* revealed that the primary risk factors for colonization with VRE were proximity to a case or exposure to a nurse who cared for a case. The epidemic strain was later isolated from 26 out of 92 samples cultured from the environmental surfaces in the rooms of case patients. The result showed that VRE spread was primarily by cross-contamination. Also, molecular epidemiologic studies revealed that cross-transmission of VRE is the main means of spread in ICU accounting for 85% of acquired colonization due to endemicity of VRE at the hospital. Screening was also conducted from 5 patients who yielded VRE. Thus most colonized patients would have gone undetected if active screening for VRE had not been done.

The Epidemiology of VRE shows different patterns. In Europe, VRE has been detected in hospital samples, in community-acquired infections, in asymptomatic carriers and farm animals (Maraha *et al*., 1992; Repizo *et al*. 2014).

Robredo *et al*. (1999) associated the emergence of such strains to the use of avoparcin, a glycopeptide growth promoter in farm animals .According to Stobberingh *et al* (1999), VRE in poultry and pigs, are probably a major reservoir of VRE for humans which suggest that infection could pass from animals to humans through food chain. As a result of this, avoparcin usage has been restricted in the European Union. On the other hand, VRE is only detected in hospitals since avoparcin has not been licensed in USA. Donskey and Rice (1999) observed that resistance is associated with high dosage of antibiotics and frequent vancomycin administration, especially through the oral route. VRE is spread through the clonal dissemination of a single resistant strain and the genetic transfer of resistance encoding plasmids and/or transposons between different strains or species. When VRE have been present in a hospital or community for months or years, molecular typing of isolates often reveals that vancomycin resistance has spread by plasmids or transposons to many different clones (Gilmore, 2002).

The epidemiology of VRE is mainly focused in hospitals than in communities in the United States (Murray, 1995).This is because investigators have failed to find any VRE in the faeces or carcasses of chickens and from healthy volunteers in two studies (Murray,1995). Friden *et al.* (1993) reported two cases of community-acquired VRE in urinary tract infections in New York City. Also, an elderly woman colonized with VRE who was cared for by her husband was diagnosed with UTI due to VRE that was not differentiated from the woman's isolate by PFGE (Shekar *et al*., 1995). Thus, there is possibility that colonized patients can transmit VRE in the community as they leave the hospital.

In Europe, VRE have been isolated from sewage and various animal sources. The *vanA* resistant *E. faecum* was isolated from frozen poultry and pork and from the faeces of 12 of 100 non-hospitalized inhabitants in a rural area. The *vanA* VRE have also been found in the faeces or intestines of other farm animals or pets, including horses, dogs, chickens, and pigs. These observations suggest that the resistance genes of VRE could reach humans through the food chain or through contact with domesticated animals (Sun et *al*., 2014).

2.12 Mortality/Morbidity

Enterococci show lower virulence than *S. aureus*. Enterococcal infections often occur in debilitated patients and as part of polymicrobial infections. These factors limit the ability of investigators to determine the independent contribution of enterococcal infections to mortality and morbidity.

DiazGranados *et al*. (2005) noted that vancomycin-resistant bacteraemia increases the length of hospital stay by an average of 2 weeks which is attributable to mortality rate of up to 37% from these infections. Mortality rates associated with enterococcal infections may exceed 50% in critically ill patients, those with solid tumours, and some transplant patients. Bacteraemia caused by VRE strains carries higher mortality rates than does bacteraemia due to vancomycin-susceptible strains (DiazGranados *et al*., 2005).

2.13 Sociodemographic factors associated with Enterococcal Infections

2.13.1 Sex

Enterococcal infections are generally distributed equally between the sexes. Although urinary tract infections are more common in healthy women than in healthy men, enterococci

are an uncommon cause of uncomplicated cystitis in this setting. In published series of enterococcal endocarditis, men often outnumber women (Fraser *et al*., 2010).

2.13.2 Age

Enterococcal infections are more common in elderly patients because of various associated factors such as urinary tract catheterization and instrumentation that are more common in elderly populations. Elderly persons commonly undergo abdominal surgery for diverticulitis or biliary tract disease and suffer more from enterococcal endocarditis (McDonald *et al*., 2005). Boyce *et al.* (1993) also noted that critically ill old patients or those with severe immunosuppression, those in oncology or transplantation wards suffer more from VRE. Enterococci occasionally cause bacteraemia and meningitis in neonates. Butler (2006) reported outbreaks of enterococcal infections, including VRE infections, in neonatal ICUs, paediatric ICUs, and haematology/oncology units, but, overall, VRE infections are less common in paediatric patients than in adults.

2.14 Treatment

2.14.1 **Antimicrobial therapy**

Ampicillin is the drug of choice for susceptible *E. faecalis infection*. For rare strains that are resistant to ampicillin because of beta-lactamase production, ampicillin plus sulbactam may be used. Vancomycin should be used in patients with a penicillin allergy or infections with strains that have high-level penicillin resistance due to altered PBPs. Nitrofurantoin is effective in the treatment of enterococcal urinary tract infections, including

many caused by vancomycin-resistant enterococci (VRE) strains. Combination therapy with a cell wall–active agent (e.g., ampicillin, vancomycin) and an aminoglycoside (e.g., gentamicin, streptomycin) is necessary to adequately treat enterococcal endocarditis. The resultant bactericidal synergism is effective against susceptible enterococcal strains. For multidrug resistant enterococci, vancomycin is used for strains showing high level resistance to ampicillin. For gentamicin-resistant strains, the only alternative is streptomycin. Gavalda *et al*. (2007) noted that prolonged therapy with ampicillin plus imipenem-cilastatin or ampicillin plus ceftriaxone may be used for multidrug resistant *E. feacalis* infection.

Vancomycin resistant enterococci (VRE) infection such as uncomplicated urinary tract infections have been treated successfully with nitrofurantoin. Isolates that are relatively susceptible to penicillin or ampicillin (MICs of 0.5-2 µg/ml) may be treated with high doses of these agents. Doxycycline, chloramphenicol, and rifampin in various combinations have been used to treat VRE infection (Cunha, 2006). Linezolid is used orally and intravenously to treat infections caused by *E. faecium* and *E. faecalis* strains, including VRE. Plosker and Figgit (2005) reported that Linezolid is useful in patients who require oral or outpatient therapy (when intravenous therapy is undesirable), who are intolerant to glycopeptides, or who have impaired renal function. Unfortunately, linezolid-resistant VRE isolates have already been reported (Kainer *et al*., 2007). Oritavancin is a new parenteral semi-synthetic glycopeptide that is active against all gram-positive pathogens, including VRE. Its advantages include once-daily dosing, good penetration into macrophages and low adverse drug reaction potential (Poulakou and Giamarellou, 2008).

2.15 Prevention and Control Measures

2.15.1 Prudent use of vancomycin

A strict vancomycin restriction policy can decrease the use of vancomycin to reduce the prevalence of VRE. Any comprehensive strategy to limit the occurrence of resistant pathogens must seek to prescribe antibiotics only when they are needed.

Cetinkaya *et al*. (2000) reported that vancomycin use is a risk factor for colonization and infection with VRE which may increase the possibility of the emergence of vancomycin-resistant *S. aureus* or *S. epidermidis*. CDC Hospital Infection Control Practices Advisory Committee (HICPAC) emphasizes the importance of education of medical staff and students about the situations in which the use of vancomycin is considered appropriate (CDC, 1995). HICPAC recommendations are as follows:(i) for treatment of serious infections due to β-lactam-resistant gram-positive microorganisms; (ii) for treatment of infections due to gram-positive microorganisms in patients with serious allergy to β-lactam antimicrobials; (iii) when antibiotic-associated colitis fails to respond to metronidazole therapy or is severe and potentially life-threatening; (iv) prophylaxis, as recommended by the American Heart Association, for endocarditis following certain procedures in patients at high risk for endocarditis; and (v) prophylaxis of major surgical procedures involving the implantation of prosthetic materials or devices, e.g., cardiac and vascular procedures and total hip placement, at institutions with a high rate of infections due to methicillin-resistant *S. aureus* (MRSA) or methicillin-resistant *S. epidermidis* (MRSE). A single dose administered immediately before surgery is sufficient unless the procedure lasts more than 6 hours in which case the dose should be repeated. Prophylaxis should be discontinued after a maximum of two doses.

Situations in which the use of vancomycin should be discouraged include the following: (i) routine surgical prophylaxis other than in a patient with life-threatening allergy to β-lactam antibiotics;(ii) empirical antimicrobial therapy for a febrile neutropenic patient, unless there is strong evidence at the outset that the patient has an infection due to gram-

positive microorganisms and the prevalence of infections due to MRSA in the hospital is substantial; (iii) treatment in response to a single blood culture positive for coagulase-negative staphylococci, if other blood cultures drawn in the same time frame are negative; (iv)continued empirical use for presumed infections in patients whose cultures are negative for β-lactam-resistant gram-positive microorganisms;(v) systemic oral or local prophylaxis of infection or colonization of indwelling central or peripheral vascular catheters; (vi) selective decontamination of the digestive tract; (vii) eradication of MRSA colonization; (viii) primary treatment of antibiotic-associated colitis; (ix) routine prophylaxis in very low-birth-weight infants; (x) routine prophylaxis in patients on continuous ambulatory peritoneal dialysis or haemodialysis;(xi) treatment (chosen for dosing convenience) of infections due to β-lactam-susceptible gram-positive microorganisms in patients with renal failure; and (xii) use of vancomycin solution for topical application or irrigation (CDC, 1995).

2.15.2 Education programs.

Continuing educational programs for hospital personnel such as physicians, medical residents, students, pharmacist, nurses, laboratory workers, and other direct patient care giver should include information about the epidemiology of VRE and the potential impact of this pathogen on the cost and outcome of patient care since detection and containment of VRE requires enlightenment (Cetinkaya *et al*., 2000).

Role of the microbiology laboratory in the detection, reporting, and control of VRE. The microbiology laboratory is the first line of defence against the spread of VRE in the hospital. The ability of the laboratory to identify enterococci and to detect vancomycin resistance promptly and accurately is essential in recognizing VRE colonization and infection (Cetinkaya *et al*., 2000). Effective control efforts will be achieved if there is cooperation and communication between the laboratory and the infection control program. Cases of VRE

have first been detected in hospitals by isolating VRE from clinical specimens submitted to the laboratory for clinical purposes (Cetinkaya *et al*., 2000). Although some hospital laboratories continue to perform antimicrobial susceptibility testing only on enterococci recovered from normally sterile body sites such as blood or urine, this practice is no longer appropriatefor areas in which VRE have been encountered (Murray, 1995). According to Cetinkaya *et al.* (2000), once VRE have been detected in a hospital, enterococci recovered from all body sites should be tested for susceptibility to vancomycin. Hospitals that have not yet detected VRE but are located in geographic areas where VRE have been isolated should performing susceptibility tests on all enterococcal isolates (CDC, 1995).

When VRE are isolated from a clinical specimen, vancomycin resistance should be confirmed by repeating antimicrobial susceptibility testing by using agar dilution, disk diffusion, E-test, agar screen plate, Vitek GPS-TA and GPS-101, and Micro Scan overnight and rapid panels (Endtz *et al*.,1998).The establishment of whether the emergence of VRE in a hospital is caused by the spread of a single strain or by the several different clones is important because this can affect the control measures (Boyce,1997). Apart from antimicrobial susceptibility testing, molecular typing methods such ribotyping, plasmid analysis, PFGE, arbitrarily primed PCR, and examination of hybridization patterns obtained with probes, such as insertion sequences (IS*6770*) or *VanA* and *VanB* gene probes should be used to establish accurate information about the number of strains present in an institution, the patterns of transmission and their control (Clark *et al*., 1993; Mato *et al*., 1996).

2.15.3 Hand hygiene

Hand hygiene by health-care workers, visitors and patients is essential to decrease VRE transmission and nosocomial infections. Hands can be better cleaned with antiseptic soaps and with an alcohol-based solution (Parienti *et al*., 2002). Workers should disinfect

their hands even after glove removal. In a research conducted by Tenorio *et al.* (2001) it was noted that despite wearing gloves, 5 of 17 health-care workers acquired their patients' VRE strain on their hands. Great care must be taken to avoid touching potentially contaminated surfaces, such as door knobs, bed rails, or countertops after hand washing.

2.15.4 Environment decontamination

There is the need for every hospital to develop a detailed policy for cleaning the environment. The hospital room or outpatient clinic is supposed to be cleaned after patients are discharged or seen (Smith *et al*., 1998). Medical equipment such as stethoscopes, blood-pressure cuffs, glucose monitors, oxymeters, and scales must be dedicated to VRE patients. In high-risk settings, fabrics and surfaces should be selected based on how easy they are to clean (Falk *et al*., 2002). Disinfectants such as isopropyl alcohol, sodium hypochlorite, phenolic and quaternary ammonium compound are effective against VRE (Saurina *et al*., 1997).

CHAPTER THREE

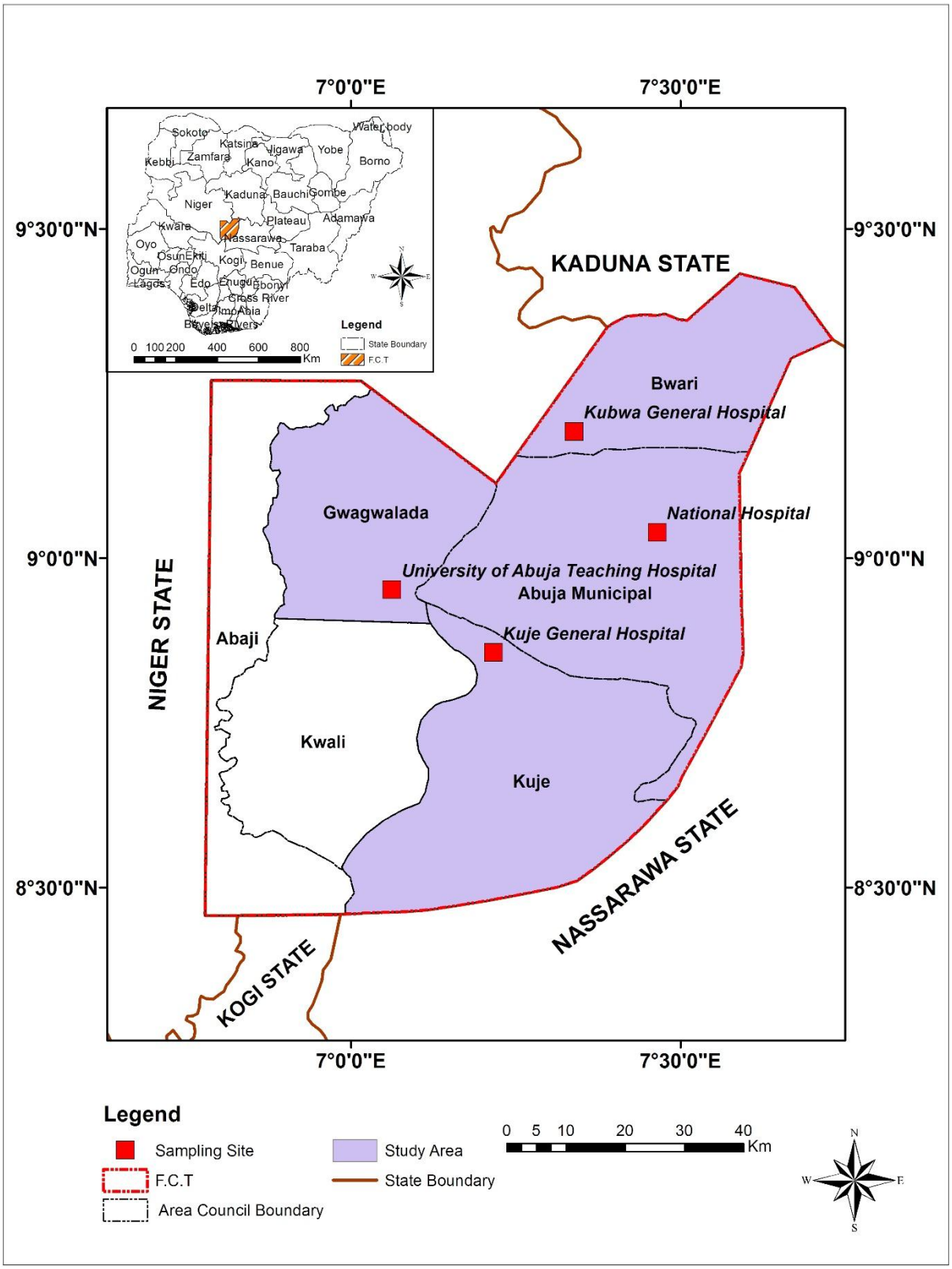
3.0 MATERIALS AND METHODS

3.1 Study Area

The study area included some Abuja area councils such as Abuja Municipal Area Council (AMAC), Bwari, Gwagwalada and Kuje all in the Federal Capital Territory (FCT), (Fig 3.1). The FCT was formed in 1976. It is in the central region of Nigeria bordered to the north by Kaduna state, to the east by Nasarawa State, to the southwest by Kogi state and to the west by Niger State.

The FCT lies between latitude 8.20 and 9.20 north of the equator and longitude 6.40 and 7.40 east of the Greenwich Meridian. The FCT has a landmass of approximately 7,315 km^2. It is situated within the Savannah region with moderate climatic conditions. It is divided into 6 area councils namely: Abaji, Abuja municipal Area Council (AMAC), Bwari, Gwagwalada, Kuje and Kwali.

The study was facility-based and was conducted in selected multi-disciplinary tertiary and general hospitals which include the National Hospital, University of Abuja Teaching Hospital, Kubwa and Kuje general hospitals. These hospitals were chosen because they serve as referral centres in providing specialized health services for residents of the FCT, Nigeria at large and for people from other parts of the world.The study enrolled patients attending University of Abuja Teaching Hospital (UATH), National Hospital Abuja (NHA), Kubwa and Kuje general Hospitals. Clinical specimens collected included urine, stool and wound swab and environmental specimens included swabs taken from the hospital environment such as blood pressure (BP) cuff, trolleys, bed, chairs, floor, cupboards, fridge top, sink, weighing balance were submitted to the medical Microbiology Laboratory.



Source: Modified from the administrative map of FCT, 2016.

Figure 3.1: Map of Federal Capital Territory showing the Sampling Sites

3.2 Ethical Consideration

Ethical approval (appendix VIII) was obtained from Health and Human Services Secretariat (HHSS) of the Federal Capital Territory Administration (FCTA), Abuja for clinical samples collected from Kubwa and Kuje General Hospitals. Also, ethical approval letters were obtained from the management of University of Abuja Teaching Hospital (Appendix VI) and National Hospital, Abuja (Appendix VII) before the commencement of the study.

3.3 Sample Size

The sample size for this study was determined using the prevalence of 29.6% as given by Lautenbach *et al* (2003).The sample size was obtained using the following equation as described by Naing *et al.* (2006).

i.e. $n = \frac{Z^2 P(1-P)}{d^2}$

Where n = sample size

Z = statistics for a level of 95% confidence interval = 1.96

P = prevalence rate = 29.6%= 0.296

d = precision (allowable error) = 5% = 0.05

Thus:

$$n = \frac{(1.96)^2 \times 0.296 \times (1 - 0.296)}{0.05^2}$$

= 320.211

≈ 320

However, a total of 500 samples was collected for the study.

3.4 Sample Collection

3.4.1 Distribution of Samples

A total of 500 hundred samples were collected from the 4 hospitals used which included 400 clinical samples and 100 environmental samples. The clinical samples included 100 stool, 240 urine and 60 wound swabs. Environmental samples were collected from the hospital environment from blood pressure (BP) cuff, trolleys, beds, chairs, floor, cupboards, fridge tops, sink, weighing balances with swab sticks soaked in sterile normal saline.

For University of Abuja Teaching Hospital (UATH), 150 samples were collected which included 30 stool, 70 urine, 20 wound and 30 environmental. For National Hospital, 30 stool, 70 urine, 20 wound swabs and 30 environmental samples were collected giving a total of 150 samples; for Kubwa General Hospital (KUBWA), 20 stool, 50 urine, 10 wound swabs and 20 stool samples were collected giving a total of 100 samples and for Kuje General Hospital (KUJE), 20 stool, 50 urine, 10 swabs and 20 environmental samples were collected giving a total of 100 samples as shown in Figure 3.2

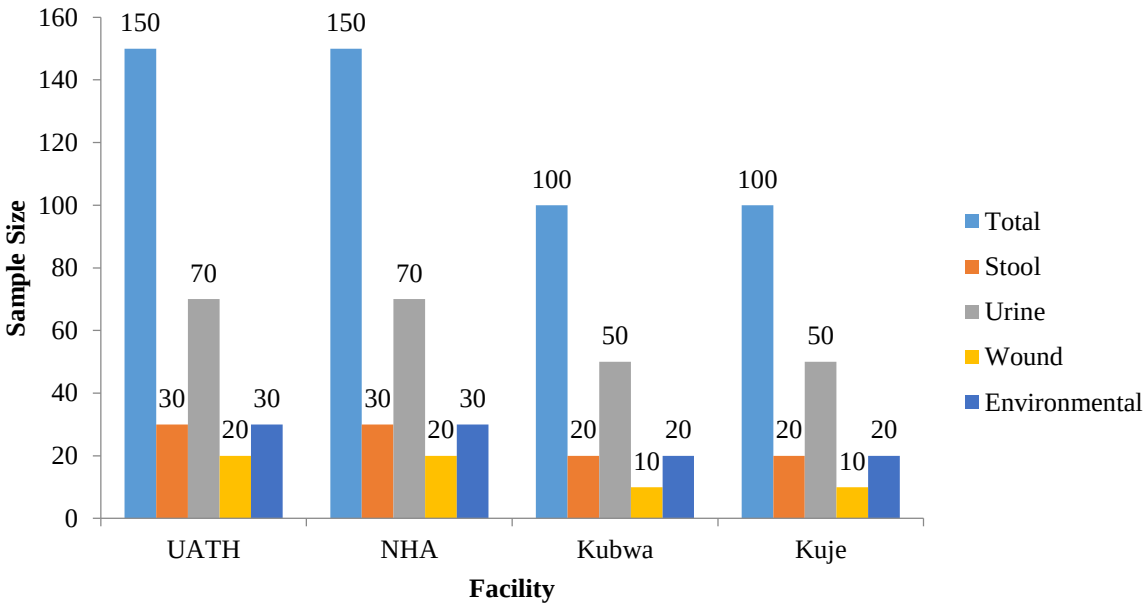


Figure 3.2: Hospital and their samples collected

3.5 Identification and speciation of Enterococcal Isolates

Identification and speciation of enterococcal strains were carried out by using conventional physiological tests (Facklam and Collins, 1989) and validation with Microgen kit. The samples were processed for the isolation of enterococci within an hour after collection. The media used were Bile Esculin Azide Agar (BEA) and blood agar (BA).

3.6 Preparation of Media for Isolation of enterococci

3.6.1 Bile Esculin Azide Agar

For the primary isolation and presumptive identification of enterococci.

Bile Esculin Agar (Oxoid, UK) was prepared according to the manufacturer's instruction. In a litre of distilled water, 44.5g of the dehydrated powder was dissolved over low heat. About 0.15g of sodium azide was added. This was brought to boil to dissolve completely. It was sterilized by autoclaving at 121°C for 15 minutes. The medium was poured into sterile petri-dishes and allowed to gel.

The samples were inoculated plated on the bile-esculin azide medium and the plates incubated at 37°C for 48 hours. Dark brown colonies due to bile esculin hydrolysis were presumptively identified as *Enterococcus* spp.

3.6.2 **Blood Agar**:

The blood agar was prepared by measuring 40g of blood agar base into a litre of distilled water and brought to boil to dissolve before being sterilized by autoclaving at 121 ^{o}C for 15 minutes. The base was allowed to cool to 50 ^{o}C before blood was added into it, mix gently and poured into petri dishes and allowed to solidify.

3.7 Gram-Staining of Enterococcal Isolates

Gram staining was done as outlined by Cheesbrough (2005). Smears of the isolated colonies were prepared on clean grease-free slides containing a drop of distilled water using a flamed wire loop. The smears were air-dried and heat-fixed. Crystal violet stain was applied on the smears for 60 seconds followed by rinsing with water and mordanting with lugol's iodine for 60 seconds. This was washed off with water and brief decolourization with acetone after which the smears were again rinsed with water and counterstained with neutral red for 1 minutes before being washed off finally with water. The smear was allowed to air-dry and examined microscopically.

3.8 Catalase Test

Two fresh colonies of the isolates were emulsified in 2 ml of hydrogen peroxide (H_2O_2) solution in test tubes using wooden applicator and observed for gas bubbling.

3.9 Growth in 6.5% Salt (NaCl) Broth

To prepare a litre of the broth, 28g of dehydrated nutrient broth powder was dissolved in 1 litre distilled water containing 65g of sodium chloride salt. This was distributed in bijou bottles and sterilized for 15 minutes at 121°C. The broth was inoculated with the isolates and incubated at 35°C for 24-48 hours. Growth was indicated by turbidity of the medium.

3.10 Growth in 40% Bile Agar (Bile Tolerance Test)

To prepare 100 ml, 2.8g of dehydrated powder of nutrient agar was dissolved in distilled water containing 40g bile salt by gentle heat. This was sterilized at 121°C for 15 minutes in an autoclave. The medium was then poured into petri-dishes and allowed to solidify. The isolates were inoculated on the medium, incubated for 24 to 48 hours and observed for growth.

3.11 Growth at 45°C

The isolates were also presumptively confirmed as *Enterococcus* spp by growing them on bile esculin agar and blood agar at 45 ^{o}C for 24 hours.

3.12 Motility Test

All the isolates were tested for motility as described by Cheesbrough (2005). A drop of the suspension in peptone water was placed on a slide and covered with a cover glass. The preparation was examined microscopically for motile strains.

3.13 Microgen Test

The Microgen Strep-ID system is made up of 12 standardised biochemical substrates in a microwell test strip and three additional tests which include Hippurate, Voges Proskauer and Pyrrolidonyl Arylamidase (PYR) to identify members of the genus Streptococcus, Enterococcus and related species of importance to medical, veterinary, food and environmental laboratories. Colour change is produced when the organisms in suspending medium metabolises the substrate during incubation or after addition of specific reagent.

Prior to identification of the isolates with microgen, Gram-stain, catalase test and haemolysis on blood agar were noted. An inoculum was prepared for the microwell test strips. Five identical colonies were emulsified in the suspending medium supplied in the kit to produce a suspension equivalent to 2.0 MacFarland standard. In an empty tube, 3 drops of Hippurate solution were added and the colonies of the bacterial isolates were emulsified and incubated at 35°C for 18 hours. For the microwell strips, 4 drops (100ul) of bacterial suspension were added and incubated aerobically for 18 hours at 35°C. Well 12 was overlayed with mineral oil after inoculation. The top of the microwell test strips were sealed with adhesive tapes, incubated at 35 ^{o}C and read after 18 hours incubation. The positive

results were recorded with the aid of the colour chart included in the booklet. To perform Voges-Proskauer (VP) test, on well 8, 1 drop of VPI was added followed by 1 drop of VPII to the well and read after 15- 30 minutes with the development of light pink to dark red colour indicating VP (Voges Proskauer) positive reaction while a clear background indicated a negative VP result. For Pyrrolidonyl Arylamidase (PYR), 1 drop of PYR reagent was added to well 11 and read 5 minutes with formation of pale pink to a very deep red colour indicating a positive result. For Hippurate test, 3drops of Ninhydrin reagent were added to overlay the inoculum. After incubation for 10 minutes without mixing, the result was read with the development of purple colour in the upper reagent layer as a positive Hippurate reaction. A clear colour indicated a negative reaction.

To identify an organism, on the microgen Strep-ID Report Form, the substrates were organised into triplets (sets of 3 reactions) with each substrate assigned a numerical value (1, 2 or 4). The sum of the positive reactions for each triplet formed a single digit of the Octal Code that was used to determine the identity of the isolate with the aid of a Microgen Indentification System Software.

The isolates were preserved at 4°C on nutrient agar slants (Oxoid) for further studies.

3.15 Screening Test to Detect Vancomycin Resistant Enterococci

Mueller-Hinton Agar (MHA) (Oxoid) was used for the antimicrobial disk diffusion susceptibility testing of the isolates by the Bauer-Kirby method as standardized by the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2014).

3.15.1 Media preparation

Thirty-eight grams (38 g) of the MHA powder was suspended in 1 L of distilled water, thoroughly mixed and brought to boil with frequent agitation and boiled for 1 minute

to completely dissolve. The medium was sterilized by autoclaved at 121°C for 15 minutes, cooled to 45°C and aseptically poured into sterile Petri dishes. The agar medium was allowed to gel on a level surface at room temperature (CLSI, 2014).

3.15.2 Antibiotic Susceptibility Testing

All the preserved isolates were sub-cultured onto blood agar (Oxoid) prior to use. Five well-isolated similar colonies were selected and transferred with a loop into 5 ml MH broth (Oxoid). These were incubated at 35°C until it achieved the turbidity of the 0.5 McFarland standards (usually 6 hours). The turbidity of the standard and the test inocula were compared by holding both tubes in front of a white background with a printed material. Within 15 minutes after adjusting the turbidity of the inoculum, a sterile cotton swab was immersed into the properly diluted inoculum and rotated firmly several times against the upper inside wall of the tube to remove excess fluid. Two plates of MHA were prepared for each isolate in order to contain the 12 antibiotics used for this research.The entire agar surface of the plates was inoculated aseptically rotating the plate 60° between streakings to obtain even inoculation before applying the antimicrobial disks. The single disks used included vancomycin (30µg), erythromycin (15µg), doxycycline (30µg), ampicillin (10µg), chloramphenicol (30µg), linezolid (30µg), rifampicin (5µg), teicoplanin (30µg), ciprofloxacin (5µg), nitrofurantoin (300µg), Gentamicin (120µg) and Streptomycin (300µg). Within 15 minutes after the discs were applied the plates were incubated at 35°C for 24 hours. Diameters of zones of inhibition were measured based on CLSI (2014) interpretative chart.

3.15.3 Confirmation by Minimum Inhibitory Concentration (MIC):

All the isolates that were vancomycin resistant by disc diffusion method were confirmed by MIC using E-test strip. Fresh isolates were used to prepare a suspension equivalent to 0.5 Mc Farland standard using MH broth.

3.15.4 Determination of Multiple Antibiotic Resistance (MAR) Index and Classification of Drug Resistance

The MAR index was determined for each isolate by dividing the number of antibiotics to which the isolate was resistant by the total number of antibiotics tested (Krumperman, 1983; Olayinka *et al*.; 2004; Olonitola *et al*., 2009). Isolates that were resistant to 3 or more antibiotics tested were classified as multidrug resistant (MDR), extremely drug resistant (XDR) and pan drug resistant (PDR).

3.16 Molecular Characterization

Molecular characterization of the vancomycin resistant enterococci selected based on their phenotypic characteristics was carried out at International Institute for Tropical Agriculture (IITA) Ibadan, Oyo state and Inqaba, South Africa. The isolates were analyzed for carriage of *van* resistant genes and *16SrRNA* of *Enterococcus* species.

3.16.1 DNA Extraction:

The DNA from the isolates was extracted with Zymo Research Fungal/Bacterial DNA MiniPrep^{TM}. The procedure was carried out according to the manufacturer`s instructions. An 18hour Luria Bertani (LB) broth cultures of the *Enterococcus* spp. was centrifuged at 10,000 rpm for 1minute and the supernatant discarded into a disinfecting Jar. Hundred (100) µg of the cells were re-suspended in 200µl of buffer in ZR bashing bead lysis tubes and added with 750µl lysis solution. Zymo Research bashing bead lysis tube was centrifuged in a microcentrifuge at 10,000 rpm for 1 minute. Up to 400µl supernatant was transferred to a

zymo-spin IV spin filter in a collection tube and centrifuged at 7,000 rpm for 1minute. Approximately 1200 µl of bacterial DNA binding buffer was added to the filtrate in the collection tube. Eight hundred (800) µl of the above mixture was transferred to a Zymo-spin IIC column in a collection tube and centrifuged at 10,000 rpm for 1minute. The flow through from the collection tube was discarded and the step repeated. Two hundred (200) µl DNA pre-wash buffer was added to the Zymo-spin IIC column in a new collection tube and centrifuged at 10,000 rpm for 1 minute, 500µl bacterial DNA wash buffer was added to the tube and centrifuged once more. The Zymo-spin IIC column was transferred to a clean 1.5ml microcentrifuge tube and 100µl DNA elution buffer was added directly to the column matrix and centrifuged at 10,000 rpm for 30 seconds to elute the DNA. The eluted DNA was ready for PCR and other molecular work.

3.16.2 Polymerase Chain Reaction (PCR) for detection of *van* genes and *16SrRNA*.

Polymerase chain reaction (PCR) mixtures were prepared under PCR workstation to prevent cross contamination. A reaction mix of 25ul volume was prepared in PCR tubes on ice, PCR components (listed in Table 3.1) were added in the right volumes after votexing and brief centrifugation. The reaction mix was gently votexed and briefly centrifuged to collect all the drops on the wall of the tubes before placing it in the Thermocycler machine and the run started. The universal forward and reverse primers targeting *16SrRNA* of bacterial isolates used in this study were:27F-AGAGTTTGATCMTGGCTCAG and 1492R-AAGGAGGTGWTCCARCCGCA. The primers allowed amplification of the *16SrRNA* genes of the enterococal isolates. *Other primer* sequences and their predicted sizes used in this single PCR to amplify the *van* genes are shown in Table 3.3. The temperatures and cycling times were optimized for each new DNA template target and primers to achieve maximum yield and specificity. The PCR steps shown in Table 3.2 started with an initial denaturation step at 94°C for 5 minutes followed by 3 steps of 9 cycles: denaturation at 94°C for 15

seconds, annealing at temperature of 55°C for 20 seconds, and extension step for 30 seconds at 72°C. This was followed by another 3 step of 35 cycles: denaturation step at 94°C for 15 seconds, annealing at 45°C for 20 second and extension step for 30 seconds at 72°C. The final extension was at 72°C for 7 minutes followed by holding temperature at 10°C for (∞) infinity. After amplification of the gene, the PCR products were subjected to electrophoresis and visualized under the UV trans-illumination /gel documentation system. The amplicons were loaded on 1.5% agarose gel. The first well was loaded with the ladder (1 kbplus gene ruler from thermoscientific) followed by the PCR products in that order.

Table 3.1: PCR mix components

Reagent	Quantity (µL)
10× PCR buffer	2.5
25mM Mgcl2	1.0
5pMol forward primer	1.0
5pMol reverse primer	1.0
DMSO	1.0
2.5Mm dNTPs	2.0
Taq 5u/ul	0.1
10ng/µl DNA	3.0
H_2O	13.4
Total	25µL

Key: PCR-Polymerase Chain Reaction

DMSO-Dimethyl sulfoxide

dNTPs-Deoxy-nucleoside triphosphate

DNA-Deoxy ribonucleic acid

Table 3.2: PCR Steps

		9 Cycles			35 Cycles			
Initial den.	Den.	Ann. tempt	extension	Den.	Ann. tempt	extension	Final Extension	Hold tempt
94˚C	94˚C	55˚C	72˚C	94˚C	45˚C	72˚C	72˚C	10˚C
5min	15sec	20sec	30sec	15sec	20sec	30sec	7min	∞

3.16.3 Gel Electrophoresis

Tri-acetic acid – EDTA buffer (TAE) gel was prepared. Three (3) gram of the agarose was added into 200 ml× 1TAE and dissolved by warming in a microwave for 2minutes, removed and allowed to stand for 10 minutes. Eight (8) µl ethidium bromide was added to stain the DNA product.

The viscous agarose was added with ethidium bromide, shaken and mixed and poured into the gel mold with well comb and allowed to stand for 30 minutes to harden. The comb was carefully pulled out of the gel before transferring the gel into the electrophoretic tank filled with 1xTAE buffer to the maximum limit. The PCR products were loaded into the gel wells and ran at 120V for 1hour. After the electrophoresis, the PCR products were viewed and the pictures of the bands taken.

Table 3.3: Primer sequences for *van* genes

S/N	Primer	Forward	Reverse	Bp	References
1	*vanA*	GGGAAAACGACAATTGC	GTACAATGCGGCCGTTA	732	Dutka-malen et al., 1995
2	*VanB*	ATGGGAAGCCGATAGTC	GATTTCGTTCCTCGACC	635	Dutka-malen et al., 1995
3	*vanC1*	GGTATCAAGGAAACCTC	CTTCCGCCATCATAGCT	822	Dutka-malen et al., 1995
4	*vanC2*/3	CTCCTACGATTCTCTTG	CGAGCAAGACCTTTAAG	439	Dutka-malen et al., 1995
5	*vanXY*	AATAGCTATTTTGATTTCCCCGTTA	TCCTGAGAAAACAGTGCTTCATTAA	543	Van Den Braak *et al*., 1998
6	*vanR*	AGCGATAAAATACTTATTGTGGA	CGGATTATCAATGGTGTCGTT	645	Anahita *et al*., 2012

3.16.4 Sequence analysis of *16S rRNA*

The purification of the PCR products was carried out by adding 240ul of the sequencing binding buffer to 20ul of the sequencing reaction. The mixture was transferred to Zymo-Spin^{TM} IB 96 plate mounted onto a collection plate. This was centrifuged for 5000x g for 2 minutes. Three hundred ul sequencing wash buffer was added to each well of the plate and centrifuged for 5 minutes at 3000xg. To the column matrix of the filter paper was 20ul of water added directly. The Zymo-Spin™ IB-96 Plate was placed on top of the supplied 96-well PCR Plate and mounted on the collection plate, then centrifuged at 3000xg for 2 minutes to elute the DNA. The ultra-pure DNA was loaded into the sequencer (ABI 3730XL DNA ANALYSER) to give the the nucleotide sequences. Sequence alignment and analysis of the data (nucleotide sequence) obtained was performed online using the Basic Local Alignment Search Tool (BLAST) program of the National Centre for Biotechnology Information (www.ncbi.nlm.nih.gov).

3.17 Data Analysis

All the data obtained from this study were analysed using Statistical Package for Social Sciences (SPSS) version 21. Chi-square was used to test difference proportion and p-values <0.05 was considered statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Distribution of Samples

A total of one hundred and two (102) enterococcal strains were isolated from the five hundred (500) samples collected from the various hospitals giving a percentage of 20.4. Out the of 100 stool samples cultured, 68(68.0%) isolates were isolated, out of the 240 urine samples cultured, 24 isolates were obtained giving a percentage of 10, wound swab yielded 5 isolates giving a percentage of 8.3 while environmental samples yielded 5 isolates giving a percentage of 5 as shown in Figure 4.1. The statistical analysis ($p < 0.001^{**}$) showed that there is a significant association between *Enterococcus* spp isolated and the type of samples with stool samples having highest yield.

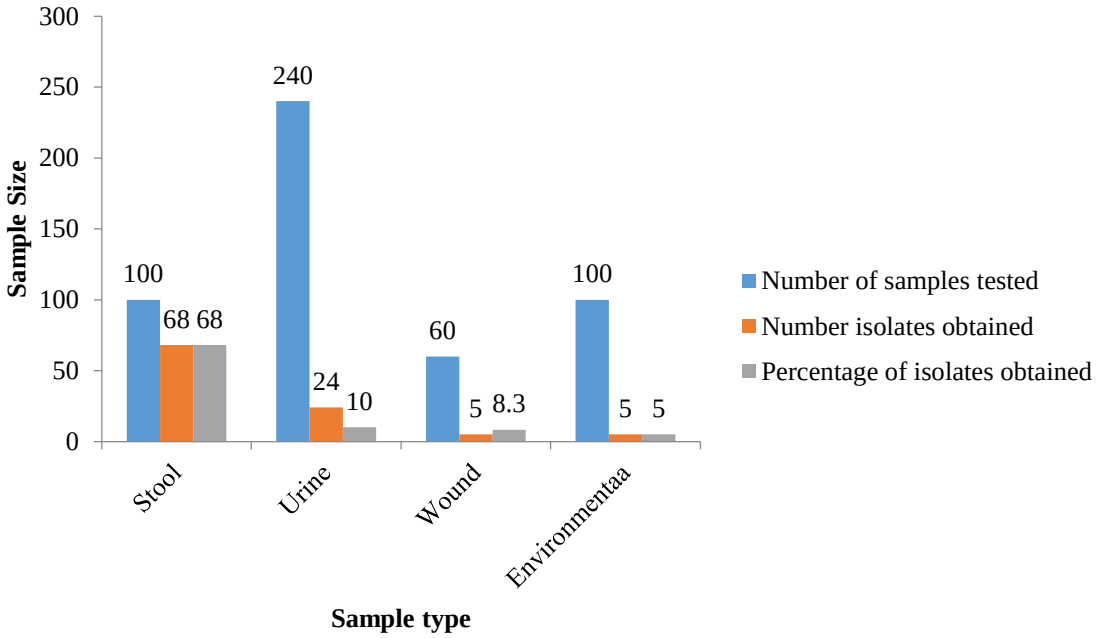


Figure 4.1: Distribution of Enterococci Isolated from clinical Samples in the Hospitals, Abuja, Nigeria

4.2 Isolates from the Samples Collected from the various hospital

Of the 150 samples collected from patients and Hospital environment from UATH, a total of 31(20.7%) enterococci were isolated. Thirty(30) stool samples yielded 22(73.3%) enterococci, 70 urine samples yielded 5(7.1%), 20 wound swabs yielded 2(10.0%) enterococci while swabs taken from the hospital environment yielded 2(6.7%) enterococcal isolate as shown in Table 4.1.

Of the 150 samples collected from patients and Hospital environment from NHA, a total of 39(26.0%) enterococci were isolated. Thirty (30) stool samples yielded 26(86.6%) enterococci, 70 urine samples yielded 11(15.7%), 20 wound swabs collected yielded 3(10.0%) enterococci while swabs from the hospital environment yielded no enterococcal isolate (Table 4.1)

Of the 100 samples obtained from patients and Hospital environment from Kubwa General Hospital, 20 were stool samples which yielded 7(35.0%) enterococci, 50 were urine samples which yielded 6(12.0%), 10 wound swabs collected yielded no isolate while swabs taken from the hospital environment yielded 2(10%) enterococci giving a total of 15(15.0%) isolates (Table 4.1).

Of the 100 samples collected from patients and Hospital environment from Kuje General Hospital, 20 stool samples yielded 14(70%) enterococci, 50 urine samples yielded 2(4.0%), 10 wound swabs yielded no isolate while 20 swabs taken from the hospital environment yielded 1(5%) enterococcal isolates giving a total of 17(17%) isolates(Table 4.1)

Table 4.1: Distribution of *Enterococcus* species among the Clinical and Environmental samples in the Study area.

Hospital	**Sample Source**	**No. of samples tested**	**No and % *Enterococcus* isolated**	**Chi-square**	**p-value**
UATH	Urine	70	5(7.1)	63.536	<0.001**
	Stool	30	22(73.3)		
	Wound	20	2(10.0)		
	Environmental	30	2(6.7)		
	Sub-total	150	31(20.7)		
NHA	Urine	70	11(15.7)	67.310	<0.001**
	Stool	30	25(83.3)		
	Wound	20	3(15.0)		
	Environmental	30	0(0.0)		
	Sub-total	150	39(26.0)		
KUBWA	Urine	50	6(12.0)	8.784	0.032*
	Stool	20	7(35.0)		
	Wound	10	0(0.0)		
	Environmental	20	2(10.0)		
	Sub-total	100	15(15.0)		
KUJE	Urine	50	2(4.0)	49.894	<0.001**
	Stool	20	14(70.0)		
	Wound	10	0(0.0)		
	Environmental	20	1(5.0)		
	Sub-total	100	17(17.0)		
TOTAL		500	102(20.4)	172.977	<0.001**

Key: UATH: University of Abuja Teaching Hospital; NHA: National Hospital, Abuja.

4.3 Prevalence of Enterococci Isolated from the Hospitals

In table 4.2, the clinical and environmental samples from UATH yielded 31 enterococci out of which *E. faecalis* were 18(58.1%), *E. faecium* 9(29.0%), *E. gallinarum* 1(3.2%), *E. mundtii* 2(6.5%) and *E. avium* 1(3.2%). Five (5) enterococci were isolated from urine samples out of which 3(60%) were *E. faecalis*, 1(20%) was *E. faecium* and 1(20%) *E. avium*. Stool samples yielded 22 enterococci out of which 12(54.5%) were *E. faecalis*, 8(36.43%) were *E. faecium*, 2(2.9%) were *E. mundtii*; wound swabs yielded 2 enterococci all (100%) of which were *E. faecalis*; environmental samples yielded 2 enterococci out of which 1(50%) was *E. faecalis* and 1(50%) was *E. gallinarum*.

At NHA, the clinical and environmental isolates yielded 39 enterococci out of which 19(48.7%) were *E. faecalis*, 11(28.2%) were *E. faecium* 1(2.6%) *E. gallinarum*, 7(17.9%) *E. mundtii* and 1(2.6%) *E. dispar*. Urine samples yielded all 11(100%) *E. faecalis*. Stool yielded 5(20%) *E. faecalis*, 11(44.0%) *E. faecium*, 1(4.0%) *E. gallinarum*, 7(28.0%) *E. mundtii* and 1(4.0%) *E. dispar* giving a total of 25 *Enterococcus* spp. Wound swabs yielded all 3(100%) *E. faecalis* while no environmental samples yielded any *Enterococcus* as shown in Table 4.3

At Kubwa General Hospital, the clinical and environmental isolates yielded 15 enterococci out of which 9(60%) were *E. faecalis*, 2(13.3%) were *E. faecium*, 2(13.3%) were *E. mundtii*, 1(6.7%) was *E. dispar*, 1(6.7%) was *E. hirae*. Urine samples yielded 6(100%) *E. faecalis*. Stool yielded 2(28.6%) *E. faecalis*, 2(28.6%) *E. faecium*, 2(28.6%) *E. mundtii* and 1(14.3%) *E. hirae* giving a total of 7 enterococcal spp. Wound Swabs yielded no isolate while environmental samples yielded 1(50.0%) *E. faecalis* and 1(50.0%) *E. dispar* giving a total of 2 *Enterococcus* spp as shown in Table 4.4.

At Kuje General Hospital, the clinical and environmental isolates yielded 17 enterococci out of which 13(76.5%) were *E. faecalis*, 2(11.8%) were *E. faecium*, 1(5.9%) was *E. casseliflavus* and 1(5.9%) *E. gallinarum*. Urine samples yielded all 2(100%) *E. faecalis*. Stool yielded 10(73.4%) *E. faecalis*, 2(14.3%) *E. faecium*, 1(7.1%) *E. casseliflavus* and 1(7.1%) *E. gallianarum* giving a total of 14 *Enterococcus* spp. Wound swabs yielded no isolate while environmental samples yielded 1(100.0%) *E. faecalis* as shown in Table 4.5

In all the 4 Hospitals, the clinical and environmental isolates yielded 102 enterococci out of which 59(57.8%%) were *E. faecalis*, 24(23.5%) were *E. faecium*, 1(1.0%) was *E. casseliflavus*, 3(2.9%) were *E. gallinarum*, 11(10.8%) were *E. mundtii*, 1(1.0%) was *E. avium*, 2(2.0%) were *E. dispar*,and 1(1.0) was *E. hirae*. Urine samples yielded 22(91.7%) *E. faecalis*, 1(4.2%) *E. faecium* and 1(4.2%) *E. avium* giving a total of 24 enterocci. Stool samples yielded 29(42.6%), *E. faecalis*, 23(33.8%) *E. faecium*, 1(1.5%) *E.casseliflavus*, 2(2.9%) *E. gallianarum*, 11(16.2%) *E. mundtii*, 1(1.5%) *E. dispar* and 1(1.5%) *E. hirae* giving a total of 68 enterococcal spp. Wound swabs yielded 5(100%) *E. faecalis* while environmental samples yielded 3(60.0%) *E. faecalis*, 1(20.0%) *E. gallinarum* and 1(20.0%) *E. dispar* giving a total of 5 *Enterococcus* spp. as shown in Table 4.6

Table 4.2: Distribution of *Enterococcus* Spp isolated from UATH based on sample type

Sample(No tested)	No. +ve for *Enterococcus*	*E.faecalis* (%)	*E.faecium* (%)	*E.gallinarum* (%)	*E.mundtii* (%)	*E.avium* (%)
Urine(70)	5	3(60.0)	1(20.0)	0(0.0)	0(0.0)	1(20.0)
Stool(30)	22	12(54.5)	8(36.43)	0(0.0)	2(9.1)	0(0.0)
Wound(20)	2	2(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Environment(30)	2	1(50.0)	0(0.0)	1(50.0)	0(0.0)	0(0.0)
Total(150)	31	18(58.1)	9(29.0)	1(3.2)	2(6.5)	1(3.2)

Key: UATH: University of Abuja Teaching Hospital

Table 4.3: Distribution of *Enterococcus* species isolated from NHA based on sample type.

Sample(No tested)	No +ve for *Enterococcus*	*E.faecalis* (%)	*E.faecium* (%)	*E.gallinarum* (%)	*E.mundtii* (%)	*E.dispar* (%)
Urine(70)	11	11(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Stool(30)	25	5(20.0)	11(44.0)	1(4.0)	7(28.0)	1(4.0)
Wound(20)	3	3(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Environment(30)	0	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total(150)	39	19(48.7)	11(28.2)	1(2.6%)	7(17.9)	1(2.6)

Key: NHA: National Hospital, Abuja.

Table 4.4: Distribution of *Enterococcus* Spp isolated from Kubwa based on sample type.

Sample (No tested)	**No. +ve for *Enterococcus***	***E.faecalis* (%)**	***E.faecium* (%)**	***E.mundtii* (%)**	***E. dispar* (%)**	***E. hirae* (%)**
Urine(50)	6	6(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Stool(20)	7	2(28.6)	2(28.6)	2(28.6)	0(0.0)	1(14.3)
Wound(10)	0	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Environment(20)	2	1(50.0)	0(0.0)	0(0.0)	1(50.0)	0(0.0)
Total(100)	15	9(60.0)	2(13.3)	2(13.3)	1(6.7)	1(6.7)

Table 4.5: Distribution of *Enterococcus* Spp isolated from Kuje based on sample type.

Sample (No tested)	**No. +ve for *Enterococcus***	***E. faecalis* (%)**	***E.faecium* (%)**	***E.casseliflavus* (%)**	***E.gallinarum* (%)**
Urine(50)	2	2(100.0)	0(0.0)	0(0.0)	0(0.0)
Stool(20)	14	10(73.4)	2(14.3)	1(7.1)	1(7.1)
Wound(10)	0	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Environmental(20)	1	1(100)	0(0.0)	0(0.0)	0(0.0)
Total(100)	17	13(76.5)	2(11.8)	1(5.9)	1(5.9)

Key: +ve: positive

Table 4.6: Distribution of *Enterococcus* Spp isolated from all the hospitals based on sample type.

Sample (No tested)	**No. +ve for *Enterococcus***	**E.f (%)**	**E.fc (%)**	**E.c (%)**	**E.g (%)**	**E.m (%)**	**E.a (%)**	**E.d (%)**	**E.h (%)**
Urine(240)	24	22(91.7)	1(4.2)	0(0.0)	0(0.0)	0(0.0)	1(4.2)	0(0.0)	0(0.0)
Stool(100)	68	29(42.6)	23(33.8)	1(1.5)	2(2.9)	11(16.2)	0(0.0)	1(1.5)	1(1.5)
Wound(60)	5	5(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Env (100)	5	3(60.0)	0(0.0)	0(0.0)	1(20.0)	0(0.0)	0(0.0)	1(20.0)	0(0.0)
Total(500)	102	59(57.8)	24(23.5)	1(1.0)	3(2.9)	11(10.8)	1(1.0)	2(2.0)	1(1.0)

Key: +ve: positive, *E.f:E.faecalis, E.fc:E.faecium, E.c:E.casselliflavus, E.g:E.gallinarum, Em: E. mundtii, E.a:E.avium, E.d: E.dispar, E.h: E.hirae,* Env: Environmental swab

4.4 Antibiotic Susceptibility Profile of Enterococcal isolates obtained from samples in UATH, Abuja.

Table 4.7 elaborated the susceptibility pattern of the different 31enterococcal species isolated from UATH. For ampicillin, all the *Enterococcus Spp* had 6 (19.4%) resistant and 25(80.6%) susceptible strains. For ciprofloxacin, 12(38.7%) were resistant, 6(19.4%) had intermediate sensitivity while 13(42.0%) had full sensitivity. For rifampicin, 19(61.3%) were resistant, 4(12.9%) had intermediate sensitivity while 8(26.0%) had full sensitivity. For linzolid, 7(22.6%) were resistant, 3(9.8%) had intermediate sensitivity while 21(67.7%) had full sensitivity. For vancomycin, 4(12.9%) strains were resistant, 27(87.1%) were sensitive. For doxycycline, 22(71.0%) were resistant, 4(12.9%) had intermediate sensitivity while 5(16.1%) had full sensitivity. For teicoplanin, 4(12.9%) were resistant, 27(87.1%) were sensitive to teicoplanin. For erythromycin, 13(42.0%) were resistant, 13(42.0%) had intermediate sensitivity while 5(16.1%) had full sensitivity. For chloramphenicol, 13(42.0%) were resistant, 3(9.8%) had intermediate sensitivity while 15(48.4%) had full sensitivity. For nitrofurantoin, 2(6.5%) were resistant, 1(3.2%) had intermediate sensitivity while 28(90.3%) had full sensitivity. For gentimicin, 14(45.2%) were resistant, 1(3.2%) was intermediately sensitive while 16(51.6%) were fully sensitive strains. For streptomycin, 24(77.4%) were resistant, while 7(22.6%) had full sensitivity.

4.5 Multiple Antibiotics Resistance Pattern and Index of Isolates from UATH

Of the 31 isolates from UATH, 29(94%) exhibited 8 resistant patterns with multiple antibiotics resistance (MAR) index ranging from 0.1 – 1.0. Out of the 29(94%) resistant strains, 23(74%) were multidrug resistant (MDR: non-susceptible to 1 agent in $\geq$ 3 antimicrobial categories) while 1(3.2%) strain was pandrug resistant as it was resistant to all the antibiotics tested. Of the 29 with resistant patterns, 24(77%) had MAR index of above 0.2

indicating that the isolates had been pre-exposed to the antibiotics tested as shown in Table 4.8.

Table 4.7: Susceptibility Profile of *Enterococcus* Spp. Isolated from UATH Hospital

Antibiotics Disk potency	Sus	*E.faecalis* 18(%)	*E.faecium* 9(%)	*E.gallinarum* 1(%)	*E.mundtii* 2(%)	*E.avium* 1(%)	Total 31(%)
AMP	R	3(16.7)	2(22.2)	1(100)	-	-	6(19.4)
10µg	I	-	-	-	-	-	-
	S	15(83.3)	7(77.8)	-	2(100)	1(100)	25(80.6)
CIP	R	5(27.8)	4(44.4)	1(100)	2(100)	-	12(38.7)
5µg	I	4(22.2)	2(22.2)	-	-	-	6(19.4)
	S	9(50)	3(33.3)	-	-	1(100)	13(42.0)
RIF	R	12(66.7)	5(55.6)	-	2(100)	-	19(61.3)
5µg	I	2(11.1)	1(11.1)	-	-	1(100)	4(12.9)
	S	4(22.2)	3(33.3)	1(100)	-	-	8(26.0)
LIN	R	4(22.2)	3(33.3)	-	-	-	7(22.6)
30µg	I	1(5.6)	1(11.1)	-	-	1(100)	3(9.8)
	S	13(72.2)	5(55.6)	1(100)	2(100)	-	21(67.7)
VAN	R	2(11.1)	2(22.2)	-	-	-	4(12.9)
30µg	I	-	-	-	-	-	-
	S	16(88.9)	7(77.8)	1(100)	2(100)	1(100)	27(87.1)
DOX	R	13(72.2)	7(77.8)	1(100)	-	1(100)	22(71.0)
30µg	I	3(16.7)	1(11.1)	-	-	-	4(12.9)
	S	2(11.1)	1(11.1)	-	2(100)	-	5(16.1)
TEIC	R	2(11.1)	2(22.2)	-	-	-	4(12.9)
30µg	I	-	-	-	-	-	-
	S	16(88.9)	7(77.8)	1(100)	2(100)	1(100)	27(87.1)
ERY	R	6(33.3)	5(55.6)	1(100)	-	1(100)	13(42.0)
15µg	I	10(55.6)	1(11.1)	-	2(100)	-	13(42.0)
	S	2(11.1)	3(33.3)	-	-	-	5(16.1)
CHL	R	7(38.9)	5(55.6)	-	-	1(100)	13(42.0)
30µg	I	1(5.6)	1(11.1)	1(100)	-	-	3(9.8)
	S	10(55.6)	3(33.3)	-	2(100)	-	15(48.4)
NIT	R	1(5.6)	1(11.1)	-	-	-	2(6.5)
300µg	I	1(5.6)		-	-	-	1(3.2)
	S	16(88.9)	8(88.9)	1(100)	2(100)	1(100)	28(90.3)
GEN	R	7(38.9)	4(44.4)	-	2(100)	1(100)	14(45.2)
120µg	I	1(5.6)		-	-	-	1(3.2)
	S	10(55.6)	5(55.6)	1(100)	-	-	16(51.6)
STR	R	14(77.8)	7(77.8)	-	2(100)	1(100)	24(77.4)
300µg	I	-	-	-	-	-	-
	S	4(22.2)	2(22.2)	1(100)	-	-	7(22.6)

Key: Sus: Susceptibility,R: Resistance, I: intermediate, S: susceptible, AMP: Ampicillin, CIP:Ciprofloxacin, RIF: Rifampicin,LIN: Linzolid, VAN:Vancomycin, DOX: Doxicyclin,

TEC:Teicoplanin,ERY:Erythromycin,CHL:Chloramphenicol,NIT:Nitrofurantoin,GEN:Genti micin,STR:Streptomycin.

Table 4.8: Antibiotic Resistance Pattern and resistance index of UATH Isolates

NA	AWP	Resistance pattern/phenotype	RI	%	CR
	4	RIF, STR	0.1	16	Nil
2	1	RIF, DOX			Nil
	2	RIF, DOX, STR	0.3	23	Mdr
	3	RIF, GEN, STR			Mdr
3	1	AMP, CIP, DOX			Mdr
	1	AMP, DOX, CHL			Mdr
	2	DOX, ERY, CHL, STR	0.3	10	Mdr
4	1	CIP, DOX, ERY, STR			mdr
	1	DOX, ERY, CHL, GEN, STR	0.4	13	mdr
5	1	CIP, DOX, ERY, CHL, STR			mdr
	2	RIF, DOX, CHL, GEN, STR			mdr
	1	RIF, DOX, ERY, CHL, GEN, STR	0.5	13	mdr
6	2	CIP, LIN, DOX, CHL, GEN, STR			mdr
	1	RIF, LIN, VAN, DOX, TEIC, ERY			mdr
	1	CIP, RIF, DOX, ERY, CHL, GEN, STR	0.6	10	mdr
7	1	AMP, RIF, LIN, VAN, DOX, TEIC, ERY			mdr
	1	AMP, CIP, LIN, DOX, CHL, GEN, STR			mdr
	1	AMP, RIF, DOX, ERY, CHL, NIT, GEN, STR	0.7	7	mdr
8	1	RIF, LIN, VAN, DOX, TEIC, ERY, GEN, STR			mdr
12	1	AMP, CIP, RIF, LIN, VAN, DOX, TEIC, ERY, CHL, NIT, GEN, STR	1.0	3	pdr

MDR: non-susceptible to ≥ 1 agent in ≥3 antimicrobial categories,
XDR: non-susceptible to ≥ 1 agent in all but ≤2 categories

PDR: non-susceptible to all antimicrobial agents listed,
NA: number of antibiotics. AWP: Antibiotics with pattern. RI: Resistance Index. CR: classification of resistance

4.6 Antibiotic Susceptibility Profile of Enterococcal isolates obtained from samples in NHA, Abuja.

Table 4.9 elaborated the susceptibility pattern of the different 39 *Enterococcus* species isolated from NHA. For ampicillin, 16(41.0%) were resistant, 23(59.0%) were sensitive. For ciprofloxacin, 9(23.1%) were resistant, 6(15.4%) had intermediate sensitivity while 24(61.5%) had full sensitivity. For rifampicin, 34(87.2%) were resistant while 5(12.8%) had intermediate sensitivity. For linzolid, 24(61.5%) were resistant while 15(38.5%) were sensitive. For vancomycin, 24(61.5%) were resistant while 15(38.5%) were sensitive. For Doxycyclin, 28(71.8%) were resistant, 4(10.3%) had intermediate sensitivity while 7(17.9%) had full sensitivity. For teicoplanin, 23(59.0%) were resistant, 1(2.6%) was intermediately sensitive while 15(38.5%) were sensitive. For erythromycin, 30 (76.9%) strains were resistant, 5(12.8%) intermediately sensitive and 4 were sensitive. For chloramphenicol, 21(53.8%) were resistant while 18(46.2%) were sensitive. For nitrofurantoin, 4(10.3%) were resistant, 5(12.8%) had intermediate sensitivity while 30(76.9%) had full sensitive. For gentimicin, 8(20.5%) were resistant, 2(5.1%) had intermediate sensitivity while 29(74.4%) were sensitive. For streptomycin, 8(20.5%) strains were resistant, 1(2.6%) intermediately sensitive while 30(76.9%) were sensitive.

Table 4.9: Susceptibility Profile of *Enterococcus* spp. Isolated from NHA

Antibiotics Disk potency	Sus	*E.faecalis* 19(%)	*E.faecium* 11(%)	*E.gallinarum* 1(%)	*E.mundtii* 7(%)	*E.dispar* 1(%)	Total 39(%)
AMP	R	3(15.8)	6(54.5)	-	6(85.7)	1(100)	16(41.0)
10µg	I	-	-	-	-	-	
	S	16(84.2)	5(45.5)	1(100)	1(14.3)	-	23(59.0)
CIP	R	4(21.1)	3(27.3)	-	2(28.6)	-	9(23.1)
5µg	I	5(26.3)	-	-	-	1(100)	6(15.4)
	S	10(52.6)	8(72.7)	1(100)	5(71.4)	-	24(61.5)
RIF	R	14(73.7)	11(100)	1(100)	7(100)	1(100)	34(87.2)
5µg	I	5(26.3)	-	-	-	-	5(12.8)
	S	-	-	-	-	-	
LIN	R	7(36.8)	8(72.7)	1(100)	7(100)	1(100)	24(61.5)
30µg	I	-	-	-	-	-	
	S	12(63.2)	3(27.3)	-	-	-	15(38.5)
VAN	R	7(36.8)	8(72.7	1(100)	7(100)	1(100)	24(61.5)
30µg	I	-	-	-	-	-	
	S	12(63.2)	3(27.3)	-	-	-	15(38.5)
DOX	R	13(68.4)	6(54.5)	1(100)	7(100)	1(100)	28(71.8)
30µg	I	2(10.5)	2(18.2)	-	-	-	4(10.3)
	S	4(21.1)	3(27.3)	-	-	-	7(17.9)
TEIC	R	6(31.6)	8(72.7)	1(100)	7(100)	1(100)	23(59.0)
30µg	I	1(5.3)	-	-	-	-	1(2.6)
	S	12(63.2)	3(27.3)	-	-	-	15(38.5)
ERY	R	12(63.2)	9(81.8)	1(100)	7(100)	1(100)	30(76.9)
15µg	I	5(26.3)	-	-	-	-	5(12.8)
	S	2(10.5)	2(18.2)	-	-	-	4(10.3)
CHL	R	13(68.4)	3(27.3)	1(100)	4(57.1)	-	21(53.8)
30µg	I	-		-	-	-	
	S	6(31.6)	8(72.7)	-	3(42.9)	1(100)	18(46.2)
NIT	R	1(5.3)	1(9.1)	-	2(28.6)	-	4(10.3)
300µg	I	1(5.3)	3(27.3)	-	1(14.3)	-	5(12.8)
	S	17(89.5)	7(63.6)	1(100)	4(57.1)	1(100)	30(76.9)
GEN	R	4(21.1)	2(18.2)	-	1(14.3)	1(100)	8(20.5)
120µg	I	-	2(18.2)	-	-	-	2(5.1)
	S	15(78.9)	7(63.6)	1(100)	6(85.7)	-	29(74.4)
STR	R	4(21.1)	2(18.2)		2(28.6)	-	8(20.5)
300µg	I	1(5.3)		1(100)	5(71.4)	1(100)	1(2.6)
	S	14(73.7)	9(81.8)	-	-	-	30(76.9)

Key: Sus: Susceptibility,R: Resistance, I: intermediate, S: susceptible, AMP: Ampicillin, CIP:Ciprofloxacin, RIF: Rifampicin,LIN: Linzolid, VAN:Vancomycin, DOX: Doxicyclin, TEC:Teicoplanin,ERY:Erythromycin,CHL:Chloramphenicol,NIT:Nitrofurantoin,GEN:Gentimicin,STR:Streptomycin.

4.7 Multiple Antibiotic Resistance Pattern and Index of Isolates from NHA

All the 39(100%) enterococcal isolates from NHA exhibited 10 different resistant patterns with MAR index ranging from 0.1 – 0.8. From the result, out of the 39(100%) resistant strains, 29(74%) were multidrug resistant, 3(8%) were extremely drug-resistant (XDR). Out of the 39 Enterococci isolated, 34(87%) had MAR index of 0.2 and above indicating that the isolates had been pre-exposed to the antibiotics tested as shown in table 4.10.

Table 4.10: Antibiotic Resistance Pattern and resistance index of NHA Isolates.

NA	AWP	Resistance pattern/phenotype	RI	%	CR
1	4	RIP	0.1	13	Nil
	1	STR			Nil
2	1	RIF, LIN	0.2	5	nil
	1	RIF, DOX			nil
3	1	CIP, GEN, STR	0.3	8	mdr
	1	RIF, DOX, ERY			mdr
	1	DOX, ERY, CHL			mdr
4	2	CIP, DOX, ERY, CHL	0.3	8	mdr
	1	RIF, DOX, ERY, CHL			mdr
5	2	RIF, LIN, VAN, TEIC, ERY	0.4	8	mdr
	1	AMP, RIF, VAN DOX, ERY			mdr
6	2	RIF, LIN, VAN, DOX, TEIC, ERY	0.5	10	mdr
	1	CIP, RIF, ERY, CHL, GEN, STR			mdr
	1	AMP, RIF, LIN, VAN, TEIC, CHL			mdr
7	2	RIF, LIN, VAN, DOX, TEIC, ERY, CHL	0.6	18	mdr
	1	CIP, RIF, DOX, ERY, CHL, GEN, STR			mdr
	4	AMP, RIF, LIN, VAN, DOX, TEIC, ERY			mdr
8	2	AMP, RIF, LIN, VAN, DOX, TEIC, ERY, CHL	0.7	18	mdr
	1	AMP, RIF, LIN, VAN, DOX, TEIC, ERY, NIT			mdr
	1	AMP, CIP, RIF, LIN, VAN, DOX, TEIC, CHL			mdr
	1	CIP, RIF, LIN, VAN, DOX, TEIC, CHL, NIT			mdr
	1	RIF, LIN, VAN, DOX, TEIC, ERY, GEN, STR			mdr
	1	AMP, RIF, LIN, VAN, DOX, TEIC, ERY, GEN			mdr
9	1	AMP, CIP, RIF, LIN, VAN, DOX, TEIC, ERY, NIT	0.8	5	mdr
	1	AMP, RIF, LIN, VAN, DOX, TEIC, ERY, GEN, STR			mdr
10	1	AMP, CIP, RIF, LIN, VAN, DOX, TEIC, ERY, GEN, STR	0.8	8	xdr
	1	AMP, CIP, RIF, LIN, VAN, DOX, TEIC, ERY, CHL, NIT			xdr
	1	AMP, RIF, LIN, VAN, DOX, TEIC, ERY, CHL, NIT, STR			xdr

MDR: non-susceptible to 1 agent in ≥ 3 antimicrobial categories, XDR: non-susceptible to ≥1 agent in all but ≤ 2 categories. NA: number of antibiotics. AWP: Antibiotics with pattern. RI: Resistance Index. CR: classification of resistance

4.8 Antibiotic Susceptibility Profile of Enterococcal isolates obtained from samples in Kubwa, Abuja.

Table 4.11 elaborated the susceptibility pattern of the different 15 *Enterococcus* Spp isolated from Kubwa General Hospital. For ampicillin, 5(33.3%) strains were resistant while 10(66.7%) were fully sensitive. For ciprofloxacin, 4 (26.7) had resistance, 2(13.3) had intermediate sensitivity while 9(60%) had full sensitivity. For rifampicin, 9(60.0%) were resistant, 2(13.3%) had intermediate sensitivity while 4(26.7%) had full sensitivity. For linzolid, 6(40%) had resistance, 2(13.3%) had intermediate sensitivity while 7(46.7%) had full sensitivity. For vancomycin, 6(40.0%) were resistant, 9(60.0%) were sensitive. For doxycyclin, 8(53.3%) had resistance, 2(13.3%) had intermediate sensitivity while 5(33.3%) had full sensitivity. For teicoplanin, 6(40.0%) were resistant, 1(6.7%) had intermediate sensitivity while 8(53.3%) were fully sensitive. For erythromycin, 9(60.0%) were resistant, 4(26.7%) had intermediate sensitivity while 2(13.3%) were fully sensitive. For chloramphenicol, 7(46.7%) were resistant, 1(6.7%) had intermediate sensitivity while 7(46.7%) were fully sensitive. For nitrofurantoin, 2(13.3%) were resistant, 1(6.7%) had intermediate sensitivity while 12(80.0%) were fully sensitive. For gentimicin, 1(6.7%) was resistant, while 14(93.3%) were fully sensitive. For streptomycin, 2(13.3%) were resistant, 1(6.7%) was intermediately sensitive while 12(80.0%) were fully sensitive.

4.9 Multiple Antibiotic Resistance Pattern and Index of Kubwa Isolates

Twelve out of 15 *Enterococcus* spp. isolated from Kubwa exhibited 6 resistant patterns with MAR index ranging from 0.1 - 1.0. From the result, out of the 12(80%) resistant strains, 10(66.7%) were multidrug resistant while 1(6.7%) had pandrug resistance. Out of the

15 enterococci isolated from the Kubwa, 9(60%) had MAR index of 0.2 as shown in Table 4.12

Table 4.11: Susceptibility Profile of *Enterococcus* spp. Isolated from Kubwa Hospital

Antibiotics	Sus	*E.faecalis* 9(%)	*E.faecium* 2(%)	*E.mundtii* 2(%)	*E.dispar* 1(%)	*E.hirae* 1(%)	Total 15(%)
AMP	R	2(22.2)	2(100)	-	-	1(100)	5(33.3)
10µg	I	-	-	-	-	-	-
	S	7(77.8)	-	2(100)	1(100)	-	10(66.7)
CIP	R	1(11.1)	2(100)	-	-	1(100)	4(26.7)
5µg	I	2(22.2)	-	-	-	-	2(13.3)
	S	6(66.7)	-	2(100)	1(100)	-	9(60.0)
RIF	R	4(44.4)	2(100)	2(100)	-	1(100)	9(60.0)
5µg	I	2(22.2)	-	-	-	-	2(13.3)
	S	3(33.3)	-	-	1(100)	-	4(26.7)
LIN	R	1(11.1)	2(100)	2(100)	-	1(100)	6(40.0)
30µg	I	2(22.2)	-	-	-	-	2(13.3)
	S	6(66.7)	-	-	1(100)	-	7(46.7)
VAN	R	1(11.1)	2(100)	2(100)	-	1(100)	6(40)
30µg	I	-	-	-	-	-	-
	S	8(88.9)	-	-	1(100)	-	9(60)
DOX	R	3(33.3)	2(100)	2(100)	-	1(100)	8(53.3)
30µg	I	2(22.2)	-	-	-	-	2(13.3)
	S	4(44.4)	-	-	1(100)	-	5(33.3)
TEIC	R	1(11.1)	2(100)	2(100)	-	1(100)	6(40.0)
30µg	I	1(11.1)	-	-	-	-	1(6.7)
	S	7(77.8)	-	-	1(100)	-	8(53.3)
ERY	R	4(44.4)	2(100)	2(100)	-	1(100)	9(60.0)
15µg	I	4(44.4)					4(26.7)
	S	1(11.1)			1(100)	-	2(13.3)
CHL	R	4(44.4)	1(50)	2(100)	-	-	7(46.7)
30µg	I	1(11.1)		-	-	-	1(6.7)
	S	4(44.4)	1(50)	-	1(100)	1(100)	7(46.7)
NIT	R	1(11.1)	1(50)	-	-	-	2(13.3)
300µg	I	1(11.1)		-	-	-	1(6.7)
	S	7(77.8)	1(50)	2(100)	1(100)	1(100)	12(80.0)
GEN	R	-	1(50)	-	-	-	1(6.7)
120µg	I	-	-	-	-	-	-
	S	9(100)	1(50)	2(100)	1(100)	1(100)	14(93.3)
STR	R	-	2(100)	-	-	-	2(13.3)
300µg	I	-	-	-	-	1(100)	1(6.7)
	S	9(100)	-	2(100)	1(100)	-	12(80.0)

Key: Sus: Susceptibility,R: Resistance, I: intermediate, S: susceptible, AMP: Ampicillin, CIP:Ciprofloxacin, RIF: Rifampicin,LIN: Linzolid, VAN:Vancomycin, DOX: Doxicyclin, TEC:Teicoplanin,ERY:Erythromycin,CHL:Chloramphenicol,NIT:Nitrofurantoin,GEN:Gentimicin,STR:Streptomycin.

Table 4.12 Antibiotic Resistance Pattern and resistance index of Kubwa Isolates.

NA	AWP	RES PATTERN	RI	%	CR
1	1	STR			
	1	AMP	0.1	20	Nil
	1	ERY			
3	1	RIF, ERY, CHL			
	1	RIF, DOX, CHL	0.2	20	MDR
	1	CIP, ERY, CHL			
6	2	RIF, LIN, VAN, DOX, TEI, ERY	0.4	13	MDR
8	2	AMP, CIP, RIF, LIN, VAN, DOX, TEIC, ERY	0.5	13	MDR
9	1	AMP, RIF, LIN, VAN, DOX, TEIC, ERY, CHL, NIT	0.6	7	XDR
12	1	AMP, CIP, RIF, LIN, VAN, DOX, TEIC, ERY, CHL, NIT, GEN, STR	0.8	7	PDR

MDR: non-susceptible to 1 agent in ≥ 3 antimicrobial categories.
XDR: non-susceptible to ≥1 agent in all but ≤ 2 categories.
PDR: non-susceptible to all antimicrobial agents listed
NA: number of antibiotics. AWP: Antibiotics with pattern. RI: Resistance Index. CR: classification of resistance

4.10 Antibiotic Susceptibility Profile of Enterococcal isolates obtained from samples in Kuje, Abuja.

Table 4.13 elaborated the susceptibility pattern of the different 17 species isolated from Kuje General Hospital. For ampicillin, the susceptibility pattern of all the *Enterococcus* spp. showed 1(5.9%) resistant strain and 16(94.1%) full sensitive strains. For Ciprofloxacin, 4(23.5%) had intermediate sensitivity while 13(76.5%) had full sensitivity. For Rifampicin, 7(41.2%) strains had resistance, 2(11.8%) strains had intermediate sensitivity while 8(47.1%) had full sensitivity. For Linzolid, 1(5.9%) strain was resistant while 16(94.1%) were sensitive. For vancomycin, the 17 enterococci were all susceptible. For Doxycyclin, 7(41.2%) strains had resistance, 1(5.9%) strain was intermediately susceptible while 9(53.0%) were fully sensitive. For Teicoplanin, the 17(100%) strains had full susceptibility. For erythromycin, 9(53.0%) had resistance, 2(11.8%) were intermediately susceptible and 6(35.3%) strains were fully sensitive. For Chloramphenicol, 5(29.4%) strains had resistance while 12(70.6%) had full sensitivity. For Nitrofurantoin, 1(5.9%) strain was resistant while 16 strains were sensitive. For Gentimicin, 4(23.5%) strains were resistant while 13(76.5%) were fully sensitive. For Streptomycin, 7(41.2%) were resistant while 10(58.8%) were fully sensitive.

Table 4.13: Susceptibility Profile of *Enterococcus* spp. Isolated from Patients in Kuje Hospital, Abuja, Nigeria.

Antibiotics Disk potency	Sus	*E.faecalis* 13(%)	*E.faecium* 2(%)	*E.casseliflavus* 1(%)	*E.gallinarum* 1(%)	Total 17(%)
AMP	R	1(7.7)	-	-	-	1(5.9)
10µg	I	-	-	-	-	-
	S	12(92.3)	2(100)	1(100)	1(100)	16(94.1)
CIP	R	-	-	-	-	-
5µg	I	3(23.1)	1(50)	-	-	4(23.5)
	S	10(76.9)	1(50)	1(100)	1(100)	13(76.5)
RIF	R	7(53.8)	-	-	-	7(41.2)
5µg	I	1(7.7)	1(50)	-	-	2(11.8)
	S	5(38.5)	1(50)	1(100)	1(100)	8(47.1)
LIN	R	1(7.7)	-	-	-	1(5.9)
30µg	I	-	-	-	-	-
	S	12(92.3)	2(100)	1(100)	1(100)	16(94.1)
VAN	R	-	-	-	-	-
30µg	I	-	-	-	-	-
	S	13(100)	2(100)	1(100)	1(100)	17(100)
DOX	R	5(38.5)	1(50)	-	1(100)	7(41.2)
30µg	I	1(7.7)	-	-	-	1(5.9)
	S	7(53.8)	1(50)	1(100)	-	9(53.0)
TEIC	R	-	-	-	-	-
30µg	I	-	-	-	-	-
	S	13(100)	2(100)	1(100)	1(100)	17(100)
ERY	R	6(46.2)	1(50)	1(100)	1(100)	9(53.0)
15µg	I	2(15.4)	-	-	-	2(11.8)
	S	5(38.5)	1(50)	-	-	6(35.3)
CHL	R	4(30.8)	1(50)	-	-	5(29.4)
30µg	I	-	-	-	-	-
	S	9(69.2)	1(50)	1(100)	1(100)	12(70.6)
NIT	R	1(7.7)	-	-	-	1(5.9)
300µg	I	-	-	-	-	-
	S	12(92.3)	2(100)	1(100)	1(100)	16(94.1)
GEN	R	3(23.1)	1(50)	-	-	4(23.5)
120µg	I	-	-	-	-	-
	S	10(76.9)	1(50)	1(100)	1(100)	13(76.5)
STR	R	6(46.2)	1(50)	-	-	7(41.2)
300µg	I	-	-	-	-	-
	S	7(53.8)	1(50)	1(100)	1(100)	10(58.8)

Key: SUS: Susceptibility, R: Resistance, I:Intermediate, S: Susceptible, AMP: Ampicillin, CIP:Ciprofloxacin, RIF: Rifampicin,LIN: Linzolid, VAN:Vancomycin, DOX: Doxicyclin, TEC:Teicoplanin,ERY:Erythromycin,CHL:Chloramphenicol,NIT:Nitrofurantoin,GEN:Gentimicin,STR:Streptomycin.

4.11 Multiple Antibiotic Resistance Pattern and Index of Kuje Isolates

Table 4.14 shows that 16 (94.1%) out of 17 *Enterococcus* spp. isolated from Kuje exhibited 6 resistant pattern with MAR index ranging from 0.1 – 0.5 of which 7 (41.0%) were multidrug resistant. Out of the 17 enterococci isolated from the Kuje Hospital, 15(88.2%) had MAR index of 0.2 and above indicating that the isolates had been pre exposed to the antibiotics tested.

Table 4.14: Antibiotic Resistance Pattern and Resistance Index from KUJE Isolates.

NA	AWP	Res pattern/phenotype	RI	%	CR
1	1	DOX	0.1	5.8	nil
2	2	RIF, DOX	0.2	47	nil
	2	RIF, ERY			nil
	1	RIF, LIN			nil
	3	GEN, STR			nil
3	2	RIF, DOX, ERY	0.3	24	MDR
	1	RIF, ERY, STR			MDR
	1	ERY, CHL, NIT			MDR
4	1	RIF, DOX, ERY, CHL	0.3	5.8	MDR
5	1	RIF, DOX, ERY, CHL, STR	0.4	5.8	MDR
6	1	AMP, DOX, CHL, NIT, GEN, STR	0.5	5.8	MDR

MDR: non-susceptible to 1≥ agent in ≥3 antimicrobial categories. Resistance pattern/phenotype
NA: number of antibiotics. AWP: Antibiotics with pattern. RI: Resistance Index. CR: classification of resistance

4.12 Antibiotic Susceptibility Profile of Enterococcal isolates obtained from samples in all Hospitals, Abuja.

Table 4.15 elaborated the susceptibility pattern of the different 102 *Enterococcus* species isolated from all the Hospitals. For ampicillin, 28(27.5%) strains were resistant while 74(72.5%) were sensitive. For Ciprofloxacin, 25(24.5%) were resistant, 18(17.6%) were intermediately sensitive while 59(57.8%) were fully sensitive. For rifampicin, 68(66.7%) strains were resistant, 13(12.7%) had intermediate sensitivity while 21(20.6%) were fully sensitive. For Linzolid, 38(37.3%) were resistant, 5(4.9%) were intermediately sensitive while 59 (57.8%) were fully sensitive. For vancomycin, 34(33.3%) were resistant while 68(66.7%) were sensitive. For doxycyclin, 65(63.7%) were resistant, 11(10.8%) were intermediately sensitive while 26(25.5%) were fully sensitive. For teicoplanin, 33(32.4%) were resistant, 2(1.96%) were intermediately sensitive while 67(65.7%) were full sensitive. For erythromycin, 61(60.0%) were resistant, 24(23.5%) intermediately sensitive while 17(16.7%) were fully sensitive. For chloramphenicol, 46(45.1%) strains were resistant, 4(3.9%) were intermediately sensitive while 52(51.0%) were fully sensitive. For nitrofurantoin, 9(8.8%) strains were resistant, 7(6.9%) were intermediately sensitive while 86(84.3%) were fully sensitive. For gentamicin, 27(26.5%) strains were resistant, 3(2.9%) intermediately sensitive while 72(70.6%) were fully sensitive. For streptomycin, 41(40.2%) strains were resistant, 2(1.96%) were intermediately susceptible while 59(57.8%) were fully sensitive.

Table 4.15: Susceptibility Profile of *Enterococcus* spp. Isolated from the Hospitals

Antibiotics Disk potency	Sus	*E.faecalis* 59(%)	*E.faecium* 24(%)	*E.cas* 1(%)	*E.ga* 3(%)	*E.mundtii* 11(%)	*E.avium* 1(%)	*E.dispar* 2(%)	*E.hirae* (1(%)	Total 102(%)
AMP	R	9(15.3%)	10(41.7)	-	1(33.3)	6(54.5)	-	1(50)	1(100)	28(27.5)
10µg	I	-	-	-	-	-	-	-	-	-
	S	50(84.7)	14(58.3)	1(100)	2(66.7)	5(45.5)	1(100)	1(50)	-	74(72.5)
CIP	R	10(16.9)	9(37.5)	-	1(33.3)	4(36.4)	-	-	1(100)	25(24.5)
5µg	I	14(23.7)	3(12.5)	-	-	-	-	1(50)	-	18(17.6)
	S	35(59.3)	12(50)	1(100)	2(66.7)	7(63.6)	1(100)	1(50)	-	59(57.8)
RIF	R	37(62.7)	18(75.0)	-	-	11(100)	-	1(50)	1(100)	68(66.7)
5µg	I	10(16.9)	2(8.3)	-	-	-	1(100)	-	-	13(12.7)
	S	12(20.3)	4(16.7)	1(100)	3(100)	-	-	1(50)	-	21(20.6)
LIN	R	13(22.0)	13(54.1)	-	1(33.3)	9(81.8)	-	1(50)	1(100)	38(37.3)
30µg	I	3(5.1)	1(4.2)	-	-	-	1(100)	-	-	5(4.9)
	S	43(72.9)	10(41.7)	1(100)	2(66.7)	2(18.2)	-	1(50)	-	59(57.8)
VAN	R	10(16.9)	12(50.0)	-	1(33.3)	9(81.8)	-	1(50)	1(100)	34(33.3)
30µg	I	-	-	-	-	-	-	-	-	-
	S	49(83.1)	12(50.0)	1(100)	2(66.7)	2(18.2)	1(100)	1(50)	-	68(66.7)
DOX	R	34(57.6)	16(66.7)	-	3(100)	9(81.8)	1(100)	1(50)	1(100)	65(63.7)
30µg	I	8(13.6)	3(12.5)	-	-	-	-	-	-	11(10.8)
	S	17(28.8)	5(20.8)	1(100)	-	2(18.2)	-	1(50)	-	26(25.5)
TEIC	R	9(15.3)	12(50.0)	-	1(33.3)	9(81.8)	-	1(50)	1(100)	33(32.4)
30µg	I	2(3.4)	-	-	-	-	-	-	-	2(1.96)
	S	48(81.4)	12(50.0)	1(100)	2(66.7)	2(18.2)	1(100)	1(50)	-	67(65.7)
ERY	R	28(47.5)	17(70.8)	1(100)	3(100)	9(81.8)	1(100)	1(50)	1(100)	61(60.0)
15µg	I	21(35.6)	1(4.2)	-	-	2(18.2)	-	-	-	24(23.5)
	S	10(16.9)	6(25.0)	-	-	-	-	1(50)	-	17(16.7)
CHL	R	28(47.5)	10(41.7)	-	1(33.3)	6(54.5)	1(100)	-	-	46(45.1)
30µg	I	2(3.4)	1(4.2)	-	1(33.3)	-	-	-	-	4(3.9)
	S	29(49.2)	13(54.1)	1(100)	1(33.3)	5(45.5)	-	2(100)	1(100)	52(51.0)
NIT	R	4(6.8)	3(12.5)	-	-	2(18.2)	-	-	-	9(8.8)
300µg	I	3(5.1)	3(12.5)	-	-	1(9.1)	-	-	-	7(6.9)
	S	52(88.1)	18(75)	1(100)	3(100)	8(72.7)	1(100)	2(100)	1(100)	86(84.3)
GEN	R	14(23.7)	8(33.3)	-	-	3(27.3)	1(100)	1(50)	-	27(26.5)
120µg	I	1(1.7)	2(8.3)	-	-	-	-	-	-	3(2.9)
	S	44(74.6)	14(58.3)	1(100)	3(100)	8(72.7)	-	1(50)	1(100)	72(70.6)
STR	R	24(40.7)	12(50.0)	-	-	4(36.4)	1(100)	-	-	41(40.2)
300µg	I	1(1.7)	-	-	-	-	-	-	1(100)	2(1.96)
	S	34(57.6)	12(50.0)	1(100)	3(100)	7(63.6)	-	2(100)	-	59(57.8)

Key: E.ca: *E.casseliflavus*, E.ga: *E.gallinarum*, Sus: Susceptibility,R: Resistance, I: intermediate, S: susceptible, Cassel: *casselliflavus*, AMP: Ampicillin, CIP:Ciprofloxacin, RIF: Rifampicin,LIN: Linzolid, VAN:Vancomycin, DOX: Doxicyclin, TEC:Teicoplanin,ERY:Erythromycin,CHL:Chloramphenicol,NIT:Nitrofurantoin,GEN:Genti micin,STR:Streptomycin.

4.13 Multiple Antibiotic Resistance Pattern and Index of Isolates from all the Hospitals

Table 4.16 shows the resistance patterns of the 96(94%) resistant out of the 102 isolates tested for antibiotics susceptibilty from the four Hospitals in Abuja. Eleven different resistant patterns were exhibited with MAR index ranging from 0.1 – 1.0. From the result, out of the 96(94%) resistant strains with patterns, 67(66%) were multidrug resistant, 3(3%) were extremely resistant (XDR), while 2(2%) had pandrug resistance. Out of the 102 Enterococci isolated, 87(85%) had MAR index of 0.2.

4.14 Minimum Inhibitory Concentration of the Resistant Isolates from all Hospitals.

Table 4.17 showes confirmation of the 34 resistant strains of the enterococcal isolates for susceptibility to vancomycin by E-test Minimum Inhibitory Concentration (M.I.C). Six isolates from Kubwa and 4 from UATH and were all susceptible with MIC of ≤ 4µg/ml except for 2 strains from Kubwa hospital that had MIC of ≤ 8µg/ml. At NHA, out of the 24 strains that had resistance to vancomycin disc, 12 had MIC of ≤ 4µg/ml while 10 had MIC of ≤8 µg. Thus, none of the isolates was extremely resistant to vancomycin with MIC of ≥32 as all were inhibited at ≤ 8ug/ml concentration which is the MIC susceptibility (4µg/ml) and intermediate (8µg/ml) interpretive breakpoint for vancomycin. Some strains with MIC of ≥ 4µg/ml were selected for molecular analysis to confirm for presence of van genes genes in them.

Table 4.16: Antibiotic Resistance Pattern and resistance index of isolates from all the hospitals

NA	AWP	PATTERN	RI	%	CR
1	1	DOX	0.1	9	nil
	2	STR			nil
	4	RIF			nil
	1	AMP			nil
	1	ERY			nil
2	4	RIF,DOX	0.2	15	nil
	2	RIF,LIN			nil
	4	RIF,STR			nil
	1	RIF, ERY			nil
	1	GEN,STR			nil
3	1	CIP,GEN,STR	0.3	17	mdr
	3	RIF,DOX,ERY			mdr
	1	DOX,ERY,CHL			mdr
	2	RIF,DOX,STR			mdr
	3	RIF,GEN,STR			mdr
	1	AMP,CIP,DOX			mdr
	1	AMP,DOX,CHL			mdr
	1	RIF,ERY,CHL			mdr
	1	RIF,DOX,CHL			mdr
	1	CIP,ERY,CHL			mdr
	1	RIF,ERY,STR			mdr
	1	ERY,CHL,NIT			mdr
	1	CIP,GEN,STR			mdr
4	2	CIP,DOX,ERY,CHL	0.3	7	mdr
	2	RIF,DOX,ERY,CHL			mdr
	2	DOX,ERY,CHL,STR			mdr
	1	CIP,DOX,ERY,STR			mdr
5	2	RIF,LIN,VAN,TEC,ERY	0.4	8	mdr
	1	AMP,RIF,VAN,DOX,ERY			mdr
	1	DOX,ERY,CHL,GEN,STR			mdr
	1	CIP,DOX,ERY,CHL,STR			mdr
	2	RIF,DOX,CHL,GEN,STR			mdr
	1	RIF,DOX,ERY,CHL,STR			mdr
6	5	RIF,LIN,VAN,DOV,TEC,ERY	0.5	11	mdr
	1	CIP,RIF,ERY,CHL,GEN,STR			mdr
	1	AMP,RIF,LIN,VAN,TEC,CHL			mdr
	1	RIF,DOX,ERY,CHL,GEN,STR			mdr
	2	CIP,LIN,DOX,CHL,GEN,STR			mdr
	1	AMP,DOX,CHL,NIT,GEN,STR			mdr
7	2	RIF,LIN,VAN,DOX,TEC,ERY,CHL	0.6	10	mdr

	2	CIP,RIF,DOX,ERY,CHL,GEN,STR			mdr
	5	AMP,RIF,LIN,VAN,DOX,TEC,ERY			mdr
	1	AMP,CIP,LIN,DOX,CHL,GEN,STR			mdr
8	2	AMP,RIF,LIN,VAN,DOX,TEC,ERY,CHL	0.7	11	mdr
	1	AMP,RIF,LIN,VAN,DOX,TEC,ERY,NIT			mdr
	1	AMP,CIP,RIF,LIN,VAN,DOX,TEC,CHL			mdr
	1	CIP,RIF,LIN,VAN,DOX,TEC,CHL,NIT			mdr
	2	RIF,LIN,VAN,DOX,TEC,ERY,GEN,STR			mdr
	1	AMP,RIF,LIN,VAN,DOX,TEC,ERY,GEN			mdr
	1	AMP,RIF,DOX,ERY,CHL,NIT,GEN,STR			mdr
	2	AMP,CIP,RIF,LIN,VAN,DOX,TEC,ERY			mdr
9	1	AMP,CIP,RIF,LIN,VAN,DOX,TEC,ERY,GEN,STR	0.8	3	mdr
	1	AMP,CIP,RIF,LIN,VAN,DOX,TEC,ERY,CHL,NIT			mdr
	1	AMP,RIF,LIN,VAN,DOX,TEC,ERY,CHL,NIT,STR			mdr
10	1	AMP,CIP,RIF,LIN,VAN,DOX,TEC,ERY,GEN,STR	0.8	3	xdr
	1	AMP,CIP,RIF,LIN,VAN,DOX,TEC,ERY,CHL,NIT			xdr
	1	AMP,RIF,LIN,VAN,DOX,TEC,ERY,CHL,NIT,STR			xdr
12	2	AMP,CIP,RIF,LIN,VAN,DOX,TEC,ERY,CHL,NIT,GEN,STR	1.0	2	pdr

MDR: non-susceptible to 1≥ agent in ≥3 antimicrobial categories,
XDR: non-susceptible to≥ 1 agent in all but ≤2 categories
PDR: non-susceptible to all antimicrobial agents listed,
NA: number of antibiotics. AWP: Antibiotics with pattern. RI: Resistance Index. CR: classification of resistance

Table 4.17: Zone diameter interpretive standard and equivalent minimum inhibitory concentration (MIC) breakpoints for *Enterococcus* species

S/N	Isolate code	Sample	Strain	R(<14mm)	Etest Van MIC(ug/ml)		
					<4	8-16	>32
1	Kw2	Stool	*E.faecium*	0	4	-	-
2	Kw3	Stool	*E.mundtii*	0	-	8	-
3	Kw4	Stool	*E.mundtii*	0	-	8	-
4	Kw5	Stool	*E.faecalis*	0	2	-	-
5	Kw6	Stool	*E.faecium*	0	2	-	-
6	Kw10	Stool	*E.hirae*	0	4	-	-
7	UA14	Stool	*E.faecalis*	0	1	-	-
8	UA15	Stool	*E.faecium*	0	1	-	-
9	UA17	Stool	*E.faecium*	0	1	-	-
10	UA18	Stool	*E.faecalis*	0	4	-	-
11	NH2	Stool	*E.gallinarum*	0	-	8	-
12	NH4	Stool	*E.mundtii*	0	2	-	-
13	NH5	Urine	*E.faecalis*	0	2	-	-
14	NH7	Stool	*E.mundtii*	0	1	-	
15	NH8	Stool	*E.faecium*	0	-	8	-
16	NH9	Stool	*E.mundtii*	0	-	8	-
17	NH10	Stool	*E.faecium*	0	2	-	-
18	NH11	Stool	*E.faecium*	0	2	-	-
19	NH12	Stool	*E.faecium*	0	4	-	-
20	NH17	Stool	*E.faecalis*	0	-	8	-
21	NH18	Stool	*E.faecium*	0	4	8	-
22	NH19	Urine	*E.faecalis*	0	2	-	
23	NH20	Urine	*E.faecalis*	0	-	8	-
24	NH21	Urine	*E.faecalis*	0	4	-	-
25	NH24	Stool	*E.faecium*	0	1	-	-
26	NH25	Stool	*E.mundtii*	0	-	8	-
27	NH26	Stool	*E.faecium*	0	4	-	-
28	NH27	Stool	*E.faecalis*	0	-	8	-
29	NH31	Stool	*E.mundtii*	0	1	-	-
30	NH32	Stool	*E.mundtii*	0	4	-	-
31	NH33	Stool	*E.faecium*	0	-	8	-
32	NH34	Stool	*E.mundtii*	0	4	-	-
33	NH35	Stool	*E.dispar*	0	-	8	-
34	NH36	Urine	*E.faecalis*	0	2	-	-

4.15 Molecular Detection of Vancomycin Resistant Genes, 16rRNA Sequence and Phylogenetic Relatedness of Isolates

DNA was extracted from 17 out of the 20 isolates that had vancomycin MIC of ≥ 4ug/ml as shown in plate I. Lane 3, 13 and 14 did not yield DNA.

Table 4.18 shows the 20 isolates that were used for DNA extraction and their codes. Out of the 20 isolates, KW2, KW10 and NH26 did not yield any DNA.

Plate II shows that 47% (8) out of the 17 extracted DNA samples harboured vanA genes. The 8 strains with *vanA* genes include samples: 1 (NH17), *E. faecalis*; 5(NH8), *E. feacium*; 6(KW3), *E. mundtii*; 7(NH35), *E. dispar*; 8(NH25), *E. mundtii*; 10(NH9), *E. mundtii*; 13(NH20), *E. faecalis*; 15(NH2), *E. gallinarum* giving a total of 2 *E. faecalis*, 1 *E. feacium*, 3 *E. mundtii*, 1 *E. dispar* and 1 *E. gallinarum*. Plate III shows that 18% (3) out of the 17 extracted DNA samples harbored the *vanB* gene. The strains with these *vanB* genes include samples: 2(KW4), *E. mundtii*; 6(KW3), *E. mundtti*; 9(NH27), *E. faecalis*.

Plate IV shows that 29% (5) out of the 17 extracted DNA samples harbored the van C gene. The strains with *vanC* genes include samples: 1(NH17), *E. faecalis*; 8(NH25), *E. mundtii*; 10(NH9), *E. mundtii*; 11(NH33), *E. faecium* and 15 (NH2) *E. gallinarum*.

Plate V shows that sample 17(NH18), *E. feacium*, harboured *vanR* out of the 17 extracted DNA samples giving a percentage of 5.8.

The nonspecific bands observed in plate II, III and V could have arisen from excessive extension time, low annealing temperature which might have caused the primers to bind nonspecifically to the template and low thermal cycler ramping speed. Also, the agarose

gel preparation could have made the amplicon on lane 1 of plate II to lie slightly below other amplicons on lane 5, 6, 7, 8, 10, 13 and 15.

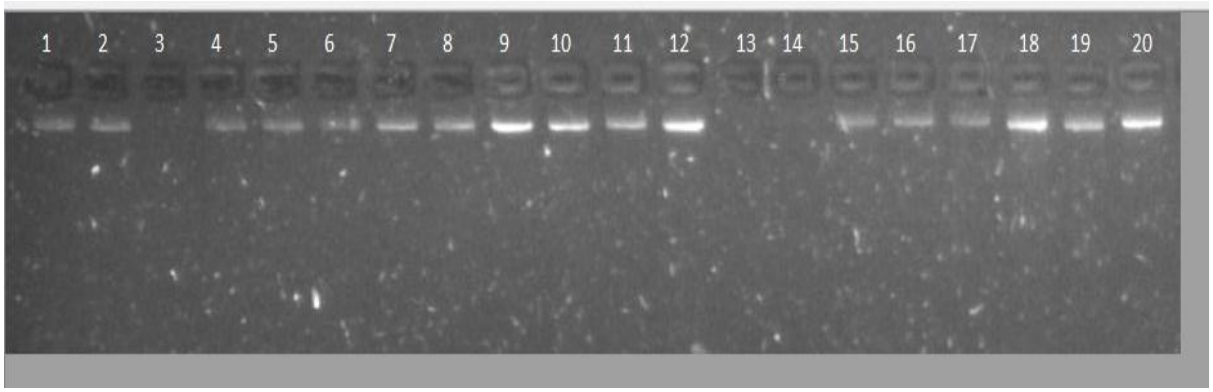


Plate I: Agarose gel electrophoresis of genomic DNA of *Enterococcus* spp. with vancomycin MIC of ≥ 4µg/ml. Lanes 1-20 shows samples.

Table 4.18: *Enterococcus* spp used for DNA extraction and their yielded DNA code:

S/N	Isolates	Code	S/N	Isolates	Code
1	*E.faecalis*	NH17	11	*E.mundtii*	NH9
2	*E.mundtii*	KW4	12	*E.faecium*	NH33
3	*E.faecium*	KW2 nil	13	*E.hirae*	KW10 nil
4	*E.mundtii*	NH32	14	*E.faecium*	NH26 nil
5	*E.faecalis*	NH21	15	*E.faecalis*	UA 18
6	*E.faecium*	NH8	16	*E.faecalis*	NH 20
7	*E.mundtii*	KW3	17	*E.mundtii*	NH34
8	*E.dispar*	NH35	18	*E.gallinarum*	NH2
9	*E.mundtii*	NH25	19	*E.faecium*	NH12
10	*E.faecalis*	NH 27	20	*E.faecium*	NH18

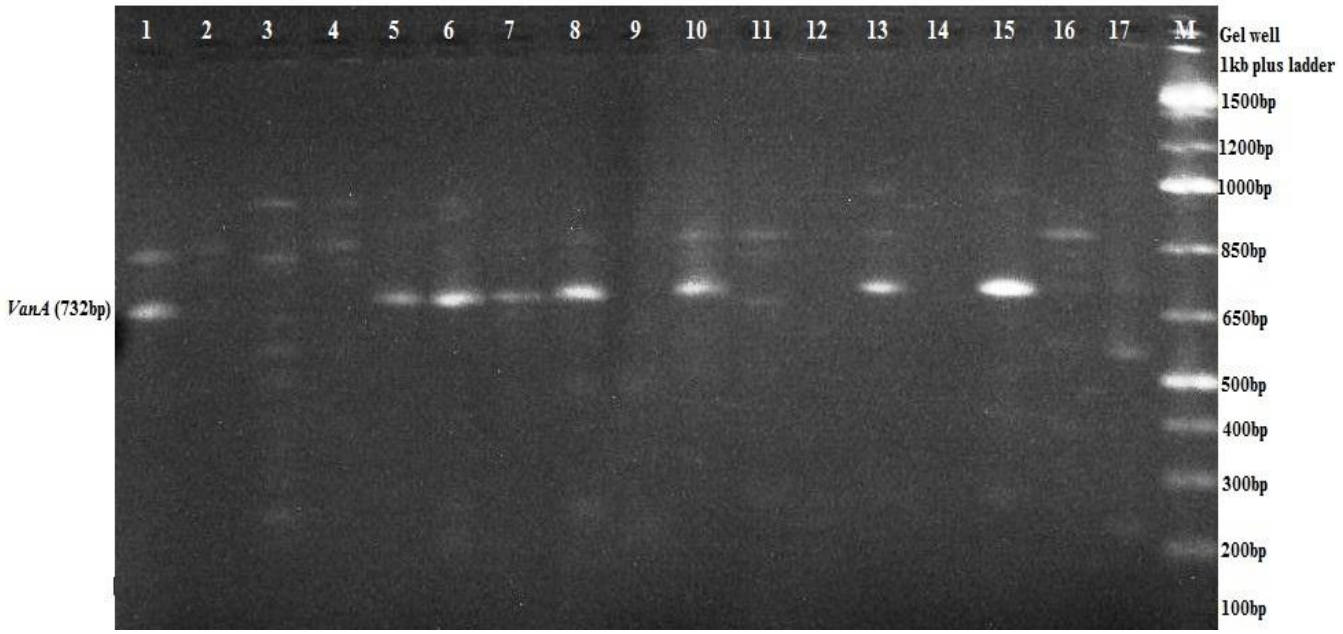


Plate II: Agarose gel electrophoresis of *vanA* amplicons (732bp) of *Enterococcus* spp. with vancomycin MIC of ≥ 4µg/ml. Lanes M = 1kb molecular ladder, lanes 1, 5, 6, 7, 8, 10, 13 and 15 show PCR products.

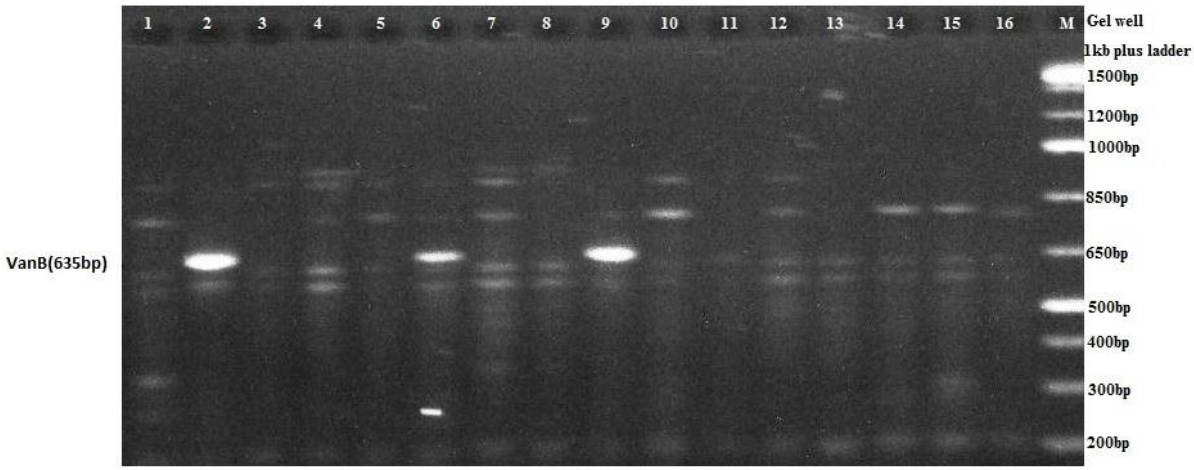


Plate III: Agarose gel electrophoresis of *vanB* amplicons (635bp) of *Enterococcus* spp with vancomycin MIC of ≥ 4µg/ml. Lanes M = 1kb molecular ladder, lanes 2, 6 and 9 show *vanB* amplicons.

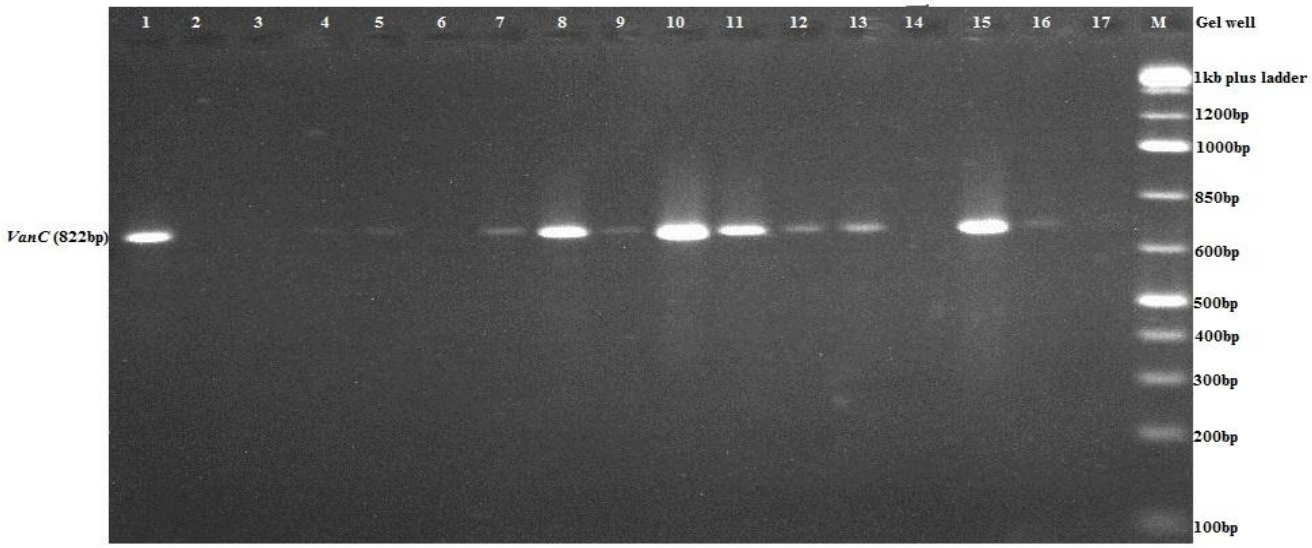


Plate IV: Agarose gel electrophoresis of *vanC* amplicons (822bp) of *Enterococcus* spp. with vancomycin MIC of ≥ 4µg/ml. Lanes M = 1kb molecular ladder, lanes 1, 8, 10, 11 and 15 show PCR products.

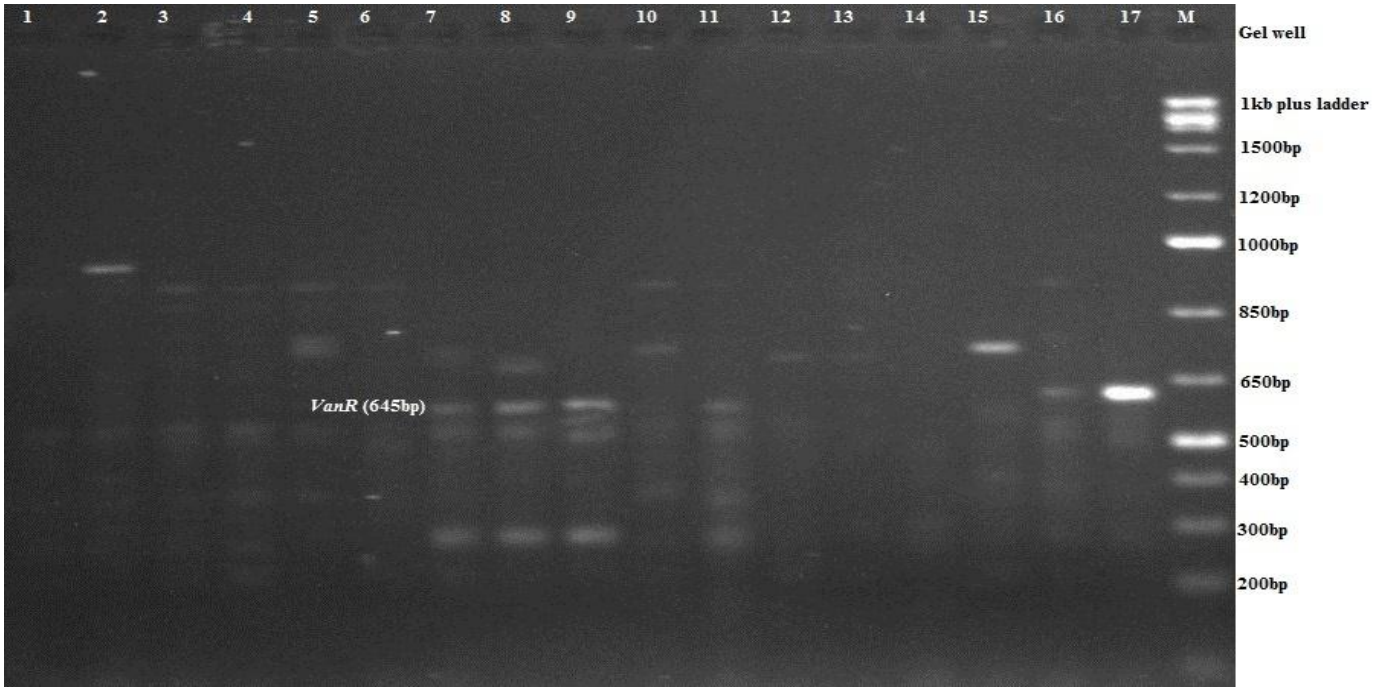


Plate V: Agarose gel electrophoresis of *vanR* amplicons (645bp) of *Enterococcus* spp. with vancomycin MIC of ≥ 4µg/ml. Lanes M = 1kb molecular ladder, lane 17 shows PCR product.

Table 4.19 shows the *Enterococcus* species with their extracted DNA and the van gene types amplified by PCR. Out of the 20 isolates subjected for DNA extraction, 17 yielded good DNA. *E. faecalis* with sample code NH17 yielded both *vanA* and *vanC* genes, *E. mundtii* (KW4) yielded *vanB, E. faecium* (NH8) yielded *vanA, E. mundtii* (KW3) yielded *vanA* and *vanB*; *E. dispar* (NH35) yielded *vanA*; *E. mundtii* (NH25) yielded *vanA* and *vanC*; *E. faecalis* (NH27) yielded *vanB*; *E. mundtii*(NH9) yielded *vanA* and *vanC*; *E. faecium*(NH33) yielded *vanC*; *E. faecalis*(NH20) yielded *vanA*; *E.gallinarum*(NH2) yielded *vanA* and *vanC* while *E. faecium*(NH18) yielded *vanR*.

Table 4.20 shows the prevalence of van genes among the Enterococcus species isolated from all the hospitals. Vancomycin resistance genes were found in 3(5.1%) of the 59 *E. faecalis*, 3(12.5%) out of 24 *E. faecium*, 4(36.4%) out of 11 *E. mundtii*, 1(33.3%) out of 3 *E. gallinarum* and 1(50%) out of 2 *E. dispar* giving a total of 12(11.8%) out of the 102 *Enterococcus* spp isolated.

Table 4.21 shows isolates distribution of vancomycin resistance genes among the isolates according to their isolation site. The distribution shows 10(25.6%) were isolated out of 39 from NHA and made up of 3(7.7%) *E. faecalis*, 3(7.7) *E. faecium*, 2(5.1%) *E. mundtii*, 1(2.6%) *E. gallinarum* and 1(2.6) *E. dispar*. Two (13.3%) *E. mundtii* from 15 isolates from Kubwa.

Table 4.19: Amplified DNA with their van genes

S/N	Code	Species	Van type
1	NH17	*E.faecalis*	A,C
2	KW4	*E.mundtii*	B
3	NH32	*E.mundtii*	
4	NH21	*E.faecalis*	
5	NH8	*E.faecium*	A
6	KW3	*E.mundtii*	A,B
7	NH35	*E.dispar*	A
8	NH25	*E.mundtii*	A,C
9	NH27	*E.faecalis*	B
10	NH9	*Emundtii*	A,C
11	NH33	*E.faecium*	C
12	UA18	*E.faecalis*	
13	NH20	*E.faecalis*	A
14	NH34	*E.mundtii*	
15	NH2	*E.gallinarum*	A,C
16	NH12	*E.faecium*	
17	NH18	*E.faecium*	R

Table 4.20: Prevalence of van genes among the *Enterococcus species* isolated from the Hospitals.

Species	**Isolates (no)**	**NHA (no)**	**UATH (no)**	**KUBWA (no)**	**KUJE (no)**	**Total VRE(%)**
E. faecalis	59	3	-	-	-	3(5.1)
E. faecium	24	3	-	-	-	3(12.5)
E. mundtii	11	2	-	2	-	4(36.4)
E. gallinarum	3	1	-	-	-	1(33.3)
E. dispar	2	1	-	-	-	1(50)
E. hirae	1	-	-	-	-	-
E.casselliflavus	1	-	-	-	-	-
E. avium	1	-	-	-	-	-
Total	102	10(9.8)	-	2(2.0)	-	12(11.8)

Table: 4.21: Distribution, frequency, and diversity of VRE species according to the site of isolation.

SITE	Isolates	VRE	E.f	E.fc	E.m	E.g	E.d
NHA	**39**	**10(25.6)**	**3(7.7)**	**3(7.7)**	**2(5.1)**	**1(2.6)**	**1(2.6)**
KUBWA	**15**	**2(13.3)**	**-**	**-**	**2(13.3)**	**-**	**-**

Key: VRE: vancomycin resistance enterococci, *E.f: E.faecalis, E.fc: E.faecium, Em: E.mundtii,*
E.g: E.gallinarum, E.d: E.dispar

Table 4.22 shows 8 *vanA* genes which included 2 from *E. faecalis*, 1 from *E. faecium*, 3 from *E. mundtii*, 1 from *E. gallinarum* and 1 from *E. dispar*; 3 *vanB* genes which included 1 from *E. faecalis* and 2 from *E. mundtii*; 5 vanC genes which included 1 from *E. faecalis*, 1 from *E. faecium*, 2 from *E. mundtii* and 1 from *E. gallinarum* and the only *vanR* gene from *E. faecium*.

The antibiotics resistance profile of the isolates with van genes shows that they were all multidrug resistant. For *E. faecium*: NH18 with *vanR* had 10 resistance pattern and was classified as extremely resistant (xdr) while NH8 with *vanA* and NH33 with *vanC* had 7 resistance pattern and were multidrug resistant (mdr). For *E. mundtii*, KW4 with *vanB* and KW3 with *vanA* and *vanB* had 6 resistance pattern, NH25 with *vanA* and *vanC* had 7 resistant pattern while NH9 with *vanA* and *vanC* had 8 resistance pattern and were all multidrug-resistant (mdr). For *E. faecalis*, NH27 with *vanB* had 10 resistant pattern and extremely resistant (xdr) while NH20 with *vanA* and NH17 with *vanA* and *vanC* had 8 resistant pattern and were multi resistant. *E. gallinarum* (NH2) with *vanA* and *vanC* had 7 resistant pattern and was classified as multidrug resistant (mdr) while *E. dispar* (NH35) with *vanA* had 9 resistance pattern and classified as multidrug resistant (mdr). All were isolated from stool samples except NH20 (*E. faecalis*) that was isolated from urine sample (Table 4.23).

Plate VI shows the amplication of 14 out of the 15 DNA samples analyzed while sample 14(NH12) did not amplify as shown by gel electrophoresis.

Table 4.22: Frequency of the van types among the isolates

Van type	*E.faecalis*	*E.faecaium*	*E.mundtii*	*E.gallinarum*	*E.dispar*	Total(%)
vanA	2	1	3	1	1	8(47%)
vanB	1		2			3(17.6%)
vanC	1	1	2	1		5(29.4%)
vanR		1				1(5.9%)
Total *van* gene type	4	3	7	2	1	17

Table 4.23: Antibiotic Resistance profile of Isolates with *Van* Genotypes

S/N	Source	Isolate Code	Species	NOA	AWP	*Van* Genotype	CR
1	Stool	NH18	*E. faecium*	10	AMP,RIF,LIN,VAN,DOX,TEIC,ERY,NIT,GEN, STR	R	XDR
2	Stool	NH8	*E. faecium*	7	RIF,LIN,VAN,DOX,TEIC,ERY,NIT	A	MDR
3	Stool	NH33	*E. faecium*	7	AMP,RIF,LIN,VAN,DOX,TEIC,ERY	C	MDR
4	Stool	KW4	*E. mundti*i	6	RIF,LIN,VAN,DOX,TEIC,ERY	B	MDR
5	Stool	KW3	*E. mundtii*	6	RIF,LIN,VAN,DOX,TEIC,ERY	A/B	MDR
6	stool	NH25	*E. mundtii*	7	AMP,RIF,LIN,VAN,DOX,TEIC,ERY	A/C	MDR
7	Stool	NH9	*E. mundti*	8	AMP,RIF,LIN,VAN,DOX,TEIC,ERY,CHL	A/C	MDR
8	Stool	NH27	*E. faecalis*	10	CIP,RIF,LIN,VAN,DOX,TEIC,ERY,NIT,GEN,STR	B	XDR
9	Urine	NH20	*E. faecalis*	8	AMP,CIP,RIF,LIN,VAN,DOX,TEIC,ERY	A	MDR
10	Stool	NH17	*E. faecalis*	8	AMP,RIF,LIN,VAN,DOX,TEIC,ERY,NIT	A/C	MDR
11	Stool	NH2	*E.gallinarum*	7	RIF,LIN,VAN,DOX,TEIC,ERY,CHL	A/C	MDR
12	Stool	NH35	*E. dispar*	9	AMP,CIP,RIF,LIN,VAN,DOX,TEIC,ERY,GEN	A	MDR

Key: S/N: serial number, NOA: number of antibiotics, AWP: antibiotics with pattern, CR: classification of resistance, AMP: Ampicillin, CHL: Chloramphenicol, CIP: Ciprofloxacin, DOX: Doxycyclin, ERY: Erythromycin, GEN: Gentamicin, LIN: Linzolid, NIT: Nitrofurantoin, RIF: Rifampicin, STR: Streptomycin, TEIC: Teicoplanin , VAN: Vancomycin.

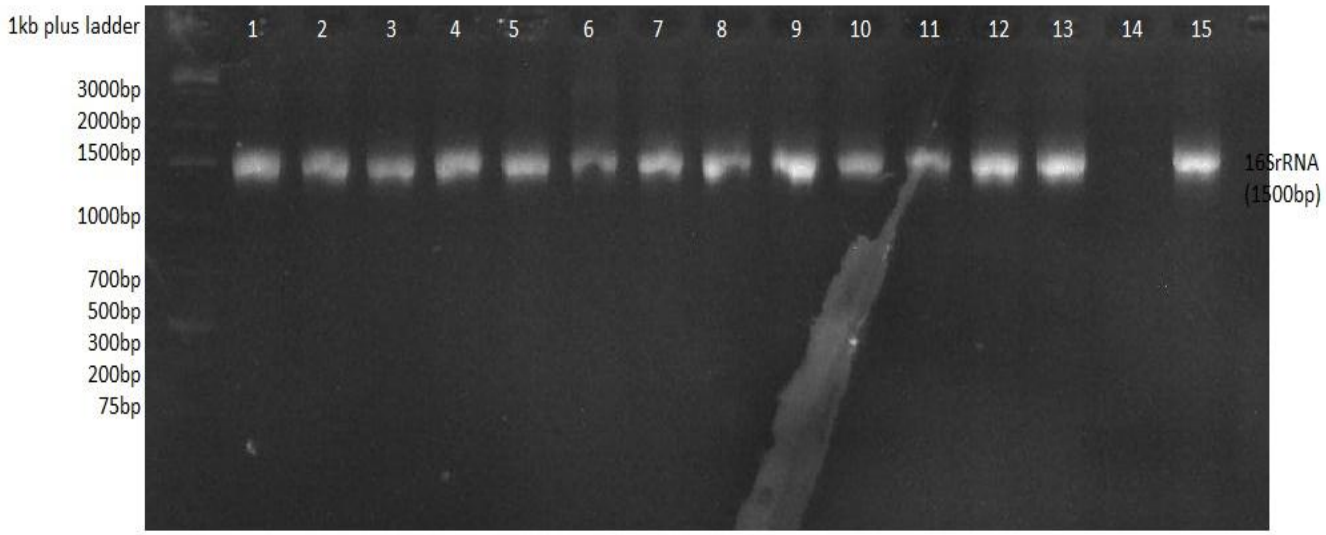


Plate VI: Agarose gel electrophoresis of *16srRNA* amplicons (1500bp) of *Enterococcus* spp. Lanes M = 1kb molecular ladder, lanes 1-15 shows PCR products.

Key:

1	**NH17**
2	**KW4**
3	**NH8**
4	**KW3**
5	**NH35**
6	**NH25**
7	**NH27**
8	**NH9**
9	**NH33**
10	**UA18**
11	**NH20**
12	**NH34**
13	**NH2**
14	**NH12**
15	**NH18**

Table 4.24 shows the 16SrRNA gene partial sequencing of some selected *Enterococcus* spp. isolated from the hospitals in Abuja. The BLAST showed 99% identity with *E. faecium* for DNA sample NH17, 93% identity with *E. mundtii* for KW4, 97% with *E. faecium* for NH8, 95% identity with *E. mundtii* for KW3, 94% identity with *E. dispar* for NH35, 95% identity with *E. mundtii* for NH25, 98% identity with *E. faecalis* for NH27, 98% identity with *E. mundtii* for NH9, 95% identity with *E. faecium* for NH33, 94% identity with *E. faecalis* for UA18, 94 % identity with *E. faecalis* for NH20, 95% identity with *E. mundtii* for NH34, 93% identity with *E.gallinarum* for NH2 and 98% identity with *E. faceium* for NH18.

The phylogenic tree (Figure 4.3) constructed from the BLAST confirmed the isolates as *Enterococcus* spp. with close relatedness with each other as obtained in the data base of National Center for Biotechnological Information (NCBI) using BLAST (Basic Local Alignment Search Tool) pairwise alignments with neighbour joining method.

Table 4.24: Closest of *16S rRNA* sequence of some *Enterococcus* spp. isolated

S/N	CODE	SEQUENCING	%	ACCESSION NO	E.Value
1	**NH17**	***E. faecium***	**99**	**NR_040789.1**	**0.0**
2	**KW4**	***E. mundtii***	**93**	**NR_113906.1**	**0.0**
3	**NH8**	***E. faecium***	**97**	**NR_113904.1**	**0.0**
4	**KW3**	***E. mundtii***	**95**	**NR_113906.1**	**0.0**
5	**NH35**	***E. dispar***	**94**	**NR_113927.1**	**0.0**
6	**NH25**	***E. mundtii***	**95**	**NR_024906.1**	**0.0**
7	**NH27**	***E. faecalis***	**98**	**NR_113901.1**	**0.0**
8	**NH9**	***E. mundtii***	**98**	**NH_113906.1**	**0.0**
9	**NH33**	***E. faecium***	**95**	**NH_114742.1**	**0.0**
10	**UA18**	***E. faecalis***	**94**	**NH_040789.1**	**0.0**
11	**NH20**	***E. faecalis***	**94**	**NH_040789.1**	**0.0**
12	**NH34**	***E. mundtii***	**95**	**NH_113906.1**	**0.0**
13	**NH2**	***E. gallinarum***	**93**	**NH_104559.2**	**0.0**
14	**NH18**	***E. faecium***	**98**	**NH_114742.1**	**0.0**

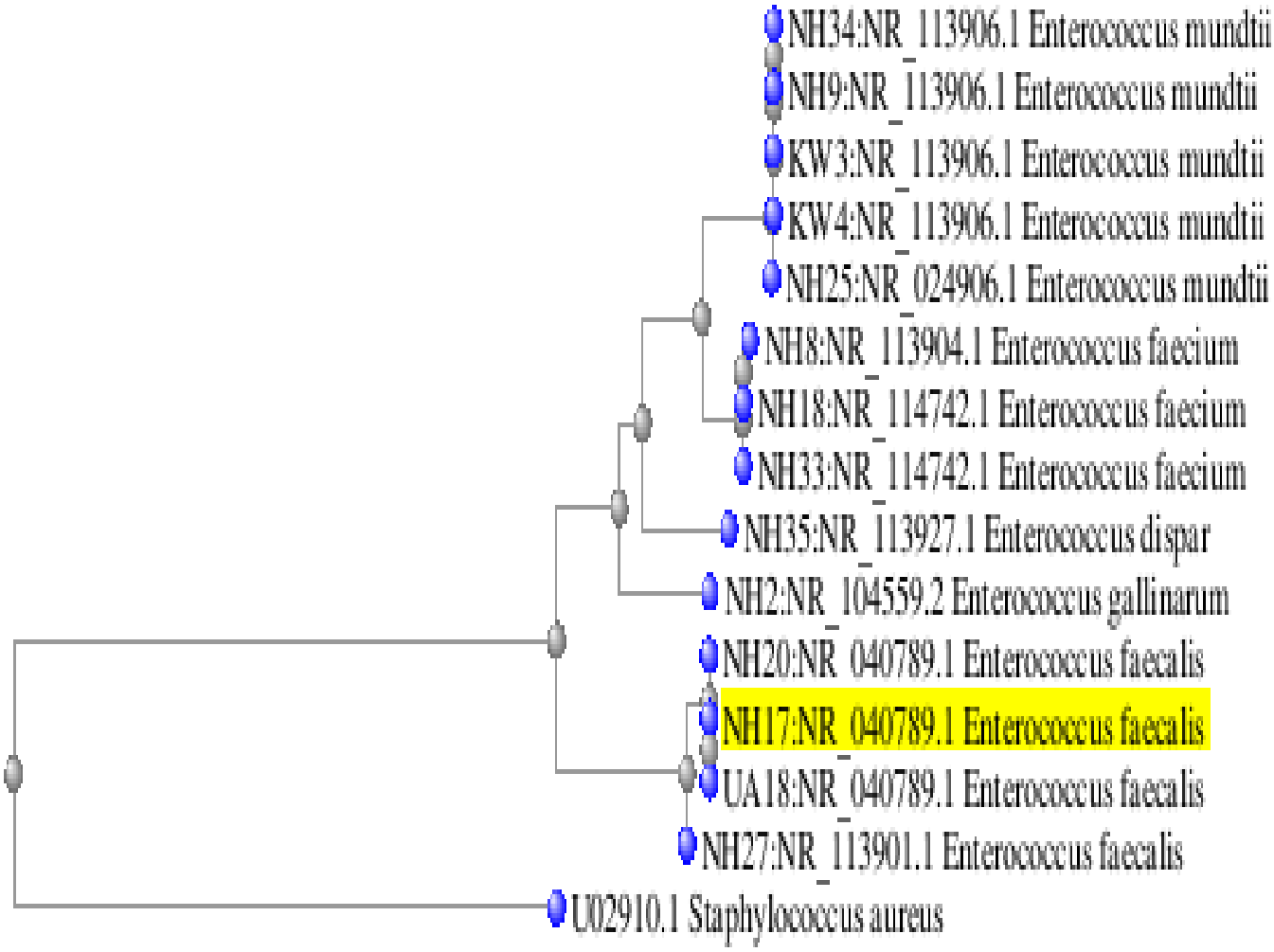


Figure 4.3: Phylogenetic relationship among 14 *Enterococcus* species based on analysis of 16S rRNA gene sequences. The tree was constructed using NCBI BLAST pairwise alignments with neighbor joining method.

CHAPTER FIVE

5.0 DISCUSSION

Enterococci are part of human and animal intestinal flora which have emerged as community acquired pathogens and a leading cause of hospital acquired infection.

More urine samples were collected than stool samples because *Enterococcus* spp. are normal flora in the gastrointestinal tract (GIT) which entails the high percentage of their isolation from stool unlike urine that is sterile and can only yield organisms if there is urinary tract infection (UTI). Wound does not occur unless the normal intact skin is broken exposing the underlying tissue which could be infected if measures are not taken.

One hundred and two (102) enterococcal strains were isolated from the five hundred (500) samples collected from the various hospitals giving a prevalence of 20.4%.The 400 clinical samples yielded 97 isolates with a prevalence of 24.3% which is comparable to the results obtained by Desai *et al*. (2001) with a prevalence of 22%, Chaudhary *et al.*, (2007) with 18% prevalence, Trivelda and Gomathi (2016) with clinical enterococcal isolates constituting 15.07%, and Sunilkumar and Karthika (2012) with a prevalence of 15.46%, Parvathy and Appalaraju (2003) reported a very low prevalence of 3.38% enterococci. The prevalence of enterococcal isolates is higher in our study because we isolated the strains from patients in order to get VRE from those colonized and infected.

Stool samples led in the number of *Enterococcus* spp. isolated with 68% of the isolates followed by urine with 10%, wound swabs with 8.3% and environmental with 5%. There was statistically significant association ($<0.001^{**}$) between *Enterococcus* spp. isolated and the samples especially stool samples with the highest yield. The high isolation of *Enterococcus* spp from stool samples is because it is a normal flora in the gastro intestinal tract of humans. The normal mixed flora in the GIT include enterococci and it is difficult to differentiate

colonization from true infection. VRE mostly colonize the GIT, but can be found to a lesser extent on the skin, in the genitourinary (GU) tract, and in the oral cavity with *E. faecalis* being the major colonizer in these sites. Once VRE colonizes the GIT, it can persist for months to years and attempt at decolonizing them is typically for a short duration as recurrence occurs days or weeks later. The most common type of enterococcal infection occurs in the urinary tract such as cystitis, prostatitis, and epididymitis. Enterococcal UTI is associated with urinary catheterisation and or instrumentation (Desai *et al.,* 2001) and diabetes mellitus (Nicolle, 2005). According to Orret and Connors, (2001) and Courvalin (2006), UTI are mostly caused by *Enterococcus* species both within and outside hospital settings. Agudelo and Huycke (2014) also noted that the most common type of enterococcal infection occurs in the urinary tract. Exposure of subcutaneous tissue due to wound provides a conducive environment for microbial colonization and proliferation. Factors such as wound type, depth, location, the level of tissue perfusion and antimicrobial efficacy of the host immunity affect the microorganism's diversity and abundance. The microflora common with clean, surgical wounds are expected to be low but the presence of foreign material and devitalized tissue in a traumatic wound may enhance microbial growth except measures such as surgical debridement and early prophylactic antibiotic treatment are taken. Low number of isolates from wound in this study could be due to preservative prophylaxis which decreases incidence of wound infection (Sands *et al.,* 1996). Environmental microorganisms can cause human diseases from the human interaction with the inanimate environment. The Five (5%) *Enterococcus* spp. isolated from the environmental samples in this study is comparable to the result obtained by Nwankwo (2012) from Murtala Mohammed Specialist Hospital, Kano where 3.33% of the isolates from fomites in the operating rooms were *E. faecalis*. The hygienic level of the various hospitals could be responsible for the low level of environmental surface contamination from microorganisms thus the reason for the low isolation

A total of 39(26.0%) strains were isolated from 150 samples at NHA, 31(20.7%) from 150 samples at UATH, 17(17.0%) from 100 samples at Kuje and 15(15%) from 100 at Kubwa. This result is similar to the result obtained by Billstrom *et.al*. (2008) who got majority of his clinical isolates from tertiary hospitals, followed by secondary and primary hospitals in a research on cross-transmission of clinical *Enterococcus faecium* in relation to *esp* and antibiotic resistance. The result showed more UTI in NHA (15.7%) and Kubwa general hospitals (12.0%) than UATH (7.1%) and Kuje (4.0%) which could be attributed to the caliber of people inhabiting the areas as NHA is situated in the heart of the federal capital territory and serves the rich and the mighty in the society as well as Kubwa general hospital that is situated in the biggest satellite town in the FCT where the lower, middle and upper class live. The life style of the rich coupled with their accessibility and willingness to access health care whenever the need arises could attribute to the high isolation of enterococci from their urine as a result of UTI. On the other hand, UATH is situated at the outskirt of the city and mainly serve as a referral and teaching center for medical and health students. There was high isolation of organisms in stool from NHA (86.6%), UATH (73.3%), Kuje (70%) and lower in Kubwa (35.0%) which could be due to intake of antibiotics prior to sample submission by Kubwa patients. For environmental isolates, 2(10.0%) strains from 20 samples in Kubwa, 1 (5.0%) strain from Kuje and 2(6.7%) strains from 30 samples in UATH were isolated while no enterococci was isolated from NHA. The low level of isolation of enterococci from the hospital environment indicated good sanitary practice and infection control because *Enterococcus* is known to survive for long on environmental surfaces when contaminated due to their wide resistance nature.

Enterococcus faecalis, E. faecium, E.mundtii, E.hirae, E.dispar, E gallinarum, E.casseliflavus and E.avium made up the 102 isolates from the clinical and environmental samples from UATH, NHA, Kubwa and Kuje general hospitals in the FCT, Abuja. The

distribution is similar to results obtained by Fathollahzadeh *et al*. (2006) with *E.faecalis* as the majority of the isolates followed by *E.faecium* and *E.mundtii*. The species prevalence from the hospitals showed that *E. faecalis* was the majority with a percentage of 57.8 followed by *E.faecium* with a percentage of 23.5, *E.mundtii* with a percentage of 10.8%, *E.gallinarum* with a percentage of 2.9, *E.dispar* with a percentage of 2.0, *E.hirae* with a percentage of 1.0, *E.casseliflavus* with a percentage of 1.0 and *E.avium* with a percentage of 1.0 in consonance with the studies of Gary and Adley (2001), Simonson *et al*. (2003), Trivelda and Gomathi (2016). This findings also confirmed the report of Cetinkaya *et al*. (2000) where *E. gallinarum, E. casseliflavus, E. dispar and E. avium* were isolated less frequently and account for less than 5% of clinical isolates.

According to Deshpande (2013), the predominance of *E. faecalis* among the normal flora of the body could be responsible for its high proportion among hospital isolates, however Willey *et al*. (1993), Mohanty *et al*. (2005), Baragundi *et al*. (2010), Anjana *et al*. (2012), Azza *et al*. (2013) reported *E. faecium* as the predominant species in their studies. According to Agudelo and Huycke (2014), *E. faecalis* and *E. faecium* majorly cause human infections, but other enterococcal species such as *E. durans, E. avium, E. casseliflavus, E. raffinosus* and *E. mundtii* rarely cause human infections. This could be the reason for their low isolation. The incidence of other *Enterococcus* species is underestimated due to frequent misidentification. The need for proper identification to species level is important for proper management and prevention of enterococcal infections.

The sources of the species from the various hospitals showed that urine with 24 isolates yielded 91.7% of *E. faecalis,* 4.2% *E. faecium* and 4.2% *E. avium*. Among *Enterococcus* species, *E. faecalis* strains are found more frequently in urine from patients with complicated UTI than are the *E. faecium* strains (Lee, 2013). The prevalence of the species from urine in this study showed that *E. faecalis* outnumbered *E. faecium* and *E. avium*

which is similar to the results of Desai *et al.* (2001), Miskeen and Doedhar (2002) and Anbumani *et al.* (2005), Chaudhary *et al*. (2007) but deviated from Baragundi *et al.* (2010) where *E. faecium* predominated followed by *E. faecalis*. Stool samples yielded 7 species out of the 8 identified. This is due to the fact that the GIT of both humans and animals serve as a habitat to enterococcus where they make up a significant part of the gut normal flora. Interaction amongst various bacteria have shown that enterococci can act synergistically with other bacteria to enhance the rate of infection (Chaudhary *et al*., 2007). *Enterococcus faecalis* was the most isolated with a percentage of 42.6 followed by *E. faecium* with a percentage of 33.8, *E. mundtii* with a percentage of 16.2, *E. gallinarum* with a percentage of 2.9 and *E. hirae, E. dispar* and *E. casseliflavus* with a percentage of 1.5 each. In many studies, *E. faecalis* has always outnumbered *E. faceium* in prevalence rate while other species are not commonly isolated (Chaudhary *et al*., 2007). The result from UATH indicated more isolation of *E. faecalis* from stool with a percentage of 54.5 than NHA with a percentage of 20.0. However, there was more isolation of *E. faecium* (44.0%) and *E. mundtii* (28.0%) from NHA than UATH and the general hospitals. This could be responsible for the high antibiotics resistance witnessed in NHA than other hospitals since most vancomycin resistant enterococci are associated to *E. faecium* which is closely related to *E. mundtii* phylogenetically (Moellering, 1992; Repizo *et al*., 2014). Wound swabs as one of the sources of the isolates yielded 100% *E. faecalis* from the two tertiary hospitals. The Centres for Disease Control and Prevention guideline for the prevention of surgical site infection has recognized *S. aureus*, coagulase-negative staphylococci, *Enterococcus* spp., *Escherichia coli*, *P. aeruginosa*, and *Enterobacter* spp. as the most frequently isolated pathogens. Wound contaminants are likely to originate from the environment, surrounding skin and endogenous sources such as gastrointestinal, oropharyngeal, and genitourinary mucosae (Bowler *et al*., 2001). Since *E. faecalis* has been reported to be the most frequently isolated among the

Enterococcus spp. from the GIT in several studies (Chaudhary *et al*., 2007), this could be the reason for its 100% isolation from wound in the present study as detailed microbiological analyses of wounds have demonstrated close correlations between the species found in the normal flora of the gut or oral cavity and microorganisms present in wounds in close proximity to those sites (Bowler *et al*., 2001). However, the 100% *E. faecalis* prevalence in wound isolates is in deviance to more isolation of *E. faecium* from wound by Rajkumari *et al*. (2014). Use of antibiotics, antiseptics such as iodine, herbs and honey (Bowler *et al*., 2001) in the self-treatment of wound might be the cause of non-isolation of *Enterococcus* spp from patients with wounds in Kubwa and Kuje general hospitals. Environmental isolates yielded 60% (3/5) *E. faecalis*, 20% (1/5) *E. dispar* and 20%(1/5) *E. gallinarum* from UATH, Kubwa and Kuje general hospitals while non was isolated from NHA. This result is similar to the result obtained by Nwankwo (2012) from Murtala Mohammed Specialist Hospital, Kano where 3.33% of the isolates from fomites in the operating rooms were *E. faecalis*. The hygienic level of the various hospitals could be responsible for the low level of environmental surface contamination from microorganisms thus the reason for the low isolation.

The antiobiotics profile of the *E. faecalis*, *E. faecium*, *E. casseliflavus*, *E. gallinarum*, *E. mundtii*, *E. avium*, *E. dispar* and *E. hirae* isolated from the 4 hospitals where samples were collected in Abuja showed nitrofurantoin as the most active antimicrobial agent with 84.3% of the isolates susceptible to it. The susceptibility rate of the isolates was 94.1% in Kuje, 80.0% in Kubwa, 90.3% in UATH and 76.9% in NHA. This high activity of nitrofurantoin against the isolates in this study could be because it is not commonly prescribed and uncommon among the populace and thus not abused. Nitrofurantoin could be used in the management of UTI, especially when the susceptibility testing shows good susceptibility (Onoh *et al*., 2013). It is a broad spectrum antimicrobial agent which is effective in the treatment of enterococcal UTIs and may be used to treat VRE infections. Studies have shown

increased susceptibility of bacterial isolates from urine of pregnant women with or without symptoms of UTI to nitrofurantoin with recommendations made for it to be used as a first line drug (drugs.com/pro/nitrofurantoin, 2015). Nitrofurantoin has excellent activity against *E. faecalis* but the majority of *E. faecium* isolates are resistant. (drugs.com/pro/nitrofurantoin, 2015). This was observed in this study as 6.8% and 12.5% of *E. faecalis* and *E. faecium* respectively were resistant.

The other effective antibiotics in this study after nitrofurantoin was ampicillin with 72.5% of the isolates susceptible. This is similar to the findings of Bonadio *et al.* (2000) and Jabalameli *et al.* (2009), where they reported 94.1% of the isolates to be susceptible to ampicillin, with 5.9% resistance but disagrees with the work of Akhi *et al.* (2009), where there was higher resistance rate. The susceptibility of the isolates was 94.1% in Kuje, 80.6% in UATH, 66.7% in Kubwa and 59.0% in NHA. The population density and economic wellbeing of the people could have a direct influence of drug usage in the FCT, Abuja. Even though all the isolates have >50% susceptibility, the result showed ease of access, affordability and abuse on the part of residents of Kubwa and Abuja Municipal Area Council (AMAC). Also, ampicillin showed lower antimicrobial activity against the isolates with 66.7% and 59.0% in Kubwa and NHA susceptible respectively unlike its higher activity in Kuje with higher susceptibility of 94.1%. This could be due to isolation of more resistant *E. faecium* with 54.5% (6/11) in NHA and 100% (2/2) in Kubwa due to penicillin binding proteins (PBS) and beta lactamase production. Ampicillin is the drug of choice for the treatment of enterococcal infection except where the isolates are found to be beta lactmase producers. The predominance of *E. faecalis* against *E. faecium* could have played role in the high susceptibility to ampicillin as most *E. faecium* are less susceptible to β-lactam agents than *E. faecalis*, because their PBPs have lower affinities for those antibiotics and some strains have plasmid-encoded β-lactamase (Zouain and Araj, 2001).

Gentamicin and Streptomycin (Aminoglycosides) showed good activity against the isolates with 72.6% susceptibility for gentamicin and 57.8% for streptomycin. The susceptibility rate for gentamicin was 93.3% against isolates from Kubwa, 76.5% for those from Kuje, 74.4% for those from NHA and 51.6% for those from UATH, while that of streptomycin was 80% for those from Kubwa, 76.9 for those from NHA, 58.8% for those from Kuje and 22.6% for those from UATH. The high activity by aminoglycosides (120ug gentamicin and 300ug of streptomycin) in this study is encouraging as ampicillin, penicillin, or vancomycin (for susceptible strains) can be combined, plus an aminoglycoside to work synergistically for the treatment of serious enterococcal infections, such as endocarditis, unless high-level resistance to both gentamicin and streptomycin is documented (CLSI, 2014). According to CLSI (2014), the use of aminoglycosides (except for high-level resistance) against enterococci should not be reported susceptible as they may appear active *in vitro,* but are not effective clinically. This is because enterococci exhibit intrinsic low-level cross resistance to all aminoglycosides due to decreased uptake of antibiotics which makes susceptibility testing of clinical enterococci to low-level aminoglycosides meaningless (Rice *et al*., 2003). Enterococci can also exhibit high level acquired resistance aminoglycosides typically mediated by aminoglycoside-modifying enzymes (Eliopoulos and Gold, 2001). Aminoglycosides activity against the isolates from UATH was discouraging as gentamicin showed a little above average of 51.6%, while streptomycin was very poor with 22.6% indicating high level aminoglycosides resistance (HLAR) in the area. This could be due to HLAR observed among *E. faecium* isolates with 44.4% (4/9) resistance to gentamicin and 77.8% (7/9) to streptomycin than other *Enterococcus* spp, which is consistent with findings in previous reports (Adhikari, 2010). The 2 *E.mundtii* from isolated in UATH also exhibited 100% (2/2) HLAR which could also be linked to its closer relatedness to *E. faecium*.

Vancomycin and teicoplanin (Glycopeptides) had been use for treatment for more than 30 years without the emergence of marked resistance. They have been in use for the treatment of methicillin-resistant staphylococci and other gram-positive bacteria due to their activity (Cetinkaya *et al*., 2000). The glycopeptides, vancomycin and teicoplanin proved to be effective in this study as 65.7% of the isolates were susceptible, while about 33.3% were resistant from the various hospitals. The susceptibility rate of the isolates was 100% in Kuje, 87.1% in UATH, >50.0% in Kubwa and 38.5% in NHA. The resistance rate of the isolates to vancomycin at NHA is contrary to the 100% susceptibility obtained by Iregbu *et al*. (2002). Factors such as accessibility, affordability and travelling exposure could have made the patients to be exposed to this drug. The resistance to vancomycin was confirmed by E-test MIC where only 12 out of the 34 resistant isolates from UATH, Kubwa and NHA had intermediate susceptibility with MIC $\leq$ 8µg/ml comparising of non from UATH, 2 from Kubwa and 10 from NHA. None had high level vancomycin resistance of $\geq$32µg/ml. Excellent susceptibility with 100% against all the isolates was observed in Kuje hospital and UATH which showed that vancomycin and teicoplanin are not abused in these study areas. Absence of resistance to vancomycin in Kuje and UATH is similar to the work of Barisica and Punda-policb (2000), Miskeen and Deodhar (2002), Srujana *et al.* (2005), Vigani *et al.* (2008) and Olawale *et al.* (2010). Vancomycin is expensive, scarce in pharmacies and medicine stores and not commonly prescribed to patients. Moreover, it is intravenously administered. Therefore, lack of exposure of the isolated enterococci to the antibiotics could have contributed to their susceptibility. However, there has to be constant monitoring as vancomycin resistant isolates have been reported to emerge sporadically (Mathur *et al.*, 2003). Two (13%) out of the 15 isolates from Kubwa and 10(25.6%) out of 39 from NHA had intermediate susceptibility indicating the likelihood of indiscriminate use of this drug due to socio-economic over residents of Kuje and Gwagwalada. Since usage of glycopeptide is

more in tertiary hospitals, this could be the reason its resistance was more in NHA than Kubwa, which is a secondary care hospital. Overuse of vancomycin in hospitalised patients in the United States has been reported to increase its prevalence (Silverman *et al.,* 1998). Apart from drug consumption, farm animals have been implicated to be reservoirs for antimicrobial drug resistance in enteric bacteria as contact with them or consumption of food products from them is the source of dissemination of resistance to humans (Ombui *et al*., 2000). Vancomycin resistant enterococci have been isolated due to the use of avoparcin in animal feed in Europe (Bates *et al.,* 1993; Aarestrup *et al.,* 1996). The findings in Kubwa and NHA is in consonance to reported incidence of VRE isolated in hospitals worldwide (Schouten *et al.,* 2000; Feizabadi *et al.,* 2004; Courvalin, 2006; Jabalameli *et al.,* 2009). The species type isolated from Kubwa could have contributed as the *E. faecium* and *E. mundtii* isolated were resistant to both vancomycin and teicoplanin by disc-diffusion, however, susceptibility testing with E-test MIC confirmed the 2(13.3%) *E. mundtii* from Kubwa to have intermediate susceptibility with MIC of 8µg/ml which have been shown to be closely related to *E. fecium* by phylogeny and has been majorly implicated to be more associated with vancomycin resistance (Moellering, 1992). *Enterococcus* spp. isolated from NHA exhibited the highest resistance to vancomycin and teicoplanin with >60% by disc-diffusion method. The species distribution showed 100% (7/7) by *E. mundtii,* 72.7% (8/11) *E. faecium*, 36.8%(7/19) *E. faecalis*, 100% (1/1) *E. gallinarum* and 100%(1/1) *E. dispar,* but confirmation by E-test MIC reduced the VRE to 10(25.6%) out of the 24 resistant isolates by disc-diffusion from NHA with 3(7.7%) *E. faecalis*, 3(7.7%) *E. faecium*, 2(5.1%) *E. mundtii*, 1(2.6%) *E. gallinarum* and 1(2.6%) *E. dispar*. Vancomycin resistant enterococci (VRE) prevalence according to species from all the hospitals showed 3(5.1%) out of 59 *E. faecalis*, 3(12.5%) out of 24 *E. faecium*, 4(36.4%) out of 11 *E. mundtii*, 1(33.3%) out of 3 *E. gallinarum* and 1(50%) out of 2 *E. dispar*. Detecting vancomycin resistance in clinically significant *Enterococcus spp. early* is

important for proper case management. The treatment of VRE is a clinical problem as resistance to antibiotics eliminates the synergism achieved by some antibiotics such as aminoglycoside combination, leaving β-lactamase as the alternative choice to combine with aminoglycosides. Many VRE are multi-drug resistant and there treatment is a problem as no drug of choice is known yet (Adhikari, 2010).

Ciprofloxacin, also showed high efficacy against 57.8% of the isolates from all the hospital susceptible. Susceptibility of the isolates from Kuje was 76.5%, 61.5% in NHA, 60% in Kubwa, 42% in UATH, showing high usage in Gwagwalada with below average susceptibility, which could be attributed to social and economic factors and the patterns of antimicrobial prescription in the area. Ciprofloxacin being a fluoroquinolone is used for the treatment of UTI because of its high urine concentrations and wide spectrum of activity. Though not a drug of first choice, but has been successfully used in the treatment of enterococcal UTIs (Gary and Adley, 2001). Ciprofloxacin has a broad spectrum activity against Gram-positive and Gram-negative bacteria. It is convenient for oral intake with few side effects. The combination of ciprofloxacin plus rifampin plus gentamicin have been used to treat infections caused by strains resistant to ampicillin, vancomycin, and teicoplanin, but still susceptible to aminoglycosides (Zervos *et al*., 2010). In this study, *E. faecium* showed higher resistance of 37.5% (9/24) than *E. faecalis* with resistance of 16.9% (10/59), showing that *E. faecium* is associated with antimicrobial resistance than other enterococci. The resistance to fluoroquinolones in many enterococci, is mediated by alterations in enzymes involved in DNA replication (Eliopoulos and Gold, 2001)

Linzolid showed its good efficacy in Kuje as 94.1% of the isolates were susceptible, followed by UATH with 67.7% susceptibility indicating they were not much abused. The susceptibity of Linzolid got below average in Kubwa with 46.7% susceptible isolates and much lower in NHA with 38.5% susceptible isolates and high resistance level of 61.5%

indicating higher exposure of the drug. However, the susceptibility of the isolates from all the hospitals to linezolid was 57.8% indicating above average activity. *E. faecium* exhibited 54.1% (13/24) resistance while *E. mundtii* exhibited 81.8% (9/11) resistance much higher than *E. faecalis* with resistance of 22.0% (13/59) to linezolid. Linezolid, an oxazolidinone antibiotic, is available orally and intravenously and is used to treat infections caused by *E. faecium* and *E. faecalis* strains, including VRE. Linezolid is useful in patients who require oral or outpatient therapy, who are intolerant to glycopeptides, or who have impaired renal function. It is recommended for infants and children. Unfortunately, linezolid-resistant VRE isolates have already been reported (Fraser and Donskey, 2016).

Chloramphenicol is used for the treatment of infections caused by VRE that are resistant to other antibiotic regimens. It is a bacteriostatic broad spectrum drug use in treating many infections. Chloramphenicol is not a first-line drug for most infections, but because of its low cost and easy administration through oral and intramuscular routes, it is widely use in developing countries. The use of chloramphenicol is generally restricted to those infections where it is clearly the most effective drug by laboratory test (CLSI, 2006). In this study, 51% of the isolates from all the hospitals were susceptible. This average susceptibility came from mostly the tertiary hospitals such as UATH with susceptibility of 48.4% and NHA with susceptibility of 46.2% and Kubwa, a secondary care hospital with 46.7% susceptible isolates. Chloramphenicol used in this study exhibited good efficacy against isolates from Kuje showing the patients are probably not used to antibiotics but resort to herbal medication most likely due to socio-economics of the people in the area. *Enterococcus faecium* was more susceptible to chloramphenicol with 54.1% (13/24) than *E. faecalis* with 49.2% (29/59) susceptibility. This susceptibility pattern of the isolates in Kuje and UATH was similar with that of Chaudhary *et al.* (2007) where *E. faecalis* was more susceptible but differ with Kubwa

and NHA where *E. faecium* was more susceptible. *Enterococcus avium* sensitivity is consistent with that of Mellman *et al.* (1992).

Doxycyclin, showed a very poor activity against the isolates with 25.5% susceptibility and high resistance of 63.7% in all the hospitals. Though resistance of the isolates was high in NHA with 71.8%, followed by UATH with 71.0%, Kubwa with 53.3% and Kuje with 41.2%. This shows that residents of Kuje with lower resistance to doxycycline are not much exposed to this drug. Also, low population in Kuje when compared to AMAC, Kubwa and Gwagwalada population as mostly indigenous/rural dwellers who are not very much exposed to western life and thus resort to their local medications when sick. However, since doxycycline is cheap and relatively available in the community, it can be seen that its efficacy was reduced as reflected in the result generally. Tetracyclines are bacteriostatic and act by inhibition of protein synthesis of both gram-negative and gram-positive bacteria. Many strains of *Enterococcus* species are known to be resistant to tetracyclines worldwide as confirmed from the above result even though CLSI (2014) recommended it due to limited alternative drug for VRE. The *Enterococcus* spp isolated exhibited resistance with 81.8% (9/11) of *E. mundtii*, 66.7% of *E. faecium*, 57.6% of *E. faecalis* and 100% (3/3) of *E. gallinarum*.

Rifampicin showed poor activity against the bacterial isolates with only 20.6% susceptible. The susceptibility of the isolates from the various hospitals showed 47.1% from Kuje, 26.7% from Kubwa, 26.0% from UATH and non from NHA. The low efficacy of rifampicin against our enterococcal isolates is similar to the result obtained by Chaudhary *et al*. (2007). It should be noted that rifampicin should never be used alone but in combination with other antibiotics such as daptomycin, linezolid, tygecyclin or quinupristin-dalfopristin. This below average susceptibility could be as a result of exposure to the drug while treating

other infections. Though rifampicin is not commonly used for the treatment of enterococcal infections, acquired enterococcal resistance to rifampicin is common as 79% of clinical isolates were found to exhibit rifampicin resistance in hospitals in the UK as well as >57% of a diverse collection of isolates from six other countries in Europe (Kristich *et al*., 2014). This could be due to the exposure of commensal enterococci to rifampicin during treatment for non-enterococcal infections with other unknown factors. Rifampicin is used for the treatment of *Mycobacterium tuberculosis* and recently has been used in the treatment of staphylococcal infections associated with indwelling medical devices, such as artificial joints. Rifampicin inhibits bacterial growth by binding to the beta subunit of RNA polymerase and preventing initiation of transcription (Kristich *et al*., 2014). Among the isolates, all the 11(100%) *E. mundtii*, 62.7% of the *E. faecalis* and 75% of the isolates were resistant.

Erythromycin also showed poor activity against the isolates in this study with resistance/intermediate susceptibility of enterococci found to be 83.5%. In the four hospitals, the isolates had intermediate/resistance of > 70 to antibiotics tested except in Kuje where intermediate/resistance of 64.8% was observed. The resistance pattern is similar to reports by Baragundi *et al.* (2010) in which more than 70% of the isolated enterococci were resistant to erythromycin. For the susceptibility pattern of the species, 59 *E. faecalis* isolates, 50.9% were resistant. *E. faecium* showed higher resistance than *E. faecalis* with 75% resistance while *E. mundtii* had 81.8% (18/11) resistance. Erythromycin is an alternative drug used for the treatment of VRE due to limited alternative therapy (CLSI, 2006). They are used mainly for the treatment of Gram-positive bacteria and are bacteriostatic agents, but could be bactericidal at high concentrations. Erythromycin is useful as a second-line drug of choice for the treatment of patients with penicillin hypersensitivity. Resistance encountered in this study could be as a result of alteration by methylation of rRNA receptor under the control of a transmissible plasmid carried by the *Enterococcus* species isolated. Since the drug is readily

available, its usage is common among the patients and therefore the resistance among the isolated strains since acquisition and transfer of resistance genes is common (Miller *et al*., 2014).

Eleven different resistant patterns were exhibited with MAR index ranging from 0.1 – 1.0 out of the 96 (94%) resistant isolates of the 102 tested for antibiotics susceptibility from the four hospitals in Abuja, From the results, out of the 96(94%) resistant strains with different antibiotics resiatance patterns, 67(66%) were multidrug resistant (MDR), 3(3%) were extremely drug resistant (XDR), while 2(2%) had pandrug resistance (PDR) as they were resistant to all tested antibiotics. According to Magiorakos *et al*. (2012), MDR is defined as non-susceptibility to at least one agent in three or more antimicrobial categories; XDR is defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories) while, PDR is defined as non-susceptibility to all agents in all antimicrobial categories (i.e. no agents tested as susceptible for that organism). Thus, a bacterial isolate that is characterized as XDR will also be characterized as MDR. Similarly, a bacterial isolate would have to be XDR in order for it to be further defined as PDR. Bacteria that are PDR carry the most absolute type of antimicrobial resistance possible, implying that there are no approved antimicrobial agents that have activity against these strains. Out of the 102 enterococci isolated, 87(85%) had MAR index of 0.2 and above, indicating that the isolates had been pre-exposed to the antibiotics tested which agrees with the works of Krumperman (1983), Olayinka *et al*. (2004) and Olonitola *et al*. (2009), where they observed that MAR index greater than 0.2 indicates that an organism must have originated from environment where antibiotics are habitually used without prescription. These antibiotics resistance in enterococci could be intrinsic or acquired. Unlike acquired resistance, intrinsic resistance is based in chromosomal genes, which typically are nontransferable while acquired antibiotic

resistance occur through exchange of resistance-encoding genes carried on conjugative transposons, pheromone-responsive plasmids, and other broad-host-range plasmids. The past two decades have witnessed the rapid emergence of multi-drug resistant enterococci and the consequent increase report of nosocomial and community acquired infections. Multidrug resistance in enterococci especially *E. faecalis* and *E. faecium* which are the common *Enterococcus* spp cause serious problems in the treatment of patients suffering from enterococcal infection as a result of misuse of antibiotics. Some of the risk factors for multiple drug resistant enterococci especially VRE include haemodialysis, receipt of corticosteroids, anti-neoplastic agents or total parenteral nutrition, surgery, severity of illness, antimicrobial administration, indwelling bladder catheters, neutropenia and mucositis which lead to prolong hospital stay (Vergis *et al*., 2001; Husni *et al*., 2002).

Five phenotypes of vancomycin resistance such as *vanA*, *vanB*, *vanC*, *vanD*, and *vanE* are recognized, but *vanA* and *vanB* are mediated by newly acquired gene clusters not previously found in enterococci. These *vanA* and *vanB* phenotypes were described primarily in *E. faecalis* and *E. faecium*. The *vanC* phenotype is chromosomally encoded by the $vanC_1$ and $vanC_{2/3}$ genes, which are intrinsic to *Enterococcus gallinarum* and *Enterococcus casseliflavus*, respectively, and therefore can be used for species identification (Moura *et al*., 2013)

The 12 intermediate strains with MIC of ≤ 8 µg and few susceptible strains with MIC of ≥4µg were chosen for molecular analysis to detect the presence of van genes in them. Single PCR was used to detect the *van* genes after molecular extraction of the DNA present in the strains. The result showed that 12(11.8%) of the strains harboured different van genes which included *vanA, vanB, vanC* and *vanR*. No $vanC_{2/3}$ and *vanXY* was found. The prevalence is similar to that of Bhatt *et al.* (2015) with VRE prevalence of 14.6% from 96

enterococci and lower than that of Deshpande (2013) with VRE prevalence of 19.6% from 291 enterococcal isolates.

The presence of *vanA* and *vanB* co-existing, *vanB*, *vanA* and *vanC* in the strains of *E.mundtii*; *vanA*, *vanB* and *vanA* and *vanC* in *E.faecalis*; *vanA*, *vanC*, and *vanR* in *E. faecium*; *vanA* in *E.dispar* and *vanA* and *vanC* in *E.gallinarum* is a clear indication that there is a slow but ongoing *van* gene transfer among the species. The detection of more than one *van*-type of gene in *Enterococcus* strains have been reported in other studies, including the presence of $vanC_1$ + *vanA* or $vanC_1$ + *vanB (Moura et al, 2013)*

The 3 *E.mundtii* strains in this study harboured *vanA* which is similar to results obtained by Praharaj *et al.* (2013) in India and Sharifi-Rad *et al*. (2016) in Iran. The co-existence of *vanA* with *vanB* in 1 *E.mundtii* strain isolated from a stool sample in Kubwa in this study is similar to the findings of Mirzaei *et al*. (2015) where *vanA* and *vanB* coexisted in 3 *E. faecium* and 5 *E. faecalis* isolated in their research even though they phenotypically exhibited *vanA*. The coexistence of *vanA* with *vanC* in 2 *E. mundtii*, 1 *E. faecalis* and 1 *E. gallinarum* strains isolated from stool samples in NHA is also similar to Praharaj *et al.* (2013) who reported the presence of *vanA* and *vanC1* in *E. gallinarum* in their findings with other reported findings where VRE possess *vanB* in addition to $vanC_1$ genes in other parts of the world. This shows that these organisms can take up other vancomycin resistance genes and are capable of spreading this resistance to other *Enterococcus* strains. The possession of both *vanA* and $vanC_1$ gene clusters will alter the resistance phenotype of a VRE isolate. In our study, the *E. mundtii*, *E. faecalis* and *E. gallinarum* isolate which possessed both the *vanA* and *vanB* genes; *vanA* and $vanC_1$ genes showed resistance to both vancomycin and teicoplanin by disk diffusion but showed low level resistance (MIC $\leq$ 8µg/ml) to vancomycin by minimum inhibitory concentration. This shows that determination of vancomycin

resistance by minimum inhibitory concentrations alone may not predict the genotype. Also, the presence of both *vanA* and *vanC₁* genes in an *E. gallinarum* isolate indicated that phenotypic and genotypic characterization of glycopeptide resistance might not always correspond. According to Cetinkaya *et al*. (2000), strains with *vanA* resistance genes possess inducible, high-level resistance to vancomycin (MIC ≥64 μg/ml) and teicoplanin (MIC, ≥16 μg/ml), however our study found that all the VRE with vanA genes had intermediate susceptibility with MIC of 8 μg/ml. Strains with *vanC* resistance possess low level resistance to vancomycin (MICs, 4 to 32 μg/ml) but susceptible to teicoplanin and are found mainly in *E. casseliflavus* and *E. gallinarum (Cetinkaya et al., 2000). VanC genes are constitutive in E.casseliflavus and E.gallinarum and are not transferable to other enterococci (Praharaj et al.,2013) but the genes in our study discovered otherwise as they were found in E. faecalis, E.faecium and E.mundtii. The vanB* isolates were thought to be resistant to more modest levels of vancomycin with MIC of 32 to 64 μg/ml and are susceptible to teicoplanin but it has been found out that levels of vancomycin resistance among vanB isolates may range from 4 to ≥1,000 μg/ml (Cetinkaya *et al*., 2000).

The *vanA* was found in 1(NH20) *E. faecalis*, 1(NH8) *E. faecium* and 1(NH35) *E. dispar* which is similar to findings of Mirzaei *et al.* (2015) where *vanA* gene was mostly isolated from *E. faecium* and *E. faecalis*. The most common genotype in VRE all over the world is vanA genotype which transfers vancomycin resistance from *Enterococcus* spp. especially vancomycin resistant *E. faecium* to *Staphylococcus aureus* (Nasaj *et al*., 2016). Glycopeptides such as vancomycin, teicoplanin, avoparcin, and ristocetin can induce resistance. Other nonglycopeptide agents such as bacitracin, polymyxin B, and robenidine, used for the treatment of infections in poultry can also induce resistance (Cetinkaya *et al*., 2000). The *vanA* gene cluster are found on transposons, or "jumping" genetic element,

Tn1546 which can be transferred from one strain of enterococcus to another (Cetinkaya *et al*., 2000).

The *vanB* gene was found in 1(KW4) *E. mundtii* from Kubwa and 1(NH27) *E. faecalis* from NHA similar to the work of Merquior *et al*. (2012) where *vanB* genes were found in *E. faecalis*. They also reported the possession of *vanB* genes in other enterococci and nonenterococal reservoirs.

An isolate of *E. faecium* (NH33) possessed *vanC* in our findings which is similar to the result of Sun *et al.* (2014) where $vanC_1$ was found in *E. faecium*. The *vanC* genotype is associated with *E. gallinarum* ($vanC_1$), *E. casseliflavus* ($vanC_2$) and *E. flavescens* ($vanC_3$). These *vanC* operons are chromosome associated and testing *Enterococcus* species for the presence of *vanC* is believed not necessary because these genes are thought to be species-specific. However, *E. faecalis* strain with $vanC_1$ gene has also been isolated from pig manure samples in Germany, sheep bulk tank milk samples in Spain and cloacal swabs of broilers in Brazil. (Sun *et al*., 2014). This *E. faecium* may have acquired the $vanC_1$ gene through a horizontal gene transfer from a natural carrier such as *E. gallinarum* or from other *Enterococcus* spp. The present study shows the importance of *E. faecium* as potent resistance gene collectors and possibly donors.

The *vanR* is a regulatory protein which activates the transcription of *vanH*, *vanA* and *vanX* in response to vancomycin which results in vancomycin resistance. It was found in 1 strain of *E. faecium* (NH18) in this study.

Many researchers have reported *E. faecium* and *E. faecalis* to be the most carrier of van genes with *E. faecium* being more resistant than *E. faecalis* like the work of Bhatt *et al*. (2015) where vancomycin resistance was more prevalent with 42.3% in *E. faecium* than in *E.*

faecalis with 4.3%. But our findings shows that vancomycin resistance genes were more in *E. mundtii* with 4 out of 11 isolates (36.4%) which is similar to the work of Willey *et al*. (1993) where *vanA* genes was found in 2 out of 3 isolates. Other parts of the world such as Iran have noted a similar shift in the carriage of *van* genes in other enterococci such as the case of *vanA* gene found in *E. raffinosus* and *E. mundtii* which was believe to be the first documented findings aside *E. faecium* and *E. faecalis* (Sharifi-Rad *et al*., 2016).

The resistance in *E.mundtii* in this study could be because of its close relatedness with *E. faecium* since they are phylogenetically close as reported by Repizo *et al*. (2014) even though they are less virulent. *Enterococcus faecium* accounted for 3 resistant strains out of 24 (12.5%) isolates while *E. faecalis* accounted for 3 strains out of 59(5.1%) isolates. *Enterococcus gallinarum* accounted for 1 strain out of 3(33.3%) isolates while *E.dispar* accounted for 1 out of 2(50%) isolates. Vancomycin genes have been known to be prevalent in *E. faecium* and *E. faecalis* but not much has been reported in other enterococci as they account for less than 5% in most samples.

The *vanA* was the most prevalent gene in this study with 8(47%) of the VRE isolates harbouring it which is in consistence to other reports (Nasaj *et al*., 2016). After *vanA*, the prevalent gene in this study was *vanC* found in 5(29.4%) strains. The *vanB* genes were found in 3(17.6%) of the VRE isolates while *vanR* was found in 1(5.9%) *E. faecium*.

The prevalence of vancomycin resistance among enterococci in this study could be due to increased vancomycin selection pressure. Most of the patients were exposed to various antibiotics such as vancomycin and cephalosporins in various hospitals, pharmacies and chemist before coming to the NHA, a tertiary hospital where most of the VRE were isolated and Kubwa general hospital where only 2 (2%) were isolated.

Treatment of infections due to VRE, especially *E. faecium*, is extremely problematic. Almost all *E. faecalis* strains are at least moderately susceptible to ampicillin. Therefore, if

vancomycin resistance emerged predominantly in *E. faecalis*, the treatment of most of these infections could be relatively easy. Unfortunately, vancomycin resistance has preferentially appeared in *E. faecium*, which is inherently more resistant to penicillin and ampicillin.

The identification of our isolates is crucial for clinical and public health surveillance in order to understand the pathobiology of infectious clinical syndromes and use of specific antibiotics and control of infections. The use of molecular methods of identification is important because the traditional identification of bacteria on the basis of phenotypic characteristics is generally not as accurate as identification based on genotypic methods. Analysis with *16S rRNA* gene sequencing can better identify poorly described, rarely isolated, or phenotypically aberrant strains of organisms. Irrespective of its accuracy, *16S rRNA* gene sequence analysis lacks widespread use beyond the large and reference laboratories because of technical and cost considerations. The *16S rRNA* gene sequence has been determined for a large number of strains. The relationship among bacteria can be measured with 16S rRNA gene. The comparison of the *16S rRNA* gene sequences helps in differentiating between organisms at the genus level across all major phyla of bacteria, in addition to classifying strains at multiple levels, such as species and subspecies level. Though occasional exceptions to the usefulness of *16S rRNA* gene sequencing usually relate to more than one well-known species having the same or very similar sequences (Clarridge, 2004). Using *16SrRNA* primer, the DNA of the 12 isolates that yielded van genes and 3(UA18, NH12 and NH34) that did not yield van genes but phenotypically showed resistance were subjected to PCR in order to generate amplicons for sequencing. The *16S rRNA* gene product amplified at 1500bp. The 16S ribosomal RNA which is a highly conserved region in the organisms was sequenced to confirm that the biochemical identification (Microgen kit) technique employed actually identified *Enterococcus* spp. The sequencing results were compared with stored sequences in the data base of the National Center for Biotechnology Information (NCBI) using Basic

Local Alignment Search Tool (BLAST). Out of these 15 DNA samples, 1(NH12) did not amplify which could be as a result of loss of DNA prior to PCR amplification. Our *16S rRNA* gene sequences confirmed 2 isolates from Kubwa as *E. mundtii* with KW3 having 95% identity and KW4 with 93% identity to strains in Genbank database with accession no of NR_113906.1. Also from NHA, 3 isolates were confirmed to be *E. faecium* with 99% identity for NH17 and accession no of NR_040789.1, 98% identity for NH18 with accession no of NR_114742.1, 97% for NH8 with accession no of NR_113904.1 and 95% identity for NH33 with accession no of NR_114742.1; 3 were confirmed to be *E. mundtii* with 98% identity for NH9 with accession no of NR_113906.1, 95% identity for NH34 with accession no of NR_113906.1 and 95% identity for NH25 with accession no of NR_024906.1; 2 were confirmed to be *E. faecalis* with 98% identity for NH27 with accession no of NR_113901.1 and 94% identity for NH20 with accession no NR_040789.1, 1 was confirmed to be *E. dispar* with 94% identity to NH35 with accession no of NR_113927.1; 1 was confirmed to be *E. gallinarum* with 98% identity for NH18. One isolate (UA18) from university of Abuja was confirmed to be *E. faecalis* with 94% identity and accession no of NR_040789.1. The multiple alignment of our sequenced enterococci and phylogenetic analysis showed good homology in the sequences of the isolates indicating their common ancestry.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In this research, the isolates from all the hospitals gave a prevalence of 102 (20.4%) with clinical (human) isolates accounting for 97(24.3%). The 5(1%) prevalence of isolation of enterococcus from the environment could be due to proper environmental cleanliness.

Enterococcus faecalis was the most frequently isolated accounting for 59(57.8%) of the isolates, followed by *E. faecium* with 24(23.5%), *E. mundtii* with 11(10.8%), *E. gallinarum* with 3(2.9%), *E. dispar* with 2(2.0%) while *E. casseliflavus*, *E. avium* and *E. hirae* had 1(1%) each. The 5 isolates from the hospital environment were all *E. faecalis*.

The high resistance rate to rifampicin 68(66.7%), doxycycline 65(63.7%), and erythromycin 61(60.0%) makes them unreliable in the management of infections caused by *Enterococcus* spp.

This study showed that the isolates exhibited 11 resistant patterns ranging from 0.1 to 1.0 resistance index. Out of the 96(94%) resistant strains, 67(66%) were multi drug-resistant, 3(3%) were extremely drug-resistant and 2(2%) were pan drug-resistant. The public health significance and consequences of these multi-drug resistant strains is in their ability to disseminate antibiotics resistance genes to members of their species and other bacteria.

Minimum inhibitory concentration by E-test method confirmed 12 out of the 34 strains that were resistant to vancomycin by disk diffusion to be intermediately susceptible based on CLSI 2014 guideline.

No vancomycin resistant enterococci was isolated from the hospital environment which ruled out their comparison with human isolates.

In this study, both the acquired genes (*vanA* and *vanB*) and the intrinsic genes (*vanC*) were detected in the genomic DNA of the isolates indicating that antibiotics resistance is both inherent and acquired in many enterococcal strains.

The *16SrRNA* sequences of the isolates confirmed the isolates to be truly *Enterococcus* spp. with greater than 90% identity.

The phylogenetic studies of the sequenced *Enterococcus* spp. in this research showed a high probability of common ancestry among the isolates.

The identification, antibiotics profile and molecular detection of vancomycin genes in *Enterococcus* species is vital because of their importance to public health.

6.2 Recommendation

i. Medical microbiology laboratories in all the hospitals should ensure that *Enterococcus* spp. are rapidly and accurately identified to species level.
ii. There should be continued surveillance in the hospitals and outside hospital settings such as the environment, water, animal and food industries for VRE.
iii. Hospital environmental cleanliness should be maintained at all times with disinfectant to eradicate or minimize cross contamination of patients with VRE.
iv. Personal hygiene such as hand washing with soap by health workers should be encouraged.
v. Use of antibiotics in animal husbandry and fisheries should be regulated to reduce in discriminate usage in order to reduce or eliminate the possibility of transfer of resistance genes such as van genes to humans via consumption of animal products.
vi. Pharmacies and patent medicine stores should be regulated with strict restriction of over-the-counter drugs sales to consumers in order to control the abuse of antibiotics and thus reduce the problem associated with antibiotics resistance.

vii. Biochemical identification and *16S rRNA* sequencing of *Enterococcus* to species level should be encouraged to know the species prevalence from samples whether clinical or environmental and to understand their pathogenicity.

Contribution to knowledge

- The prevalence of *Enterococcus* spp. from all the samples in the study was found to be 102(20.4%).
- *Enterococcus* spp. yield from the samples remained higher in stool than other samples such as urine, wound and environmental swabs.
- *Enterococcus faecalis* remain the most frequently isolated among the *Enterococcus* species followed by *E. faecium*.
- *Enterococcus mundtii* exhibited highest level of resistance to antibiotics tested.
- Nitrofurantoin was the most active antibiotic against enterococci in this study. The study also demonstrated that ampicillin, gentamicin, vancomycin, teicoplanin, streptomycin, linezolid, ciprofloxacin and chloramphenicol showed above average activity indicating that they can be used singly or in combination for synergistic treatment of enterococcal infections such as endocarditis, bacteremia and UTI.
- This study demonstrated that 87(85%) of the isolates from all the hospitals had MAR index of 0.2 and above, showing that antibiotics are heavily used and abused in FCT, Abuja.
- This study also showed 67(66%) of the isolates are multi drug-resistant, 3(3%) extremely drug-resistant and 2(%) pan drug-resistant.
- Most van genes were also found in *E. mundtii.*
- Phylogenetic relationship confirmed that *E. mundtii* is more closely related to *E. faecium* than other species.

- The study also showed the co-habitation of van genes such as presence of *vanA* and *vanB* or *vanA* and *vanC* in a single strain.
- The study also showed that *vanC* gene which was believed to be intrinsic in *E. gallinarum* and *E. casseliflavus* could be found in other species such as *E.faecalis*, *E. faecium* and *E. mundtii*.

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APPENDICES

Appendix I: Antibiotic profile of Isolates from Kuje General Hospital

Code no/Sample	Isolated spp		S ≥17 I R≤16 **AMP** (5µg)	S ≥21 I=16-20 R≤15 **CIP** (5µg)	S ≥20 I=17-19 R≤16 **RIF** (5µg)	S ≥23 I=21-22 R=20 **LIN** (30µg)	S ≥17 I=15-16 R≤14 **VAN** (30µg)	S ≥16 I=13-15 R≤12 **DOX** (5µg)	S ≥14 I=11-13 R≤10 **TEIC** (5µg)	S ≥23 I=14-22 R≤13 **ERY** (5µg)	S ≥18 I=13-17 R≤12 **CHL** (5µg)	S ≥17 I=15-16 R≤14 **NIT** (5µg)	S ≥10 I=7-9 R=6 **GEN** (5µg)	S ≥10 I=7-9 R=6 **SIR** (5µg)
kj1stool	*E. faecalis*	R	-	-	-	-	-	-	-	8	0	-	-	6
		I	-	-	-	-	-	15	-	-	-	-	-	-
		S	30	24	21	40	20	-	20	-	-	29	15	-
kj2stool	*E. faecalis*	R	-	-	8	-	-	7	-	-	-	-	-	-
		I	-	-	-	-	-	-	-	20	-	-	-	-
		S	24	26	-	32	23		19		28	21	13	11
kj3stool	*E. galli narium*	R	-	-	-	-	-	7	-	-	-	-	-	-
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	26	25	22	27	20	-	18	-	18	17	12	11
kj4stool	*E. faecalis*	R	-	-	14	-	-	6	-	0	10	-	-	5
		I	-	20	-	-	-	-	-	-	-	-	-	-
		S	30	-	-	27	19	-	18	-	-	25	14	-
kj5stool	*E. faecalis*	R	-	-	10	-	-	12	-	-	-	-	-	-
		I	-	18	-	-	-	-	-	-	-	-	-	-
		S	26	-	-	25	18	-	16	30	25	22	12	12
kj6stool	*E. faecalis*	R	-	-	10	-	-	7	-	7	10	-	-	-
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	30	30	-	31	18	-	15	-	-	24	10	14

Sample No.	Isolated spp		S ≥17 I R≤16 **AMP** (5µg)	S ≥21 I=16-20 R≤15 **CIP** (5µg)	S ≥20 I=17-19 R≤16 **RIF** (5µg)	S ≥23 I=21-22 R=20 **LIN** (30µg)	S ≥17 I=15-16 R≤14 **VAN** (30µg)	S ≥16 I=13-15 R≤12 **DOX** (5µg)	S ≥14 I=11-13 R≤10 **TEIC** (5µg)	S ≥23 I=14-22 R≤13 **ERY** (5µg)	S ≥18 I=13-17 R≤12 **CHL** (5µg)	S ≥17 I=15-16 R≤14 **NIT** (5µg)	S ≥10 I=7-9 R=6 **GEN** (5µg)	S ≥10 I=7-9 R=6 **SIR** (5µg)
kj7stool	*E. faecium*	R	-	-	13	-	-	9	-	0	-	-	-	-
		I	-	20	-	-	-	-	-	-	-	-	-	-
		S	29	-	-	30	19	-	19	-	25	26	10	15
kj8stool	*E. faecalis*	R	-	-	6	-	-	-	-	10	-	-	-	0
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	28	22	-	28	18	25	17	-	27	27	18	-
kj9stool	*E. casseliflavis*	R	-	-	8	-	-	-	-	7	-	-	-	-
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	27	22	-	26	20	24	16	-	25	24	14	15
kj10stool	*E. faecalis*	R	-	-	10	-	-	-	-	13	-	-	-	-
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	25	23	-	25	19	25	17	-	26	23	13	12
kj11stool	*E. faecalis*	R	-	-	8	20	-	-	-	-	-	-	-	-
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	21	22	-	-	18	22	17	22	20	18	12	12
kj12stool	*E. faecium*	R	-	-	-	-	-	-	-	-	-	-	5	6
		I	-	-	17	-	-	-	-	-	-	-	-	-
		S	24	22	-	24	20	22	18	28	24	22	-	-

Sample No.	Isolated spp		S ≥17 I R≤16 **AMP** (5µg)	S ≥21 I=16-20 R≤15 **CIP** (5µg)	S ≥20 I=17-19 R≤16 **RIF** (5µg)	S ≥23 I=21-22 R=20 **LIN** (30µg)	S ≥17 I=15-16 R≤14 **VAN** (30µg)	S ≥16 I=13-15 R≤12 **DOX** (5µg)	S ≥14 I=11-13 R≤10 **TEIC** (5µg)	S ≥23 I=14-22 R≤13 **ERY** (5µg)	S ≥18 I=13-17 R≤12 **CHL** (5µg)	S ≥17 I=15-16 R≤14 **NIT** (5µg)	S ≥10 I=7-9 R=6 **GEN** (5µg)	S ≥10 I=7-9 R=6 **SIR** (5µg)
kj13stool	*E. faecalis*	R	-	-	-	-	-	-	-	-	-	-	6	6
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	24	24	21`	24	18	17	15	24	18	19	-	-
kj14stool	*E. faecalis*	R	-	-	-	-	-	-	-	-	-	-	5	0
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	18	22	24	27	19	18	16	27	19	19	-	-
kj15urine	*E. faecalis*	R	-	-	-	-	-	-	-	13	12	18	-	-
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	19	21	23	24	17	17	18	-	-	-	12	10
kj16urine	*E. faecalis*	R	16	-	-	-	-	12	-	-	0	14	4	5
		I	-	18	19	-	-	-	-	22	-	-	-	-
		S	-	-	-	23	19	-	17	-	18	-	-	-
kj17enviro ment	*E. faecalis*	R	-	-	-	-	-	-	-	-	-	-	-	-
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	18	22	24	27	19	18	16	27	19	19	14	16

Appendix II: Antibiotic profile of Isolates from Kubwa General Hospital

Sample No.	Isolated spp		S ≥17 I R≤16 **AMP** (5µg)	S ≥21 I=16-20 R≤15 **CIP** (5µg)	S ≥20 I=17-19 R≤16 **RIF** (5µg)	S ≥23 I=21-22 R=20 **LIN** (30µg)	S ≥17 I=15-16 R≤14 **VAN** (30µg)	S ≥16 I=13-15 R≤12 **DOX** (5µg)	S ≥14 I=11-13 R≤10 **TEIC** (5µg)	S ≥23 I=14-22 R≤13 **ERY** (5µg)	S ≥18 I=13-17 R≤12 **CHL** (5µg)	S ≥17 I=15-16 R≤14 **NIT** (5µg)	S ≥10 I=7-9 R=6 **GEN** (5µg)	S ≥10 I=7-9 R=6 **SIR** (5µg)
kw1urine	*E. faecalis*	R	-	-	8	-	-	-	-	-	-	-	-	-
		I	-	-	-	-	-	14	-	22	-	-	-	-
		S	33	22	-	31	20	-	18	-	30	29	15	14
kw2stool	*E. faecium 9*	R	0	10	0	0	0	0	0	0	-	-	-	4
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	-	-	-	-	-	-	-	-	24	27	14	-
kw3stool	*E. mundtii*	R	-	-	0	0	0	0	0	11	-	-	-	-
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	20	23	-	-	-	-	-	-	25	22	15	15
kw4stool	*E. mundtii*	R	-	-	7	0	0	0	0	0	-	-	-	-
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	20	22	-	-	-	-	-	-	18	18	12	11
kw5stool	*E. faecalis 35*	R	0	-	0	0	0	0	0	0	0	13	-	-
		I	-	20	-	-	-	-	-	-	-	-	-	-
		S	-	-	-	-	-	-	-	-	-	-	15	13
kw6stool (PDR)	*E. faecium 3*	R	0	0	10	0	0	0	0	0	0	12	7	13
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	-	-	-	-	-	-	-	-	-	-	-	-

Sample No.	Isolated spp		S ≥17 I R≤16 **AMP** (5µg)	S ≥21 I=16-20 R≤15 **CIP** (5µg)	S ≥20 I=17-19 R≤16 **RIF** (5µg)	S ≥23 I=21-22 R=20 **LIN** (30µg)	S ≥17 I=15-16 R≤14 **VAN** (30µg)	S ≥16 I=13-15 R≤12 **DOX** (5µg)	S ≥14 I=11-13 R≤10 **TEIC** (5µg)	S ≥23 I=14-22 R≤13 **ERY** (5µg)	S ≥18 I=13-17 R≤12 **CHL** (5µg)	S ≥17 I=15-16 R≤14 **NIT** (5µg)	S ≥10 I=7-9 R=6 **GEN** (5µg)	S ≥10 I=7-9 R=6 **SIR** (5µg)
kw7urine	*E. faecalis*	R	0	-	-	-	-	-	-	-	-	-	-	-
		I	-	-	19	-	-	-	13	14	13	-	-	-
		S	-	21	-	22	19	20	-	-	-	19	10	10
kw8urine	*E. faecalis*	R	-	-	-	-	-	-	-	0	-	-	-	-
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	35	22	23	27	20	26	19	-	24	25	13	11
kw9urine	*E. faecalis*	R	-	-	8	-	-	0	-	0	0	-	-	-
		I	-	-	-	-	-	-	-	-	-	19	-	-
		S	23	22	-	24	20	-	19	-	-	-	14	13
kw10stool	*E. hirae*	R	0	8	0	0	0	12	0	18	-	-	-	-
		I	-	-	-	-	-	-	-	-	-	-	-	8
		S	-	-	-	-	-	-	-	-	21	20	18	-
kw11stool	*E. faecalis*	R	-	-	-	-	-	-	-	-	-	-	-	-
		I	-	20	-	22	-	-	-	22	-	-	-	-
		S	22	-	20	-	20	20	19	-	23	23	17	14
kw12urine	*E. faecalis*	R	-	-	13	-	-	10	-	-	10	-	-	-
		I	-	-	-	22	-	-	-	22	-	-	-	-
		S	25	23	-	-	19	-	18	-	-	20	11	10

Sample No.	Isolated spp		S ≥17 I R≤16 **AMP** (5µg)	S ≥21 I=16-20 R≤15 **CIP** (5µg)	S ≥20 I=17-19 R≤16 **RIF** (5µg)	S ≥23 I=21-22 R=20 **LIN** (30µg)	S ≥17 I=15-16 R≤14 **VAN** (30µg)	S ≥16 I=13-15 R≤12 **DOX** (5µg)	S ≥14 I=11-13 R≤10 **TEIC** (5µg)	S ≥23 I=14-22 R≤13 **ERY** (5µg)	S ≥18 I=13-17 R≤12 **CHL** (5µg)	S ≥17 I=15-16 R≤14 **NIT** (5µg)	S ≥10 I=7-9 R=6 **GEN** (5µg)	S ≥10 I=7-9 R=6 **SIR** (5µg)
kw13urine	*E. faecalis*	R	-	10	-	-	-	-	-	0	10	-	-	-
		I	-	-	17	-	-	14	-	-	-	-	-	-
		S	22	-	-	27	20	-	18	-	-	24	10	10
kw14environmental	*E. dispar*	R	-	-	-	-	-	-	-	-	-	-	-	-
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	23	25	24	25	20	19	16	24	25	20	13	13
kw15environment	*E. faecalis*	R	-	-	-	-	-	-	-	-	-	-	-	-
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	25	26	26	27	21	20	19	25	23	19	20	11

Appendix III: Antibiotic profile of Isolates from UATH

Sample No.	Isolated spp		S ≥17 I R≤16 **AMP** (5µg)	S ≥21 I=16-20 R≤15 **CIP** (5µg)	S ≥20 I=17-19 R≤16 **RIF** (5µg)	S ≥23 I=21-22 R=20 **LIN** (30µg)	S ≥17 I=15-16 R≤14 **VAN** (30µg)	S ≥16 I=13-15 R≤12 **DOX** (5µg)	S ≥14 I=11-13 R≤10 **TEIC** (5µg)	S ≥23 I=14-22 R≤13 **ERY** (5µg)	S ≥18 I=13-17 R≤12 **CHL** (5µg)	S ≥17 I=15-16 R≤14 **NIT** (5µg)	S ≥10 I=7-9 R=6 **GEN** (5µg)	S ≥10 I=7-9 R=6 **SIR** (5µg)
UA1urine	*E. faecium*	R	-	-	15	-	-	10	-	0	10	-	0	0
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	27	23	-	30	18	-	19	-	-	25	-	-
UA2urine	*E. avium*	R	-	-	-	-	-	10	-	0	8	-	6	6
		I	-	-	17	17	-	-	-	-	-	-	-	-
		S	30	24	-	-	19	-	16	-	-	25	-	-
UA3stool	*E. faecalis*	R	-	-	11	-	-	9	-	-	-	-	-	-
		I		-	-	-	-	-	-	21	-	-	-	-
		S	27	27	-	32	20	-	18	-	25	26	10	10
UA4urine	*E. faecalis*	R	-	-	-	-	-	-	-	-	-	-	-	-
		I	-	-	-	-	-	14		16	-	-	-	-
		S	25	24	22	27	19	-	19	-	23	22	7	9
UA5urine	*E. faecalis*	R	-	-	-	-	-	10	-	0	6	-	-	0
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	24	22	20	27	19	-	18	-	-	21	16	-
UA6urine	*E. faecalis*	R	0	-	0	-	-	0	-	0	0	0	0	0
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	-	23	-	28	20	-	19	-	-	-	-	-

Sample No.	Isolated spp		S ≥17 I R≤16 **AMP** (5µg)	S ≥21 I=16-20 R≤15 **CIP** (5µg)	S ≥20 I=17-19 R≤16 **RIF** (5µg)	S ≥23 I=21-22 R=20 **LIN** (30µg)	S ≥17 I=15-16 R≤14 **VAN** (30µg)	S ≥16 I=13-15 R≤12 **DOX** (5µg)	S ≥14 I=11-13 R≤10 **TEIC** (5µg)	S ≥23 I=14-22 R≤13 **ERY** (5µg)	S ≥18 I=13-17 R≤12 **CHL** (5µg)	S ≥17 I=15-16 R≤14 **NIT** (5µg)	S ≥10 I=7-9 R=6 **GEN** (5µg)	S ≥10 I=7-9 R=6 **SIR** (5µg)
UA7stool	*E. faecalis*	R	-	0	-	-	-	8	-	0	0	-	-	6
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	28	-	23	30	18	-	17	-	-	20	18	-
UA8stool	*E. faecalis*	R	-	-	8	-	-	-	-	-	-	-	-	0
		I	-	-	-	-	-	14	-	18	-	-	-	-
		S	23	24	-	24	18	-	16	-	20	17	17	-
UA9stool	*E. faecalis*	R	-	-	0	-	-	10	-	-	-	-	-	5
		I	-	18	-	-	-	-	-	16	-	-	-	-
		S	23	-	-	24	18	-	16	-	20	20	18	-
UA10stool	*E. faecalis*	R	-	-	13	-	-	-	-	-	-	-	0	0
		I	-	-	-	-	-	-	-	17	-	-	-	-
		S	21	22	-	27	19	20	18	-	21	20	-	-
UA11wound Swab	*E. faecalis*	R	-	0	-	20	-	0	-	-	7		6	5
		I	-	-	18	-	-	-	-	17	-	-	-	-
		S	24	-	-	-	18	-	16	-	-	22	-	
UA12stool	*E. faecalis*	R	-	-	10	-	-	0	-	-	7	-	0	5
		I	-	20	-	-	-	-	-	17	-	-	-	-
		S	22	-	-	24	18	-	16	-	-	22	-	-

Sample No.	Isolated spp		S ≥17 I R≤16 **AMP** (5µg)	S ≥21 I=16-20 R≤15 **CIP** (5µg)	S ≥20 I=17-19 R≤16 **RIF** (5µg)	S ≥23 I=21-22 R=20 **LIN** (30µg)	S ≥17 I=15-16 R≤14 **VAN** (30µg)	S ≥16 I=13-15 R≤12 **DOX** (5µg)	S ≥14 I=11-13 R≤10 **TEIC** (5µg)	S ≥23 I=14-22 R≤13 **ERY** (5µg)	S ≥18 I=13-17 R≤12 **CHL** (5µg)	S ≥17 I=15-16 R≤14 **NIT** (5µg)	S ≥10 I=7-9 R=6 **GEN** (5µg)	S ≥10 I=7-9 R=6 **SIR** (5µg)
UA13stool	*E. faecalis*	R	-	12	12	-	-	10	-	0	10	-	6	6
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	24	-	-	24	19	-	17	-	-	20	-	-
UA14stool	*E. faecalis 783/8?*	R	-	-	0	0	0	0	0	0	-	-	-	-
		I	-	-	-	-	-	-	-	-	-	15	-	-
		S	25	26	-	-	-	-	-	-	22	-	15	14
UA15stool	*E. faecium 695*	R	16	-	7	0	0	8	0	8	-	-	-	-
		I	-	18	-	-	-	-	-	-	17	-	-	-
		S	-	-	-	-	-	-	-	-	-	18	13	13
UA16stool	*E. faecium*	R	-	-	-	-	-	-	-	-	-	-	-	-
		I	-	-	17	-	-	-	-	-	-	-	-	-
		S	32	24	-	33	21	28	21	32	22	28	12	11
UA17stool (PDR)	*E. faecium 714/2?*	R	0	0	0	0	0	0	0	0	0	0	0	0
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	-	-	-	-	-	-	-	-	-	-	-	-
UA18stool	*E. faecalis*	R	-	-	7	0	0	8	0	8			6	5
		I	-	-	-	-	-	-	-	-	17	-	-	-
		S	18	25	-	-	-	-	-	-	-	18	-	-

Sample No.	Isolated spp		S ≥17 I R≤16 **AMP** (5µg)	S ≥21 I=16-20 R≤15 **CIP** (5µg)	S ≥20 I=17-19 R≤16 **RIF** (5µg)	S ≥23 I=21-22 R=20 **LIN** (30µg)	S ≥17 I=15-16 R≤14 **VAN** (30µg)	S ≥16 I=13-15 R≤12 **DOX** (5µg)	S ≥14 I=11-13 R≤10 **TEIC** (5µg)	S ≥23 I=14-22 R≤13 **ERY** (5µg)	S ≥18 I=13-17 R≤12 **CHL** (5µg)	S ≥17 I=15-16 R≤14 **NIT** (5µg)	S ≥10 I=7-9 R=6 **GEN** (5µg)	S ≥10 I=7-9 R=6 **SIR** (5µg)
UA19stool	*E. faecium*	R	-	-	-	-	-	8	-	0	0	-	-	6
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	28	26	23	30	18	-	17	-	-	20	18	-
UA20stool	*E. faecalis*	R	-	-	8	-	-	-	-	-	-	-	-	0
		I	-	-	-	-	-	14	-	18	-	-	9	-
		S	23	24	-	24	18	-	16	-	20	17	-	-
- UA21wound	*E. faecalis*	R	-	-	0	-	-	10	-	-	-	-	-	5
		I	-	-	-	-	-	-	-	16	-	-	-	-
		S	23	26	-	24	18	-	16	-	20	20	18	-
UA22stool	*E. faecalis*	R	-	-	13	-	-	-	-	-	-	-	0	0
		I	-	18	-	-	-	-	-	17	-	-	-	-
		S	21	-	-	27	19	20	18	-	21	20	-	-
UA23stool	*E. faecalis*	R	14	0	-	20	-	0	-	-	7	-	6	5
		I	-	-	18	-	-	-	-	-	-	-	-	-
		S	-	-	-	-	18	-	16	17	-	22	-	-
UA24stool	*E. faecium*	R	-	-	10	-	-	0	-	-	7	-	0	5
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	22	26	-	24	18	-	16	17	-	22	-	-

Sample No.	Isolated spp		S ≥17 I R≤16 **AMP** (5µg)	S ≥21 I=16-20 R≤15 **CIP** (5µg)	S ≥20 I=17-19 R≤16 **RIF** (5µg)	S ≥23 I=21-22 R=20 **LIN** (30µg)	S ≥17 I=15-16 R≤14 **VAN** (30µg)	S ≥16 I=13-15 R≤12 **DOX** (5µg)	S ≥14 I=11-13 R≤10 **TEIC** (5µg)	S ≥23 I=14-22 R≤13 **ERY** (5µg)	S ≥18 I=13-17 R≤12 **CHL** (5µg)	S ≥17 I=15-16 R≤14 **NIT** (5µg)	S ≥10 I=7-9 R=6 **GEN** (5µg)	S ≥10 I=7-9 R=6 **SIR** (5µg)
UA25stool	*E. faecium*	R	-	0	-	-	-	8	-	0	-	-	-	6
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	28	-	23	30	18	-	17	-	25	20	18	-
UA26stool	*E. faecium*	R	-	-	8	-	-	-	-	-	-	-	-	0
		I	-	20	-	22	-	14	-	18	-	-	-	-
		S	23	-	-	-	18	-	16	-	20	17	17	-
UA27stool	*E. mundtii*	R	-	-	0	-	-	-	-	-	-	-	-	5
		I	-	18	-	-	-	-	-	16	-	-	-	-
		S	23	-	-	24	18	17	16	-	20	20	18	-
UA28stool	*E. mundtii*	R	-	-	13	-	-	-	-	-	-	-	0	0
		I	-	-	-	-	-	-	-	17	-	-	-	-
		S	21	22	-	27	19	20	18	-	21	20	-	-
UA29stool	*E. faecium*	R	-	0	-	20	-	0	-	-	7	-	6	5
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	24	-	24	-	18	-	16	17	-	22	-	-
UA30environmental	*E. faecalis*	R	14	12	-	-	-	0	-	-	-	-	-	-
		I	-	-	-	22	-	-	-	-	-	-	-	-
		S	-	-	24	-	18	-	16	23	26	25	15	14
UA31environmental	*E. gallinarium*	R	12	-	-	-	-	10	-	0	-	-	-	-
		I	-	-	16	-	-	-	-	-	14	-	-	-
		S	-	27	-	27	20	-	16	-	-	18	14	10

Appendix IV: Antibiotic profile of Isolates from NHA

Sample No.	Isolated spp		S ≥17 I R≤16 **AMP** (5µg)	S ≥21 I=16-20 R≤15 **CIP** (5µg)	S ≥20 I=17-19 R≤16 **RIF** (5µg)	S ≥23 I=21-22 R=20 **LIN** (30µg)	S ≥17 I=15-16 R≤14 **VAN** (30µg)	S ≥16 I=13-15 R≤12 **DOX** (5µg)	S ≥14 I=11-13 R≤10 **TEIC** (5µg)	S ≥23 I=14-22 R≤13 **ERY** (5µg)	S ≥18 I=13-17 R≤12 **CHL** (5µg)	S ≥17 I=15-16 R≤14 **NIT** (5µg)	S ≥10 I=7-9 R=6 **GEN** (5µg)	S ≥10 I=7-9 R=6 **SIR** (5µg)
NH1stool	*E. faecium 698*	R	-	15	11	-	-	-	-	0	10	-	4	5
		I	-	-	-	-	-	13	-	-	-	-	-	-
		S	33	-	-	34	20	-	18	-	-	27	-	-
NH2stool	*E. gallinarium*	R	-	-	9	0	0	9	0	13	6	-	-	-
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	24	29	-	-	-	-	-	-	-	24	14	14
NH3stool	*E. faecium*	R	-	-	10	-	-	-	-	-	-	-	-	-
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	18	24	-	30	24	18	18	20	30	25	13	12
NH4stool	*E. mundtii*	R	-	-	13	18	0	9	0	10	-	-	-	-
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	23	27	-	-	-	-	-	-	28	23	14	6
NH5urine	*E. faecalis*	R	-	-	0	0	0	-	0	10	-	-	-	-
		I	-	-	-	-	-	18	-	-	-	-	-	-
		S	21	23	-	-	-	-	-	-	28	23	10	14
NH6stool	*E. faecalis*	R	-	0	-	-	-	-	-	-	-	-	6	6
		I	-	-	17	-	-	-	-	-	-	-	-	-
		S	32	-	-	33	21	28	21	32	25	28	-	-

Sample No.	Isolated spp		S ≥17 I R≤16 **AMP** (5µg)	S ≥21 I=16-20 R≤15 **CIP** (5µg)	S ≥20 I=17-19 R≤16 **RIF** (5µg)	S ≥23 I=21-22 R=20 **LIN** (30µg)	S ≥17 I=15-16 R≤14 **VAN** (30µg)	S ≥16 I=13-15 R≤12 **DOX** (5µg)	S ≥14 I=11-13 R≤10 **TEIC** (5µg)	S ≥23 I=14-22 R≤13 **ERY** (5µg)	S ≥18 I=13-17 R≤12 **CHL** (5µg)	S ≥17 I=15-16 R≤14 **NIT** (5µg)	S ≥10 I=7-9 R=6 **GEN** (5µg)	S ≥10 I=7-9 R=6 **SIR** (5µg)
NH7stool	*E. mundtii*	R	0	7	0	0	0	10	0	7	-	-	5	0
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	-	-	-	-	-	-	-	-	25	26	-	-
NH8stool	*E. faecium 668*	R	-	-	8	0	0	-	0	0	-	-	-	-
		I	-	-	-	-	-	15	-	-	-	16	-	-
		S	23	24	-	-	-	-	-	-	28	-	15	15
NH9stool	*E. mundtii 648*	R	0	-	9	0	0	0	0	12	0	-	-	-
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	-	25	-	-	-	-	-	-	-	23	14	13
NH10stool	*E. faecium*	R	0	-	10	0	0	12	0	0	10	-	-	-
		I	-	-	-	-	-	-	-	-	-	-	8	-
		S	-	23	-	-	-	-	-	-	-	26	-	13
NH11stool	*E. faecium 702*	R	0	13	6	0	0	11	0	13	-	13	-	-
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	-	-	-	-	-	-	-	-	25	-	10	10
NH12stool	*E. faecium*	R	0	-	0	0	0	0	0	0	-	-	-	-
		I	-	-	-	-	-	-	-	-	-	-	7	-
		S	-	23	-	-	-	-	-	-	25	17	-	15

Sample No.	Isolated spp		S ≥17 I R≤16 **AMP** (5µg)	S ≥21 I=16-20 R≤15 **CIP** (5µg)	S ≥20 I=17-19 R≤16 **RIF** (5µg)	S ≥23 I=21-22 R=20 **LIN** (30µg)	S ≥17 I=15-16 R≤14 **VAN** (30µg)	S ≥16 I=13-15 R≤12 **DOX** (5µg)	S ≥14 I=11-13 R≤10 **TEIC** (5µg)	S ≥23 I=14-22 R≤13 **ERY** (5µg)	S ≥18 I=13-17 R≤12 **CHL** (5µg)	S ≥17 I=15-16 R≤14 **NIT** (5µg)	S ≥10 I=7-9 R=6 **GEN** (5µg)	S ≥10 I=7-9 R=6 **SIR** (5µg)
NH13urine	*E. faecalis 1803*	R	-	-	15	-	-	12	-	10	-	-	-	-
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	27	21	-	30	19	-	17	-	24	24	14	12
NH14stool	*E. faecalis*	R	-	0	16	-	-	12	-	0	8	-	5	4
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	26	-	-	30	20	-	18	-	-	29	-	-
NH15stool	*E. faecalis*	R	-	-	8	-	-	11	-	-	-	-	-	-
		I	-	19	-	-	-	-	-	15	-	-	-	-
		S	28	-	-	28	18	-	18	-	28	25	12	11
NH16stool	*E. faecium 667*	R	-	-	7	-	-	-	-	-	-	-	-	-
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	24	24	-	33	24	28	19	32	26	26	14	13
NH17stool	*E. faecalis 644*	R	0	-	0	0	0	7	0	0	-	14	-	-
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S		25	-	-	-	-	-	-	20	-	16	16
NH18stool	*E. faecium*	R	0	-	0	0	0	10	0	0	-	-	0	0
		I	-	-	-	-	-	-	-	-	-	15	-	-
		S	-	25	-	-	-	-	-	-	19	-	-	-

Sample No.	Isolated spp		S ≥17 I R≤16 **AMP** (5µg)	S ≥21 I=16-20 R≤15 **CIP** (5µg)	S ≥20 I=17-19 R≤16 **RIF** (5µg)	S ≥23 I=21-22 R=20 **LIN** (30µg)	S ≥17 I=15-16 R≤14 **VAN** (30µg)	S ≥16 I=13-15 R≤12 **DOX** (5µg)	S ≥14 I=11-13 R≤10 **TEIC** (5µg)	S ≥23 I=14-22 R≤13 **ERY** (5µg)	S ≥18 I=13-17 R≤12 **CHL** (5µg)	S ≥17 I=15-16 R≤14 **NIT** (5µg)	S ≥10 I=7-9 R=6 **GEN** (5µg)	S ≥10 I=7-9 R=6 **SIR** (5µg)
NH19urine	*E. faecalis*	R	-	-	6	0	0	10	0	6	8	-	-	-
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	20	24	-	-	-	-	-	-	-	25	17	14
NH20urine	*E. faecalis*	R	0	12	10	0	0	6	0	10	-	-	-	-
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	-	-	-	-	-	-	-	-	27	24	15	14
NH21urine	*E. faecalis 707*	R	-	-	6	0	0	8	0	10	-	-	-	-
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	22	21	-	-	-	-	-	-	28	25	14	12
NH22urine	*E. faecalis 691*	R	-	-	-	-	-	-	-	-	-	-	-	5
		I	-	-	18	-	-	-	-	19	-	-	-	-
		S	26	24	-	25	18	22	16	-	24	25	13	-
NH23urine	*E. faecalis*	R	-	-	15	-	-	-	-	-	-	-	-	-
		I	-	20	-	-	-	-	-	21	-	-	-	-
		S	25	-	-	24	17	24	16	-	24	25	13	13
NH24stool	*E. faecium*	R	12	-	10	10	0	-	0	12	-	-	-	-
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	-	22	-	-	-	19	-	-	24	18	19	18

Sample No.	Isolated spp		S ≥17 I R≤16 **AMP** (5µg)	S ≥21 I=16-20 R≤15 **CIP** (5µg)	S ≥20 I=17-19 R≤16 **RIF** (5µg)	S ≥23 I=21-22 R=20 **LIN** (30µg)	S ≥17 I=15-16 R≤14 **VAN** (30µg)	S ≥16 I=13-15 R≤12 **DOX** (5µg)	S ≥14 I=11-13 R≤10 **TEIC** (5µg)	S ≥23 I=14-22 R≤13 **ERY** (5µg)	S ≥18 I=13-17 R≤12 **CHL** (5µg)	S ≥17 I=15-16 R≤14 **NIT** (5µg)	S ≥10 I=7-9 R=6 **GEN** (5µg)	S ≥10 I=7-9 R=6 **SIR** (5µg)
NH25stool	*E. mundtii*	R	0	-	6	0	0	0	0	0	-	-	-	-
		I	-	-	-	-	-	-	-	-	-	15	-	-
		S	-	21	-	-	-	-	-	-	23	-	11	11
NH26stool	*E. faecium*	R	-	13	0	0	0	0	0	0	12	-	-	-
		I	-	-	--	-	-	-	-	-	-	16	-	-
		S	24	-	-	-	-	-	-	-	-	-	15	12
NH27stool	*E. faecalis*	R	-	-	15	0	0	0	0	0	-	-	5	6
		I	-	20	-	-	-	-	-	-	-	16	-	-
		S	26	-	-	-	-	-	-	-	23	-	-	-
NH28urine	*E. faecalis*	R	-	0	-	-	-	10	-	0	10	-	-	-
		I	-	-	18	-	-	-	-	-	-	-		
		S	22	-	-	28	18	-	16	-	-	22	15	14
NH29urine	*E. faecalis*	R	-	-	0	0	-	-	-	-	-	-	-	-
		I	-	-	-	-	-	13	-	-	-	-	-	-
		S	21	20	-	-	18	-	16	25	20	22	19	19
NH30wound Swab	*E. faecalis*	R	-	-	0	-	-	-	-	-	-	-	-	-
		I	-	-	-	-	-	-	-	21	-	-	-	-
		S	30	22	-	28	19	24	19	-	25	26	14	12

Sample No.	Isolated spp		S ≥17 I R≤16 **AMP** (5µg)	S ≥21 I=16-20 R≤15 **CIP** (5µg)	S ≥20 I=17-19 R≤16 **RIF** (5µg)	S ≥23 I=21-22 R=20 **LIN** (30µg)	S ≥17 I=15-16 R≤14 **VAN** (30µg)	S ≥16 I=13-15 R≤12 **DOX** (5µg)	S ≥14 I=11-13 R≤10 **TEIC** (5µg)	S ≥23 I=14-22 R≤13 **ERY** (5µg)	S ≥18 I=13-17 R≤12 **CHL** (5µg)	S ≥17 I=15-16 R≤14 **NIT** (5µg)	S ≥10 I=7-9 R=6 **GEN** (5µg)	S ≥10 I=7-9 R=6 **SIR** (5µg)
NH31stool	*E. mundtii*	R	0	0	0	0	0	0	0	0	9	14	-	-
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	-	-	-	-	-	-	-	-	-	-	16	16
NH32stool	*E. mundtii*	R	0	-	0	0	0	0	0	8	8	0	-	0
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	-	23	-	-	-	-	-	-	-	-	12	-
NH33stool	*E. faecium*	R	0		7	0	0	7	0	0	-	-	-	-
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	-	24	-	-	-	-	-	-	25	23	17	15
NH34stool	*E. mundtii*	R	0	-	0	0	0	12	0	13	-	-	-	-
		I	-	-	-	-	-	-	-	-	-	-	-	-
		S	-	22	-	-	-	-	-	-	24	25	14	15
NH35stool	*E. dispar*	R	0		0	0	0	0	0	0	-	-	0	-
		I	-	20	-	-	-	-	-	--	-	-	-	-
		S	-	-	-	-	-	-	-	-	25	20	-	14
NH36urine	*E. faecalis*	R	0	-	8	-	0	0	-	9	-	-	-	-
		I	-	-	-	-	-	-	12	-	-	-	-	-
		S	-	25	-	23	-	-	-	-	27	23	15	15

Sample No.	Isolated spp		S ≥17 I R≤16 **AMP** (5µg)	S ≥21 I=16-20 R≤15 **CIP** (5µg)	S ≥20 I=17-19 R≤16 **RIF** (5µg)	S ≥23 I=21-22 R=20 **LIN** (30µg)	S ≥17 I=15-16 R≤14 **VAN** (30µg)	S ≥16 I=13-15 R≤12 **DOX** (5µg)	S ≥14 I=11-13 R≤10 **TEIC** (5µg)	S ≥23 I=14-22 R≤13 **ERY** (5µg)	S ≥18 I=13-17 R≤12 **CHL** (5µg)	S ≥17 I=15-16 R≤14 **NIT** (5µg)	S ≥10 I=7-9 R=6 **GEN** (5µg)	S ≥10 I=7-9 R=6 **SIR** (5µg)
NH37wound Swab	*E. faecalis*	R	-	-	10	-	-	12	-	-	12	-	0	-
		I	-	20	-	-	-	-	-	20	-	-	-	-
		S	22	-	-	23	18	-	16	-	-	22	-	12
NH38wound Swab	*E. faecalis*	R	-	0	-	-	-	10	-	0	10	-	-	-
		I	-	-	18	-	-	-	-	-	-	-	-	8
		S	22	-	-	23	18	-	17	-	-	20	10	-
NH39urine	*E. faecalis*	R	-	-	-	-	-	10	-	0	8	-	-	-
		I	-	20	18	-	-	-	-	-	-	-	-	-
		S	19	-	-	24	18	-	17	-	-	20	13	12

Appendix V: 16SrRNA sequence of the isolates

1: NH17

Enterococcus faecalis strain JCM 5803 16S ribosomal RNA gene, partial sequence

Sequence ID: NR_040789.1 Length: 1517 Number of Matches: 1

Score	Expect value	Identities	Gaps	Strand
1572 bits(851)	0.0	872/884(99%)	5/884(0%)	Plus/Minus

```
Query  7    GGARTGCTT-ATGCGTTTGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAGCACY  65
            ||| ||||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  891  GGAGTGCTTAATGCGTTTGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAGCACT  832

Query  66   CWTYGKTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGAG  125
            | | | ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  831  CATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGAG  772

Query  126  CCTCAGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATATATCT  185
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  771  CCTCAGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATATATCT  712

Query  186  ACGCATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCCCAGT  245
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  711  ACGCATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCCCAGT  652

Query  246  TTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTG  305
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  651  TTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTG  592

Query  306  CGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTG  365
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  591  CGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTG  532

Query  366  CTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTCAGGGGACGTTCAGTTACTA  425
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  531  CTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTCAGGGGACGTTCAGTTACTA  472

Query  426  ACGTCCTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTCACGC  485
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  471  ACGTCCTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTCACGC  412

Query  486  GGCGTTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCCCGTAG  545
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  411  GGCGTTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCCCGTAG  352

Query  546  GAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTATGCAT  605
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  351  -AGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTATGCAT  293

Query  606  CGTGGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCATCCATCA  665
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  292  CGTGGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCATCCATCA  233

Query  666  GCGACACCCGAAAGCGCCTTTCACTCTTATGCCATGCGGCATAAACTGTTATGCGGTATT  725
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  232  GCGACACCCGAAAGCGCCTTTCACTCTTATGCCATGCGGCATAAACTGTTATGCGGTATT  173

Query  726  AGCACCTGTTTCCAAGTGTTATCCCCCTCTGATGGGTAGGTTACCCACGTGTTACTCACC  785
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  172  AGCACCTGTTTCCAAGTGTTATCCCCCTCTGATGGGTAGGTTACCCACGTGTTACTCACC  113

Query  786  CGTCCGCCACTCCTCTTTCCAATTGAGTGCAAGCACCTCGGGAGGAAAGAASCGTTCGAC  845
            |||||||||||||||||||||||||||| |||||||||||| |||||||
Sbjct  112  CGTCCGCCACTCCTCTTTCCAATTGAGTGCAAGCAC-TCGGGAGGAAAGAAGCGTTCGAC  54

Query  846  TTGCATGTATTAAGCAMCGCCGCCAGCGTTCGTCCYTGAGCCAG  889
            |||||||||| ||| |||||||||||||| |||||||
Sbjct  53   TTGCATGTATTAGGCA-CGCCGCCAGCGTTCGTCC-TGAGCCAG  12
```

2: KW4

Enterococcus mundtii strain NBRC 100490 16S ribosomal RNA gene, partial sequence

Sequence ID: NR_113906.1 Length: 1486 Number of Matches: 1

Score	Expect value	Identities	Gaps	Strand
1262 bits (683)	0.0	795/854(93%)	8/854(0%)	Plus/Minus

```
Query  17   GCTT-ATGCGTTTGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAGCACTYATYT  75
            |||| ||||||| ||||||||||||||||||||||||||||||||||||||||||||| ||
Sbjct  867  GCTTAATGCGTTAGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAGCACTCAT-C  809

Query  76   GKTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGAGCCTC  135
            | ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  808  GTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGAGCCTC  749

Query  136  AGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATATATCTACGC  195
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  748  AGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATATATCTACGC  689

Query  196  ATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCCCAGTTTCC  255
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  688  ATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCCCAGTTTCC  629

Query  256  AATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGCT  315
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  628  AATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGCT  569

Query  316  CGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGG  375
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  568  CGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGG  509

Query  376  CACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTCAGGGGACGTTCAGTTACTAACGT  435
            |||||||||||||||||||||||||||||||| |||  |  |||||||  |
Sbjct  508  CACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTCAAGGGGTGAACAGTTACTCTCAC  449

Query  436  CCTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTCACGCGGCG  495
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  448  CCTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTCACGCGGCG  389

Query  496  TTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCCCGTAGGAGT  555
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  388  TTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCCCGTAGGAGT  329

Query  556  CTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTATGCATCGTG  615
             |||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||
Sbjct  328  TTGGGCCGTGTCTCAGTCCCAATGTGGCCGATCACCCTCTCAGGTCGGCTATGCATCGTG  269

Query  616  GCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCATCCCATCAGCG  675
            |||||||||||||||||||||||||||||||||||||||||||||| ||||||
Sbjct  268  GCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCATCC-ATCAGCG  210

Query  676  ACACCCGAAAGCGCCTTTCACTCTTATGCCATGCGGCATAAACTGTTATGCGGTATTAGC  735
             ||||  ||||||||||||    | |||||||  |  | |||||| ||||||||
Sbjct  209  GCACCGTAAAGCGCCTTTCAAAACGAAACCATGCGGTTTCGATTGTTATACGGTATTAGC  150

Query  736  ACCTGTTTCCAAGTGTYATCCCCCTCTGATGGGTAGGT-ACCCACGTGTTACTCACC-GT  793
            |||||||||||||| |||||| |||||||| |||| ||||||||||||||| ||
Sbjct  149  ACCTGTTTCCAAGTGTTATCCCCTTCTGATGGGCAGGTTACCCACGTGTTACTCACCCGT  90

Query  794  CCGYCACTCCTTCTTTYCCATTGAGTGCAASCACTCGSGGAGGAAAGAARCGSTTCGACT  853
             || |||||||  ||| ||  || | |||| | | ||  | | |||||| || |||||||
Sbjct  89   TCGCCACTCCTCTTTTCCCGGTG-GAGCAAGCTC-CGGTGGGAAAAGAAGCG-TTCGACT  33

Query  854  TGCATGWWTTAGGC  867
            ||||||  ||||||
Sbjct  32   TGCATGTATTAGGC  19
```

3: NH8

Enterococcus faecium strain NBRC 100486 16S ribosomal RNA gene, partial sequence

Sequence ID: NR_113904.1 Length: 1485 Number of Matches: 1

Score	Expect	Indentities	Gaps	Score
1402 bits (759)	0.0	804/829(97%)	7/829(0%)	Plus/Minus

```
Query  8    CGGARTGCTTTAATGCGTTAGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAGCA  67
            |||| ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  873  CGGAGTGC-TTAATGCGTTAGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAGCA  815

Query  68   CCYWTTYGKTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTC  127
            |   | | ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  814  C-TCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTC  756

Query  128  GAGCCTCAGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATATA  187
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  755  GAGCCTCAGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATATA  696

Query  188  TCTACGCATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCCC  247
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  695  TCTACGCATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCCC  636

Query  248  AGTTTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGC  307
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  635  AGTTTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGC  576

Query  308  CTGCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGG  367
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  575  CTGCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGG  516

Query  368  CTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTCAAGGGATGAACAGTTA  427
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  515  CTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTCAAGGGATGAACAGTTA  456

Query  428  CTCTCATCCTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTCA  487
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  455  CTCTCATCCTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTCA  396

Query  488  CGCGGCGTTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCCCG  547
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  395  CGCGGCGTTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCCCG  336

Query  548  TAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATCACCCTCTCAGGTCGGCTATG  607
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  335  TAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATCACCCTCTCAGGTCGGCTATG  276

Query  608  CATCGTGGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCATCCA  667
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  275  CATCGTGGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCATCCA  216

Query  668  TCAGCGACACCCGAAAGCGCCTTTCAAATCAAAACCATGCGGTTTTGATTGWTATACGGT  727
            |||||||||||||||||||||||||||||||||||| ||||| ||||||||
Sbjct  215  TCAGCGACACCCGAAAGCGCCTTTCAAATCAAAACCATGCGGTTTNGATTGTTATACGGT  156

Query  728  ATTAGCACCTGTTTCCAAGTGT-ATCCCCTTCTGATGR-CAGGTAACCCACGTGYTACTC  785
            |||||||||||||||||| ||||||||||||  ||||| |||||||| |||||
Sbjct  155  ATTAGCACCTGTTTCCAAGTGTTATCCCCTTCTGATGGGCAGGTTACCCACGTGTTACTC  96

Query  786  CRCCCGWTCGCCATTT-TCTTTTTCTGG-GGAGCTAGCTCCGGAGGAAA  832
             |||| ||||||  |  |||||||| || ||||| ||||||| |||||
Sbjct  95   A-CCCGTTCGCCACTCCTCTTTTTCCGGTGGAGCAAGCTCCGGTGGAAA  48
```

4: KW3

Enterococcus mundtii strain NBRC 100490 16S ribosomal RNA gene, partial sequence

Sequence ID: NR_113906.1 Length: 1486 Number of Matches: 1

Score	Expect	Indentities	Gaps	Score
1053 bits (570)	0.0	629/665(95%)	6/665(0%)	Plus/Minus

```
Query  8    GGAGGTGCTTAATGCGTTTGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAGCAC  67
            ||| ||||||||||||| |||||||||||||||||||||||||||||||||||||||||
Sbjct  872  GGA-GTGCTTAATGCGTTAGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAGCAC  814

Query  68   YYATYKTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGA  127
             ||  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  813  TCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGA  754

Query  128  GCCTCAGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATATATC  187
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  753  GCCTCAGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATATATC  694

Query  188  TACGCATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCCCAG  247
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  693  TACGCATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCCCAG  634

Query  248  TTTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCT  307
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  633  TTTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCT  574

Query  308  GCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCT  367
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  573  GCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCT  514

Query  368  GCTGGCACGTAGTTAGCCGTGRCTTTCTGGTTAGATACCGTCAGGGGACGTTCAGTTACT  427
            |||||||||||||||| |||||||||||||||| |||  |  ||||||||
Sbjct  513  GCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTCAAGGGGTGAACAGTTACT  454

Query  428  AACGTCCTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTCACG  487
             |  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  453  CTCACCCTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTCACG  394

Query  488  CGGCGTTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCCCGTA  547
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  393  CGGCGTTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCCCGTA  334

Query  548  GGAGTCTGGGCCGTGTCTCAGTCCCMGKGTGGGCCGATCACCCCTCTCCAGGYCGSCTAT  607
            ||||| ||||||||||||||||   |||| |||||||||| ||| |||| || ||||
Sbjct  333  GGAGTTTGGGCCGTGTCTCAGTCCCAATGTGG-CCGATCACCC-TCT-CAGGTCGGCTAT  277

Query  608  GCMTCGKGGSCCTKGGTGAGCCGTTACCTCACCAACTASCTAATGMWCCCCGGGKTCCWT  667
            || ||| || ||| |||||||||||||||||||| ||||||  || |||| ||| |
Sbjct  276  GCATCGTGG-CCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGG-TCCAT  219

Query  668  CCCTC  672
            || ||
Sbjct  218  CCATC  214
```

5: NH35

Enterococcus dispar strain NBRC 100678 16S ribosomal RNA gene, partial sequence

Sequence ID: NR_113927.1Length: 1484Number of Matches: 1

Score	Expect	Indentities	Gaps	Score
1206 bits (653)	0.0	745/790(94%)	16/790(2%)	Plus/Minus

```
Query  10   ggRGTGCTTTATGCGTTTGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAGCACC  69
            || |||||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  870  GGAGTGCTTAATGCGTTTGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAGCACT  811

Query  70   YWTYTGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGA  129
             | ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  810  CAT-CGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGA  752

Query  130  GCCTCAGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATATATC  189
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  751  GCCTCAGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATATATC  692

Query  190  TACGCATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCCCAG  249
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  691  TACGCATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCCCAG  632

Query  250  TTTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCT  309
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  631  TTTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCT  572

Query  310  GCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCT  369
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  571  GCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCT  512

Query  370  GCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTCAGGGGACGTTCA-GTTAC  428
            |||||||||||||||||||||||||||||||||| |||| |  ||  ||||
Sbjct  511  GCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTCAAGGGATGAACATTTTAC  452

Query  429  TAACGTCCTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTCAC  488
            | | ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  451  TCTCATCCTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTCAC  392

Query  489  GCGGCGTTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCCCGT  548
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  391  GCGGCGTTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCCCGT  332

Query  549  AGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTATGC  608
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  331  AGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTATGC  272

Query  609  ATCGTGGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCATCCAT  668
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |
Sbjct  271  ATCGTGGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCATCC-T  213

Query  669  CA-GCGACACCCGAAAGCGCCTTTCACTCTTATGC-CATGCGG-CATAAACTGTTATGC-  724
            || | ||| |  |||||||||||| | | ||  |||| |  ||| || | ||||||
Sbjct  212  CAAGTGACGCA--AAAGCGCCTTTCAAT-TAATAAACATGAGTTCATTAA-TATTATGCG  157

Query  725  GTATTAGCACCTGTTTCCAAGTGT-ATCCCCC-CTCTGATGGGTAGG-TACCCMCGKGTT  781
            |||||||||||||||| ||| ||||||| || ||| ||||||| ||||| || |||
Sbjct  156  GTATTAGCACCTGTTTCCAAATGTTATCCCCCGCT-TGA-GGGTAGGTTACCCACGTGTT  99

Query  782  ACTCMCCCGT  791
            |||| |||||
Sbjct  98   ACTCACCCGT  89
```

6: NH25

Enterococcus dispar strain NBRC 100678 16S ribosomal RNA gene, partial sequence

Sequence ID: NR_113927.1 Length: 1484 Number of Matches: 1

Score	Expect	Indentities	Gaps	Score
1273 bits (689)	0.0	765/806(95%)	2/806(0%)	Plus/Minus

```
Query  6    GGCGGGAGTGCTTAATGCGTTTGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAS  65
            ||| ||||||||||||||| ||||||||||||||||||||||||||||||||
Sbjct  874  GGC-GGAGTGCTTAATGCGTTAGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAG  816

Query  66   CMCTCATYGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTT  125
            | ||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  815  CACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTT  756

Query  126  CGAGCCTCAGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATAT  185
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  755  CGAGCCTCAGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATAT  696

Query  186  ATCTACGCATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCC  245
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  695  ATCTACGCATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCC  636

Query  246  CAGTTTCCAATGACCCTCCRSGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCG  305
            |||||||||||||||  ||||||||||||||||||||||||||||||||
Sbjct  635  CAGTTTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCG  576

Query  306  CCTGCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCG  365
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  575  CCTGCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCG  516

Query  366  GCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTCAGGGGACGTTCAGTT  425
            |||||||||||||||||||||||||||||||||||| ||| | |||||
Sbjct  515  GCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTCAAGGGGTGAACAGTT  456

Query  426  ACTAACGTCCTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTC  485
            ||| | ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  455  ACTCTCACCCTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTC  396

Query  486  ACGCGGCGTTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCCC  545
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  395  ACGCGGCGTTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCCC  336

Query  546  GTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTAT  605
            ||||||| |||||||||||||||| |||||||||||||||||||||||||
Sbjct  335  GTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATCACCCTCTCAGGTCGGCTAT  276

Query  606  GCATCGTGGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCATCC  665
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  275  GCATCGTGGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCATCC  216

Query  666  ATCAGCGACACCCGAAAGCGCCTTTCACTYTTATGCCATGCGGCATAAACTGTTATGCGG  725
            ||||||| ||||  ||||||||||||    | ||||||||  | | ||||||  |||
Sbjct  215  ATCAGCGGCACCGTAAAGCGCCTTTCAAAACGAAACCATGCGGTTTCGATTGTTATACGG  156

Query  726  TATTAGCMCCTGTTTCCAAGTGTTATCCCCCTCTGATGGGTAGGTYACCCACGTGTYACT  785
            ||||||| |||||||||||||||||| |||||||| |||| |||||||||  |||
Sbjct  155  TATTAGCACCTGTTTCCAAGTGTTATCCCCTTCTGATGGGCAGGTTACCCACGTGTTACT  96

Query  786  CACCCCGTCCGCCACTCCTCTTTTCC  811
            ||||| || ||||||||||||||||
Sbjct  95   CACCC-GTTCGCCACTCCTCTTTTCC  71
```

7: NH27

Enterococcus faecalis strain NBRC 100480 16S ribosomal RNA gene, partial sequence

Sequence ID: NR_113901.1Length: 1426Number of Matches: 1

Score	Expect	Indentities	Gaps	Score
1458 bits (789)	0.0	815/833(98%)	3/833(0%)	Plus/Minus

```
Query  3    GGCGGGRGTGSCTTAATGCGTTTGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTA  62
            ||| || ||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  875  GGC-GGAGTG-CTTAATGCGTTTGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTA  818

Query  63   GCACTYWTCGKTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTT  122
            |||||  ||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  817  GCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTT  758

Query  123  TCGAGCCTCAGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATA  182
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  757  TCGAGCCTCAGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATA  698

Query  183  TATCTACGCATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTC  242
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  697  TATCTACGCATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTC  638

Query  243  CCAGTTTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACC  302
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  637  CCAGTTTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACC  578

Query  303  GCCTGCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGC  362
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  577  GCCTGCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGC  518

Query  363  GGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTCAGGGGACGTTCAGT  422
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  517  GGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTCAGGGGACGTTCAGT  458

Query  423  TACTAACGTCCTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACT  482
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  457  TACTAACGTCCTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACT  398

Query  483  CACGCGGCGTTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCC  542
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  397  CACGCGGCGTTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCC  338

Query  543  CGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTA  602
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  337  CGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTA  278

Query  603  TGCATCGTGGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCATC  662
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  277  TGCATCGTGGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCATC  218

Query  663  CATCAGCGACACCCGAAAGCGCCTTTCACTCTTATGCCATGCGGCATAAACTGTTATGCG  722
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  217  CATCAGCGACACCCGAAAGCGCCTTTCACTCTTATGCCATGCGGCATAAACTGTTATGCG  158

Query  723  GTATTAGCMCCTGTTTCCMAGTGTTATCCCCCTCTGATGGKWAGGWTACCCMCGTGTTAC  782
            |||||||| |||||||| ||||||||||||||||||  ||| ||||| ||||||||
Sbjct  157  GTATTAGCACCTGTTTCCAAGTGTTATCCCCCTCTGATGGGTAGGTTACCCACGTGTTAC  98

Query  783  TCACCCGTCCGCCACTYCTCTTTCCAATTGASTGCCCGCACTCCKGGAGGAAA  835
            |||||||||||||| |||||||||||| |||  ||||||  |||||||
Sbjct  97   TCACCCGTCCGCCACTCCTCTTTCCAATTGAGTGCAAGCACTCG-GGAGGAAA  46
```

8: NH9

Enterococcus mundtii strain NBRC 100490 16S ribosomal RNA gene, partial sequence

Sequence ID: NR_113906.1Length: 1486Number of Matches: 1

Score	Expect	Indentities	Gaps	Score
1517 bits (821)	0.0	852/870(98%)	2/870(0%)	Plus/Minus

```
Query  11   GTGSCTT-ATGCGTTAGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAGCACYCA  69
            ||| ||| ||||||||||||||||||||||||||||||||||||||||||||||| ||
Sbjct  869  GTG-CTTAATGCGTTAGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAGCACTCA  811

Query  70   TYGKTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGAGCC  129
            | | ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  810  TCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGAGCC  751

Query  130  TCAGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATATATCTAC  189
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  750  TCAGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATATATCTAC  691

Query  190  GCATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCCCAGTTT  249
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  690  GCATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCCCAGTTT  631

Query  250  CCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGCG  309
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  630  CCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGCG  571

Query  310  CTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCT  369
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  570  CTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCT  511

Query  370  GGCACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTCAAGGGATGAACAGTTACTCTC  429
            |||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct  510  GGCACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTCAAGGGGTGAACAGTTACTCTC  451

Query  430  ATCCTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTCACGCGG  489
            | ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  450  ACCCTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTCACGCGG  391

Query  490  CGTTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCCCGTAGGA  549
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  390  CGTTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCCCGTAGGA  331

Query  550  GTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATCACCCTCTCAGGTCGGCTATGCATCG  609
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  330  GTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATCACCCTCTCAGGTCGGCTATGCATCG  271

Query  610  TGGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCATCCATCAGC  669
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  270  TGGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCATCCATCAGC  211

Query  670  GACACCCGAAAGCGCCTTTCAAATCAAAACCATGCGGTTTCGATTGTTATACGGTATTAG  729
            | ||||  |||||||||||||| | ||||||||||||||||||||||||||||||||||
Sbjct  210  GGCACCGTAAAGCGCCTTTCAAAACGAAACCATGCGGTTTCGATTGTTATACGGTATTAG  151

Query  730  CACCTGTTTCCAAGTGTTATCCCCTTCTGATGGGCAGGTTACCCACGTGTTACTCACCCG  789
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  150  CACCTGTTTCCAAGTGTTATCCCCTTCTGATGGGCAGGTTACCCACGTGTTACTCACCCG  91

Query  790  TTCGCCACTCYTCTTTTTCCGGTGGAGYMAGCTCCGGTGGAAAAAGAAGCGTACGACTTG  849
            |||||||||| |||||| |||||||||  |||||||||| |||||||||| |||||||
Sbjct  90   TTCGCCACTCCTCTTTTCCCGGTGGAGCAAGCTCCGGTGGGAAAAGAAGCGTTCGACTTG  31

Query  850  CATGTATTAGGCACGCCGCCAGCGTTCGTC  879
            |||||||||||||||||||||||||||||
Sbjct  30   CATGTATTAGGCACGCCGCCAGCGTTCGTC  1
```

9: NH33

Enterococcus faecium strain DSM 20477 16S ribosomal RNA gene, complete sequence

Sequence ID: NR_114742.1Length: 1533Number of Matches: 1

Score	Expect	Indentities	Gaps	Score
1443 bits (781)	0.0	839/882(95%)	5/882(0%)	Plus/Minus

```
Query  15   GTGSCTTAATGCGTTWGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAGCACTYW  74
            ||| |||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  877  GTG-CTTAATGCGTTAGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAGCACT-C  820

Query  75   TTYGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGAGC  134
             | ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  819  ATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGAGC  760

Query  135  CTCAGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATATATCTA  194
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  759  CTCAGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATATATCTA  700

Query  195  CGCATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCCCAGTT  254
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  699  CGCATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCCCAGTT  640

Query  255  TCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGC  314
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  639  TCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGC  580

Query  315  GCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGC  374
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  579  GCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGC  520

Query  375  TGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTCARGGGAYGAWCAGTTACTMT  434
             |||||||||||||||||||||||||||||||||| |||| || ||||||| |
Sbjct  519  TGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTCAAGGGATGAACAGTTACTCT  460

Query  435  CRTCCTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTCACGCG  494
             | ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  459  CATCCTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTCACGCG  400

Query  495  GCGTTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCCCGTAGG  554
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  399  GCGTTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCCCGTAGG  340

Query  555  AGTYTGGGCCGTGTCTCAGTCCCARTGTGGCCGATCACCCTCTCAGGTCGGCTATGCATC  614
             ||| |||||||||||||||| |||||||||||||||||||||||||||||||||||||||
Sbjct  339  AGTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATCACCCTCTCAGGTCGGCTATGCATC  280

Query  615  GTGGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCATCCATCAS  674
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  279  GTGGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCATCCATCAG  220

Query  675  CGACACCCGAAAGCGCCTTTCamwyywawsCCATGCGGYWTMRAYTGTTATRCGGTATTA  734
             ||||||||||||||||||    | |||||||  | | ||||| |||||||
Sbjct  219  CGACACCCGAAAGCGCCTTTCAAATCAAAACCATGCGGTTTCGATTGTTATACGGTATTA  160

Query  735  GCACCCTGTTTCCAAGTGTTATCCCCYTCTGATGGGCAGGWTACCCCACGTGTTACTCAC  794
             ||||| |||||||||||||||| |||||||||||| ||||| ||||||||||||
Sbjct  159  GCACC-TGTTTCCAAGTGTTATCCCCTTCTGATGGGCAGGTTACCC-ACGTGTTACTCAC  102

Query  795  CCGTTCGCCACTCTTTCTTTTYCCKGKGGAGCAAGCTCCYSGKGAAAAAGAAGCGTACGA  854
             |||||||||||| | ||||| || | ||||||||||    |||||||||||| |||
Sbjct  101  CCGTTCGCCACTCCT-CTTTTTCCGGTGGAGCAAGCTCCGGTGGAAAAAGAAGCGTTCGA  43

Query  855  CTWGCATGTATTAGGCACGCCGCCAGCGTTCGTCCTGAGMCA  896
             || ||||||||||||||||||||||||||||||||| ||
Sbjct  42   CTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCCTGAGCCA  1
```

10: UA18

Enterococcus faecalis strain JCM 5803 16S ribosomal RNA gene, partial sequence

Sequence ID: NR_040789.1Length: 1517Number of Matches: 1

Score	Expect	Indentities	Gaps	Score
1384 bits (749)	0.0	848/900(94%)	12/900(0%)	Plus/Minus

```
Query  6    GGCGGGAGTGCTT-ATGCGTTAGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAG  64
            ||| |||||||| ||||||| ||||||||||||||||||||||||||||||||||||||
Sbjct  894  GGC-GGAGTGCTTAATGCGTTTGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAG  836

Query  65   CMCYYATYGKTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTT  124
            | |  || | ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  835  CACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTT  776

Query  125  CGAGCCTCAGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATAT  184
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  775  CGAGCCTCAGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATAT  716

Query  185  ATCTACGCATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCC  244
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  715  ATCTACGCATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCC  656

Query  245  CAGTTTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCG  304
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  655  CAGTTTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCG  596

Query  305  CCTGCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCG  364
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  595  CCTGCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCG  536

Query  365  GCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTCARGGGATGAACAGTT  424
            |||||||||||||||||||||||||||||||||||| |||| |  |||||
Sbjct  535  GCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTCAGGGGACGTTCAGTT  476

Query  425  ACTCTCRTCCTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTC  484
            |||  | ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  475  ACTAACGTCCTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTC  416

Query  485  ACGCGGCGTTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCCC  544
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  415  ACGCGGCGTTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCCC  356

Query  545  GTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATCACCCTCTCAGGTCGGCTAT  604
            |||| ||| |||||||||||||||| ||||||||||||||||||||||||||
Sbjct  355  GTAG-AGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTAT  297

Query  605  GCATCGTGGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCATCC  664
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  296  GCATCGTGGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCATCC  237

Query  665  ATCAGCGACACCCGAAAGCGCCTTTCAMATCAAAR-CCATGCGGTTTCGATTGTTATACG  723
            |||||||||||||||||||||  ||  |  |||||||  |  | ||||| ||
Sbjct  236  ATCAGCGACACCCGAAAGCGCCTTTCAC-TCTTATGCCATGCGGCATAAACTGTTATGCG  178

Query  724  GTATTAGCACCTGTTTCCAAGTGTTATCCCCTTCTGATGGGCAGGTTACCCACGTGTTAC  783
            |||||||||||||||||||||||||| |||||||| ||||||||||||||||
Sbjct  177  GTATTAGCACCTGTTTCCAAGTGTTATCCCCCTCTGATGGGTAGGTTACCCACGTGTTAC  118

Query  784  TCACCCGTTCGCCACTCTTCTTTTTCCGKTGGAG--CAAGCTC-CGGTGGAAAAAGAAGC  840
            ||||||| ||||||| |||||  ||  | |||  ||||| | |||  |  ||||||| 
Sbjct  117  TCACCCGTCCGCCACTCCTCTTT--CCAATTGAGTGCAAGCACTCGGGAGG-AAAGAAGC  61

Query  841  GTACGACTTGCATGTATTASGCACGCCGCCAGCGYTCGYC-TGAGCCAGGATCAAACTCT  899
            || ||||||||||||| ||||||||||||| ||| | ||||||||||||||||
Sbjct  60   GTTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCCTGAGCCAGGATCAAACTCT  1
```

11: NH20

Enterococcus faecalis strain JCM 5803 16S ribosomal RNA gene, partial sequence

Sequence ID: NR_040789.1Length: 1517Number of Matches: 1

Score	Expect	Indentities	Gaps	Score
1339 bits (725)	0.0	845/903(94%)	19/903(2%)	Plus/Minus

```
Query  8    GGARGTGCTTAATGCGTTTGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAGCAC  67
            ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  891  GGA-GTGCTTAATGCGTTTGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAGCAC  833

Query  68   YYATYTGKTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCG  127
             ||  | |||||||||||||||||||||||||||| ||||||||||||||
Sbjct  832  TCAT-CGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCG  774

Query  128  AGCCTCAGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATATAT  187
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  773  AGCCTCAGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATATAT  714

Query  188  CTACGCATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCCCA  247
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  713  CTACGCATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCCCA  654

Query  248  GTTTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCC  307
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  653  GTTTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCC  594

Query  308  TGCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGC  367
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  593  TGCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGC  534

Query  368  TGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTCAAGGGATGAACATTTTA  427
            |||||||||||||||||||||||||||||||||||| |||| |  || || |
Sbjct  533  TGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTCAGGGGACGTTCAGTT-A  475

Query  428  CTCTCATCCTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTCA  487
            ||  | ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  474  CTAACGTCCTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTCA  415

Query  488  CGCGGCGTTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCCCG  547
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  414  CGCGGCGTTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCCCG  355

Query  548  TAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTATG  607
            ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  354  TAG-AGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTATG  296

Query  608  CATCGTTGCCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCATCC  667
            |||||| ||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  295  CATCGTGGCC-TTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCATCC  237

Query  668  ATCAGTGACGCA--AAAGCGCCTTTCAACT-TTCTTCCATGCGGAAAATAGTGTTATACG  724
            ||||| ||| |   |||||||||||| || || | ||||||| | | | ||||| ||
Sbjct  236  ATCAGCGACACCCGAAAGCGCCTTTCA-CTCTTATGCCATGCGGCATAAACTGTTATGCG  178

Query  725  GTATTAGCACCTGTTTTCCAAGTGTTATCCCCTTCTGATGGGCAGGTTACCCACGTGTTA  784
            |||||||||||||| |||||||||||||| |||||||| ||||||||||||||||||||
Sbjct  177  GTATTAGCACCTGTTT-CCAAGTGTTATCCCCCTCTGATGGGTAGGTTACCCACGTGTTA  119

Query  785  CTCACCCGTTCGCCACTCTT-TTTYCTTTYCGGTGGA-GCAAGCTCCGGTGAAAGAAAAA  842
            |||||||| ||||||| | ||| | |   ||| | |||  ||| || ||  |||| |
Sbjct  118  CTCACCCGTCCGCCACTCCTCTTTCCAATTGAGTGCAAGCA--CTC-GG-GAG-GAAAGA  64

Query  843  AGCG-TCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCCTGASC-AGAATCAAAC  900
            |||| |||||||||||||||||||||||||||||||||||| | || ||||||
Sbjct  63   AGCGTTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCCTGAGCCAGGATCAAAC  4

Query  901  TCT  903
            |||
```

Sbjct 3 TCT 1

12: NH34

Enterococcus mundtii strain NBRC 100490 16S ribosomal RNA gene, partial sequence

Sequence ID: NR_113906.1Length: 1486Number of Matches: 1

Score	Expect	Indentities	Gaps	Score
1341 bits (726)	0.0	817/865(94%)	4/865(0%)	Plus/Minus

```
Query 11  TGCTTAATGCGTTTGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAGCWCTYWTY 70
          |||||||||||| |||||||||||||||||||||||||||||||| ||  |
Sbjct 868 TGCTTAATGCGTTAGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAGCACTCATC 809

Query 71  GKTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGAGCCTC 130
          | ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 808 GTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGAGCCTC 749

Query 131 AGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATATATCTACGC 190
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 748 AGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATATATCTACGC 689

Query 191 ATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCCCAGTTTCC 250
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 688 ATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCCCAGTTTCC 629

Query 251 AATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGCT 310
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 628 AATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGCT 569

Query 311 CGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGG 370
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 568 CGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGG 509

Query 371 CACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTCAGGGGACGTTCAGTTACTAACGT 430
          |||||||||||||||||||||||||||||||| |||  |  |||||||  |
Sbjct 508 CACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTCAAGGGGTGAACAGTTACTCTCAC 449

Query 431 CCTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTCACGCGGCG 490
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 448 CCTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTCACGCGGCG 389

Query 491 TTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCCCGTAGGAGT 550
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 388 TTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCCCGTAGGAGT 329

Query 551 CTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTATGCATCGTG 610
           |||||||||||||||| ||||||||||||||||||||||||||||||||||
Sbjct 328 TTGGGCCGTGTCTCAGTCCCAATGTGGCCGATCACCCTCTCAGGTCGGCTATGCATCGTG 269

Query 611 GCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCATCCATCAGCGA 670
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 268 GCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCATCCATCAGCGG 209

Query 671 CACCCGAAAGCGCCTTTCACTCTTATGCCATGCGGCATAAACTGTTATGCGGTATTAGCA 730
          ||||  ||||||||||||    | |||||||  | | |||||| ||||||||||
Sbjct 208 CACCGTAAAGCGCCTTTCAAAACGAAACCATGCGGTTTCGATTGTTATACGGTATTAGCA 149

Query 731 CCTGTTTCCAAGTGTTATCCCCCTCTGATGGGTAGGTTACCCACGTGTTACTCACCCGTC 790
          |||||||||||||||||| |||||||| |||||||||||||||||||||||
Sbjct 148 CCTGTTTCCAAGTGTTATCCCCTTCTGATGGGCAGGTTACCCACGTGTTACTCACCCGTT 89

Query 791 CGCCACTCCTCTTT-CCAGTTGAGTGCAAGCACTCGG-GAGGAAAGAAGCGTTCGACTTG 848
          |||||||||||||| || | || | |||||| | ||| | | ||||||||||||||||||
Sbjct 88  CGCCACTCCTCTTTTCCCGGTG-GAGCAAGCTC-CGGTGGGAAAAGAAGCGTTCGACTTG 31

Query 849 CATGTATTAGGCMCGCCGCCMGCGT 873
          |||||||||||| ||||||| ||||
```

Sbjct 30 CATGTATTAGGCACGCCGCCAGCGT 6

13: NH2

Enterococcus gallinarum strain LMG 13129 16S ribosomal RNA gene, complete sequence

Sequence ID: NR_104559.2Length: 1553Number of Matches: 1

Score	Expect	Indentities	Gaps	Score
1258 bits (681)	0.0	803/862(93%)	25/862(2%)	Plus/Minus

```
Query 17  GCTT-ATGCGTTTGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAGCACTYATYT 75
          |||| |||||||||||||||||||||||||||||||||||||||||||||||||||| ||
Sbjct 886 GCTTAATGCGTTTGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAGCACTCAT-C 828

Query 76  GKTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGAGCCTC 135
          | |||||||||||||||||||||||||||||| ||||||||||||||||||
Sbjct 827 GTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGAGCCTC 768

Query 136 AGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATATATCTACGC 195
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 767 AGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATATATCTACGC 708

Query 196 ATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCCCAGTTTCC 255
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 707 ATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCCCAGTTTCC 648

Query 256 AATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGCT 315
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 647 AATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGCT 588

Query 316 CGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGG 375
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 587 CGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGG 528

Query 376 CACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTCA-GGG--G-ACGTTCAGTTACTA 431
          |||||||||||||||||||||||||||||||| |||  | |||||  |||||
Sbjct 527 CACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTCAAGGGATGAACGTT---TTACTC 471

Query 432 ACGTCCTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTCACGC 491
           | ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 470 TCATCCTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTCACGC 411

Query 492 GGCGTTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCCCGTAG 551
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 410 GGCGTTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCCCGTAG 351

Query 552 GAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTATGCAT 611
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 350 GAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTATGCAT 291

Query 612 CGTGGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCATCCCATC 671
          |||||||||||||||||||||||||||||||||||||||||||||||||| |||
Sbjct 290 CGTGGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCATCC-ATC 232

Query 672 AGCGACACCCGAAAGCGCCTTTCA-CTCTTATGCCATGCGGCATAAACTGTTATGCGGTA 730
          || ||| |  |||||||||||| || || | |||||||| | | | ||||| |||||
Sbjct 231 AGTGACGCA--AAAGCGCCTTTCAACT-TTCTTCCATGCGGAAAATAGTGTTATACGGTA 175

Query 731 TTAGCACCTGTTTCCAAGTGTYATCCCCCTCTGATGGGTAGGT-ACCCACGTGTTACTCA 789
          |||||||||||||||||| |||||| |||||||| |||| ||||||||||||||
Sbjct 174 TTAGCACCTGTTTCCAAGTGTTATCCCCTTCTGATGGGCAGGTTACCCACGTGTTACTCA 115

Query 790 CC-GTCCGYCACTCCTT--CTTTYCCATTGAGTGCAASCACTCGSGGAG-GAAAGAARCG 845
          || || || ||||| ||  |||| | | |||  ||| | | ||  ||  |||| || ||
Sbjct 114 CCCGTTCGCCACTCTTTTTCTTT-CGGTGGAG--CAAGCTC-CGGTGAAAGAAA-AAGCG 60

Query 846 STTCGACTTGCATGWWTTAGGC 867
           |||||||||||  ||||||
```

Sbjct 59 -TTCGACTTGCATGTATTAGGC 39

14: NH18

Enterococcus faecium strain DSM 20477 16S ribosomal RNA gene, complete sequence

Sequence ID: NR_114742.1Length: 1533Number of Matches: 1

Score	Expect	Indentities	Gaps	Score
1554 bits (681)	0.0	866/884(98%)	3/884(0%)	Plus/Minus

Query 6 GGCGGGAGTGCTT-ATGCGTTAGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAG 64
||| |||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct 883 GGC-GGAGTGCTTAATGCGTTAGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAG 825

Query 65 CMCYYATYGKTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTT 124
| | || | ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 824 CACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTT 765

Query 125 CGAGCCTCAGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATAT 184
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 764 CGAGCCTCAGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATAT 705

Query 185 ATCTACGCATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCC 244
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 704 ATCTACGCATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCC 645

Query 245 CAGTTTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCG 304
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 644 CAGTTTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCG 585

Query 305 CCTGCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCG 364
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 584 CCTGCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCG 525

Query 365 GCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTCARGGGATGAACAGTT 424
|||||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct 524 GCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTCAAGGGATGAACAGTT 465

Query 425 ACTCTCRTCCTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTC 484
|||||| ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 464 ACTCTCATCCTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTC 405

Query 485 ACGCGGCGTTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCCC 544
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 404 ACGCGGCGTTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCCC 345

Query 545 GTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATCACCCTCTCAGGTCGGCTAT 604
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 344 GTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATCACCCTCTCAGGTCGGCTAT 285

Query 605 GCATCGTGGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCATCC 664
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 284 GCATCGTGGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCATCC 225

Query 665 ATCAGCGACACCCGAAAGCGCCTTTCAMATCAAARCCATGCGGTTTCGATTGTTATACGG 724
|||||||||||||||||||||||| |||||| ||||||||||||||||||||||
Sbjct 224 ATCAGCGACACCCGAAAGCGCCTTTCAAATCAAAACCATGCGGTTTCGATTGTTATACGG 165

Query 725 TATTAGCACCTGTTTCCAAGTGTTATCCCCTTCTGATGGGCAGGTTACCCACGTGTTACT 784
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 164 TATTAGCACCTGTTTCCAAGTGTTATCCCCTTCTGATGGGCAGGTTACCCACGTGTTACT 105

Query 785 CACCCGTTCGCCACTCTTCTTTTTCCGKTGGAGCAAGCTCCGGTGGAAAAAGAAGCGTAC 844
|||||||||||||| ||||||||| |||||||||||||||||||||||| |
Sbjct 104 CACCCGTTCGCCACTCCTCTTTTTCCGGTGGAGCAAGCTCCGGTGGAAAAAGAAGCGTTC 45

Query 845 GACTTGCATGTATTASGCACGCCGCCAGCGYTCGYC-TGAGCCA 887
|||||||||||||| |||||||||||| ||| | |||||||
Sbjct 44 GACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCCTGAGCCA 1

Appendix VI: Ethical Approval letter from UATH

UNIVERSITY OF ABUJA TEACHING HOSPITAL

P.M.B. 228, ABUJA - F.C.T. NIGERIA
07040045614, 09-2905535, 09-2904040
www. uath. ng.org.

Chief Medical Director
Dr. Peter Alabi
BM. BCH, FMCP

Chairman, Board of Management
Valentine I. Attah Ph D

Director of Administration
Musa Abdullahi
MPA, AHAN

Chairman Medical Advisory Committee
Dr. A.S. Haruna
MBBS, FWACP

Our Ref: FCT/UATH/HREC/PR/408

Your Ref: _____

Date: 13/5/15

Ndubuisi John Chima
Department of Microbiology
Ahmadu Bello University
Zaria

RE: Molecular Characterisation of Vancomycin Resistant Eneterococcus SPP Isolated from Hospitals and Hospital Effluent Impacted Environment

I am happy to inform you that approval has been given to conduct the above named study.

The approval is for one year and will lapse on 12/5/16. It can be renewed on request. If for any reason the study is not commenced as per the expected date, the committee should be appropriately informed.

Any changes to the protocol would necessarily require an approval from committee.

Best wishes.

Edith Akanya (Mrs
Secretary, UATH HREC

Appendix: VII: Ethical approval letter from National Hospital

NATIONAL HOSPITAL

(Established by Act No 36 of 1999).

ABUJA

BOARD CHAIRMAN:
Dr. Tony Okam

DIRECTOR OF ADMINISTRATION
S. U. Gyaranya, B.A (Comb. Hons), AHSAN, MNIM
Member/Sec. to the Board

CHIEF MEDICAL DIRECTOR / CEO
Dr. J. A. F. Momoh, MBBS, MSC, FWACP(LM)

Ag. DIRECTOR OF CLINICAL SERVICES/CMAC
Dr. Ogugua Osi-Ogbu, MB,BS., D.A.B

NHA/ADMIN/236/V.VII/ 17th February, 2015

RE: MOLECULAR CHARACTERIZATION OF VANCOMYCIN RESISTANT ENTEROCOCCUS SPP ISOLATED FROM HOSPITAL AND HOSPITAL EFFLUENT IMPACTED ENVIRONMENT

HREC Assigned number: NHA/EC/034/2014

Name of Principal Investigator: Ndubuisi John

Address of Principal Investigator: Department of Microbiology, A.B.U Zaria, Kaduna State

Date of Receipt of Valid Application: 19th November, 2014

Notice of Approval

This is to inform you that the research described in the submitted protocol, the consent forms, advertisements and other participant information materials have been reviewed and *given full approval by the Institute Review Board (IRB) Committee, National Hospital Abuja.*

This approval dates from **17th February, 2015** to **16th February, 2017.** If there is delay in starting the research, please inform the HREC National Hospital Abuja so that the dates of approval can be adjusted accordingly. Note that no participant accrual or activity related to this research may be conducted outside of these dates. *All informed consent forms used in this study must carry the HREC assigned number and duration of HREC approval of the study.*

The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the Code including ensuring that all adverse events are reported promptly to the HREC. No changes are permitted in the research without prior approval by the HREC except in circumstances outlined in the Code. The HREC reserves the right to conduct compliance visit to your research site without previous notification.

Dr. O. Osi-Ogbu
(Ag. CMAC)
For: Chairman, HREC, National Hospital

Plot 132 Central District (Phase II) P.M.B. 425, Garki - Abuja Nigeria
Telephone: 0803-787-9543, 0809-751-9764, 0809-752-0012
E-mail: info@nationalhospitalabuja.net www.nationalhospitalabuja.net

AppendixVIII: Ethical Approval Letter from FCT HREC

FEDERAL CAPITAL TERRITORY
HEALTH RESEARCH ETHICS COMMITTEE

Research Unit, Room 10, Block A Annex, HHSS
FCT Secretariat No. 1 Kapital Street Area 11, Garki, Abuja - Nigeria

Name of Principal Investigator	Ndubuisi John Chima
Address of Principal Investigator:	Dept. of Microbiology, Ahmadu Bello University Zaria, Kaduna State.
Date of receipt of valid application:	26/11/2014

NOTICE OF RESEARCH APPROVAL

Protocol Approval Number: FHREC/2014/01/76/16-12-14

Study Title: Molecular Characterization of Vancomycin Resistant Enterococcus SPP Isolated From Hospitals and Hospital Effluent Impacted Environment.

This is to certify that the FCT Health Research Ethics Committee (FCT HREC) has fully approved the research described in the above stated protocol.

Approval Date: - 16/12/2014
Expiration Date: - 15/12/2015

Note that no activity related to this research may be conducted outside of these dates. Only the FCT HREC approved informed consent forms may be used when written informed consent is required. They must carry FCT HREC assigned protocol approval number and duration of approval of the study.

The National Code of Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the code. The FCT HREC reserves the right to conduct compliance visit to your research site without previous notification.

Modifications: Subsequent changes are not permitted in this research without prior approval by the FCT HREC.

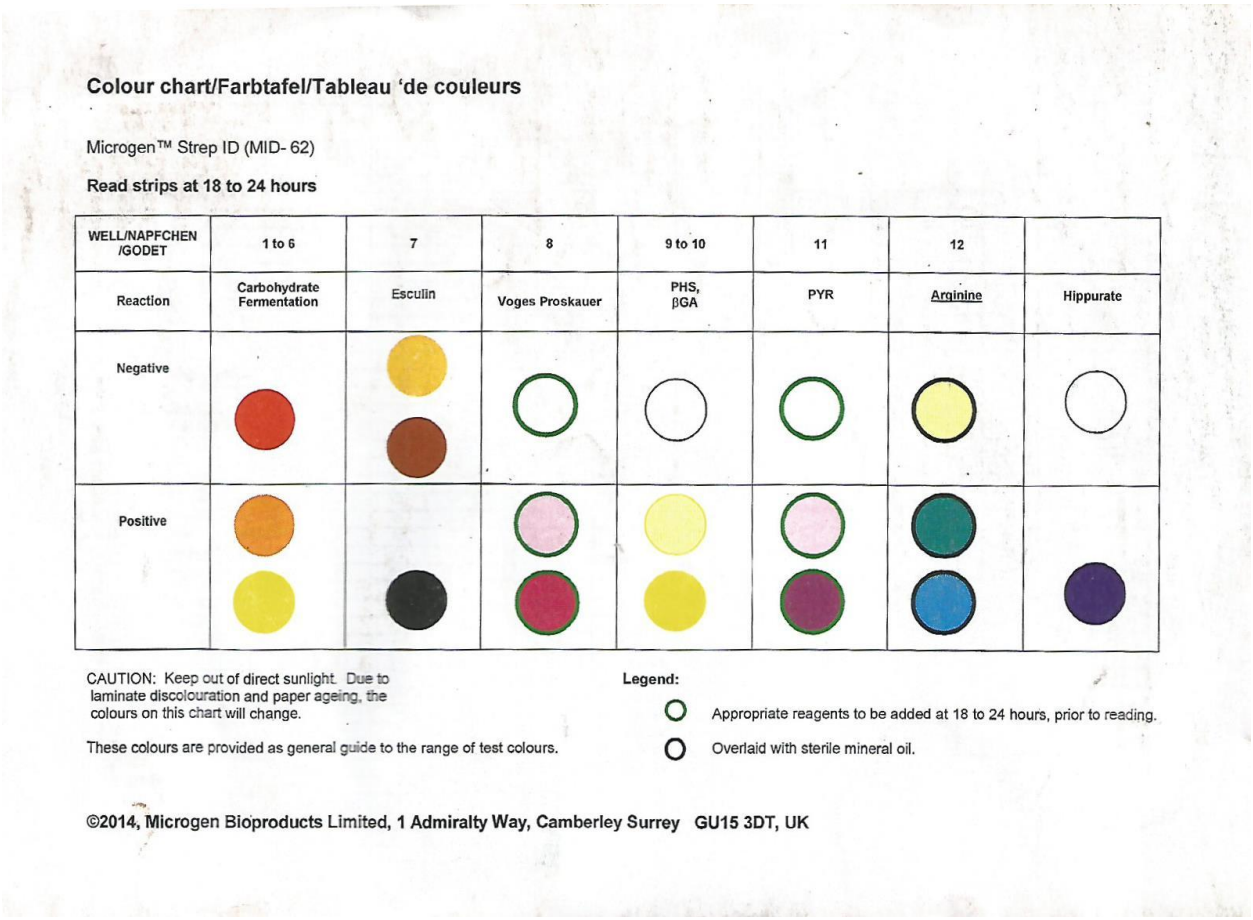
Problems: All adverse events or unexpected side effects arising from this project must be reported promptly to FCT HREC.

Renewal: This approval is valid until the expiration date. If you are continuing your project beyond the expiration date, endeavor to submit your annual report to FCT HREC early, and request for renewal of your approval to avoid disruption of your project.

Closure of Study: At the end of the project, a copy of the final report of the research should be forwarded to FCT HREC for record purposes, and to enable us close the project.

Ikwubiela S. Adem
Secretary, FCT HREC
December 16. 2014

Appendix IX: Microgen colour chart



Colour chart/Farbtafel/Tableau 'de couleurs

Microgen™ Strep ID (MID- 62)

Read strips at 18 to 24 hours

WELL/NAPFCHEN /GODET	1 to 6	7	8	9 to 10	11	12	
Reaction	Carbohydrate Fermentation	Esculin	Voges Proskauer	PHS, βGA	PYR	Arginine	Hippurate
Negative							
Positive							

CAUTION: Keep out of direct sunlight. Due to laminate discolouration and paper ageing, the colours on this chart will change.

These colours are provided as general guide to the range of test colours.

Legend:

Appropriate reagents to be added at 18 to 24 hours, prior to reading.

Overlaid with sterile mineral oil.

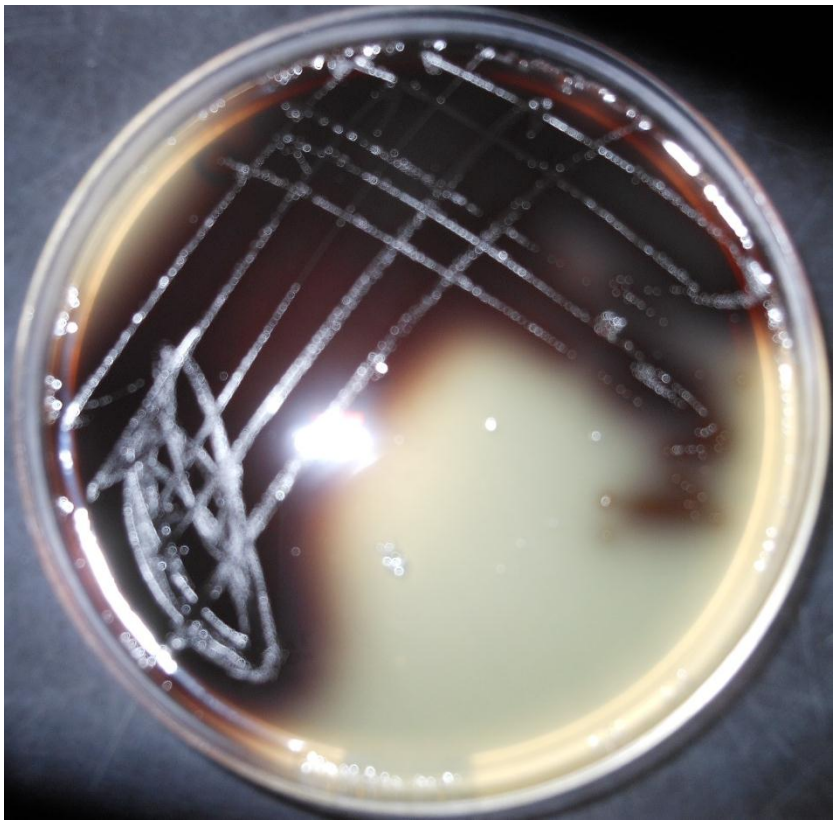
©2014, Microgen Bioproducts Limited, 1 Admiralty Way, Camberley Surrey GU15 3DT, UK

Appendix X: Microgen Test Kit




laminate discolouration and paper ageing, the
colours on this chart will change.
These colours are provided as general guide to the range of test colours.
©2014, Microgen Bioproducts Limited, 1 Admiralty Way, Cambe
MEL
SOR
INU
LAC
ARA
RIB
ESC
VP
PHS
BGA
PYR
ARG
STREP
28
666
1074
MEL
SOR
INU
LAC
ARA
RIB
ESC
VP
PHS
BGA
PYR
ARG
STREP
STREP

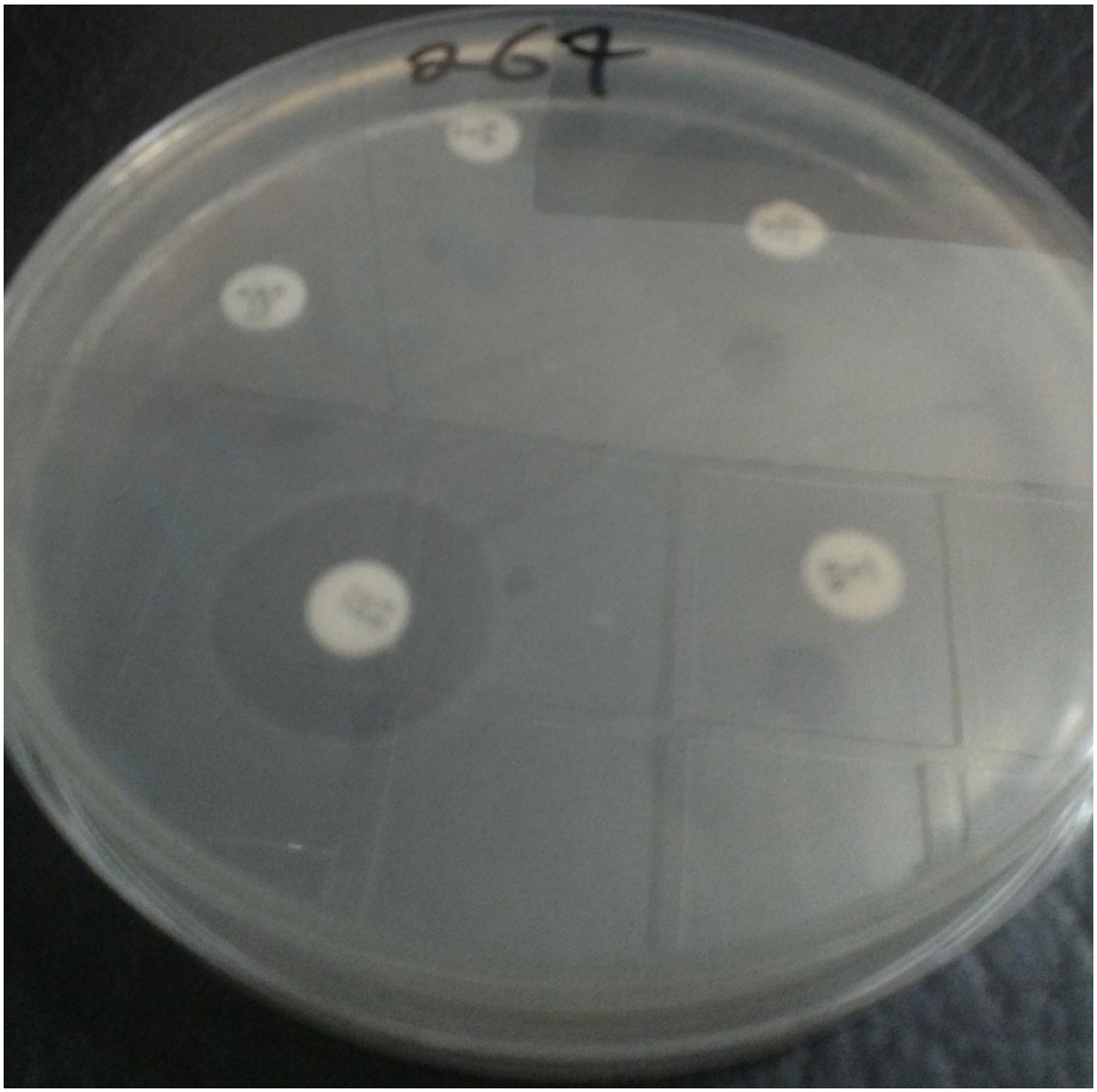
Appendix XI: Enterococcus growth on Bile Esculin Agar



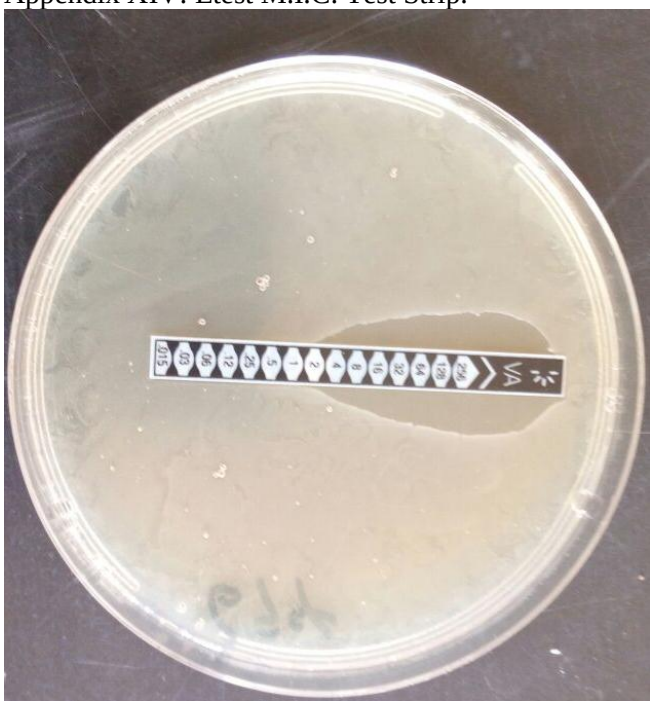
Appendix XII: Enterococcus growth on Blood Agar



Appendix XIII: Antibiotics zones of inhibition



Appendix XIV: Etest M.I.C. Test Strip.



Appendix XV: Work Bench



Appendix XVI: microgen^{tm} Strep ID Characterization of the Isolated *Enterococcus* spp.

No of Isolates	Hippurate	α-Haemolysis	B-Haemolysis	Melibiose	Sorbitol	Inulin	Lactose	Arabitol	Ribose	Esculin	Voges Proskauer	Alkaline Phosphotase	B-Galactosidase	PYR	Arginine	Octal code
49	+	-	-	-	+	-	+	-	+	+	+	-	-	+	+	42563
2	+	-	-	-	+	-	+	-	+	+	+	-	+	+	+	42567
3	+	-	-	-	-	-	+	-	+	+	+	+	-	+	+	40573
1	-	-	-	-	+	-	+	-	+	+	+	-	-	+	+	12563
2	+	-	+	-	+	-	+	-	+	+	+	-	+	+	+	52567
2	+	-	-	-	+	-	+	-	+	+	+	+	+	+	+	42577
16	+	-	-	+	-	-	+	+	+	+	+	-	+	+	+	44767
3	+	-	-	+	+	-	+	+	+	+	+	-	-	+	+	46763
1	+	-	-	+	-	-	+	+	+	+	+	+	+	+	+	44777
1	-	-	-	+	+	-	+	+	+	+	+	-	-	+	+	06763
1	+	-	-	+	+	-	+	+	+	+	+	-	+	+	+	46767
1	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+	56767
1	+	-	-	-	+	-	+	+	+	+	+	-	-	+	+	42763
5	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	06777
6	-	-	-	+	+	-	+	+	+	+	+	-	+	+	+	06767
1	+	-	-	+	-	-	+	-	+	+	-	-	-	+	+	44543
2	-	-	-	+	-	-	+	-	+	+	+	-	-	+	+	04567
2	+	-	-	+	-	+	+	+	+	+	+	-	-	+	+	45763
1	+	-	-	+	+	+	+	+	+	+	+	-	+	+	+	47767
1	-	-	-	+	-	-	+	+	+	+	-	-	+	+	-	04746
1	-	-	-	-	+	-	+	+	+	+	+	-	+	+	-	02766