Sudan University Of Science And Technology

College Of Graduate Studies

Phenotypic and Genotypic Detection of High Level Strepomycin Resistant Genes among *Enterococcus* species Isolated from Various Clinical Specimens in Khartoum State, Sudan

الكشف المظهري والوراثي للجينات المقاومة للستربتومايسين عالي التركيز بين أنواع المكورات المعوية المعزولة من عينات سريرية مختلفة في ولاية الخرطوم

A Dissertation Submitted in Partial Fulfillment of The Requirement of M.Sc Degree in Medical Laboratory Sciences (Microbiology)

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January, 2023

الآية

بِسَمِ ٱللهِ الرَحمنِ الرَحِيم

((يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِير))

صدق الله العظيم

سورة المجادلة، آية(11)

EDICATION

To my father,

Who taught me the meaning of life

To my mother,

Praying for me to successful

To my brother and sister,

For their support and kindness

To my friends and colleagues,

The persons, whom I love, respect and appreciate

To everyone from whom I learned

ACKNOWLEDGEMENTS

I thank the Almighty Allah for making it possible for me to complete this work successfully. Also I need to stress out my heartfelt gratitude to my supervisor **Prof.Yousif Fadlallah Hamedelni**l, **Emad Shawkat**, **Dr.Asgad Mohammed**, **Dr. Samar Mohammed** and my colleague **Enaam mokhlis** for their unlimited support.

ABSTRACT

Enterococci are not generally regarded as highly virulent bacterial pathogens; however, resistance to many antimicrobial drugs complicates treatment of Enterococcal infections, acquired resistance to high concentrations of glycopeptide antibiotics, has exacerbated this problem. The main issue related to enterococci is their antibiotic resistance, in fact, members of the genus *Enterococcus* are intrinsically resistant to many antimicrobials, and they have the ability to acquire and transfer genes encoding for resistance to different molecules. this cross sectional Study was aim to investigate high-level Stretomycin resistance genes in *Enterococcus* species by phenotypic and genotypic methods among hospitalized patients in Khartoum State . A total of four hundred (n=400) different clinical samples (urine, blood, wound swabs and body fluids) according to sites of infection were collected from patients attended to Ahmed Gasim Hospital, Almoalim Medical City and Rebat National Hospital during the period between June and August 2022.Their age ranged from1-75 years and mean of age 44.6years.

Then these samples were inoculated on MacConkey , blood agar and blood cuture bottle. Bacterial identification was carried out by different conventional methods. in this study 30 out of 400 (7.5%) were cofirmed as *E.faecalis* and *E.faecium* by primary and secondry biochemical tests. ,Antimicrobial susceptibilities of strains were investigated by kirby- Bauer method ,For detection of high-level Stretomycin resistance (HLSR) genes , polymerase chain reaction was used,Statistical analysis was done using SPSS version 25 .The current results found that prevalence of *E.feacalis* and *E.faecium* were 14 (3.5%) and 16 (4%) respectively, resistatance of *E.faecalis* and *E.faecium* to streptomycin were 6 (16.7%) and 11 (36.7%) respectively .*7 (23.3*%) and 1 (3.3%) was the frequency of *Aph(2)-1c* and *Aph(2)-1d* genes in isolated *Enterococcus,* and it was noticed that *Aph(2)-1c* and *Aph(2)-1d* were higher in *E.faecium* more than *E.faecalis.*

In conclusion, the study demonstrated that *E.faecium* was found to be more resistant to Streptomycin than *E.faecalis*, *Aph(2)-1c* frequency was high in *E.faecium* more than *E.faecalis* with significant statistical difference while in significant statistica difference was noticed with

Aph(2)-1d

مخلص الدراسة

لا تعتبر المكورات المعوية بشكل عام من مسببات الأمراض البكتيرية شديدة الضراوة ؛ ومع ذلك ، فإن المقاومة للعديد من الأدوية المضادة للميكروبات تعقد علاج عدوى المكورات المعوية ، وقد أدت المقاومة المكتسبة لتركيزات عالية من المضادات الحيوية غليكوببتيد إلى تفاقم هذه المشكلة. القضية الرئيسية المتعلقة بالمكورات المعوية هي مقاومتها للمضادات الحيوية ، في الواقع ، فإن اعضاء جنس المكورات المعوية مقاومون جوهريًا للعديد من مضادات الميكروبات ، ولديهم القدرة على اكتساب ونقل الجينات المشفرة لمقاومة الجزيئات المختلفة.

هدفت هذه الدراسة المقطعية إلى التحقيق في جينات مقاومة الاستربتومايسين عالي التركيز في أنواع المكورات المعوية بالطرق المظهرية والجينية بين المرضى في المستشفى في ولاية الخرطوم. تم جمع مجموعه أربعمائة (ن = 400) عينة سريرية مختلفة (بول ودم ومسحات جروح وسوائل الجسم) حسب مواقع الإصابة من المرضى الذين حضروا إلى مستشفى أحمد قاسم ومدينة المعلم الطبية ومستشفى الرباط الوطني خلال الفترة ما بين حزيران وأغسطس 2022 تراوحت أعمارهم بين1-75 سنة ومتوسط أعمارهم 44.6 سنة.

تم تلقيح هذه العينات في اجار ماكونكي واجار الدم وزجاجة قطع الدم. تم التعرف على البكتيريا بطرق تقليدية مختلفة. في هذه الدراسة ، تم تأكيد 30 من أصل 400 (7.5) على أنها *E.faecalis* و *E.faecium* عن طريق الاختبارات الكيميائية الحيوية الأولية والثانوية. ، تم فحص حساسية السلالات بمضادات الميكروبات بطريقة كيربي- باور ، للكشف عن مقاومة الجينات للاستربتومايسين عالي التركيز تم استخدام تفاعل البوليميراز المتسلسل ، تم إجراء التحليل الإحصائي باستخدام SPSS الإصدار 25.

وجدت النتائج الحالية أن انتشار بكتريا *E.feacalis* و *E .faecium* 14 (3.5%)و(4%)16 على التوالي ، وكانت مقاومة بكتريا *E.faecalis* و *E.faecium* للستربتومايسين (16.7%)6 و(36.7%) 11 على التوالي ، ووجد أن *E.faecium* أكثر كانت مقاومة الستربتومايسين أكثر من بكتريا *E.faecalis* .(23.3%) 7 و (3.3%) 1 كانت نسبة تواتر الجينات *Aph(2)-1c* و*Aph(2)-1d* في المعوية المعزولة ، ولوحظ أن *Aph(2)-1c* و *Aph(2)-1d* كانا أعلى في *E.faecium* أكثر من *E.faecalis* .

في الختام ، أوضحت الدراسة أن بكتيريا *E.faecium* كانت أكثر مقاومة للستربتومايسين من *E.faecalis* ، وكان تردد *Aph(2)-1c* مرتفعًا في *E.faecium* أكثر من *E.faecalis* مع وجود فرق إحصائي معنوي بينما لوحظ اختلاف إحصائي ضئيل مع *Aph(2)-1d*

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1. INTRODUCTION

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1. INTRODUCTION

1.1 Introduction

Enterococci are Gram-positive bacteria widely distributed in nature, mainly on the mucosal surfaces of humans and animals, but they are also found in soil, water, dairy and other food products, and on plants. Under certain circumstances they cause a variety of infections in humans. Most infections occur in hospitalised patients and these bacteria have recently been recognised as one of the most common causes of nosocomial.(García *et al* ,2019)

The main issue related to Enterococci is their antibiotic resistance, in fact, members of the genus *Enterococcus* are intrinsically resistant to many antimicrobials, and they have the ability to acquire and transfer genes encoding for resistance to different molecules. Previous investigations have been carried out on antibiotic resistance in *Enterococcus spp*. isolated from domestic and wild mammals. Moreover, the role of poultry as a source of antibiotic-resistant Enterococci has been documented, too (Cagnoli *et al.*, 2022). Because the use of antimicrobial agents, such as Cephalosporin, in hospitals has rapidly increased since 1980, *Enterococcus spp*., which shows a relative resistance to these agents, has been highlighted as an important causative bacterium of nosocomial infections. It ranked second to *E. coli* as a nosocomial pathogen in the US in 1995 (Song *et al.,* 2005). According to the National Nosocomial Infection Surveillance Study by the Korean Society of Nosocomical Infection Control in 1996, *Enterococcus spp*. accounts for 7.6% among the causative bacteria of nosocomial infections and ranks the fifth most frequently isolated pathogen. With the increasing isolation of *Enterococcus spp*., Enterococci resistant to vancomycin, which has very effectively been used in treating *Enterococcus spp*. infection until recently, were reported in France for the first time in1986 (Song *et al.,* 2005).However they can also be signifcant pathogens, causing surgical wound infection, bacteraemia, endocarditis, neonatal sepsis and rarely meningitis, the most common nosocomial infection caused by these organisms are urinary tract infection (associated with instrumentation and antimicrobials administration), followed by intra-abdominal and pelvic infection (Sood *et al*., 2008). The relative importance of *Enterococcus* as a pathogen has increased with the occurrence

of high-level resistance to multiple antimicrobial drugs, such as ampicillin, aminoglycosides and vancomycin. Several trends have been identified in the epidemiology of enterococcal infections: an increasing incidence of enterococcal infections particularly among the severely ill hospitalized patients, an increasing proportion of nosocomial Enterococcal infections caused by *Enterococcus faecium* and an increasing level of resistance to Ampicillin,Aminoglycosides,and Glycopeptides (Abamecha *et al*., 2015). The global problem of Enterococci is two-fold: an increasing rate of infection; and increasing resistance to antimicrobial agents such as β-lactams as well as high-level resistance to aminoglycosides and, more recently, Glycopeptides, particularly in *E. faecium* (Zouain and Araj,2001) Resistance to Aminoglycosides, especially high-level Aminoglycoside resistance, abolishes the synergy between cell-wall-active agents and aminoglycosides that is required for treatment of Enterococcal sepsis (Murray,1998)

Aminoglycoside resistance in Enterococci occurs in several ways, including decreased cell permeability, alteration of the ribosome binding site and production of aminoglycoside-modifying enzymes (AMEs). The most common mechanism of resistance is the acquisition of Aminoglycoside resistance genes encoding various AMEs that result in high-level resistance to aminoglycosides (MICs usually ≥2000 μg/mL), with the most clinically important of these genes being the bifunctional aac(6′)-Ie–aph(2″)-Ia gene (Gold, 2001). Aminoglycoside-modifying enzymes are mainly responsible from high-level Streptomycin resistance. So far detected Aminoglycoside resistance genes in Enterococci encoding Aminoglycoside-modifying enzymes are *aac (6')-Ie-aph (2'')-Ia, aph(2'')-Ib, aph(2'')-Ic, aph(2'')-Id, aph(3')-IIIa, aac(6')-Ii,ant(3'')-Ia, ant(4')-Ia, ant(6')-Ia* (ShepardandGilmo re,2002).

1.2. Rationale

Enterococcus, especially *E. faecalis* and *E. faecium,* have in recent years become one of the most common etiological factors in nosocomial infections. Although these bacteria are part of the normal flora of the gastrointestinal and genitourinary tracts and they are characterized by low pathogenicity, they can lead to serious infections such as bacteraemia, endocarditis, and infections of wounds and the urinary tract. *Enterococcu*s can survive in a hospital environment because of their resistance to a variety of antimicrobials. In addition to their intrinsic resistance to cephalosporins, lincosamides, low levels of aminoglycosides, and many β-lactams, *Enterococcus* are also able to acquire resistance to many antibiotics by means of mutations or as a result of the transfer of genes located in plasmids/transposons or due to the incorporation of integrons(Chen and Zerros,2009).The largest threats are strains resistant to glycopeptides (vancomycin-resistant *Enterococcus*,VRE) and high-level aminoglycoside resistance (HLAR) (Chow JW,2000) .The increasing role of *Enterococcus* infections and their increasing resistance to antibiotics call for constant monitoring of their susceptibility (Wieczorek *et al.,* 2014). Several studies have documented that Enterococcal infections are most commonly caused by the patient'sown commensal flora.Colonization may occur lon gbefore or immediately before infection, but either way, it plays a major role in the development of nosocomial infection (Abamecha *et al*., 2015). The aim of the study was to investigate high-level Stretomycin resistance genes in *Enterococcus species* by phenotypic and genotypic methods among hospitalized patients in khartoum state .

1.3. Objectives

1.3.1 General objective

To detect Streptomycin resistant genes of Enterococc*i* isolates from different clinical samples among hospitalized patients in Khartoum State.

1.3.2 Specific objectives

1- To isolate and identify Enterococci and other organisms from different clinical samples.

2- To assess the antibiotics profile susceptibility of Enterococci using kirby_Bauer method.

3- To detect the presence of Streptomycin resistant encoding genes (*aph21C* and *aph21D*) by multiplex PCR assay.

CHAPTER II
LITERATURE REVIEW

CHAPTER II

2. Literature Review

2.1. Enterococci

Enterococci are normal inhabitants of the alimentary canal and cause urinary tract infections, bacteremia, and endocarditis. They are also commonly recovered from infections of the abdomen, the pelvis, the biliary tract, and wounds, settings in which polymicrobial flora are common. Enterococci less frequently cause infections of other sites, for example, bone, joints, and the meninges (EL-Ghazawy,2016) . *E.faecalis* causes the majority of enterococcal infections overall. *E. faecium* causes a substantial proportion of enterococcal infections, particularly infections acquired in the hospital setting (Said and Abdelmegeed,2020). Data collected by the National Nosocomial Infections Surveillance System on infections in patients in intensive care units from 1989 through 1998 showed that enterococci were the third most common bloodstream isolate, the third most common urinary isolate, the most common isolate from surgical site infections,and the fourth most common isolate from all sites. Enterococci are primarily opportunistic pathogens. The increasing severity of illness in hospitalized patients has contributed to the ascendance of Enterococci as nosocomial pathogens. Progress in medical technology and treatment, such as the use of various intravascular access devices, implanted prosthetic devices, cytotoxic chemotherapy, and immunosuppression, has magnified the impact of organisms of relatively low virulence, such as enterococci. Of critical import is the intensive use of relatively broad-spectrum antibiotics in the hospital, which provides selective pressure favoring the growth of intrinsically drug-resistant commensal organisms such as Enterococci (Gold, 2001).

Enterococcus is a Gram-positive and Catalase-negative bacterium. It is an important gastrointestinal tract normal flora of most warm-blooded animals and humans, however, different species of Gram-positive cocci could be an opportunistic pathogen causing various infectious diseases, *Enterococcus* species especially *Enterococcus faecium* and *Enterococcus faecalis* are two common causes of urinary tract infection (Rostkowska *et al.*, 2020), inflammation of the lining of the heart and its valves, intra-abdominal abscesses, wound infections, bacteremia, and sepsis in human,. The inherent resistance to several antibiotics and

their ability to cause infections has placed Enterococci on the pedestal as an important hospital-acquired pathogen (Wada *et al.,* 2020)

Significant level of Enterococci colonization might be found among persons not related to the health care settings because Enterococci from animal sources and humans foods of animal origin play a huge part in colonization and infection in humans (Mathur and Singh., 2013). Infections due to enterococci have been reported to be linked with increased rates of morbidity, mortality, a longer length of hospital stays, higher healthcare expenses due to the reduced number of therapeutic options and the patients infected with resistant organisms require a higher frequency of surgical interventions for infection control (Shrestha *et al.,* 2021).

In humans, Enterococcal infections may be caused by at least 12 species but most clinical infections are due to either *Enterococcus faecalis* or *E. faecium* (Sood *et al.,* 2008). *E. faecalis* is the most common cause (80–90%) followed by *E. faecium* (10–15%). Occasional infections are due to *Enterococcus gallinarum*, *Enterococcus rafnosus*, *Enterococcus casseliflavus*, *Enterococcus avium*, *Enterococcus pseudoavium*, *Enterococcus malodoratus*, *Enterococcus mundtii*, *Enterococcus durans*, and *Enterococcus hirae*. The proportion of isolates of motile Enterococci (*E. gallinarum*, *E. casseliflavus*) remained low, i.e. less than two per cent. It is important to probably recognize the motile Enterococci because they are intrinsically resistant to vancomycin (low level) and inappropriate treatment with vancomycin may contribute to morbidity and mortality (Abamecha *et al*., 2015).

2.1.1 *E.Feacalis*

Several virulence factors have been found to render specific *E. faecalis* strains more apt to cause disease or worsen disease symptoms. Enterococcal surface protein (*esp*) has been found to further adherence and colonization of cells and abiotic surfaces. Gelatinase (*gelE*) is an extracellular metalloprotease, able to hydrolyze gelatin, collagen and hemoglobin, which has also been reported to contribute to bacterial adherence and biofilm formation (Shrestha *et al.,* 2021).

Aggregation substance (AS) has also been reported to increase adherence and invasion of eukaryotic cells as well as promote biofilm formation. Hyaluronidase (*hyl*), has been associated with virulence of Enterococci in host tissue invasion. Furthermore, *E. faecalis* endocarditis antigen A (e*faA*) has been presumed to contribute to the adhesion of *E. faecalis* to heart cells in endocarditis Finally, cytolysin (*cyl*, beta-hemolysin) is a potent bacteriocin that exacerbates

Enterococcal infections in humans. It is capable of lysing many prokaryotic cells, as well as erythrocytes and other eukaryotic cells (Anderson *et al.,* 2016).

2.1.2 *E. faecium*

E. faecium has rapidly evolved as a worldwide nosocomial pathogen by successfully adapting to conditions in a nosocomial setting and acquiring resistance against glycopeptides. The resistance genes against glycopeptides are organized in van operons located on mobile genetic elements (MGEs). The operons include regulatory genes controlling the expression of ligase genes conferring resistance to glycopeptides, of which th*e vanA and vanB* genes are the most common. Although the alcohol tolerance experiments were established with a concentration of 23%, lower than the 70% which is used in hand alcohols, these tolerant *E. faecium* isolates still survived better than the less tolerant isolates after the 70% isopropanolol surface disinfection. This exemplifies how *E. faecium* can adapt to environmental changes such as an increased use of hand alcohols (Zhou *et al*., 2020).

2.2. Virulence of Enterococci

Infections associated with enterococci used to be treated successfully with antibiotic treatments, but many antibiotics are currently less effective resulting in longer hospitalization periods, treatment failure and significant financial burdens (Diaz Granados *et al*, 2005). This rapid and global dissemination of multidrug resistant strains were attributed to the imprudent and overuse of antibiotics in human and veterinary practices as well as inappropriate use in animal production (Kürekci *et al*, 2016)

Enterococci are nosocomial pathogen with multiple-drug resistance by intrinsic and extrinsic mechanisms. aminoglycosides along with cell wall inhibitors are given clinically for treating enterococcal infections (Padmasini *et al.,* 2014). Enterococci possess virulence genes including *ace, PAI, asa1, sprE, cylA, efaA, esp, gelE and hyl* encoding collagenbinding protein, pathogenicity islands, aggregation substance, serine protease, cytolysin, endocarditis antigen, enterococcal surface protein, gelatinase and hyaluronidase, respectively. The gelatinase is an extracellular metalloprotease that hydrolyzes collagen, gelatin, and small peptides. The enterococcal cytolysin is a member of bacteriocin family which lyses bacterial and eukaryotic cells in response to quorum sensing signals (Ferguson *et al.,* 2016). The enterococcal surface protein seems to contribute in the colonization and persistence of Enterococci in ascending

infections of the urinary tract and biofilm formation. Hyaluronidase is an important factor in nasopharyngeal colonization and pneumonia (Haghi *et al.*, 2019).

The virulence of Enterococci is known to be conferred by various factors including but not limited to cytolysin (CylLLLSM), enterococcal surface protein (Esp), aggregation substance (AS), gelatinase (GelE), *E. faecium* cell wall adhesion factors and sex pheromones Cob and Ccf. The enterococcal surface protein is hypothesised to be involved in immune evasion. Cytolysin has a role in progression of enterococcal infection by its haemolytic activity as well as bactericidal activity against Gram positive bacteria. As helps in mating and conjugation at the site of infection, resulting in accumulation of bacteria at the site of infection. GelE hydrolyses haemoglobin and other peptides resulting in inflammation, and the sex pheromones can transfer plasmid carrying one or more antibiotic resistant genes (Jahan and Holly, 2014). Various factors that increase the risk of infection with VRE in a medical intensive care unit (ICU) include prolonged hospitalisation, younger age, use of Ceftriaxone and vancomycin.18 Hospital workers can also transmit VRE as it can survive on fingers for about 30 minutes even after washing hands. Companion animals and pets can also be a reservoir for VRE. A recent report revealed the frequency of vancomycin-resistant enterococci to be 11.3% from a tertiary care hospital of Pakistan (Raza *et al.*, 2018). Although Enterococci are intrinsically resistant to low levels of aminoglycosides, high level resistance to aminoglycosides (MIC $\geq$ 2000ug/ml) is mediated by acquisition of genes encoding AMEs, high level Gentamicin resistance (MIC $\geq$ 500 ug/ml) in Enterococci eliminates the synergistic activity of Gentamicin when combined with a cell wall active agent, such as Ampicillin or Vancomycin, high level Streptomycin and Kanamycin resistance in Enterococci are mediated by *aph(3)*-IIIa gene encoding aminoglycoside phosphotransferase enzyme *APH(3)*-IIIa, high level aminoglycoside resistance has been reported, however studies on prevalence of these resistance genes are limited (Padmasini *et al.*, 2014).

2.3. *Enterococcus* resistance genes

Enterococci have changed from commensal intestine organisms in human beings to a significant cause of infection. These bacteria are important causes of nosocomial infections, such as urinary tract infections, bacteremia, and endocarditis. Recently, Enterococci have become considerably resistant to a broad range of antimicrobial agents, particularly Glycopeptides, β-lactam, and Aminoglycosides. Due to inappropriate use of antibiotics in nosocomial infections, the resistance

rate is increasing. The prevalence of antibiotic resistance among *E. faecium* is higher than in *E. faecalis*. Enterococci are either intrinsically resistant to antibiotics or acquire the resistance genes. The resistance is due to inadequate transfer of antibiotics across the cytoplasmic membranes of bacteria. Unfortunately, Enterococci with high-level aminoglycoside-resistance (HLAR) (MIC > 500 µg/mL) do not seem to be sensitive to this synergistic effect, making treatment more difficult. HLAR in Enterococci is due to aminoglycoside-modifying enzymes (AMEs). The most common genes coding AME are *aac(6')-aph(2")* and *aph(3)-IIIa*. AMEs eliminate the synergism effect of aminoglycosides when combined with a cell-wall-active agent. *Aac(6')aph(2")* is the most common gene causing HLGR in Enterococci, and *aph(3)-IIIa* is common in high-level Kanamycin and Streptomycin resistance (Khani *et al.*, 2016).

Aminoglycoside-modifying enzymes are mainly responsible from high-level Streptomycin resistance.So far detected aminoglycoside resistance genes in Enterococci encoding Aminoglycoside-modifying enzymes are *aac (6')-Ie-aph (2'')-Ia, aph(2'')-Ib, aph(2'')-Ic, aph(2'')-Id, aph(3')-IIIa, aac(6')-Ii,ant(3'')-Ia, ant(4')-Ia, ant(6')-Ia* (Shepardand Gilmore,2002).

2.4. Epidemiology

Enterococcus species have become the second or third leading cause of nosocomial urinary tract infections (UTIs), wound infections (mostly surgical, decubitus ulcers, and burn wounds), and bacteremia in the United States. UTIs are the most common of the Enterococcal infections: *Enterococcus* species have been implicated in approximately 10% of all UTIs and in up to approximately 16% of nosocomial UTIs. Enterococcal bacteremia is frequently associated with metastatic abscesses in multiple organs and high mortality rates. *Enterococcus* have also been considered an important cause of endocarditis; they are estimated to account for about 20% of the cases of native valve bacterial endocarditis and for about 6–7% of prosthetic valve endocarditis. Endocarditis remains among the most difficult to treat Enterococcal infections because of limitations on bactericidal antimicrobial therapy for Enterococcal infections, especially when caused by vancomycin-resistant *Enterococcus* (VRE). There is also a growing concern about the role of the *Enterococcus* species in endodontic and implant- and medical device-associated infections (Ferede *et al*., 2018).

The distribution of HLAR genes varies depending on the different geographical areas and is widely distributed across Asia, North America, and Europe. These genes have been reported to

be either integrated within the mobile genetic elements or plasmid-encoded, in which both are transferable via horizontal gene transfer (Moussa *et al.,* 2019).

The epidemiology of Enterococci is not fully understood since there are striking diferences among different species of resistant isolates obtained from various geographic locations. Despite the fact that Enterococci have been considered to be relatively low virulent in the past few years, they are among all nosocomial pathogens that have emerged as a signifcant concern, the prevalence rate in Egypt (3.3%), in Bangladesh (3.2%), in India (2.3%) and in Asian pacifc (3.6%), in Kenya (0.22%), while the prevalence in USA and Canada, 18.0% and 21.2%, respectively (Ferede *et al*., 2018).

2.5. Diagnosis

The facultative anaerobic Gram-positive *Enterococcus spp*. normally colonize the gastrointestinal tract, oral cavity, and vaginal tract. Enterococci are among the major agents associated with nosocomial infections particularly in burn patients presenting with bacteremia, urinary tract infections and endocarditis (Hashem *et al,*2017). The US National Nosocomial Infection Surveillance (NNIS) system, has ranked Enterococci among the top three most common pathogens of nosocomial infections and the leading cause of nosocomial infections in burn patients (Labibzadeh *et al.,* 2018).

The recent introduction of many of these media has led to improved accuracy and faster detection of target microorganisms. In this sense, a new selective and differential medium (Chromocult Enterococci agar; Merck, Darmstadt, Germany) has been designed by Merck for the isolation and enumeration of *Enterococcus spp*., using a chromogenic mix in a selective agar. Enterococci cleave chromogenic substrates in this medium. Owing to the b-Dglucosidase activity present in enterococci, the chromogenic mix is cleaved and the red colour of the colonies indicates the presence of Enterococci. Other b-D-glucosidase-producing organisms are suppressed by the sodium azide content of the media, sodium azide being an inhibitor of enzyme systems (catalase, cytochrome c oxidase) in electron transport (Miranda *et al.,* 2005).

A range of different media has been used to isolate Enterococci, including 10% horse blood agar with Aztreonam and Amphotericin, Colistin Nalidixic acid agar, Mueller–Hinton agar with polymyxin and Streptomycin, Kanamycin Aesculin azide agar, 5% horse blood agar with neomycin, bile aesculin azide agar, Campylobacter blood agar with Clindamycin, Cefalexin Aztreonam arabinose agar and Colistin Nalidixic acid aesculin azide agar. PCR methods of

screening for GRE in faeces have the advantage that results can be available within a few hours of receipt of the sample. However, extraction of DNA is necessary to remove inhibitory substances in the sample, and the method may not be cost-effective when dealing with large numbers of specimens or when the prevalence is low. In these situations, the use of PCR methods on enrichment broths or colonies on selective medium may be appropriate. If typing of isolates for epidemiological purposes is required, culture will also be necessary. There are no widely accepted standards for evaluation of selective media for use in screening for GRE. However ,enterococcosel agar (EA) and broth (EB), which are bileaesculin-azide formulations, have been used in several studies. Oxoid vancomycin-resistant E*nterococcus* enrichment broth (VEB) and selective agar (VSA) are novel in that they are Aesculin-azide media with Meropenem as an additional selective agent (Brown and Walpole, 2003).

2.6. Treatment

Enterococci became recognized as important major nosocomial pathogens due to their natural intrinsic resistance to several antimicrobials (e.g., Penicillin, Ampicillin, and most Cephalosporins) and capacity to quickly acquire virulence and multidrug resistance determinants. In fact, enterococci can rapidly develop resistance following the clinical introduction of antimicrobial agents, including last resort antimicrobials used to treat Glycopeptide and multidrug resistance (such as Quinupristin-Dalfopristin, Linezolid, Daptomycin, and Tigecycline) (Mohamed and Keith, 2018).

In the treatment of enterococcal infections, the use of a cell wall active agent such as a Penicillin or Vancomycin with an aminoglycoside results in synergistic bactericidal activity, the increasingly frequent occurrence of HLAR strains, caused by production of aminoglycoside-modifying enzymes (AMEs), makes standard therapy with aminoglycosides and β- lactams impossible. Two of the most prevalent AME genes, *aac(6')-Ie* and *aph(2'')-Ia*, are located on mobile genetic elements and are widespread among *Enterococcus*. These genes encode a bifunctional enzyme, *AAC(6')-Ie-APH(2'')-Ia*, that confers resistance to a broad spectrum of aminoglycosides. Recently, new AME genes such as *aph(2'')-Ib*, *aph(2'')-Ic* and *aph(2'')-Id* have been detected and they are responsible for Gentamicin resistance; high-level Streptomycin and Kanamycin resistance are mediated by the *aph(3')- IIIa* gene. At present, over 70 such enzymes have been discovered. Therefore, distinguish three different phenotypes: HLSR (high-level Streptomycin resistance), which determines resistance only for Streptomycin, HLGR (high-

level Gentamicin resistance), which determines resistance to all aminoglycosides except streptomycin, and HLAR (high-level aminoglycoside resistance), which means resistance to all aminoglycosides. Heretofore, testing HLGR, HLSR, and HLAR in *Enterococcus* has required only the use of high concentrations of Gentamicin and Streptomycin (Wieczorek *et al.*, 2014).

Enterococci are prime examples of organisms with an impressive array of genetic versatility and unparalleled ability to recruit and express antimicrobial resistance determinants. These organisms have adapted through time to outcompete other bacteria in a specific biological niche such as the GI tract of eukaryotic organisms. From a simple commensal and tamed member of the intestinal microbiota, enterococci now have risen in importance and have become one of the leading causes of intra-hospital infections (Miller *et al*., 2014).

2.6.1. Aminoglycosides

The treatment of choice for serious enterococcal infections is an aminoglycoside in combination with a cell wall active agent. However, high-level aminoglycoside resistance (HLAR) is responsible for loss of synergy between agents active on the cell wall and aminoglycosides. In enterococci, HLAR is mediated by aminoglycoside-modifying enzymes (AMEs). There are three classes of AMEs: Nacetyltransferases (AAC), O-adenylyltransferases (ANT), O-phosphotransferases (APH). The rate of Enterococci with HLAR and the distribution of AMEs vary across countries. Knowledge of the frequency of these resistance genes is important to inform the management of enterococcal infections (El-Mahdy *et al.,* 2018).

Aminoglycosides are regarded as vital drugs for the treatment of life-threatening infections (United States Pharmacopeial Convention 2008). High level resistance to aminoglycosides in bacteria may lead to ineffective therapeutic crisis. Many countries have banned the administration of certain antibiotics in animal husbandry due to their preferred usage in human medicine. However, aminoglycosides are recommended for therapy and prophylaxis in farm animals owing to their efficient bactericidal mode of action against Gram negative and Gram-positive bacteria (United States Pharmacopeial Convention 2008) (Jaimee and Halami, 2016).

2.6.2. Resistance to Aminoglycosides

Reports on the role of Enterococci in infections dates back as early as 1906 from a case of endocarditis. Enterococci have now emerged as nososcomial pathogens. In-spite of their low virulence, they are now being reported in nososcomial infections. Their multidrug resistance limits the scope of specifc antimicrobial therapy. Enterococci need to be identifed to

the species level to establish the epidemiological patterns in hospitals. Importance of Enterococci lies in their resistance to β-lactams and amino-glycosides; in particularly carrying intrinsic & acquired resistance determinants leading to life threatening infections (Shanmukhappa *et al*., 2015). Enterococci display intrinsic tolerance (manifested by the lack of bactericidal activity) to the aminoglycosides. This phenomenon seems to be mediated by two main factors: poor uptake of the antibiotic requiring higher concentrations to promote entrance into the intracellular space and inactivation by covalent modification of the hydroxyl or amino groups of the aminoglycoside molecule carried out by naturally occurring enterococcal enzymes, creating a steric hindrance and decreasing the binding to the ribosomal target. Indeed, *E. faecium* possess a chromosomally encoded 6′-acetyltransferase enzyme (*AAC*(6′)-Ii) capable of modifying tobramycin, sisomicin, kanamycin and netilmicin (Miller *et al*., 2014).

The main mechanism of glycopeptide resistance in Enterococci involves the alteration of the peptidoglycan synthesis pathway, specifically the substitution of D-Alanine-D-Alanine (D-Ala-D-Ala), to either D-AlanineD-Lactate (D-Ala-D-Lac) or D- Alanine-D-Serine (D-Ala-DSer) (Courvalin, 2006). Such alterations can lead to variable expressions of glycopeptide resistance. For example, the respective altered D-Ala-D-Lac and D-Ala-D-Ser leads to less binding affinity of glycopeptide drugs compared to the normal cell wall precursors D-Ala-D-Ala; *1000-fold decreased binding affinity for D-Ala-D-lac and *7-fold for D-Ala-D-Ser. The ability to induce such alterations is related to several genes harbored on mobile genetic elements and/or chromosomally encoded regions of different *Enterococcus* species (Mohamed and Keith, 2018). Bacterial resistance to aminoglycosides occurs due to mutations, impaired transport and acquired resistance. The most common mode of aminoglycoside resistance in Gram-positive bacteria is the acquisition of aminoglycoside-modifying genes. Clinically, the bifunctional gene aac(6′)Ie-aph(2″)Ia confers resistance to almost all aminoglycosides except Streptomycin. It has been associated with high-level Gentamicin resistance (HLGR) and high level kanamycin resistance (HLKR) with minimum inhibitory concentration (MIC) values >500 μg/mL. This bifunctional gene is highly prevalent among clinical strains of *Staphylococcus* and *Enterococcus spp,* and its frequent spread among Gram-positive organisms has been attributed to its lower G+C content (Jaimee and Halami, 2016).

Only two aminoglycosides (Gentamicin and Streptomycin) are reliably used in clinical practice (for synergism with β-lactams) due to the fact that these compounds are not readily affected by

intrinsic enzymes produced by Enterococci. However, high-level resistance to aminoglycosides, defined as an MIC >2000 μg/ml for Streptomycin and 500 μg/ml for Gentamicin (agar dilution method), abolishes the synergistic effect of these compounds. Resistance to Streptomycin occurs by one of two mechanisms. 'Absolute' inhibition at the level of the ribosome was demonstrated in clinical isolates that possessed MICs to Streptomycin >128,000 μg/ml by precipitating the ribosomal complex and showing that they were able to translate polyU RNA (through the quantification of radiolabeled phenyl-alanine) in the presence of the drug. Enzymatic inactivation due to acquisition of a Streptomycin adenyltransferase confers high-level resistance and abolishes synergy (Miller *et al* , 2014). Enterococci develop resistance to aminoglycoside by two different mechanisms. The moderate level of resistance usually develops due to low permeability. This type of resistance can be eliminated by using aminoglycoside with beta lactam group antibiotics, which inhibit cell wall synthesis. High-level resistance (HLR) occurs due to the result of changes in the ribosome binding site of aminoglycosides or the synthesis of enzymes that inactivate aminoglycoside. HLR is often dependent on the production of transferable plasmid-mediated aminoglycoside inactivating enzymes. The most common aminoglycoside-modifying enzyme in enterococci is *APH (2'')-AAC (6')* and this enzyme is encoded by *aac(6') -aph(2'')* genes and consists of two enzymes fused together. This enzyme is responsible for resistance to all aminoglycosides except streptomycin (Shanmukhappa *et al*,2015). The aminoglycoside resistance in enterococci leads to abortion of treatment wherefore this resistance causes elimination of synergistic effect between beta-lactams and aminoglycosides. As well as, recently major problems have been encountered in the treatment of emerging vancomycin-resistant Enterococci (VRE). New pathogens resistant to vancomycin generally lead to difficulty in treatment because they are also resistant to other antibiotics. Resistance to Glycopeptide group antibiotics was first reported in 1988 and then high-level vancomycin-and teicoplanin-resistant strains have spread worldwide (Miranda *et al.*, 2005)..

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

3. Materials and Methods

3.1. Study design

This study is descriptive cross sectional hospital based study

3.2. Study area

This study was conducted in khartoum state (Ahmed Gasim Hospital, Almoalim Medical City and Rebat National Hospital).

3.3. Study population and duration

Out-patient and in-patients admitted to Ahmed Gasim Hospital, Almoalim Medical City and Rebat National Hospital were selected for this study in the period between May and December 2022.

3.4. Inclusion criteria

Out-patients with syptom of infection, Each inpatient who have been hospitalized, patients who have received long-term antibiotics, patients who have undergone invasive procedures such as catheterization , patients with a history of surgery in the chest or abdomen and the patient who sign the written consent form was included in this study.

3.5. Exclusion criteria

The patient in coma and the patient who donot sign the written consent forms was exclueded from this study.

3.6. Ethical considerations

Ethical approval to conduct the study was granted by the Ethics committee of Sudan University of Science and Technology and Ministry of health. Information was given to study participants about the proposed and procedure of study, informed consents were obtained at the beginning of the study.

3.7.Sampling

3.7.1.Sample size

Four hundred clinical samples were collected from different hospital during June and August 2022.(n=400).

3.7.2.Sampling technique

Clinical samples were collected from each study participant aseptically. About 5 ml of the blood sample was collected from children and dispensed into blood culture bottle prepared with 25 ml of Tryptic Soya Broth (FL Medical, Italy) aseptically. Ten ml of freshly voided midstream urine specimen was collected using wide mouth,leak-proof, sterile, plastic container under the supervision of the principal investigator and processed within 2 hours of collection. Approximately 5 ml of cerebrospinal fluid (CSF) sample was collected aseptically into sterile tube by lumbar or ventricular puncture performed by a physician and processed within one hour of collection. Wound swab, pus, eye, and ear discharges were obtained using sterile cotton tip applicator stick aseptically.

3.8. Data collection

A structural questionnaire was conducted to collect demographical and clinical information (Appendix).

3.9 EXperimental work

3.9.1 Sample collection

Four hundred spcimens of different clinical samples (urine, blood, wound swab and body fluids) according to sites of infection were collected from patients and cultured directly in the lab.

Enterocci isolated from the sample was peserved in 80％ Glycerol and freezed into －70℃ For further analysis.

3.9.2. Laboratory procedures

3.9.2.1.Culture procedure

Samples were culture on MacConkey agar and Blood agar using ordinary streaking method. Inoculated plates were incubated at 37˚C and examine after 48 to 72 hrs .

3.9.2.2. Gram's stain

Smears were made from each sample and stained using the Gram staining procedure. Fixed dry smear was covered with crystal violet stain, for 30–60 seconds, rapidly washed off the stain with clean water, then covered with lugol’s iodine, for 30–60 seconds, washed off and decolorized rapidly (few seconds) with acetone–alcohol, then washed immediately with clean water, the smear was covered with safranin stain, for 2 minutes and wash off, the back of the slide was wiped , and examined microscopically(Cheesbrough, 2006)

3.9.2.3 Biochemical Tests for Enteococci

3.9.2.3.1 Catalase Test

Procedure (tube method)

drop of the catalase reagent 3% Hydrogen peroxide was placed on test tube. Using wooden stick, a small amount of bacteria from 24-hour pure culture was placed into the test tube. An immediate bubbles formation indicated a positive result and no bubbles formation indicated catalase negative result (Cheesbroug, 2006).

3.9.2.3.2. Bile Esculin Test

Procedure: The test organism was inoculated into the slope surface of bile esculin medium using sterile straight wire, the medium was incubated at 37 °C for 24 hrs, change in color of the indicator to black means a positive result (Tille and Forbes ,2014).

3.9.2.3.3. Litmus Milk Test

Procedure: The test organism was inoculated with 4 drops of a 24-hour broth culture into litmus milk medium Incubated at 35°-37°C in ambient air for 24-48hours. Observe daily for seven days for alkaline reaction (litmus turns blue),acid reaction (litmus turns pink), indicator reduction, acid clot, rennet clot, and peptonization (clearing). (Fobers *et al*,2007)

3.9.2.3.4. Mannitol salt agar :

Enterococcus faecalis is one of the few types of bacteria that can grow in a very salty environment, which then helps prevent it from being crowded out by other bacteria. Because *Enterococcus faecalis* produces lactic acid as part of its metabolism, when it uses the mannitol for energy (mannitol is a type of sugar), acid is secreted. This acid secretion changes the pH of the surrounding agar, which causes it to change from a pink color to yellow. As a result, *Enterococcus faecalis* will cause spots of yellow to appear on a mannitol salt plate..(Forbes *et al*., 2007)

3.9.2.4. In vitro antibiotic sensitivity testing

Kirby-Bauer method was used in the current study,Prepared the inoculum from the primary culture plate by touching with a loop the tops of each of 3 – 5 colonies of similar appearance of the organism to be tested and transfered this growth to atube of saline, Compared the tube with the 0.5 McFarland turbidity standard, the antibiotic discs used were from Himedia (Himedia Laboratories Pvt. Ltd, Mumbai 400086, India), (Nitrofrontoin 300ug, Ampicillin 10ug, Streptomycin 300ug, Levofloxacin 5ug, Penicillin 10ug, Vancomycin 30ug, Gentamycin 120ug,

Ciprofloxacin 5ug, Norofloxacin 10ug), the antibiotic discs were placed into Muller Hinton Agar (Himedia Laboratories Pvt. Ltd, Mumbai 400086, India). The distance between two adjacent discs was at least 20 mm and from the edge of the plate was 15 mm, media were incubated aerobically for 24 hrs at 37 ℃, after 24 hrs of incubation the diameter of zone inhibition was measured and compared with *Enteroccus faecais* (VRE ATCC 51575) and the pushed tables of the control strains according to CLSI guidelines.

3.9.2.5.DNA extraction

Bacterial genomic DNA was extracted manually, by boiling methods from fresh overnight incubated nutrient slope, a loop full of culture was suspended in 200 µl of dH2O in 1.5 Eppendorf tube, boiled for 10 min at 100 ℃ in thermal block incubator, vortexed then cooling at -20 ℃ for 10 min following by centrifugation for 10 min at 12000xg. Supernatant carefully was collected and store at -20 ℃ for further use.

3.9.2.6. Pcr analysis

The reaction mix with a total volume of 25 µl included: 5 µl of master mix solution (Qiagen HotStarTaq) and 2 µl from each specific antisense primers, for *Aph(2)-1c* and *Aph(2)-1d*, 1µl of the template DNA, and 15 µl of distilled water and the mixture was gently mixed, and then cycled in automated thermocycler. (Qu *et al* , 2006).

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The pcr for *aph(2)1c* gene was performed using the forward primer

- *Aph(2)-1c* : **F 5′** _GAAGTGATGGAAATCCCTTCGTG_ **3′**

and reverse primer

R 3'- GCTCTAACCCTTCAGAAAACATCTCTGCT-**5'**

and for *Aph(2)1d* gene was performed using forward

- *Aph(2)-1d*: **F 5'**_ GGTGGTTTTTACAGGAATGCCATC_**3'** and reverse primer

R 3'- CCCTCTTCATACCAATCCATATAACC_**5'** .(Qu *et al* , 2006).

Amplification was done in PCR machine Biorad included initial denaturation at 95 ºC for 2 min followed by 40 cycles with the following program: 94 ºC for 30 sec, 50 ºC for 30 sec and 72 ºC for 30 sec, and final extension step at 72 ºC for 5 min.

3.9.2.7.Visualization of product

Absence or presence of PCR products was visualized by electrophoresis. The PCR products were loaded in 2% Agarose gel. The gel was prepared as followed: 1.0 g of the agarose was added to 50 ml of 1x Tris Borate EDAT buffer. The mixture was heated until a homogenous solution was formed, then 2.5 µl of 10 µg/µl ethedium bromide was added to the mixture. 35 ml of the gel were added to the gel box and 8 µl of PCR amplified DNA loaded into the agarose gel well. 5µl of the ladder was added to the first well. After that the gel was run at 60 V, current 35 A for 50 min. The gel was then examined in Gel documentation system.

3.10. Statistical analysis

The data were analyzed using statistical package for social science (SPSS). Categorical variables were described by number and percentage (N,%) where continuous variable were described by mean and standard deviation (mean, SD).chi square test was done for the analysis of categorical variables. A p-value of <0.05 was considered statistically significant .

CHAPTER IV
RESULTS AND DISCUSSION

CHAPTER IV

4. Results and Discussion

4.1 Results

The current study was cross sectional study enrolled 400 different clinical samples from patients attended Ahmed Gasim Hospital, Almoalim Medical City and Rebat National Hospital, during June and August 2022, their age range < 1 year and > 45 years old, 230 (57.50%) males and 170 (42.50%) females the mean of their age was 44.6 years , to detect the genes responsible for resistant of Aminoglycoside in *Enterococcus* species isolated from various clinical specimens

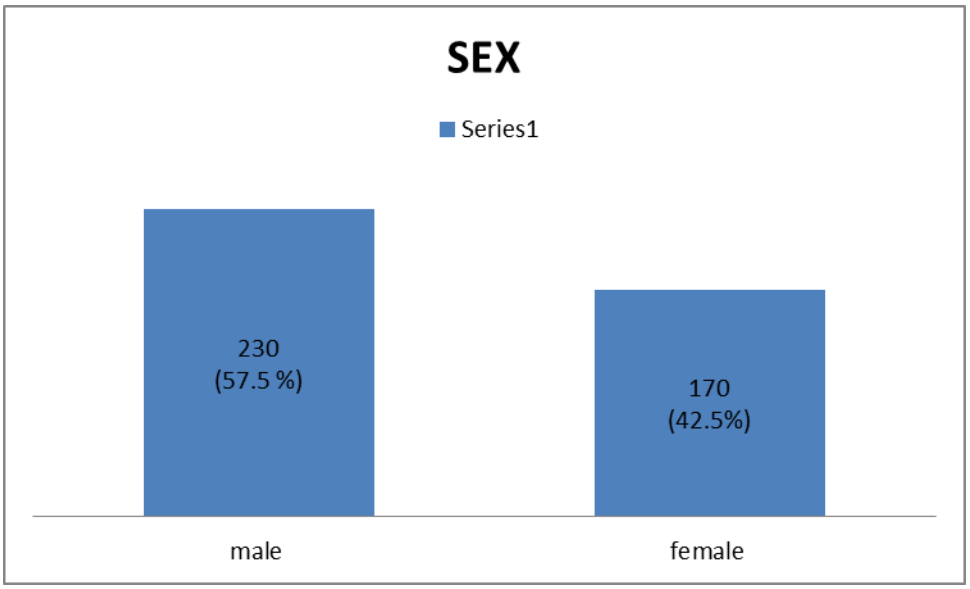


Fig.(4.1): Frequency of males and females in study population.

Most frequent age group in t1he study population was more than 45 years old as represented in fig 4.2.

Infant :1day-2years

Children::more than2years-18year Adult:more than 18year

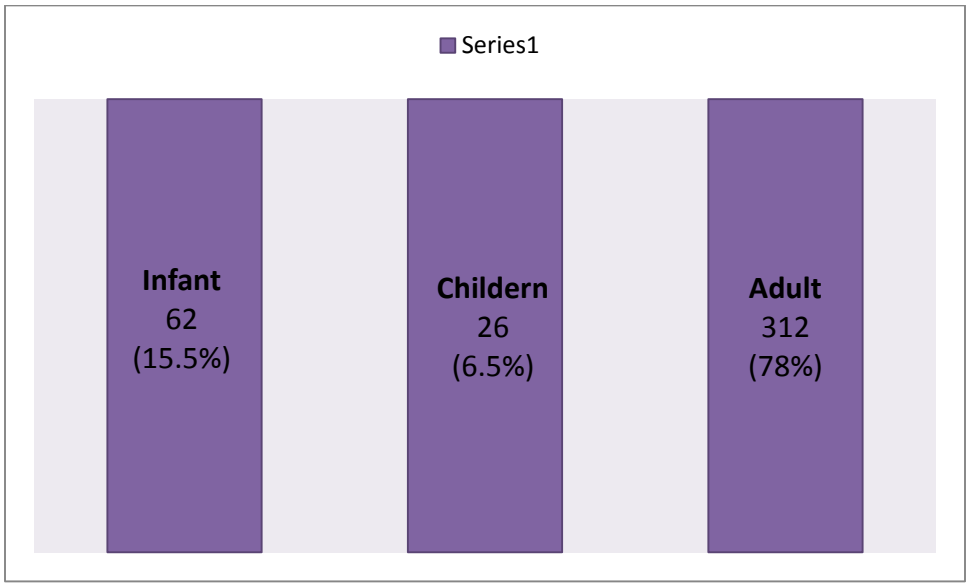


Fig.(4.2): Age distribution among study population

the most collected sample was urine as shown on fig 4.3

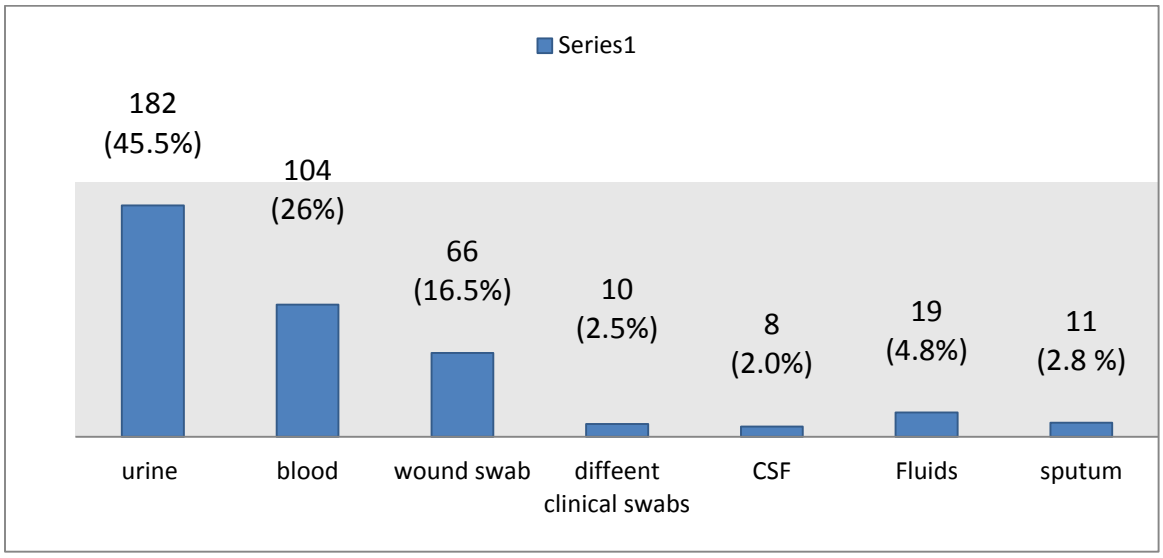


Fig. (4.3): Tye of sample collected from the study population

Patients under antibiotic treatment were 43.50%, while those were not using treatment were 56.5 % as shown in Fig 4.4

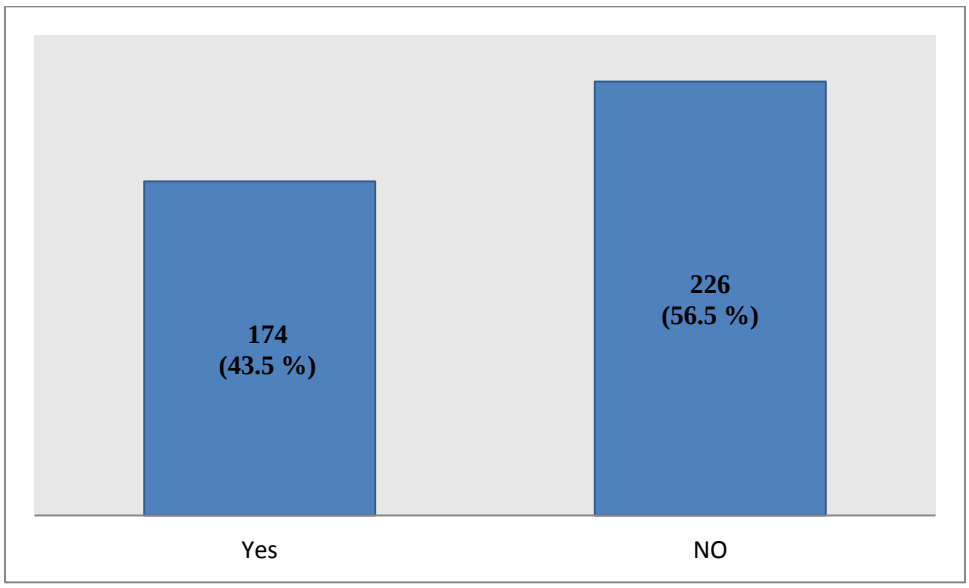


Fig.(4.4): Patients under antibiotic treatment

The duration of antibiotics used by study population represented in f Fig.4.5.

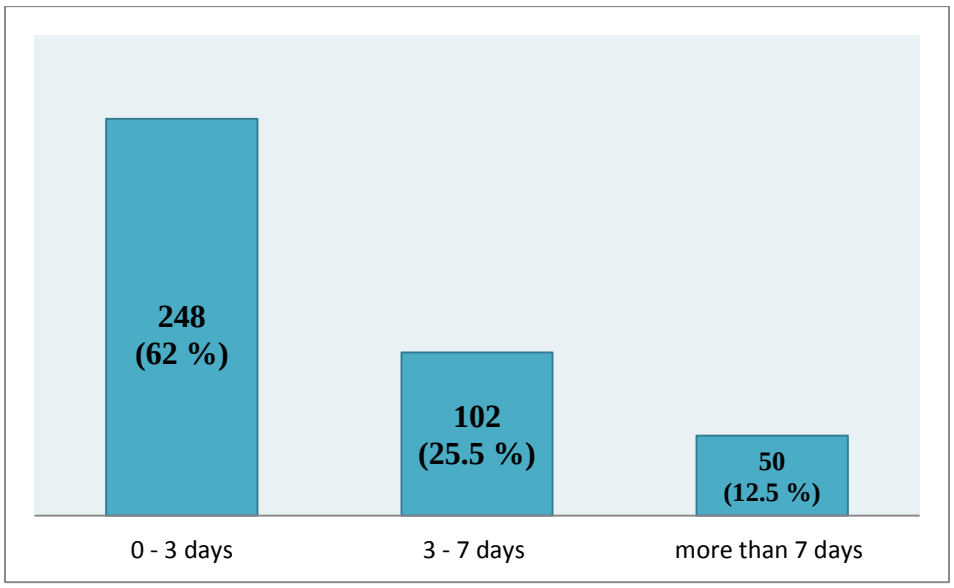


Fig.(4.5): Duration of antibiotics used by study population

The most Risk factors in study population was uinary catheter as shown in figure 4.6

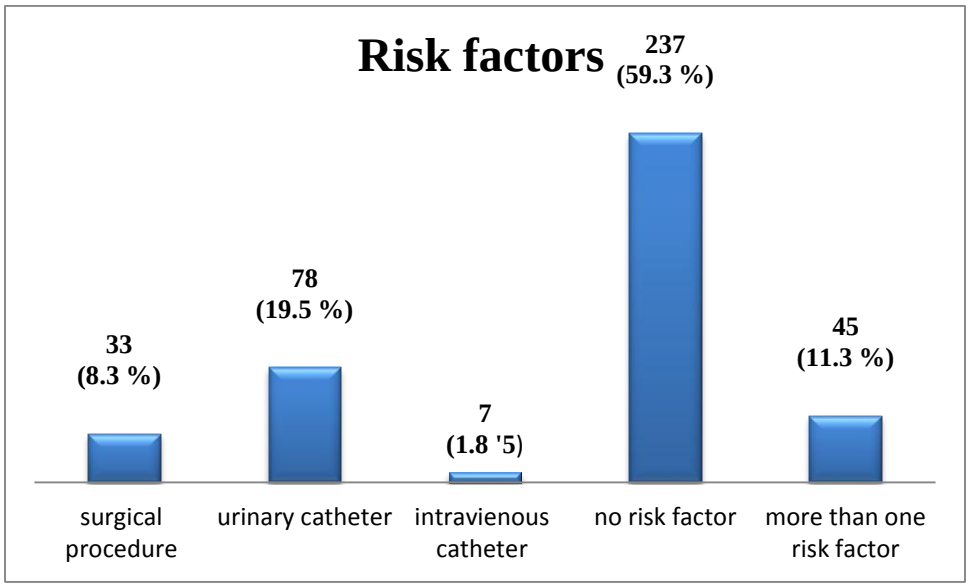
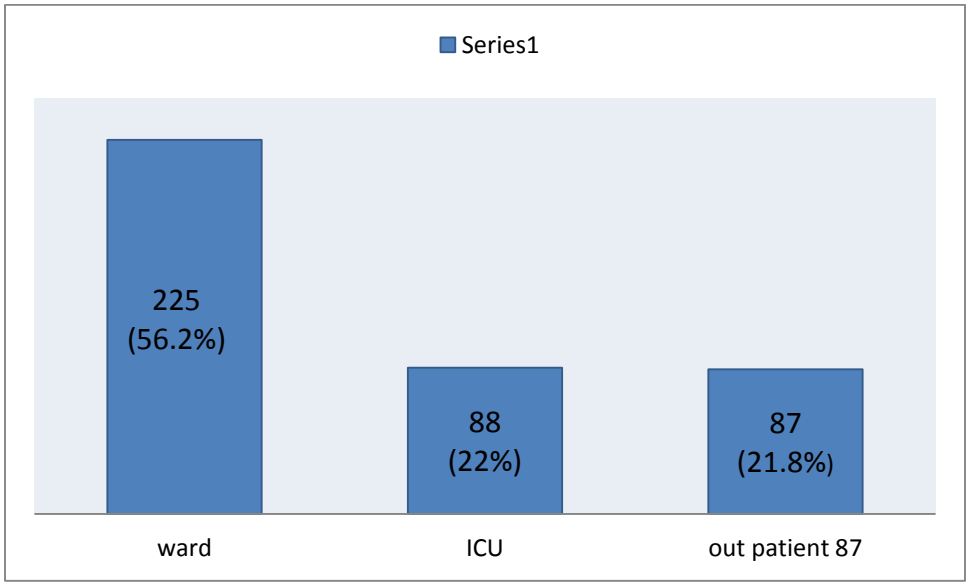


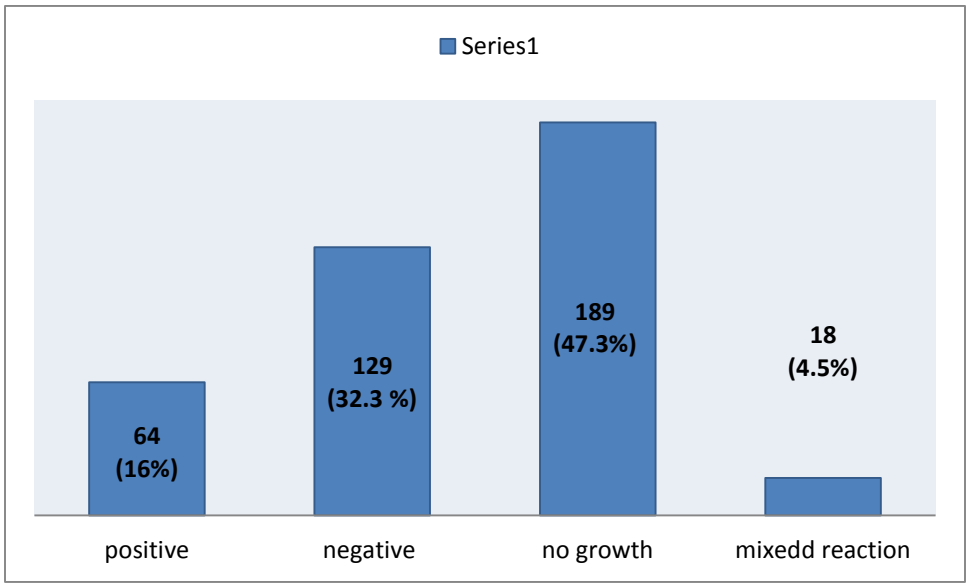
Fig. (4.6): Distribution of risk factors among the study population.

Most of study population was from ward figure 4.7 distributed patients into ward, ICU and outpatient.



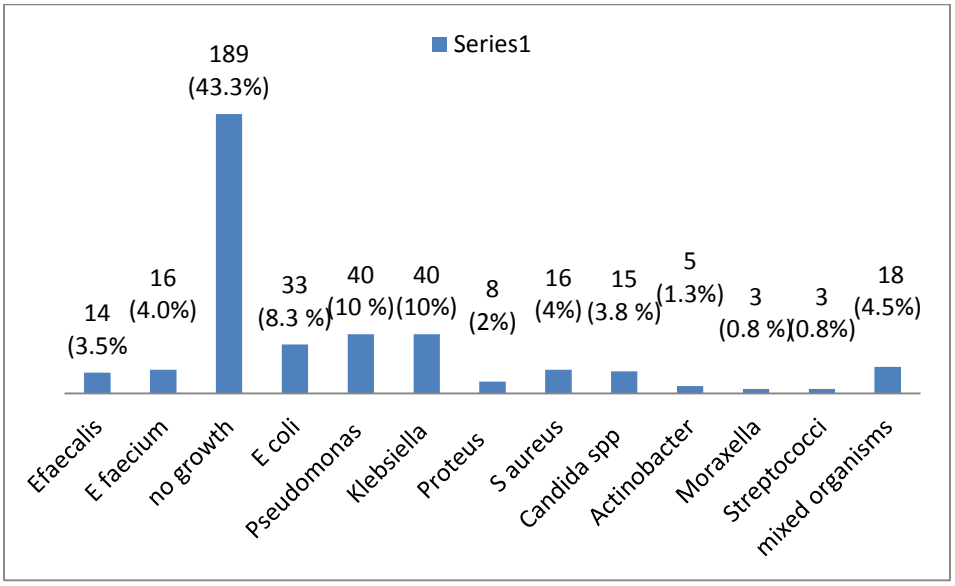
Fig(4.7): Patients distribution according to Hospital stay.

According to Gram stain, most isolated bacteria were Gram negative 32.30% while Gram positive was 16% *Enterococcus* included in.



Fig(4.8): Gram's stain result of isolated bacteria.

Species isolated from the different clinical samples were represented in figure 4.9, *E.feacalis* and *E.faecium* were 3.5% and 4% respectively.



Fig(4.9): Percentage distribution of isolated species

4..1.1:Enterococci antimicobial susceptibiity testing

Table 4.1: Sensetivity profile for all antibiotics used.

Isolate	Vancomycin		Ampicillin		Streptomycin		Gentamycin	
	Sensitive NO (%)	Resistant NO (%)	Sensitive NO (%)	Resistant NO (%)	Sensitive NO (%)	Resistant NO (%)	Sensitive NO (%)	Resistant NO (%)
E. faecalis	13 (43.3)	1 (3.3)	14 (46.7)	0 (0)	9 (30)	6 (16.7)	10 (33.3)	4 (13.3)
E. faecium	13 (43.3)	3 (10)	0 (0)	16 (53.3)	5 (16.7)	11 (36.7)	8 (26.7)	8 (26.7)

Isolate	Penicillin		Ciprofloxacin		Norfloxacin	
	senstive	resistant	sensitive	resistant	Sensitive	resistant
E. faecalis	1 (3.3)	13 (43.3)	11 (36.7)	3 (10)	10 (33.3)	4 (13.3)
E. faecium	1 (3.3)	15 (50)	4 (13.3)	12 (40)	1 (3.3)	15 (50)

4.1.3 Detection of Streptomycin Resistan Genes

The *Aph(2)-1c* found predominating as the gene was detected in 7(23.3%) of the isolates as Shown in Table **4:3** 1 (3.3 %)was found positive for *Aph(2)-1d* as in Tabe **4.3**

Table(4.3):Frequancy and percentage of streptomycin resistant Genes among *Enterococcus* species.

Isolate	*Aph(2)-1c*		*Aph(2)-1d*	
	Positive NO (%)	Negative NO (%)	Positive NO (%)	Negative NO (%)
E faecalis	1 (3.3)	13 (43.3)	0 (0)	14 (46.7)
E faecium	6 (20)	10 (33.4)	1 (3.3)	15 (50)
TOTAL	7 (23.3)	23 (76.7)	1 (3.3)	29 (3.3)

Table(4.4): The relationship between Streptomycin susceptibility and gender, age, clinical samples, hospital stay, antibiotic use and duration, and genes.

Terms		**Streptomycin**		**P.value**
		Sensitive No(%)	**Resistance No(%)**	
Gender	Male Female	8 (26.7) 6 (20.0)	6 (20.0) 10 (33.3)	0.2
Age	Infant Children Elderly	1 (3.3) 3 (10.0) 10 (33.0)	0 (0.0) 3 (10.0) 23 (43.3)	0.5
Samples	Urine Blood Wound	10 (33.3) 0 (0.0) 4 (13.3)	12 (40.0) 2 (6.7) 6 (6.7)	0.2
Hospital stay	Ward ICU Out patient	10 (33.3) 0 (0.0) 4 (13.3)	13 (43.3) 1 (3.3) 2 (6.7)	0.3
Antibiotic use	Yes No	3 (10.0) 11 (36.7)	9 (30.0) 7 (23.3)	0.05
Antibiotic duration (days)	1-3 3-7 >7	11 (36.7) 2 (6.7) 1 (3.3)	6 (20.0) 6 (20.0) 5 (13.3)	0.07
Risk factors	Surgery Urinary Catheter No risk factor Multiple risk factor	1 (3.3) 1 (3.3) 9 (30.) 3 (10.0)	0 (0.0) 6 (20.0) 6 (20.0) 4 (13.3)	.15
Aph(2)-1C	Positive Negative	3 (10.0) 11 (36.7)	4 (13.3) 12 (40.0)	0.8
Aph(2)-1d	Positive Negative	1 (3.3) 13 (43.3)	0 (0.0) 16 (53.3)	0.2

Table (4.5):The relationship between *Aph(2)-1d* and gender, age, clinical samples, Hospital stay, antibiotic use and duration, and risk factors.

Terms		***Aph(2)-1d***		**P.value**
		Positive No(%)	**Negative No(%)**	
Gender	Male	1 (3.3)	13 (43.3)	0.2
	Female	0 (0.0)	16 (53.3)	
Age	Infant	0 (0.0)	1 (3.3)	0.8
	Children	0 (0.0)	6 (20.0)	
	Elderly	1 (3.3)	22 (73.3)	
Samples	Urine	1 (3.3)	21 (70.0)	0.8
	Blood	0 (0.0)	2 (6.7)	
	Wound	0 (0.0)	6 (20.0)	
Hospital stay	Ward	1 (3.3)	22 (73.3)	0.8
	ICU	0 (0.0)	1 (3.3)	
	Out patient	0 (0.0)	6 (20.0)	
Antibiotic use	Yes	1 (3.3)	11 (36.7)	0.2
	No	0 (0.0)	18 (60.0)	
Antibiotic duration (days)	1-3	1 (3.3)	16 (53.3)	0.6
	3-7	0 (0.0)	8 (26.7)	
	>7	0 (0.0)	5 (16.7)	
Risk factors	Surgery	0 (0.0)	1 (3.3)	0.3
	Urinary Catheter	0 (0.0)	7 (23.3)	
	No risk factor	0 (0.0)	15 (50.0)	
	Multiple risk factor	1 (3.3)	6 (20.0)	

Table (4.6): The relationship between *Aph(2)-1c and* gender, age, clinical samples, patients status, antibiotic use and duration, and risk factors

Terms		*Aph(2)-1c*		**P.value**
		Positive No(%)	**Negative No(%)**	
Gender	Male	2 (6.7)	12 (40.0)	0.2
	Female	5 (16.7)	11 (36.7)	
Age	Infant	0 (0.0)	1 (3.3)	0.7
	Children	1 (3.3)	5 (16.7)	
	Elderly	6 (20.0)	17 (56.7)	
Samples	Urine	6 (20.0)	16 (53.3)	0.2
	Blood	1 (3.3)	1 (3.3)	
	Wound	0 (0.0)	6 (20.0)	
Hospital stay	Ward	5 (16.7)	18 (60.0)	0.7
	ICU	0 (0.0)	1 (3.3)	
	Out patient	2 (6.7)	4 (13.3)	
Antibiotic use	Yes	2 (6.7)	10 (33.3)	0.4
	No	5 (16.7)	13 (43.3)	
Antibiotic duration (days)	1-3	5 (16.7)	12 (40.0)	0.3
	3-7	2 (6.7)	6 (20.0)	
	>7	0 (0.0)	5 (16.7)	
Risk factor	Surgery	0 (0.0)	1 (3.3)	0.8
	Urinary Catheter	1 (3.3)	6 (20.0)	
	No risk factor	4 (13.3)	11 (36.7)	
	Multiple risk factor	2 (6.7)	5 (16.7)	

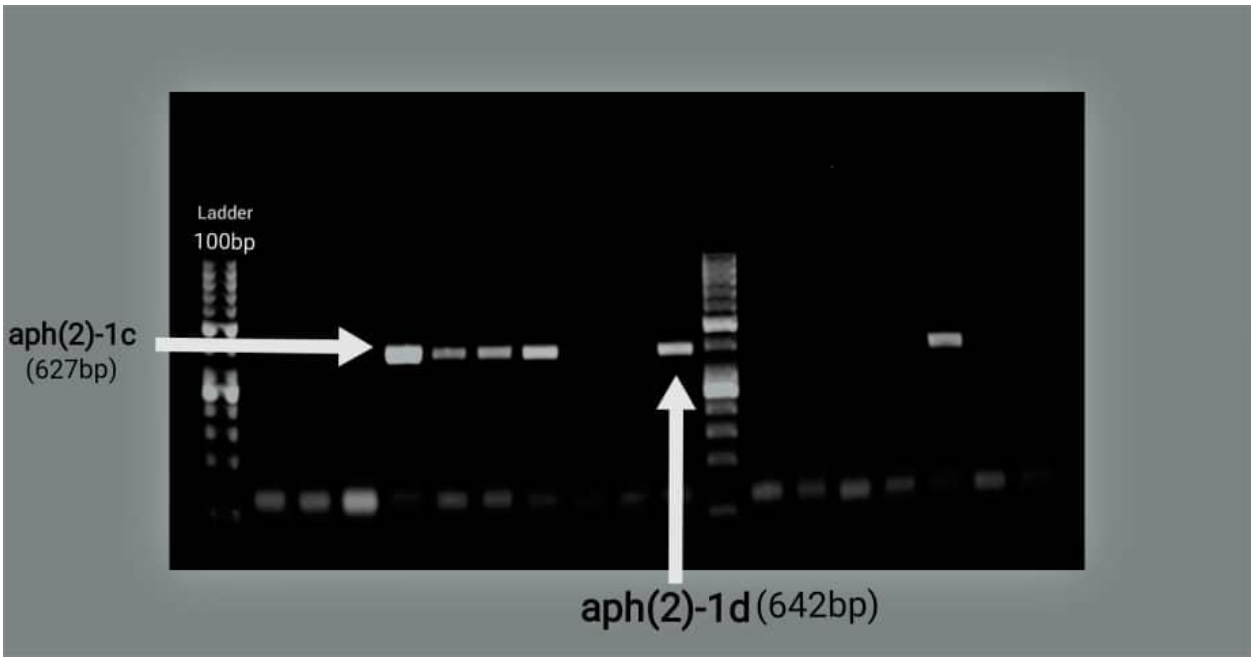


Fig 4.10.:Gel electrophoresis of *Aph(2)1C* and *Aph(2)-1d*, PCR product. . Lane no. 1 contains 100-bp DNA ladder. Lane no. 2 contains control negative, other lanes contains positive and negative samples for *Aph(2)1C* and *Aph(2)-1d* band appear at 627 bp and 642 bp respectively.

4.2 Discussion

Enterococci are part of the normal intestinal flora. They used to be classified as Group D Streptococci but are now considered a separate genus. Enterococci cause a variety of infections including, most frequently, infections of the urinary tract, catheterized urinary tract, bloodstream, wounds and surgical sites, and heart valves in endocarditis, there are more than 17 species, but *Enterococcus faecalis* and *Enterococcus faecium* most commonly cause infections in humans (Shahad and Hussein, 2020). Aminoglycosides are considered efficient in treating serious infections caused by both Gram-positive and Gram-negative organisms. However, the acquisition of extrinsic resistance to high-level aminoglycoside antibiotics in Enterococci renders these strains a serious challenge in clinical settings (Diab *et al.*, 2019).

Many researchers reported prevalence of *Enterococcus*, the current results found that prevalence of Enterococci was 30 (7.5) .This result was comparable with studies by Kabew *et al* (2013)in Ethiopia,Olawale *et a*l,(2013) in Nigeria and Marcus *et al*, (2011) who reported the ranges between 5.0 and 7.6%. The rate in our study is higher than results conducted by Ferede *et al* ,*(*2018). in Ethiopia, the prevalence of Enterococci among different clinical samples was 3.5%, the prevalence rate in Egypt (3.3%), in Bangladesh (3.2%)(Lowde *et al*,2002), in India (2.3%) (Paul *et al,* 2017) and in Asian pacific (3.6%)(Sreeja ,*et a*l 2012) , However, it was lower than other studies 11.0% reported from Malaysia(Nor *et al*,2015),20.8% from Pakistan (Gulz *et al*, 2015) and 15.3% from Tanzania (Aamodt *et al,* 2015) These differences in prevalence might be due to methodological design used , study area, study period in previous studies and might be explained due to the different chracterstics in the study participants (Niu *et al* ,2016).

In this study *E faecium* was the most prevalent species detected .accounting for 16 (53.3%), while 14 (46.6%) were identified as *E faecalis* .like a study conducted in Michigan by Vakulenko *et a l* ,(2003) in which *E. faecium* was the predominant species. in contrast with previous studies in Iran by Feizabadi *et al* ,(2006) reported the prevalence of the two species to be 63% *E. faecalis* and 33% *E. faecium* in 2002–2004 ,Saifi *et al*, (2008) in iran reported an outbreak consisting of 77.8% *E. faecalis* and 22.2% *E. faecium* in 2005–2006 , and Dallal *et al* ,(2008) reported the prevalence of the two species as 70% *E. faecalis* and 30% *E. faecium*, in 2005–2006 .These differences might be due differences in climate and bacterial prevalence, the distributions of *Enterococcus* species differ between regions. Unlike countries such as India and Japan, where *E.*

faecium is dominant in Iran, as in the USA, UK and some European countries,(Sharif *et al,* 2020) varies from one country to another depending on various contributing factors such as host dynamism, environmental conditions, or due to clinical conditions that were presented to the hospitals, genetic diversity, as well as the presence of specific virulence factors (Weng *et al,* 2013)

In the current study, the highest number of *Enterococcus* isolates was attributed to UTIs. The higher rate of occurrence of these urinary isolates confirms the prevalence of Enterococci as a cause of UTIs. this result is similar with previous studies in Iran by Sharif *et al,* (2020) reported the highest number of *Enterococcus* isolates was attributed to UTIs

In this study, a higher level of resistance to Streptomycin was observed among *E. faecium* than *E. faecalis* isolates. This resut is similar to report from Europe where high level aminoglycoside resistance has been more frequently found in *E. faecium* than in *E. faecalis* (Zarrilli *et al,*2005). And study by (Garim *et a*l, 2017)showed HLAR was found to be more in *E. faecium* (51.8%) than *E. faecalis* (27.8 %)**.** in constrast with study by Schmitz *et a*l ,(2009) in European Sentry HLAR was found to be more in *E. faecalis* than *E. faecium*

The resistance of Enterococci to antimicrobial agents has been increasing over the last two decades, and the ease of acquisition and transfer of antimicrobial resistance genes has led to the emergence of high-level Aminoglycoside-resistant and Stretomycin -resistant Enterococci strains (Miller *et a*l,2013).

In the current study resistance of *E.faecalis* and *E.faecium* to Streptomycin was 16.7% and 36.7% respectively, and *E.faecium* was found to be more resistant to Streptomycin than *E.faecalis.*

In This study investigated the detection of high level resistance to streptomycin was 53.3%. These results are slightly similar to those from Kuwait and another report from Iran and higher than Turkey, 53%,40.2% and 36% respectively (Feizabadi *et al*., 2006 ,Kaçmaz *et al* ,2005, Udo *et al* ,2004). Compared with isolates, in the Spanish *Enterococcus* isolates HLR to Streptomycin was (42%) (del Campo *et al* , 2000). and study in Iran by Sharif *et al*,(2020) the prevalence of HLSR to *E. faecalis* was 40.16%, and that of HLSR *E. faecium* was 50.49%, indicating a significant difference in resistance to high levels Streptomycin between the two species. which is lower than those reported by Padmasini *et al,(*2014)in india (77%)and by Li *et al* ,*(*2015) (56%) in china, The differences in the detection rate could possibly be due to the

horizontal transfer of the resistance factors, since HLAR genes are located on plasmid and conjugative transposons.,(Li *et al,* 2015).

7(23.3)% and 1(3.3%) was the frequency of *aph(2)1C* and *Aph(2)-1d* genes in isolated *Enterococcus species* in the current findings,similar to result by Sharif *et al*,(2020) reported 7 (23.3%) isolates harboured the *aph(2'')-Ic* gene and differ in whereas no isolates with *the aph(2'')-Id* gene were observed in isolates .And higher than result by Faizabadi *et al*,(2006) in Iran who reported the *aph(2'')-Ic* gene in 2 (6.6%) *E. faecium* strains but did not detect any strain containing the *aph(2'')-Id* genes .*Aph (2'')-Ic* and *aph(2'')-Id* genes gene were not detected in the strains of any Enterococci by Kobayash *et al, (*2001)

Distribution of HLAR genes depends on the geographical region, and the same gene is not necessarily found in the same Enterococci species.(Zarrilli *et al*.,2005)

The study found that most of the resistance to Streptomycin occurred among patients from ward section, patients in course of antibiotic treatment, in urine samples more than other clinical samples, in females more than in males and in patient more than 42 years old

And accordingly, it was noticed that, *aph(2)1C* was presented in higher percentage in females more than in males, in patient more than 42 years old , in urine samples, in patients inside ward section, patients was not under treatment course , while *aph(2)1C* was higher in *E.faecium* more than *E.faecalis* .

Moreover, *Aph(2)-1d* was noticed in higher percentage in males more than in females, in patient more than 42 years old , in urine samples, in patients inside ward section, patients was under treatment course and *aph(2)1d* higher in *E.faecium* more than *E.faecalis*

CHAPTER V

CONCLUSION AND RECOMMENDATION

CHAPTER V

5. Conclusion and Recommendations

5.1 Conclusion

In conclusion, the study demonstrated that *E.faecium* was found to be more resistant to Streptomycin than *E.faecalis*, *Aph(2)1c* frequency was high in *E.faecium* more than *E.faecalis* with significant statistical difference while insignificant statistical difference was noticed with *Aph(2)1d*.

5.2 Recommendations

1. Aminoglycoside susceptibility test to *Enterococcus* should be conducted in routine microbiology labs.

2. The presence of this percentage in resistant Enterococci in the current study should be considered as alarm.

2. Appropriate surveillance and control measures are essential to prevent the emergence and transmission of *Enterococcus* in hospitals.

3. Further studies should be carried out for a better understanding of the association between the presence of these genes and emergence of resistant Enterococci.

REFERENCES

Aamodt H, Mohn SC, Maselle S, Manji KP, Willems R, Jureen R, *et a*l.(2015) Genetic relatedness and risk factor analysis of ampicillin-resistant and high-level Gentamicin-resistant Enterococci species causing bloodstream infections in Tanzanian children.*BMC Infect Dis*.**;**15:2–9.

Abamecha, A., Wondafrash, B. and Abdissa, A. (2015). Antimicrobial resistance of *Enterococcus* species isolated from intestinal tracts of hospitalized patients in Jimma, Ethiopia, *BioMed Central Research Notes,* 8:213.0

Anderson, AC., Jonas, D., Huber, I., Karygianni, L., Wölber, J. *et al.* (2016) *Enterococcus* faecalis from Food, Clinical Specimens, and Oral Sites: Prevalence of Virulence Factors in Association with Biofilm Formation. *Front. Microbiol*ogy 6:1534.

Brown, D. and Walpole, E. (2003). Evaluation of selective and enrichment media for isolation of glycopeptide-resistant enterococci from faecal specimens. *Journal of Antimicrobial Chemotherapy,* 51: 289–296.

Cagnoli, G.; Bertelloni, F.; Interrante, P.; Ceccherelli, R.; Marzoni, M.; Ebani, V. (2022). Antimicrobial Resistant *Enterococcus spp*. in Wild Avifauna from Central Italy. *Antibiotics*, 11**:** 852.

Cheesbrough,M.(2006). summay of the clinical and laoratory feature of microorganism in Distric Laboratory Practice in Tropical Countries**,** part 2**,** cambridge unviersity press**,** UK**,**106－266

Chen AY, Zerros JM.(2009). Antimicrobial Drug Resistance and Infectious Disease: Humana Press; Chapter 49, *Enterococcus*: Antimicrobial Resistance in Enterococci. Epidemiology, Treatment and Control; pp. 715-33.

Chow JW (2000). Aminoglycoside Resistance in Enterococci. Clin Infect Dis. Aug; 31 (2):586-9.

Courvalin, P. (2006). Vancomycin resistance in gram positive cocci. *Clinical of Infectious Diseases,* 42(1):S25–S34.

Dallal, M. Saifi, M.R. Pourshafie, M.R. Eshraghian (2008)High-level Gentamicin-resistant Enterococcal isolates from urinary tract infection in Iran Infect Dis Clin Pract, 16 p 41-45.

Del Campo R., C. Tenorio, C. Rubio, J. Castillo, C. Torres and R. Go' mez-Lus.(2000)Aminoglycoside-modifying enzymes in high-level Streptomycin and Gentamicin resistant *Enterococcus spp*.in Spain. Int. J. *Antimicrob. Agents* 15: 221226.

Diab, M; Salem , D ; EL-Shenawy , A; EL-Far, A; Adelghany, A; and *et al*; (2019),Detection of high level aminogycoside resistance genes among clinical isolates of *Enterococcus* species ,*Egyptian Journal of Medical Human Genetics* ,20:28

DiazGranados, C. A., Zimmer, S. M., Mitchel, K., and Jernigan, J. A. (2005) Comparison of mortality associated with vancomycin-resistant and vancomycin-susceptible enterococcal bloodstream infections: A meta-analysis. *Clinical Infectious Diseases,* 41: 327-333.

El-Ghazawy, I; Okasha, H; and Mazloum S . (2016) A study of high level Aminoglycoside resistant Enterococci. Afr J *Microbiol Res,* 10(16):572–577

El-Mahdy, R., Mostafa A. and El-Kannishy, G. (2018). High level Aminoglycoside resistant Enterococci in hospital-acquired urinary tract infections in Mansoura, Egypt. *Germs*, 8(4): 186.

Feizabadi, P. Maleknejad, A. Asgharzadeh, S. Asadi, L. Shokrzadeh, S. Sayadi (2006) Prevalence of Aminoglycoside-modifying enzymes genes among isolates of *Enterococcus faecalis* and *Enterococcus faecium* in Iran Microb Drug Resist, 12 pp. 265-268.

Ferede, Z., Tullu, K., Solomon, G. and Addisu, G. (2018), Prevalence and antimicrobial susceptibility pattern of *Enterococcus* species isolated from different clinical samples at Black Lion Specialized Teaching Hospital, Addis Ababa, Ethiopia. *BMC Research Notes,* 11:793

Ferguson DM, Talavera GN, Hernández LA, Weisberg SB, Ambrose RF, Jay JA. (2016). Virulence genes among *Enterococcus faecalis* and *Enterococcus faecium* isolated from coastal beaches and human and nonhuman sources in Southern California and Puerto Rico. *Journal of Pathogens*. 16: 3437214-34

Forbes BA ,Sahm DF, Weissfeld, AS.(2007). *Streptococcus* , *Enterococcus,* and Similar Organisms. In Bailey & Scotts Diagnostic Microbiology , 12th ed . Mosby Elsevire ;pp . 265-79.

García-Solache, L.B. Rice (2019)**.** The *Enterococcus*: a model of adaptability to its environment *Clin Microbiol Rev.(*32).58-18.

Garima Yadav, Bhaskar Thakuria, Molly Madan, Vivek Agwan4, Anita Pandey(2017) Linezolid and Vancomycin Resistant Enterococci: A Therapeutic Problem.26:369-71**.**

Gold, H. (2001), Vancomycin-Resistant Enterococci: Mechanisms and Clinical Observations, *Clinical Infectious Diseases*, 33:210–9.

Gul Z, Jan AZ, Liaqat F, Qureshi MS.(2015) Causative organisms and antimicrobial sensetivity pattern of pediatric urinary tract infection. Gomal J Med Sci. ;13(2).

Haghi, F., Vahid Lohrasbi, V., and Habib Z. (2019). High incidence of virulence determinants, Aminoglycoside and vancomycin resistance in Enterococci isolated from hospitalized patients in Northwest Iran, *BMC Infectious Diseases*, 19:744.

Hashem Y, Amin H, Essam T, Yassin A, Aziz R. (2017). Biofilm formation in enterococci: genotype-phenotype correlations and inhibition by vancomycin. *Science Rep*, 7:5733.

Jahan, M, Holley, RA. (2014), Incidence of virulence factors in Enterococci from raw and fermented meat and biofilm forming capacity at 25°C and 37°C. *International Journal of Food and Microbiology*, 170: 65-9.

Jaimee, G. and Halami, P. (2016), High level Aminoglycoside resistance in *Enterococcus*, Pediococcus and Lactobacillus species from farm animals and commercial meat products. *Annual Microbiology* ,66:101–110.

Kabew G, Abebe T, Miheret A .(.2013) A retrospective study on prevalence and antimicrobial susceptibility patterns of bacterial isolates from urinary tract infections in Tikur Anbessa Specialized Teaching Hospital Addis Ababa, Ethiopia, 2011. Ethiop J Health Dev. **;**27(2):111–7.

Kaçmaz B. and A. Aksoy. (2005). Antimicrobial resistance of Enterococci in Turkey. Int. J. Antimicrob. Agents 25: 535538.

Khani, M., Fatollahzade, M., Pajavand, H., Bakhtiari, S. and Abiri, R. (2016), Increasing Prevalence of Aminoglycoside-Resistant *Enterococcus* faecalis Isolates Due to the aac(6')-aph(2'') Gene: A Therapeutic Problem in Kermanshah, Iran. *Jundishapur Journal of Microbiology*,9(3).

Kobayashi N, Alam M, Nishimoto Y, *et al* .(.2001) Distribution of Aminoglycoside resistance genes in recent clinical isolates of *Enterococcus faecalis*, *Enterococcus faecium* and *Enterococcus avium*. *Epidemiol Infect* **;**126:197-204.

Kürekci, C., Pehlivanlar, S., Mustafa, Y., Aslanta¸ O., and Aycan G. (2016). Characterization of Phenotypic and Genotypic Antibiotic Resistance Profile of Enterococci from Cheeses in Turkey, *Korean Society for Food Science of Animal Resources*, 36(3): 352-358.

Labibzadeh, M., Abbas, G., Savar, M and Ekrami A. (2018), Emergence of High-level Gentamicin Resistance among Enterococci Clinical Isolates from Burn Patients in South-west of Iran: Vancomycin Still Working, *Polish Journal of Microbiology*, 67(4): 401–406.

Li W, Li J, Wei Q, *et al*.(2015); Characterization of Aminoglycoside resistance and virulence genes among *Enterococcus spp*. isolated from a hospital in China. Int J *Environ Res Public*; *Health* 12(3):30143025.

Low DE, Keller N, Barth A, Jones RN.(2002) .Clinical prevalence, antimicrobial susceptibility, and geographic resistance patterns of Enterococci: results from the SENTRY antimicrobial surveillance program, 1997–1999. Clin Infect Dis. ;**32**(Supplement_2):S133–45.

Marcus N, Ashkenazi S, Samra Z, Cohen A, Livni G.. (2011) Community-acquired Enterococcal urinary tract infections in hospitalized children. Pediatr Nephrol;**27**(1):109–14.

Mathur, P. and Singh, S. (2013). Multidrug resistance in bacteria: a serious patient safety challenge for India. *Journal of Lab Physicians*, **5**(1):5–10.

Miller, B.E. Murray, C.A. Arias (2013). Emergence and management of drug-resistant Enterococcal infectionsV. Krcméry (Ed.), Microbial drug resistance, Future Medicine, London, UK, pp. 42-54.

Miller, W., Jose, M. and Arias, C. (2014). Mechanisms of antibiotic resistance in Enterococci, *Expert Rev Anti Infect Ther*apy, **12**(10): 1221–1236.

Miranda, M., Franco, B., Fente, V., Vela´zquez, B. and A. Cepeda, A. (2005), Evaluation of Chromocult enterococci agar for the isolation and selective enumeration of *Enterococcus spp*. in broilers, *Letters in Applied Microbiology*, 41:153-156.

Mohamed, A. and Keith, B. (2018), Vancomycin-Resistant Enterococci: A Review of Antimicrobial Resistance Mechanisms and Perspectives of Human and Animal Health. *Micobial Drug Resisance* , 24(5):120-135.

Moussa, A., Nordin, A., Hamat, A., and Jasni, S. (2019). High Level Aminoglycoside Resistance And Distribution Of The Resistance Genes In *Enterococcus faecalis* And *Enterococcus faecium* From Teaching Hospital In Malaysia. *Infection and Drug Resistance*, 12: 3269–3274.

Murray (1998). Diversity among multidrug-resistant Enterococci.*Emerg Infect Dis*, 4 , pp. 37-4

Niu, H; Yu H; Hu T; Tian G; Zhang L; and Guo X *et al* .(2016) .The prevalence of aminoglycoside- modifying enzyme and virulence genes among Enterococci with high-level aminoglycosides resistance in Inner Mongolia. China. Braz J Microbiol, 47:691–696

Nor NSM, Abu NA, Rashid MA, Ismail MM, Razak RKRA.(2015) Bacterial pathogens and antibiotic resistance patterns in children with urinary tract infection in a Malaysian tertiary hospital. Med J Malays. **;**70(3):153–7.

Olawale KO, Fadiora SO, Taiwo SS.(2013) Prevalence of hospital acquired *Enterococcus species* infections in two primary-care hospitals in Osogbo, Southwestern Nigeria. *Afr J Infect Dis* ;5(2).

Padmasini, E., Padmaraj, R. and Rames, S. (2014), High Level Aminoglycoside Resistance and Distribution of Aminoglycoside Resistant Genes among Clinical Isolates of *Enterococcus* Species in Chennai, India, *The Scientific World Journal.* Article ID 329157, 5 .

Paul M, Nirwan PS, Srivastava P.(2017) Isolation of *Enterococcus* from various clinical samples and their antimicrobial susceptibility pattern in a tertiary care hospital. *Int J Curr Microbiol App Sci*. ;6(2):1326–32.

Qu T, Chen Y, Yu Y.(2006). Genotypic diversity and epidemiology of high-level gentamicin resistant *Enterococcus* in a Chinese hospital. J *Infect* ,52:124-30.

Raza,T., Sidra, R., U., Khalid, M. and Saadia, A. (2018). Vancomycin resistant Enterococci: A brief review. *Journal of the Pakistan Medical Association* , 68(5): 768-772.

Rostkowska, O.M.; Kuthan, R.; Burban, A.; Sali´nska, J.; Ciebiera, M.; Mlynarczyk, G.; Durlik, M. (2020). Analysis of Susceptibility to Selected Antibiotics in Klebsiella pneumoniae, Escherichia coli, *Enterococcus* faecalis and *Enterococcus* faecium Causing Urinary Tract Infections in Kidney Transplant Recipients over 8 Years: Single-Center Study. *Antibiotics*, 9: 284.

Said HS, Abdelmegeed ES ..(2020). Emergence of multidrug resistance and extensive drug resistance among enterococcal clinical isolates in Egypt. Infect Drug Resist,12:1113-25.

Saifi, M.R. Pourshafie, M.R. Eshraghian, M.M. Soltan Dallal(2008) Anti-microbial resistance of enterococci isolated from urinary tract infections in Iran Iran Biomed J**,**12 p. 185-190.

Schmitz EJ, Verhoef J, Fluit AC (2009) Prevalence of aminoglycoside resistance in 20 European University hospitals participating in the European SENTRY antimicrobial surveillance programme. *Eur J Clin Microbiol Infec Dis* 18(6):414–421.

Sharifi Y, Hasani A, Ghotaslou R, Naghili B, Aghazadeh M, Milani M. **;**.(2013) Virulence and antimicrobial resistance in enterococci isolated from urinary tract infections. *Adv Pharm Bull* ,3:197-201.

Shahad, A. and Hussein, O. (2020), Antimicrobial resistance profile among *Enterococcus* species isolated from patients with cystitis. *Drug Invention Today*, 13(2).

Shanmukhappa, D., Venkatesha, S., Mrutha, B, MalliKarjun, K. (2015), Isolation, Identification and Speciation of *Enterococci* by Conventional Method and their Antibiogram, *National Journal of Laboratory Medicine*, 4(2): 1-6.

Sharifzadeh PeyvastiaR.Hosseini Doustc (2020),High-level aminoglycoside resistance and distribution of aminoglycoside resistance genes among *Enterococcus sp*. clinical isolates in Tehran, Iran(20) P 318-323.

Shepard B, Gilmore M(2002) Antibiotic resistant **.** enterococci: the mechanisms and dynamics of drug introduction and resistance Microbes Infect;4:215-24.

Shrestha, S., Kharel, S., Homagain, S., Aryal, R., Mishra, SK.. (2021). Prevalence of vancomycin-resistant enterococci in Asia—A systematic review and meta-analysis. *Journal of Clinical Pharmacy and Therapeutics*, 46:1226–1237.

Song, Y. Hwang, I., Eom, J., Cheong, H., Bae, W., Park, Y. and Ki, W. (2005). Prevalence and Molecular Epidemiology of Vancomycin-Resistant Enterococci (VRE) Strains Isolated from Animals and Humans in Korea. *The Korean Journal of Internal Medicine*, 20:55-60.

Sood, S., Das, M., Kapil, A. (2008) Enterococcal infections and antimicrobial resistance. *Indian Journal of Medical Research*, 128:111–121.

Sreeja S, Sreenivasa Babu PR, Prathab AG.(2012) The prevalence and the characterization of the *Enterococcus species* from various clinical samples in a tertiary care hospital. *J Clin Diagn Res JCDR.* ;6(9):1486.

Tille ,P. M. &Forbes, B.A.(2014).Bailey & and scott's diagnostic microbiology (Thirteenth edition). St. Louis, Missouri: Elsevie.

Udo E.E., N. Al-Sweih, P. John, L.E. Jacob and S. Mohanakrishnan.(2004). Characterization of high-level aminoglycosideresistant Enterococci in Kuwait Hospitals. Microb..Drug. Resist **10**: 139145.

Vakulenko SB, Donabedian SM, Voskresenskiy AM, Zervos MJ,Lerner SA, Chow JW.(2003) Multiplex PCR for detection of aminoglycoside resistance genes in Enterococci. Antimicrob Agents Chemother ;47(04):1423–1426.

Wada, Y., Harun, A., Yean, C., and Zaidah, A. (2020). Vancomycin-Resistant Enterococci (VRE) in Nigeria: The First Systematic Review and Meta-Analysis. *Antibio.tics*, 9: 565.

Weng PL, Ramli R, Shamsudin MN, Cheah YK, Hamat RA(.2013) High genetic diversity of *Enterococcus faecium* and *Enterococcus faecalis* clinical isolates by pulsed-field gel electrophoresis and multilocus sequence typing from a hospital in Malaysia. *Biomed Res Int.*:938937.

Wieczorek, P., Wieczorek, A., Sacha, P., Majewski, P., Ojdana, D., Michalska, A. (2014). Occurrence Aminoglycoside resistance among *Enterococcus* species strains, *Progress Health Sciences*, **1**(4): 179-189.

Zarrilli R., M.F. Tripodi, A. Di Popolo, R. Fortunato,M. Bagattini, M. Crispino, . Florio,M. Triassi and R. Utili. (2005). Molecular epidemiology of high-level Aminoglycoside-resistant Enterococci isolated from patients in a university hospital in southern Italy. J. *Antimicrob. Chemother*. 56: 827835.

Zhou, X., Willems, R.J.L., Friedrich, A.W. *et al*. (2020). *Enterococcus faecium*: from microbiological insights to practical recommendations for infection control and diagnostics. *Antimicrobial Resistance Infection Control*, 9**:** 130.

Zouain, G. Araj (2001). Antimicrobial resistance of Enterococci in Lebanon Int J Antimicrob Agents,(17) 209-213.

APPENDIXS

Appendix I

Sudan University for Science and Technology

College of Graduate Studies

Phenotypic and Genotypic Detection of High Level Streptomycin Resistant Genes among *Enterococcus* species Isolated from Various Clinical Specimens in Khartoum State

Questionnaire

1- ID NO. …….

2- Age …… years /months

3- Gender Male ☐ Female ☐

4- Ward……...

5- Receiving antibiotic /s

Yes ☐ NO ☐

If yes specify.....

DURATION OF TREATMENT……

6-.HISTORY OF SURGICAL PROCEDURE

Yes ☐ NO ☐

7-use of urinary catheter: Yes ☐ NO ☐

8- Use of Intravenous catheter: Yes ☐ NO ☐

9-Duration of hospital stay……

10-Type of sample……..……..……

Laboratory results

Culture: Isaolate(s)……..……..……

Sensitive to: ……..

Resistant to: ……...

Molecular results

……

……..

.

Appendix II

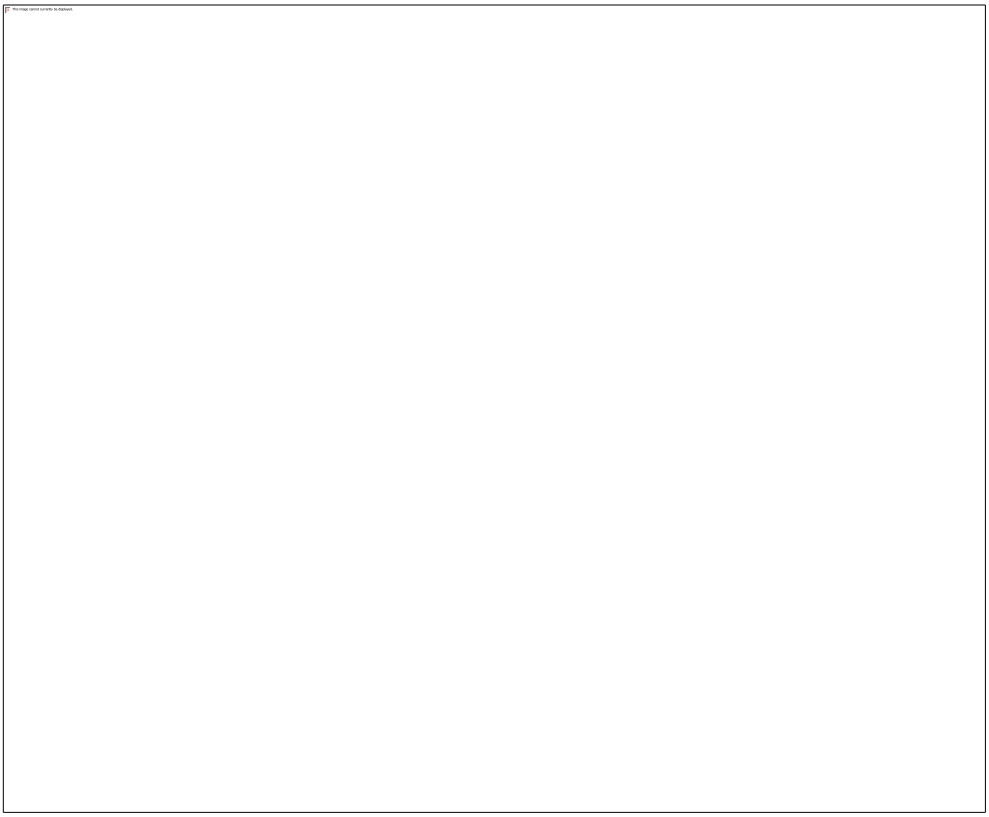


Fig 5:D Litmus Milk and Aesculin Hydrolysis Tests

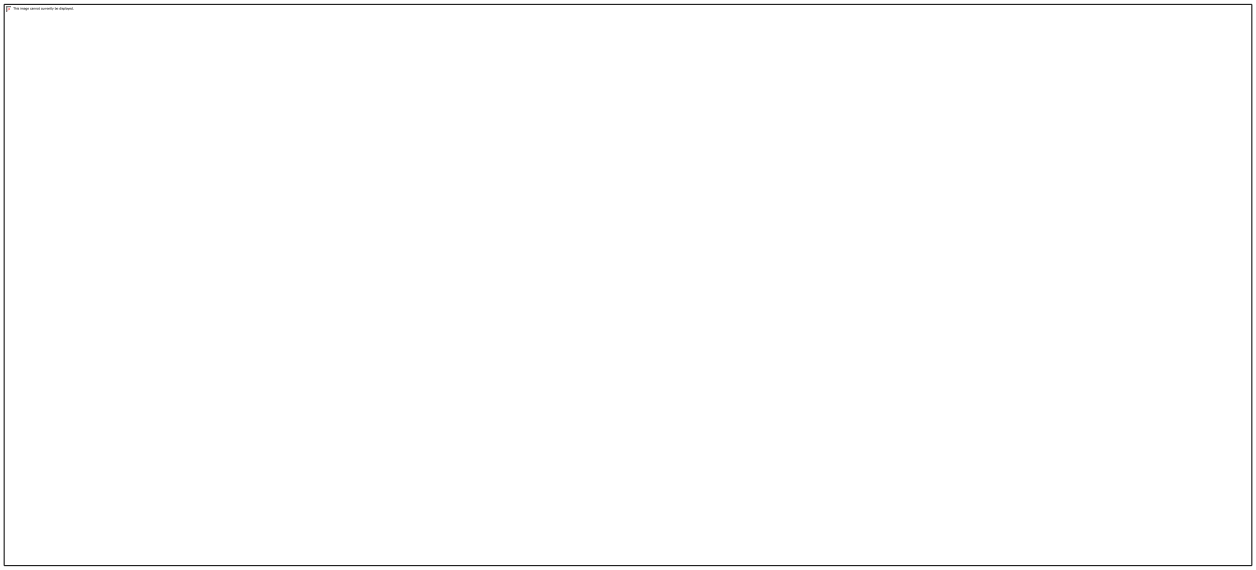


Fig 6: Antimicrobial Susceptibility tests of *Enterococcus species*

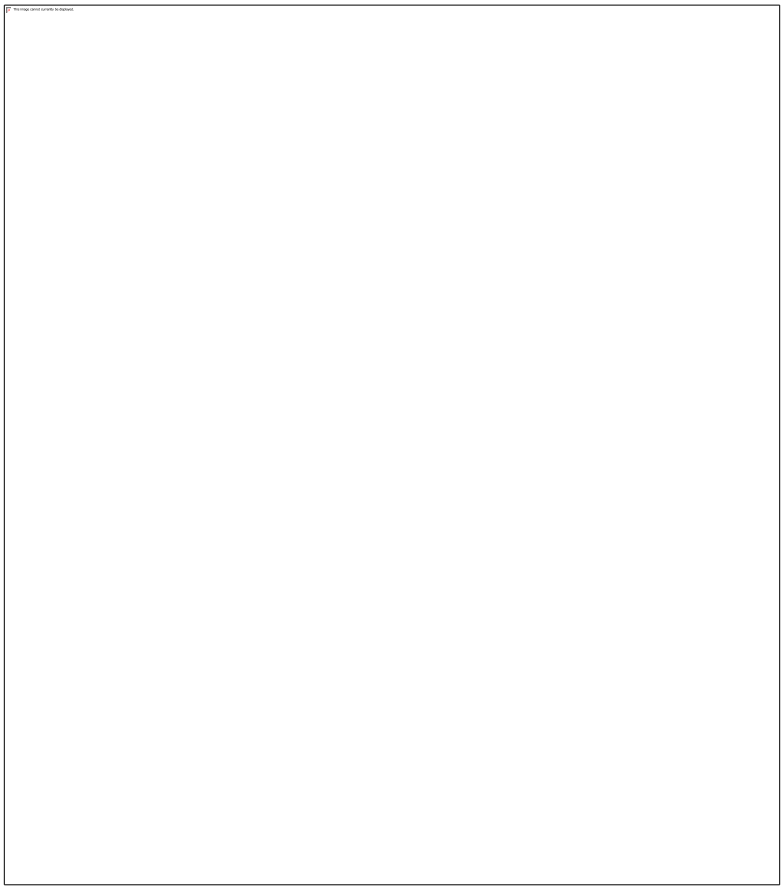


Fig7:Thermal block incubator.

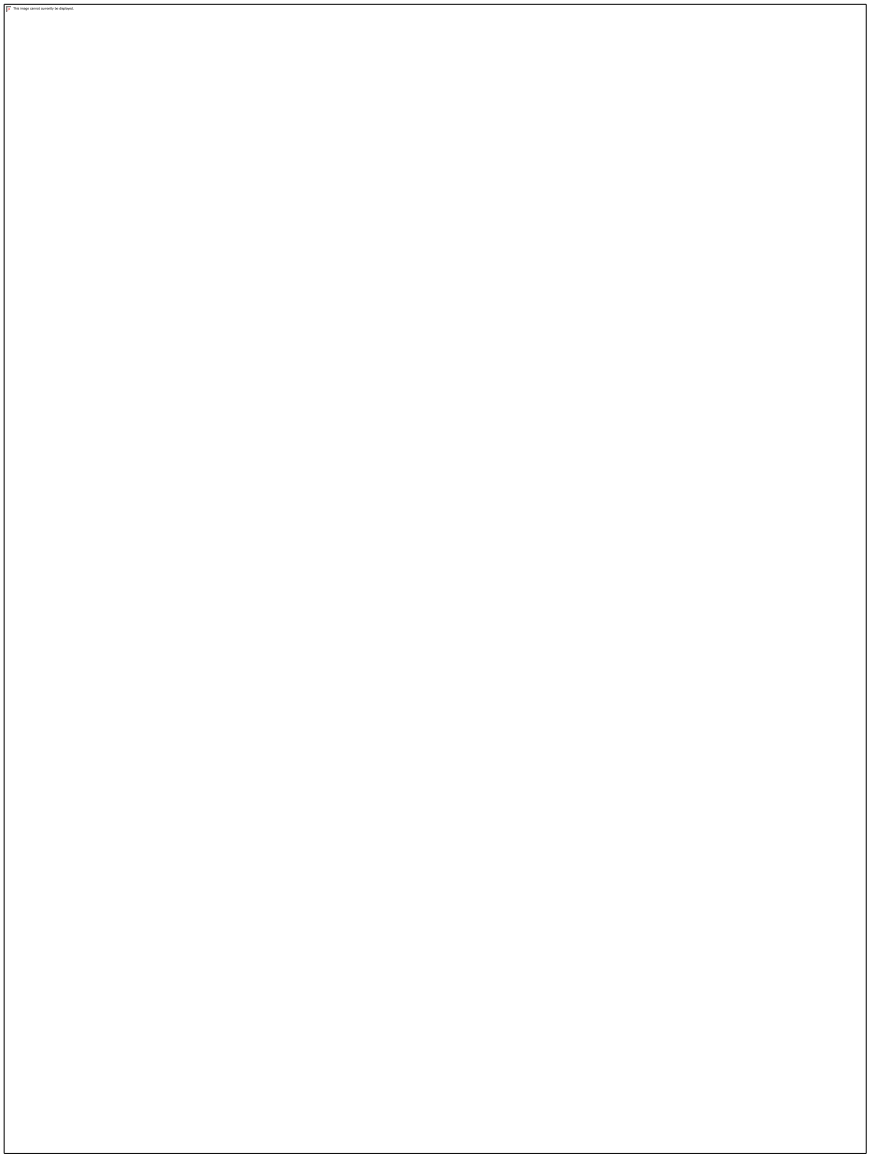


Fig 8:Thermo-Cycler machine (Bio-Rad).