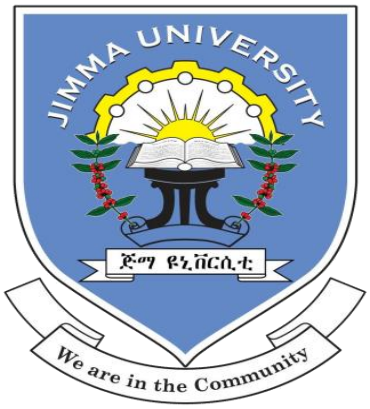
INTESTINAL COLONIZATION OF VANCOMYCIN-RESISTANT *ENTEROCOCCI* AND ITS ASSOCIATED FACTORS AMONG PATIENTS ATTENDING ANTICANCER TREATMENT AT ONCOLOGY WARDS OF JIMMA MEDICAL CENTER, SOUTHWEST ETHIOPIA: A COMPARATIVE CROSS-SECTIONAL STUDY



BY:

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A RESEARCH THESIS SUBMITTED TO THE, SCHOOL OF MEDICAL LABORATORY SCIENCES, FACULTY OF HEALTH SCIENCE, INSTITUTE OF HEALTH, JIMMA UNIVERSITY, IN PARTIAL FULFILLMENT OF MASTER OF SCIENCE DEGREE IN MEDICAL MICROBIOLOGY

MAY 2022

JIMMA, ETHIOPIA

JIMMA UNIVERSITY

INSTITUTE OF HEALTH

FACULTY OF HEALTH SCIENCE

SCHOOL OF MEDICAL LABORATORY SCIENCE

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May 2022

Jimma, Ethiopia

ABSTRACT

Background: *Vancomycin-resistant Enterococcus (VRE) is the genus Enterococcus that retains either intrinsic or acquired resistance to the antibiotic vancomycin, which is used to treat serious infections caused by these bacteria. VRE has increasingly become a major public health threat globally, due to limited therapeutic options. Even though it becomes the most public health threat, there is insufficient data in the study area as well as in Ethiopia. VRE causes severe infections among patients with weakened immune systems such as cancer patients who, undergo anticancer treatment and these infections are usually preceded by gastrointestinal colonization. Therefore, to prevent VRE-associated infections, it is crucial to identify a patient colonized with VRE.*

Objectives: *This study is aimed at determining the magnitude of intestinal colonization with VRE and associated factors among patients attending anticancer treatment at oncology wards of Jimma Medical Center (JMC).*

Methods: *A comparative cross-sectional study was conducted from April 2021 to September 2021 on a total of 226 study participants at JMC, among 113 patients attending anticancer treatment at oncology wards and an equal number of "apparently healthy clients" in Jimma, Southwest Ethiopia. Pretested structured questionnaires were used to collect sociodemographic and clinical data. Stool samples were collected for both groups and inoculated onto Bile Esculin Azide Agar with and without 6 µg/ml of vancomycin plates. Enterococcus species were identified based on their colony characteristics, gram stain, catalase test, salt tolerance, temperature tolerance, and PYR tests. Antibiotic susceptibility tests were done using the Kirby–Bauer disk diffusion and the Minimum Inhibition Concentration (MIC) was determined for vancomycin by the E-test strips. The data were entered into Epidata v4.6 and were exported to SPSS v26 for analysis. Descriptive statistics, bivariate, and multivariate logistic regression analyses were performed to evaluate the association with the outcomes of interest at a 95% confidence interval, and a P-value <0.05 was considered as statistically significant.*

Result: *In this study, a total of 226 study participants were enrolled. The overall colonization of Enterococci species was seen in 78.8% (178 /226). Among these, VRE colonization was 8.4% (95% CI = 4.3–12.5),15/226. VRE among patients attending anticancer treatment at oncology wards and "apparently healthy clients" was 11/87 (12.6%) and 4/91 (4.4%), respectively. Those* patients

attending anticancer treatment *did not have a statistically significant association (p = 0.058) with the colonization of VRE.*

The present study showed that multidrug resistance was observed in 66.7% of VRE isolates. Prior antibiotic exposure in the last three months (AOR = 4.33; 95% CI: [1.129–16.6], P = 0.033) and history of hospital admission in the last three months (AOR = 4.088; 95% CI: [1.083–15.438], P = 0.038) showed statistically significant association with VRE colonization.

Conclusion: *In this study overall prevalence of VRE colonization was 8.4 %. A patient attending anticancer treatment did not have a statistically significant association with the colonization of VRE. Prior antibiotic exposure and a history of hospital admission in the past three months were significantly associated with VRE colonization. The observed VRE with multidrug resistance colonization needs, rational use of antibiotics, more detailed study, and implementation of infection prevention protocols to reduce colonization by VRE among patients attending anticancer treatment or admitted to oncology wards.*

Keywords: Vancomycin-resistant enterococci, Cancer, Oncology, Enterococci, JMC.

ACKNOWLEDGMENTS

My first and heartfelt thanks go to the School of Medical Laboratory Sciences, Institute of Health, and Jimma University for giving me the chance and logistic support to conduct this research.

I would like to express my appreciation and deepest acknowledgment to my advisors Dr. Mulualem Tadesse, Mrs. Rahel Tamrat, and Dr. Diriba Fufa for their, valuable comments, unreserved support, encouragement, and guidance throughout the work. I am thankful for both the personal and academic guidance you have offered me, beginning from the title selection until the write-up of this thesis. It was a privilege to work with you all and learn a lot from your experience.

I am also very grateful and would like to extend my heartfelt thanks and appreciation to the study participants for their willingness to be part of the study. Finally, I would like to thank the Jimma medical center, Microbiology laboratory staff for their moral and technical support throughout the entire research process.

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AST Antimicrobial Susceptibility Testing

ATCC American Type Culture Collection

BHI Brain heart infusion

BSI Blood Stream Infection

CBC Complete Blood Count

CDC Centers for Diseases Control and Prevention

CLSI Clinical and Laboratory Standard Institute

ETB Ethiopian Birr

FNAC Fine Needle Aspiration Cytology

ICU Intensive Care Unit

JMC Jimma Medical Center

MIC Minimum Inhibition Concentration

NaCl Sodium chloride

SOP Standard operating procedure

SPSS Statistical Package for Social Sciences

VRE Vancomycin-Resistant Enterococcus

WHO World Health Organization

1. INTRODUCTION

1.1 Background

Enterococci are gram-positive, facultative anaerobic, catalase-negative cocci, arranged in pairs and short chains of various lengths, which colonize the gastrointestinal tracts of humans and animals (1). The *Enterococci* were previously classified as group D *streptococci*, but in 1984, they were reclassified into the new genus *Enterococcus*, which belongs to the family *Enterococcaceae* (2). Currently, there are 59 validly published species with the correct name. Among them, *E. faecalis* and *E. faecium* are responsible for the majority of infections (3). *Enterococci* can grow under a wide range of temperatures (10°C–45°C) and pH (4.6–9.9). They are also characterized by their ability to grow at 6.5% concentrations of sodium chloride (NaCl) and 40% bile salts. Moreover, they can hydrolyze esculin and L-pyrrolidinyl-β-naphthylamide (PYR) (1).

Enterococci were previously considered commensal organisms of little clinical importance but have emerged as serious hospital-acquired pathogens responsible for several infections. However, they display low levels of virulence, as evidenced by their presence as natural colonizers of the gastrointestinal (GI) tract in most humans and animals. Its virulence is mediated by its ability to adhere to tissues and form biofilms, and antibiotic resistance. Numerous factors mediate adherence and biofilm formation, such as surface proteins, membrane glycolipids, pili, and gelatinase (2,4).

Enterococci become resistant to a variety of antimicrobial agents through intrinsic and acquired mechanisms. Moreover, *Enterococci* acquire resistance to currently available drugs either by mutation or receipt of foreign genetic materials through the transfer of plasmids and transposons, to efficiently attain and transfer mobile resistance elements, facilitating the dissemination of resistance genes (5).

Vancomycin, a glycopeptide antibiotic that had been in use since the 1950s, was used as the last line of defense for the treatment of multidrug-resistant gram-positive bacterial infections, including *Enterococci* (6). However, the emergence of vancomycin-resistant *enterococci* (VRE) poses a major global public health problem since it was first reported in England and France in 1986 (7). VRE is defined as members of the genus *Enterococcus* that retain either intrinsic or acquired resistance to the antibiotic vancomycin, which is used to treat serious infections caused by these bacteria (8). Vancomycin resistance in *enterococci* is mediated by van genes, which code

for enzymes that make low-affinity precursors that modify the vancomycin-binding target. Currently, there are nine known vancomycin-resistant phenotypes: *van A, van B, van C, van D, van E, van G, van L, van M, and Van N*. These are distinguishable by their degree of reduced susceptibility to vancomycin, transferability, and inducibility (9). Among the vancomycin resistance gene clusters, the two, *vanA* and *vanB,* are the most prevalent globally and are responsible for acquired vancomycin resistance (9). The main mechanism of vancomycin resistance in *Enterococci* involves the alteration of the peptidoglycan synthesis pathway, specifically the substitution of the normal amino acid D-Alanine-D-Alanine to either D-Alanine-D-Lactate or D-Alanine-D-Serine (10). Such modifications can result in variable expressions of vancomycin resistance. For example, the respective altered D-Alanine-D-Lactate and D-Alanine-D-Serine lead to the less binding affinity of glycopeptide drugs compared to the normal cell wall precursors D-Alanine-D-Alanine; approximately 1000-fold decreased binding affinity for D-Alanine-D-Lactate and nearly 7-fold for D-Alanine-D-Serine (10).

In 2017, the World Health Organization (WHO) published the priority list of antibiotic-resistant bacteria and categorized VRE as a high-priority pathogen for which new and effective treatments are required (11). Also in 2019, the Centers for Disease Control and Prevention (CDC), categorized the organisms based on the burden of antibiotic-resistance threats; hence, they classified VRE under Serious Threats (12).

There are several risk factors for VRE infections, such as GI colonization, prolonged or repeated hospitalization, exposure to antibiotics, such as vancomycin, third-generation cephalosporin, and severe underlying disease, such as cancer patients receiving treatment for certain types of cancer (11,12). Following gastrointestinal colonization, *enterococci* can lead to bloodstream infections, infective endocarditis, urinary tract infections, surgical wound infections, and pelvic abscesses in critically ill patients, such as cancer patients, because of prolonged experiences of anticancer treatment and repeated antibiotic exposures (13). To prevent VRE-associated infections, it is important to determine the source of infection and routes of transmission, as well as identify patients colonized with VRE. However, there is no information about this critical problem among cancer patients in Ethiopia as well as in the study area. Therefore, this study aims to determine the magnitude of intestinal colonization by VRE and associated factors among patients attending anticancer treatment at oncology wards of JMC.

1.2 statement of the problem

Vancomycin-resistant *enterococcus* (VRE) is now becoming the most important public health concern and threat since it was first reported in Europe in 1986 and has alarmed the global infectious diseases community due to the few treatment options left (4). *Enterococcus* has remarkable genome plasticity and utilizes plasmids, transposons, and insertion sequences to efficiently attain and transfer mobile resistance elements, between the species as well as to other gram-positive bacteria, such as *Staphylococcus aureus* to form a vancomycin-resistant *Staphylococcus aureus* (VRSA) (14). VRE exhibits public health threat and concern not only due to intrinsic and acquired resistance but also in their ability to transfer resistance genes to other multi-drug resistant pathogens such as Methicillin-resistant *Staphylococcus aureus* (MRSA), which significantly limits therapeutic options for such diseases, especially in the immunocompromised such as cancer patients (3).

There are two mechanisms of vancomycin resistance in enterococci: intrinsic and acquired. Acquired resistance is primarily found in *E. faecium* and *E. faecalis* and is typically encoded by the *van A and van B genes* (9). In humans, most clinical infections are due to *E. faecalis* (80–90%), followed by *E. faecium* (10–15%) (4). several studies have shown that *Enterococca*l infections are most commonly caused by the patient's commensal flora and that colonization may occur before infection. Following gastrointestinal colonization, *Enterococci* can lead to bloodstream infections, urinary tract infections, infective endocarditis, intra-abdominal/pelvic abscesses, and rarely meningitis in critically ill patients, such as cancer patients, due to the likelihood associated with the highest odds of hospital stay, prolonged episodes of neutropenia, and repeated antibiotic exposures (13).

Patients undergoing chemotherapy and other anticancer treatments are at particular risk because of their compromised immune systems, including diminished innate immunity and permeable mucosal barriers, which facilitate colonization of the intestinal tract with VRE (15). Moreover, cancer patients visit hospitals and use antibiotics and chemotherapy repeatedly, all of which are factors that contribute to developing vancomycin and other antimicrobial-resistant *Enterococci* (16).

VRE-colonized cancer patients tend to develop VRE infections more frequently than the general population of hospitalized patients (17). Patients with cancer are at high risk of acquiring VRE and

developing VRE infections. VRE-colonized patients were as much as 24 times more likely to develop infections, particularly bloodstream infections (BSI), and up to 13% of colonized patients develop such infections over an approximate median period of 5 weeks (18). Bloodstream infections (BSIs) are a predominant cause of morbidity and mortality among cancer patients, with, a mortality rate of nearly 37% (19). Another study showed that among colonized patients, the VRE infection rates vary widely from 0–45% with the risk of VRE bacteremia being reported from 0–16% but less than 2% among non-colonized patients (20).

In 2017, WHO categorized VRE as a high-priority pathogen among three levels of the WHO priority list: critical, high, and medium, based on mortality, with a (21–40%) mortality rate, healthcare burden, community burden, prevalence of resistance, and 10-year trend of resistance, transmissibility, preventability in the community setting, preventability in the health-care setting, treatability, and pipeline(21).

In 2019, CDC assessed antibiotic resistance threats and categorized VRE under serious antibiotic-resistant threat among three levels of categories: urgent, serious, and concerning based on clinical impact, economic impact, incidence, and 10-year projection of incidence, transmissibility. availability of effective antibiotics, and barriers to prevention (12). In the same year,2019 the CDC report shows that VRE causes an estimated 54,500 hospitalizations and 5,400 deaths per year in the United States, with $539 million in estimated healthcare costs (12).

VRE has risen markedly in the last two decades, with increasing colonization and infection in many countries of the world. Due to its clinical and public health significance, the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) listed VRE as a high-priority and serious pathogen for which direct research and development of new and effective treatments are needed (11,12). The European Center for Disease Prevention and Control defined and concerned multidrug-resistant *enterococcus* because of its epidemiological significance, emerging antimicrobial resistance, and the importance of these bacteria within the healthcare system (22).

The rates of VRE are at their highest in the USA, and they rank as the second most common cause of hospital-acquired infections (23). In Europe, the proportion of VRE also increased from 8.1%

in 2012 to 19.0% in 2018 (24). According to a systematic review and meta-analysis conducted in Africa, the overall prevalence of VRE was 26.8% (25). Another systematic review and meta-analysis conducted in Ethiopia indicate the rise of VRE with a pooled prevalence of 14.8% (6). In addition to that study conducted in Jimma, Ethiopia, to determine the antimicrobial resistance profile of Enterococcus species from the intestinal tracts of hospitalized patients, the prevalence of VRE was observed in 5% of the isolates (26), and three years later, in the study conducted among pediatric patients in the same study area, VRE dramatically increased to 22.7% (27). Besides that, a report on the emerging development of resistance to newer agents, such as daptomycin and linezolid, which are being used for treating VRE infections (4) could clarify the threats and challenging nature of these bacteria in the current treatment as well as in the future.

In April 2017, Ethiopia established the National Antimicrobial Resistant Surveillance Centers and identified national priority surveillance pathogens under the mandate of the Ethiopian Public Health Institute (EPHI), with the objective of prevention and control of antimicrobial resistance. Jimma Medical Center is one of the sentinel surveillance sites (28). Even if Jimma medical center is one of the surveillance sites and the rising rates of VRE infections are being reported elsewhere in the world, and WHO recommends VRE for research, VRE is not included in the national priority surveillance pathogens. Hence, there is a shortage of national and local data in Ethiopia as well as in the study area, particularly among cancer patients.

1.3 Significance of the study

VRE has been emerging and is posing a therapeutic challenge to physicians due to the few treatment options left as well as the ease of acquiring and transferring resistant genes within the same species and between different species. Nearly all VRE infections happen in patients with healthcare exposures, antibiotic use, and receiving treatment for certain types of cancer, which is common among patients attending oncology wards (12). Hence, the CDC recommends screening high-risk patients such as those attending anticancer treatment or admitted to oncology wards. However, in Ethiopia, as well as in the study area, there is no published data that indicates the colonization rates of VRE and associated factors among cancer patients. Therefore, this study aims to determine the magnitude of intestinal colonization by VRE and associated factors among patients attending anticancer treatment at oncology wards of JMC. Knowing the magnitude is important for developing appropriate infection prevention and setting effective control measures to prevent the spread of this threat in the hospital and the community. In addition to that, the result of this study will serve as a data source for policymakers and other stakeholders to understand the problem and management of infection caused by VRE among patients attending anticancer treatment at oncology wards. Furthermore, to the best of our knowledge, this study is the first in Ethiopia as well as in the study area; hence, it can serve as a baseline study for further related studies to be conducted in the country.

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2. LITERATURE REVIEW

2.1 Prevalence of VRE colonization

Vancomycin-resistant enterococci are a global challenge currently, as reported by the WHO and CDC. They can cause serious hospital-acquired infections, especially in the immunocompromised, such as cancer patients. The growing rates of VRE infections are being reported elsewhere worldwide. A Systematic Review and Meta-Analysis were conducted worldwide to determine the prevalence of colonization with VRE and the risk for bloodstream infection (BSI) among patients with malignancy. Overall, the pooled prevalence of VRE gastrointestinal colonization was 20% (18).

In Brazil, Latin America, a study conducted on febrile neutropenia following chemotherapy, between March 2014 and 2015, found *Enterococcus spp*. in 27% of patients, and of that, 25% of the isolates were VRE (29). In the same country, Brazil, a case-control study done to determine the incidence and the risk factors associated with VRE colonization among ICU patients found that 23.1% of the study group were colonized by VRE (30). On the same continent, in South America, a case-control study conducted in Chile among hospitalized pediatric patients with an oncological diagnosis showed that 52% of the patients were colonized with VRE (16).

In the United States, a retrospective study was done to determine the frequency, risk factors, and outcomes of VRE colonization and infection in patients with newly diagnosed acute leukemia showed that 38% were colonized with VRE (31). Another systematic review and meta-analysis conducted in the same country in the United States in oncology units indicate that 23% of study participants were colonized with VRE (32).

In Europe, A study conducted in Turkey to determine VRE colonization and VRE-related infections in patients with hematological malignancies showed that 39.68% of patients were colonized with VRE (33). Another study conducted in Germany under the title "Prospective infection surveillance and systematic screening for VRE in hematologic and oncologic patients" shows that 23.8% of patients had intestinal VRE-colonization (34). In the same country, Germany, another study showed that intestinal colonization of VRE in patients with hematological and oncological malignancies was 9.9% (35). Another cross-sectional study in Ireland on vancomycin-

resistant enterococci carriage in an acute Irish hospital showed that 19.1% of specimens were positive for VRE (36).

According to a systematic review and meta-analysis conducted to estimate the pooled prevalence of VRE in Asia from January 1, 2000, to September 20, 2020, the overall VRE was 8.10% (37). A cross-sectional study was performed in Iran to identify VRE colonization and related risk factors among hematological malignancies after hematopoietic stem cell transplant. It showed that VRE was in 33% of the study groups (38). A prospective study in India to determine the colonization rate of community-acquired multidrug-resistant organisms in children with cancer showed that 12.68% of the patients were colonized with VRE (39).

A systematic review and meta-analysis conducted in Africa from a one-health perspective indicated that the overall pooled prevalence of VRE was 26.8% (25). Another systematic review and meta-analysis conducted in Nigeria showed that the pooled prevalence of VRE was 25.3% (40). In Morocco, the cross-sectional study done to determine the rate of intestinal carriage of VRE showed that the rate of fecal carriage of VRE in the community was 21% (41). Another retrospective study in Egypt on the pediatric oncology unit showed that VRE represented 75% of the isolated Enterococci among the study participants (42).

According to a systematic review and meta-analysis conducted in Ethiopia to estimate the pooled prevalence of VRE and antimicrobial resistance profiles of enterococci, the pooled prevalence of VRE was 14.8% (6). Another cross-sectional study was carried out in Gondar, Ethiopia, showing that 5.5% of the study group were colonized with VRE (43). A comparative cross-sectional study conducted in Dessie, Ethiopia, found that the overall prevalence of VRE colonization was 6.3%(44). A cross-sectional study conducted in West Amhara Government Hospitals in Ethiopia to determine the prevalence of VRE colonization among HIV-infected patients indicates that the prevalence of VRE colonization was 7.7% (45). Another study in Arba Minch, Ethiopia, showed that 11.4% of study participants were colonized with VRE (46).

The study conducted among hospitalized patients at Jimma, Ethiopia to determine the antimicrobial resistance profile of *Enterococcus species* from the intestinal tracts of hospitalized patients showed that the Overall, *Enterococci* were isolated from 76% of the study subjects. Among them, VRE was observed in 5% of the isolates (26). Another cross-sectional study was conducted in Jimma, Ethiopia in 2016 to determine the prevalence and phenotypic characterization

of *Enterococcus species* isolated from clinical samples of pediatric patients and showed that 22.7% of the isolates were VRE (27).

2.2 Risk factors for VRE

Nearly all VRE infections happen in patients with healthcare exposures and in immunosuppressed patients who have received multiple courses of antibiotics in the past (7). According to a 2019 CDC report, there are various risk factors related to VRE colonization. These include a long period of hospitalization or admission in intensive care units, undergoing organ transplants, or receiving treatment for certain types of cancer (12).

A Systematic Review and Meta-Analysis conducted to determine the risk for bloodstream infection among malignancy patients' colonization with VRE indicated that vancomycin use, hospitalization within 3 months, and being a patient with acute leukemia were associated with an increased colonization risk for VRE (18).

A systematic review and meta-analysis conducted in the United States in oncology units showed that previous use of vancomycin and ceftazidime were risk factors for VRE colonization (32). Another prospective cohort study carried out on the hematology-oncology unit at Northwestern Memorial Hospital in the United States showed that prolonged length of hospital stay, prior hospitalization, previous ICU admission, and receipt of amikacin were risk factors associated with VRE acquisition and colonization (47). Another prospective cohort study conducted on adult patients with hematological malignancy shows renal insufficiency, aminoglycoside use, ant-anaerobic antibiotic use, plus gastrointestinal disturbance, severe neutropenia, and prior beta-lactam antibiotic use were risk factors for VRE colonization and BSI infections (48).

A case-control study conducted in Chile among hospitalized pediatric patients with an oncological case shows that days of hospitalization prior to the study, neutropenia, treatment with antibiotics within 30 days prior to the study, and microsites were significant risk factors associated with VRE colonization(16). A case-control study done in Brazil’s to determine the incidence and the risk factors associated with VRE colonization found that. prior antibiotic use, carbapenems use, and nephropathy as comorbidity were associated with VRE colonization (30).

A cross-sectional study was performed in Iran to identify VRE colonization and related risk factors among hematological malignancies found that antibiotic prophylaxis and hospitalization were

independent risk factors for the acquisition of VRE (38). Another prospective cross-section study in India shows that increased duration of hospital stay, younger age, consumption of ceftriaxone and vancomycin were found to be significant risk factors for VRE colonization (49).

A cross-sectional study conducted in West Amhara, Ethiopia, shows that antibiotic treatment (for >2 weeks) and history of hospital admission in the last six months were found to be statistically associated with VRE colonization (45). Another study in Arba Minch, Ethiopia showed that prior antibiotic exposure for more than two weeks and hospitalization for the last six months were significantly associated with VRE colonization (46). Another cross-sectional study carried out in Gondar, Ethiopia showed that antibiotic treatment for the last 2 weeks was found to be the risk factor for VRE colonization (43).

2.3 Antibiotic-resistant pattern of *Enterococcus*

Enterococci have the potential to develop resistance to almost all antibiotics used in clinical practice. Antibiotic resistance mechanisms in *enterococci* may be intrinsic to the species or acquired by mutation of intrinsic genes or horizontal transmission of genetic material encoding resistance determinants (5). Today many reports indicate the rise of VRE as well as multidrug-resistant *enterococcus.*

A cross-sectional study conducted in Shiraz, Southern Iran to determine VRE colonization and related risk factors among patients with hematological malignancies showed that the isolated VRE were resistant to Ampicillin (86%), Penicillin (93%), Erythromycin (100%), chloramphenicol (57%), Tetracycline (71%), with 85% overall multidrug-resistant (38).

A comparative cross-sectional study conducted in Dessie Northeast Ethiopia indicates that (34.8%) of isolated enterococci were resistant to ampicillin and penicillin. (22.3%) of isolates were resistant to erythromycin;(7.1%), and (8.9%) of the isolate were resistant to ciprofloxacin and Chloramphenicol respectively with 22.3% of overall multiple drug resistance (44).

Another cross-sectional study conducted in West Amhara Government Hospitals showed that the isolated enterococcus was resistant to Ampicillin(20.9%), Chloramphenicol (30.9%), erythromycin (42.7%), Ciprofloxacin (37.7%) and Tetracycline (28.6%) with an overall (64.5%) multidrug resistance(45).

An institution-based cross-sectional study conducted in Arba Minch, Ethiopia showed that the isolated enterococci species showed various resistances to the tested antibiotics; namely, 69.9% to ampicillin, 54.5% to penicillin, 49.6% to erythromycin, 59.3% to tetracycline, 28.5% to ciprofloxacin and 21.1% to chloramphenicol with (49.59) Multidrug resistance (46).

Another cross-sectional study carried at the University of Gondar Teaching Hospital found that (79.6%) of Ampicillin, (33.8%) of Ciprofloxacillin, (63.2%) of Erythromycin, (12.4%) of Chloramphenicol were resistant to enterococcus isolate respectively, with (90%) overall multidrug-resistant (43).

Another cross-sectional study conducted among hospitalized patients at Jimma University Specialized Hospital found that (36%) of ampicillin, (74.6%) Penicillin, (50%) Ciprofloxacin,(63.2%) Erythromycin, (34.2%) Chloramphenicol, and (64.9%) Tetracycline were resistant to isolated Enterococcus species with 89.5% Multiple drug resistance (26).

A systematic review and meta-analysis conducted in Ethiopia showed that (44.5%) of tested Ampicillin, (32.9%) Chloramphenicol, (36.5%) Ciprofloxacin, (49.6%) Erythromycin, (60.7%) Penicillin, (53.7%) of Tetracycline, were resistance to isolated enterococci to with (60%) multidrug resistance (6).

Generally, all the above literature shows that decreased immunity, prior antimicrobial exposure, and history of hospitalization, which are commonly encountered in cancer patients, are well-recognized risk factors for VRE colonization. However, in our study area, no data show the magnitude and possible risk factors of vancomycin-resistant enterococcus colonization among patients attending anticancer treatment at the oncology ward. Therefore, this study aims to fill this gap.

3. OBJECTIVE OF THE STUDY

3.1 General Objective

- To determine the magnitude of intestinal colonization of vancomycin-resistant *enterococci* and its associated factors among patients attending anticancer treatment at oncology ward and apparently healthy clients at Jimma medical center, southwest, Ethiopia in 2021.

3.2 Specific Objectives

- To determine the magnitude of intestinal colonization of VRE among Patients attending anticancer treatment at oncology wards and apparently healthy clients at JMC.
- To determine factors associated with VRE colonization of patients attending anticancer treatment at oncology wards.
- To assess the antibiotic resistance patterns of the isolated VRE.

4. MATERIALS AND METHODS

4.1 Study Area

The study was conducted at Jimma Medical Center (JMC) among patients attending anticancer treatment at oncology ward and “apparently healthy clients”. The Jimma Medical Center is located in Jimma Town, which is located 354 km away from the capital city, Addis Ababa, in the southwestern direction of Ethiopia. The town is located at an altitude of about 1780 meters above sea level and has a latitude and longitude of 7°40′N, 36°50′E. Jimma town has a warm and humid climate with a mean annual maximum temperature of 33°C and a mean annual minimum temperature of 10°C. It lies in the climatic zone locally known as' Woyna Daga ', which is considered ideal for agriculture as well as human settlement (50).

JMC is the only referral and teaching medical center in the region. The various sections of the university hospital provide service for an inpatient and outpatient department with a projected population of over 20 million people in the catchment area of the southwestern parts of Ethiopia. Among the primary services, the oncology ward is the one that provides services to cancer patients in pediatric and adult oncology units. Based on the type of the case, either chemotherapy, surgery, or a combination of them is given as the current therapeutic approach of the JMC oncology unit. In addition to that, JMC is one of the six hospitals selected to provide radiation therapy in the country, and more recently, it has been launching a radiotherapy service since October 2021.

4.2. Study period

The study was conducted in JMC from April 1, 2021, to September 30, 2021.

4.3. Study design

A comparative cross-sectional study was conducted to determine the magnitude of VRE colonization and its associated factors.

4.4 Population

4.4.1 Source population

The source population was all clients who had visited the oncology unit and candidates for any type of anticancer treatment at JMC during the study period.

4.4.2. Study population

The study population was all patients who had been taking any type of anticancer treatment, either admitted to the hospital as inpatients or visiting the hospital as an outpatient in JMC, oncology wards during the study period.

4.5. Inclusion and exclusion criteria

4.5.1. Inclusion

All age and sex patients who were admitted and/ or outpatients attending anticancer treatment at the oncology wards of JMC during the study period were included.

4.5.2. Exclusion Criteria

Patients who were critically ill and could not provide a sample and had a sign and symptoms of infections during data collection were excluded from this study.

4.6 Sample size determination and sampling technique

The sample size was determined by a double population proportion formula, using Epi-info V 7. 50% of the prevalence was given to "apparently healthy comparatives," while the proportion given to patients attending anticancer treatment at oncology wards (cases) was 70%. This was dependent on an assumption that patients attending anticancer treatment at the oncology ward have a 20% higher VRE carriage rate than "apparently healthy clients"(18). By using the power of 80% and confidence level of 95% for the two-sample sizes with a 1:1 ratio, the initial sample size was 206. By considering the 10% non-response rate, the final sample size was 226. Of those, 113 were patients attending anticancer treatment at oncology wards and another 113 were health clients. A consecutive sampling method was used to recruit the study participants until the intended sample size was reached.

4.7. Study Variables

4.7.1. Dependent variables

- Colonization of Vancomycin-resistant Enterococci
- Antimicrobial susceptibility pattern of isolated Vancomycin-resistant enterococci

4.7.2. Independent variables

Socio-demographic data

- Age
- Sex
- Residence
- Educational status

Clinical data

- Being a Cancer patient vs apparently healthy individual.
- Duration of hospital stay before the onset of sample collection
- Type of cancer
- Duration of cancer
- Type of cancer treatment
- Duration of treatment
- Cycles of treatment
- Comorbidities (renal failure, diabetic Mellitus…)
- Antibiotic taking during the previous three months of data collections
- Types of Antibiotics
- Any invasive procedures within the prior 1 month such as Vascular or urinary catheter, Gastric tube, Nasal catheter, and Central venous catheter.
- Results of hematological parameters (CBC)

4.8 operational definitions

- ✓ **Anticancer treatment**: any of one or more therapeutic strategies to stop, prevent and fight, against malignant, or cancerous, disease. It includes chemotherapy, radiation therapy, surgery, and Combination therapy, depending upon the location, and the stage of the disease.
- ✓ **Attending oncology ward:** a client diagnosed with any of the oncological cases in the oncology ward and confirmed to have the case or abnormal cell by physicians and starting taking either one or a combination of anticancer treatments.
- ✓ **"Apparently healthy Client":** is an individual or group of clients who do not visit oncology wards for a diagnosis of cancer or oncological cases and who have not been admitted and taken an antibiotic within the past three months.
- ✓ **Cancer Patient**: either Solid or hematologic malignancy diagnosed based on history, physical examination, imaging modalities such as CT scan, X-ray, ultrasound, laboratory tests, and pathologic results such as FNAC and biopsy.
- ✓ **Clients visiting oncology wards:** a client visiting oncology wards for oncological cases and is a candidate for either one or a combination of anticancer treatments.
- ✓ **MDR**: was defined as non-susceptibility to at least one agent in three or more antimicrobial categories.
- ✓ **VRE colonization**: was defined as a patient or an individual carrying VRE in the gastrointestinal tract and positive for fecal samples but who did not have clinical signs or symptoms of infection.

4.9. Study procedure

4.9.1 Data collection procedures

Data were collected using a short interview guided by a pre-tested structured questionnaire consisting of the participant information like socio-demographic information, clinical information, and data on associated factors from each informed and consented, or assented study participant, who were either admitted or outpatients attending anticancer treatment at the oncology ward of JMC.

Data collectors were selected and oriented on how to collect the data as per the structured questionnaire. Then data was collected from the clinical record and from the interview by oriented/trained data collectors (nurses) who speak the local language. The data collection process was closely monitored and followed daily by the principal investigator. The questionnaire was initially prepared in English and then translated into the local languages of Amharic, Afan, oromo, and then re-translated back to English to check the reliability and conceptual consistency of items.

Moreover, the study participants and /or guardians were informed about their rights. Study participants were not included in the study when they were unwilling to share their personal data and when they were not able to give specimens.

Recruitment of comparator groups- apparently healthy individuals

Comparative groups, which are apparently healthy individuals, were Jimma university students, who were attendants or friends of Jimma university students, who attended Jimma university student clinics, and patient attendants in JMC during the study period. The study participant that was "apparently healthy group" were recruited and selected after agreeing to participate in the study and signing informed written consent and/or assent in which the objective of the study was explained to them clearly. Then the study participants who do not visit oncology wards for a diagnosis of any cancer/malignancy and who have not been admitted and taken an antibiotic within the past three months were enrolled in the study as comparative groups.

4.9.2. Stool sample collection

Each patient or the legal guardians of patients and healthy control were instructed how to collect stool specimens, and 5mg of a fecal specimen was collected in a sterile wide-mouth screw-capped container from each consented patient and labeled with the unique ID number, date, and time of collection. The samples were delivered to JMC Microbiology Laboratory for culture within 30 minutes. The collected samples were kept in the refrigerator for 1-2 hours if unprocessed immediately (51). The principal investigator was leading each stage of sample collection and processing, which are the pre-analytical, analytical, and post-analytical stages.

4.9.3. Sample processing, culture, and identifications

The collected stool specimens were inoculated on two sterile Bile Esculin azide agar (Hardy Diagnostics, USA) plates (one plate with 6 µg/ml of vancomycin and the other without 6 µg/ ml of vancomycin) and incubated at 35–37°C for 24–48 hours. Plates were observed for the appearance of characteristic colonies of growth and blackening. Those with a black background and gray hallow colonies were selected and picked for characterization and identified presumptively as *Enterococci Spss* by the following phenotypic tests: Gram stains, Catalase test, Salt tolerance test, Heat tolerance test, and PYR tests respectively.

Gram staining: Only plates that yield gram-positive cocci in pairs or short chains were studied further. **Catalase test**: a catalase test was performed on suspected gram-positive cocci colonies and only microbial growth that yielded a negative result for catalase production was considered further. **Growth in 6.5% NaCl broth**: similar presumptive pure colonies from each plate were picked and inoculated into brain heart infusion (BHI) (Oxoid, UK). broth containing 6.5% NaCl and incubated at 37°C for 24–48 hours, and growth in the medium was indicated by turbidity. **Growth at 45°C**: presumptive colonies which fulfilled the above criteria were picked, inoculated into BHI broth, and incubated at 45°C for 24 hours, and growth in the medium was indicated by turbidity. Finally, after all the above procedures the biochemical test was done using a rapid **Pyrrolidonyl Arylamidase (PYR) test:** colonies that fulfilled the above criteria were smeared onto a PYR disk and Bright pink or cherry-red color within 1-2 minutes was considered as a PYR positive, and Enterococcus was Positive for PYR. An isolate fulfilling the above criteria was considered an Enterococcus species (52–54).

4.9.4. Antimicrobial susceptibility test

The antimicrobial susceptibility testing of Enterococci isolates was performed by the Kirby Bauer disk diffusion technique on Muller-Hinton agar (Oxoid, UK). as modified by the Clinical and Laboratory Standard Institute in 2021(CLSI) (55). From the growth on BHI (Oxoid, UK). broth, the bacteria was refreshed onto nutrient agar for 18-24 hours. Then 3-5 pure colonies from nutrient agar were taken and transferred to a tube containing 5 ml normal saline and mixed gently to make a homogenous suspension. The turbidity of this suspension was adjusted by comparing it with 0.5 McFarland standards. A sterile cotton swab was used to streak the plates and the excess suspension

was removed by gentle pressing and rotation of the swab against the inside wall surface of the tube. Then the swab was used to distribute the bacteria evenly over the entire surface of Mueller Hinton agar (MHA). The inoculated plates were left in a room temperature to dry for 3-5 minutes and with the aid of sterile forceps, the following concentration of antibiotic discs was impregnated on the surface of Mueller hinter agar: vancomycin (30 μg), penicillin (10 IU), ampicillin (10 µg), erythromycin (15 µg), tetracycline (30 µg), ciprofloxacin (5 µg), and chloramphenicol (30 µg)[all from (Oxoid, UK).] based on 2021 CLSI guidelines and local availability of antibiotic disks. Then, the plates were incubated at 37ºC for 24 hours and the results were interpreted as sensitive, intermediate, and resistant according to the most recent version of CLSI, 2021(55). The minimum inhibitory concentration (MIC) of vancomycin was determined by the Epsilometer-test (E-test) method (BioMérieux, France), for all the VRE isolates which Initial determined by the Kirby-Bauer disc diffusion method and grown on a VRE screen agar (Bile Esculin Azide Agar with 6µg/ml vancomycin); as per the CLSI of 2021(55). Vancomycin MIC breakpoints were as follows: ≤4 µg/ml for sensitivity, from 8 to 16 µg/ml for intermediate, and ≥32 µg/ml for resistance. Interpretations of vancomycin disc diffusion tests were as follows: Resistant if zone diameter ≤14 mm, intermediate if zone diameter 15–16, and sensitive if zone diameter ≥17 mm according to CLSI 2021 criteria (55).

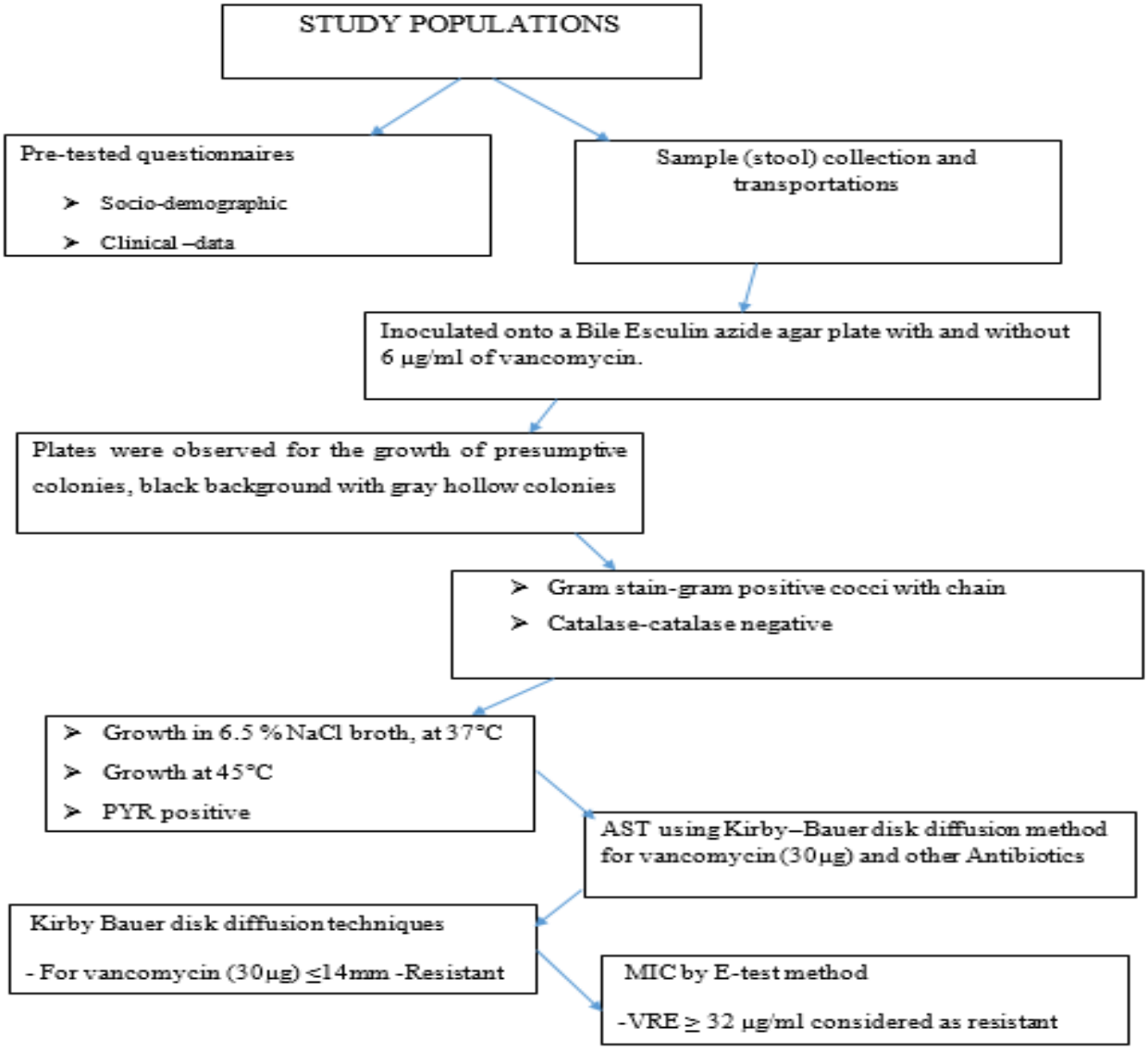

Figure 1. Flow chart for the data collection procedures, bacteria identification, and antimicrobial susceptibility testing for VRE.

4.9.5. Data Quality Assurance

The quality of the data was maintained using a questionnaire which was initially prepared in the English version and translated to Afan Oromo and Amharic then translated back to English to confirm the correctness of the translation. Data quality was also ensured through the use of standardized data collection materials, pretesting of the questionnaires, proper training or orientation of data collectors before the start of data collection, and intensive supervision during data collection, and the collected data were checked out daily for the completeness, accuracy, and clarity by the principal investigator and amendments were done before the next data collection.

The reliability of the findings was guaranteed by implementing quality control measures throughout the whole process of the laboratory work. All materials, equipment, and procedures were adequately controlled. Pre-analytical, analytical, and post-analytical stages of quality assurance and Standard Operating Procedures (SOPs) of the institution were strictly followed. Each of the Culture media was prepared and sterilized according to the manufactures instruction. The standard reference bacterial strains such as the Reference strain of *S. aureus* American Type Culture Collection (ATCC) ATCC25923 and *E. faecalis* ATCC 29212 were used as negative and positive controls, respectively. The quality and performance of culture media, biochemical tests, and potency of antimicrobial discs were checked using these reference strains. The sterility and performance of culture media were tested prior to the actual work. The sterility of culture media was checked by incubating 5 % of each batch of the prepared media at 37 °C for 24 hours and was evaluated for possible contamination. To standardize the density of inoculums of bacterial suspension for susceptibility testing, the 0.5 % McFarland standard was used (55).

4.9 Data analysis

The Collected data were checked for completeness and cleaned, coded, and entered onto Epi-Data version 4.6.0.6 and exported to SPSS version 26 for further cleaning and analysis purposes. Descriptive statistics such as frequency, and percentage, were used to present the findings. Bivariate logistic regression analysis was done to see the association between the dependent variable and independent variables. The variables with a p-value of less than 0.2 in the bivariate analysis were entered into a multivariate logistic regression analysis to control the influence of possible confounding variables. Finally, multivariable logistic regression analysis with AOR, CI at 95%, and the significance level was set at P-value < 0.05.

4.10 Ethical consideration

The study was conducted after it was ethically reviewed and approved. Institutional ethical clearance was obtained from Jimma University Health Research Ethics Review Committee. Written informed consent was obtained from each individual after the purpose of the study was explained using the common language they speak and hear. For children, consent was obtained from the parent/ guardian of the child, and assent was obtained from the children themselves. Participants were notified about the purpose and objective of the study, their right to refuse to participate in the study, and the anonymity and confidentiality of the information gathered. For VRE colonized individuals the results weres reported to the physical and appropriate communication was done.

4.11 Dissemination of results

After conducting the research, results will be presented to the school of medical laboratory sciences, Institute of Health, Jimma University, and other concerned bodies such as professional society's conferences and workshops. The manuscript can be also submitted to peer-reviewed journals for publication.

5. RESULTS

5.1. Sociodemographic Characteristics

A total of 226 study participants were enrolled in this study, of which 116 (51.3%) were males, while 110 (48.67%) were females. The mean age and standard deviation were 23.2 ± 18.5, with an age range from 1 to 82 years. Of the 226 study participants, 113 were patients attending anticancer treatment at oncology wards (with a mean age and standard deviation of 24.4 ± 22.7, ranging from 1 to 82 years) and 113 were "apparently healthy clients" (with a mean age and standard deviation of 21.99 ± 13, age-range 1–56 years). The majority of the study participants were primary education level, 70/226 (30.97%) and more than half of the participants were 124/226 (54.8%) urban residents (**Table-1**).

Table 1: Sociodemographic characteristics of patients attending anticancer treatment at oncology ward and "apparently healthy clients" at Jimma Medical Center, Southwest Ethiopia, 2021.

Socio-demographic characteristics	**Status of Clients**		**Total (n=226), Frequency (%)** N (%)
	Patients attending anticancer at oncology wards **Frequency (%)** (n=113), N (%)	"Apparently healthy clients **"** **Frequency (%)** (n=113), N (%)	
Sex			
Male	53(46.9)	63(55.8)	116(51.3)
Female	60(53.1)	50(44.2)	110(48.67)
Age			
≤ 9	40(35.4)	15(13.3)	55(24.3)
10-18	25(22.1)	29(25.7)	54(23.9)
19-27	5(4.4)	39(34.5)	44(19.5)
28-59	30(26.5)	30(26.5)	60(26.5)
≥ 60	13(11.5)	0(0.0)	13(5.8)
Mean (SD) Age (range)	24.40±22.713(1-82)	21.99±13(1-65)	23.19±18.5(1-82)
Educational status			
Unable to read and write	49(43.4)	14(12.4)	63(27.87)
able to read and write	7(6.2)	5(4.4)	12(5.3)
primary (1-8)	34(30.1)	36(31.9)	70(30.97)
secondary (9-10)	14(12.4)	15(13.3)	29(12.8)
college and above	9(8.0)	43(38.1)	52(23)
Residence			
Urban	57(50.4)	67(59.3)	124(54.87)
Rural	56(49.6)	46(40.7)	102(45.1)

5.2 Clinical characteristics of the study participant attending anticancer treatment at oncology wards

A total of 113-study participants who attended anticancer treatment at oncology wards of JMC were enrolled. The greater proportions of cancer types of the study participants were Acute lymphoblastic leukemia (ALL) 22(19.5%), followed by Non-Hodgkin lymphoma (NHL) 15 (13.3%). The majority of the study participants 92(81.4%) had less than or equal to the one-year duration of cancer.

About 26(23%) of the study participant had a history of hospitalization in the last three months, Out of that about 12(46.2%) were admitted to the hospital for 7-to 14 days. During the time of data collection, all 113(100%) of the study participants had started anticancer treatment. Out of that 106 (93.8 %) of the study participant used chemotherapy and 34(32%) of them took only one cycle of chemotherapy. About 33(29.2%) of the study participant had a history of previous treatment with antibiotics within the last three months and about 6 (5.3%) had a history of an indwelling medical device. Of the total, only 16(14.2%) of the study participants had abnormal complete blood count (CBC) results at the time of data collection (**Table-2**).

Table 2: Clinical characteristics of study participants, attending anticancer treatment at oncology wards of JMC 2021 (n=113).

Clinical features	**Category**	**Number**	**Percent (%)**
Type of Cancer	ALL	22	19.5
	NHL	15	13.3
	Breast cancer	13	11.5
	HL	10	8.8
	Wilms tumor	7	6.2
	Retinoblastoma	7	6.2
	Others	39	34.5
Duration of Cancer	≤1 Year	92	81.4
	>1 Year - ≤ 2 Years	16	14.2
	> 2 Years	5	4.4
History of hospitalization in the last three months	Yes	26	23.0
	No	87	77.0
Duration of hospital admission or stay	≥48 to ≤ 7 days	4	15.4
	>7 to 14 days	12	46.2

	15 to 30 days	6	23
	>30 days	4	15.4
Number of study participants start anticancer	Yes	113	100
	No	0	00
Type of anticancer treatment used	chemotherapy	106	93.8
	surgery	3	2.7
	combination of chemotherapy and surgery	4	3.5
	Radiotherapy	0	0
	others	0	0
Number of cycles did take chemotherapy	1 Cycles	34	32
	2 Cycles	24	22.6
	3 Cycles	10	9.4
	4 Cycles	16	15.1
	5 Cycles	6	5.7
	6 Cycles-12 Cycles	16	15.1
Usage of antibiotics in the past three months	Yes	33	29.2
	No	80	70.8
invasive procedures within the prior 4 weeks	yes	6	5.3
	No	107	94.7
Result of any hematological abnormality (CBC)	Yes	16	14.2
	No	97	85.8

Key Abbreviation: ALL -Acute lymphoblastic leukemia, CBC-Complete Blood Count HL-Hodgkin's lymphoma, NHL-Non-Hodgkin's lymphoma, UTI-Urinary tract infection

5.3. Prevalence of Enterococci and VRE colonization among patients attending anticancer treatment at oncology wards and "apparently healthy clients"

From the total of 226 study participants, colonization of *enterococci species* was seen on 178 (78.8%) stool specimens (95% CI = 73–83.6%). Of these, 87 (77%) (95% CI = 68.4–83.8%) of patients attending anticancer treatment at oncology ward and 91 (80.5%) (95% CI = 72–86.8%) of apparently healthy clients were colonized by *enterococci species.*

In turn, among 178 isolates of *enterococci*, 15(8.4%) were vancomycin-resistant *enterococci* (VRE) (95% CI = 4.3–12.5). Of these, VRE among patients attending anticancer treatment at oncology wards and apparently healthy clients was 11/87 (12.6%) (CI = 5.7–19.6) and 4/91 (4.4%) (95% CI = 0.2–8.6), respectively (**Figure 2**).

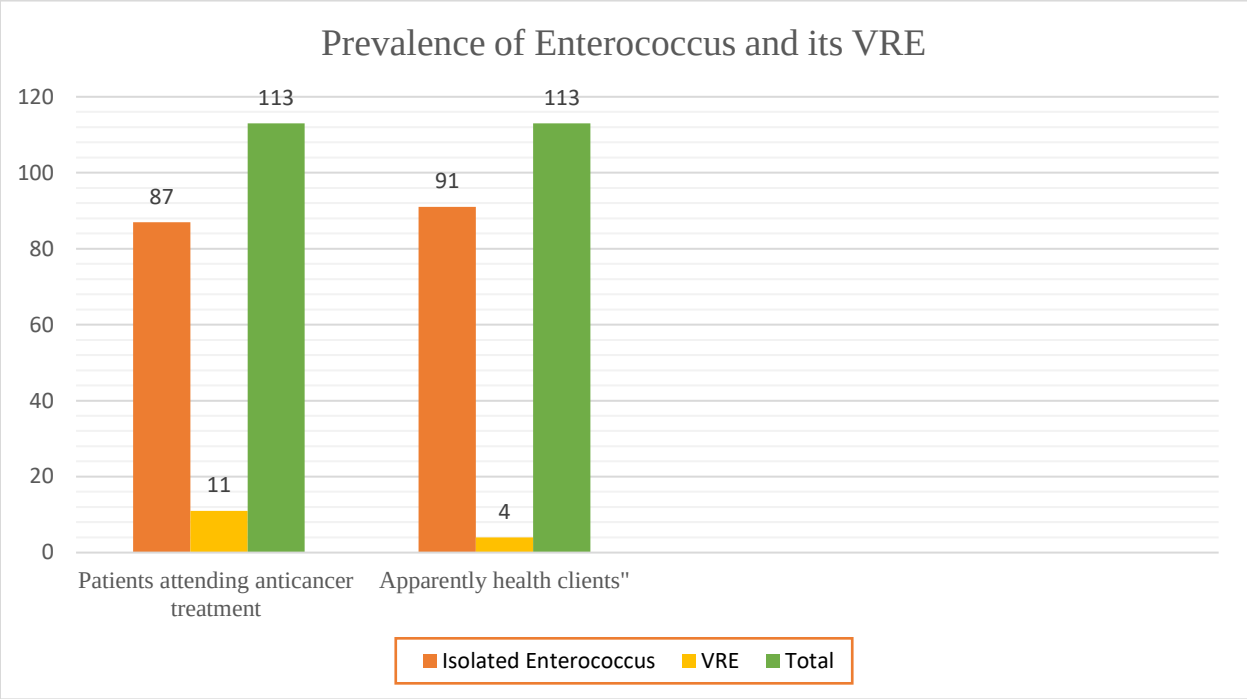

Figure 2 . Prevalence of *enterococci* and VRE colonization among patients attending anticancer treatment at oncology wards and "apparently healthy clients" at JMC, Southwest Ethiopia, 2021.

In this study, there was no statistically significant association observed between VRE colonization and patients attending anticancer treatment at oncology words (P-value = 0.058, OR = 0.32, 95% CI = 0.097-1.04) (**Table -3**).

Table 3: Prevalence of VRE among patients attending anticancer treatment at oncology wards and "apparently healthy clients" at JMC, Southwest Ethiopia, 2021.

Study participant	**Isolated enterococcus species=178**				
	Total N (%)	**VRE N (%)**	**VSE N (%)**	**OR (95% CI)**	**P-value**
Patient attending anticancer treatment at oncology wards	87 (77%)	11 (12.6%)	76(87.4)	0.32(0.097-1.04)	0.058
"Apparently healthy clients"	91(80.5%)	4 (4.4%)	87(95.6)	1.00	

VRE= Vancomycin-resistant *enterococci*, **VSE**=Vancomycin susceptible *enterococci* **N**=Number

5.4. Antimicrobial Susceptibility Patterns of Isolated VRE to other antibiotics

Antimicrobial susceptibility testing of the isolated Enterococci was evaluated against 7 antimicrobial agents using the Kirby-Bauer disk diffusion technique, and a Minimum Inhibition concentration (MIC) was performed for Vancomycin using the E-test as modified by the CLSI 2021 (55). Among antimicrobials tested, the isolated VRE had a 7/15 (46.7%) resistance pattern for each of ampicillin, penicillin, and ciprofloxacin. Of these, 5/11 (45.5%) and 2/4 (50%) isolates were from patients attending anticancer treatment at oncology wards and" apparently healthy clients," respectively.

8/15 (53.3%) of the isolated VRE were resistant to erythromycin, of which 6/11 (54.5%) were from patients attending anticancer treatment at oncology wards and 2/4 (50%) were from apparently healthy clients. 10/15 (66.7%) of VRE isolates were resistant to tetracycline, among which 6/11 (54.5%) of the isolates were from patients attending anticancer treatment at oncology wards and 4/4 (100) were from apparently healthy clients. 2/15 (13.3%) of VRE isolates were resistant to chloramphenicol; one of the isolates was from patients attending anticancer treatment at oncology wards 1/11 (9.1%) and 1/4 (25%) was from apparently healthy clients.

In this study, 86.7% of VRE isolates were sensitive to chloramphenicol while 66.7% are resistant to tetracycline. The general susceptibility pattern of VRE (susceptible, intermediate, and resistance) for all antibiotics is shown in **Table 4**.

Table 4*:* Antimicrobial susceptibility patterns of VRE among patients attending anticancer treatment at oncology ward and "apparently healthy clients" at JMC, Southwest Ethiopia, 2021.

Antibiotics pattern	Isolated Vancomycin-resistant Enterococci			
		Patient attending oncology wards (n=11), N (%)	"Apparently Healthy clients" (n=4), *N* (%)	Total (n=15), N (%)
Penicillin (10 IU)	S	6(54.5)	2(50)	8(53)
	I	0 0	0 0	0
	R	5(45.5)	2(50)	7(46.7)
Ampicillin (10 µg)	S	6(54.5)	2(50)	8(53)
	I	0 0	0 0	0
	R	5 (45.5)	2(50)	7(46.7)
Vancomycin (30 µg)	S	0 0	0 0	0
	I	0 0	0 0	0
	R	11 (100)	4 (100)	15(100)
Erythromycin (15µg)	S	3 (27.3)	1(25)	4(26.7)
	I	2(18.2)	1(25)	3(20)
	R	6 (54.5)	2(50)	8(53)
Tetracycline (30 µg)	S	3(27.3)	0 0	3(20)
	I	2(18.2)	0 0	2(13)
	R	6(54.5)	4(100)	10(66.7)
Ciprofloxacin (5 µg)	S	4(36.4)	1(25)	5(33)
	I	2(18.2)	1(25)	3(20)
	R	5(45.5)	2(50)	7(46,7)
Chloramphenicol(30 µg)	S	10(90.9)	3(75)	13(86.7)
	I	0 0	0 0	0
	R	1(9.1)	1(25)	2(13.3)

S = susceptible; I = intermediate; R = resistance.

The minimum inhibitory concentration (MIC) of vancomycin was determined by the E-test strip for all VRE isolates. In the present study, all 15/178 (8.4%) VRE isolates by disk diffusion were also resistant by a MIC method using E-test strips (MICs > 32 g/ml). MIC for vancomycin ranged from 0.016 to 256 µg/mL. 3/15 (20%) of VRE isolates had a MIC level of 48µg/mL. Out of that 2/3(66.7%) and 1/3(33.3) were from a comparative group and a patient attending anticancer treatment respectively. 7/15 (46.7%) of isolated VRE had a MIC level of 64µg/mL, from that 2/7(28.6) and 5/7(71.4) were from the comparative group and patients attending anticancer treatment respectively. Out of a total of VRE isolates 3/15 (20%) of VRE isolates showed MIC 256 µg/ml by E-test strips in which all of them were isolated from patients attending anticancer treatment.

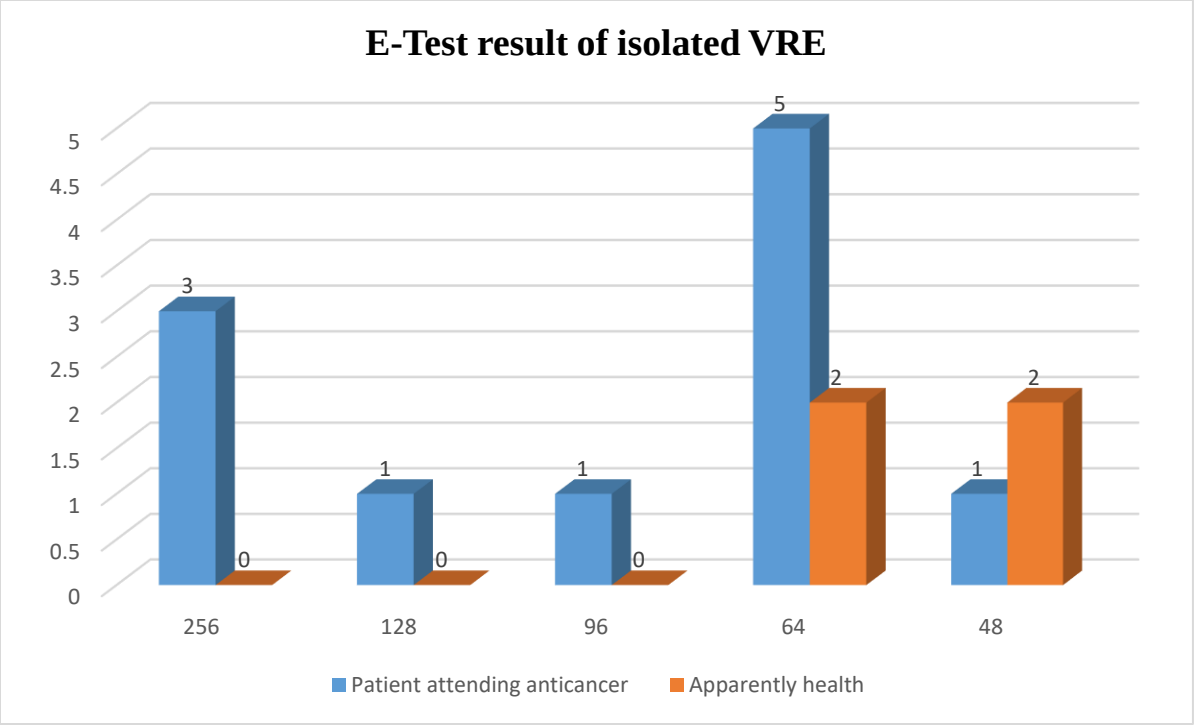

Figure 3. E-Test results of isolated VRE

The isolated Vancomycin-resistant enterococci were resistant to one or more antibiotics; of those isolates, 3 (20%), 2 (13.3%), 3 (20%), and 7 (46.7%) were resistant to one, two, three, and greater than or equal to four antibiotics, respectively. In this study, the overall prevalence of multiple drug resistance (MDR) was observed in more than half 10/15 (66.7%) of the isolated vancomycin-resistant *Enterococci*. Only 1/15 (6.7%) of the isolates were resistant to all antimicrobials tested (**Table-5**).

Table 5: Multidrug-resistant Pattern of VRE isolates of patients attending anticancer treatment at oncology ward and "apparently healthy clients" at JMC, Southwest Ethiopia, 2021.

No. of antibiotics	Combination of Antibiotics	Total number of MDR (%) of VRE isolates
R3	G, P, MAC	6(40)
R4	(G, P, MAC)+ TTC	6(40)
R5	(G, P, MAC, TTC) + F	5(33.3)
R6	(G, P, MAC ,TTC) + F+ PH	1(6.7)

Key; MDR non-susceptible to ≥1 agent in ≥3 antimicrobial categories, G-glycopeptides (vancomycin), P-penicillin's (ampicillin and/or penicillin), MAC-macrolides (erythromycin), TTC-tetracycline's (tetracycline), F-fluoroquinolones (ciprofloxacin), PH-phenicols (chloramphenicol), and R3-R6 Number of categories of antibiotics resistance from 3 to 6, antibiotics respectively

5.5. Factors Associated with the colonization of Vancomycin-Resistant Enterococci

In this study, a total of 12 independent variables were considered during the bivariate analysis of factors associated with VRE colonization. Variables with p-values of less than 0.2 in the bivariate analysis were exported to the multivariate logistic regression model to control confounders and identify the factor. The strength of association and statistical significance was declared using the adjusted odds ratios with their corresponding 95% CI and p-value ≤ 0.05, respectively. Accordingly, being a history of hospital admission in the past three months and a history of antibiotics use in the past three months were significantly associated with vancomycin-resistant enterococci colonization (AOR = 4.1, 95% CI = (1.08–15.44), P-value = 0.038) and (AOR = 4.33, 95% CI = (1.13–16.6), P-value = 0.033) respectively. Clients with hospital admission and history of antibiotics used were 4 and 4.33 times more likely to be colonized with VRE as compared with their counterparts; however, no statistically significant association between VRE colonization and socio-demographic characteristics such as age, sex, educational status, and residence in bivariate and multivariate analysis (P > 0.05) (**Table 6, and 7**).

Table 6: Bivariate logistic regression analysis of sociodemographic characteristics with the colonization of VRE among patients attending anticancer treatment at oncology wards at JMC, Southwest Ethiopia, 2021.

Socio-demographic characteristics	VRE GIT Colonization			
	Yes No **(%)**	No No (%)	P-value	COR (95% CI)
Sex				
Male	4(7.5)	49(92.5)	0. 872	1.09(0.38-3.1)
Female	7(11.7)	53(88.3)		1
Age				
≤ 9	7(17.5)	33(82.5)	0.405	2.55(0.28-22.9)
10-18	1(4)	24(96)	0.634	0.5(0.029-8.7)
19-27	0(0.0)	5(100)	0.999	NA
28-59	2(6.7)	28(93.3)	0.904	0.86(0.07-10.34)
≥ 60	1(7.7)	12(92.3)		1
Educational status				
Unable to read and write	6(12.2)	43(87.8)	0.059	0.21(0.04-1.0)
able read and write	2(28.6)	5(71.4)	0.999	NA
primary (1-8)	2(5.9)	32(94.1)	0.151	0.39(0.1-1.4)
secondary (9-10)	1(7.1)	13(92.9)	0.376	0.476(0.09-2.46)
college and above	0(0.0)	9(100)		1
Residence				
Urban	6(10.5)	51(89.5)	0. 68	1.25(0.4-3.6)
Rural	5(8.9)	51(91)		1

Table 7: Bivariate and multivariate logistic regression analysis of clinical characteristics, associated with the colonization of VRE among Patients attending anticancer treatment at oncology wards and "apparently healthy clients", at JMC, Southwest Ethiopia, 2021.

Variables	VRE GIT Colonization					
	Yes No **(%)**	No, No (%)	P-value	COR (95% CI)	P-value	AOR (95%CI)
Type of Cancer						
ALL	4(18.2)	18(81.8)	0.121	4.1(0.69-24.5)	0.419	2.3(0.3-18.3)
NHL	3(20)	12(80)	0.115	4.6(0.69-31)	0.057	9.9(0.93-106)
Breast Cancer	1(7.7)	12(92.3)	0.733	1.54(0.3-18.5)	0.557	2.3(0.15-34)
HL	0(0.0)	10(100)	0.999	NA	NA	
Wilms Tumor	1(14.3)	6(85.7)	0.387	3.0(0.24-39.5)	0.4	3.6(0.17-76)
Retinoblastoma	0(0.0)	7(100)	0.999	NA	NA	
Others	2(5.1)	37(94.9)		1		
Duration of Cancer						
≤1 Year	8(8.7)	84(91.3)	1.000	NA	NA	
>1 Year-≤2 Years	2(12.5)	14(87.5)	0.999	NA	NA	
> 2 Years	1(20)	4(80)		1		
History of hospital admitted						
YES	6(23)	20(76.9)	**0.015***	4.9(1.36-17.76)	**0.038***	4.088(1.08-15.5)
NO	5(5.7)	82(94.2)		1		
Type of Anticancer treatment						
Chemotherapy	11(10.4)	95(89.6)	0.999	NA	NA	

Radiotherapy	0(0.0)	(0.0)	1.000	NA	NA	
Surgery	0(0.0)	3(100)	0.999	NA	NA	
combination of chemotherapy and	0(0.0)	4(100)	1.000	1	NA	
History of antibiotic use						
Yes	7(63.6)	26(78.8)	**0.014***	5.12(1.39-18.89)	**0.033***	4.33(1.129-16.6)
No	4(5)	76(95)		1		
History of underlying medical conditions?						
Yes	1(8.3)	11(91.7)	0.863	0.83(0.096-7.09)		
No	10(10)	91(90)		1		
Are there any invasive procedures within the prior 4 weeks						
Yes	1(16.7)	5(83.3)	0.563	1.94(0.21-18.29)		
No	10(9.3)	97(90.7)		1		
Is there any hematological abnormality (CBC)						
Yes	2(12.5)	14(87.5)	0.688	1.397(0.273-7.148)		
No	9(9.3)	88(90.7)		1		

Note: - * statistically significant ($p<0.05$), **COR**: Crude Odds Ratio, **AOR**: Adjusted Odds Ratio, **95%CI**: 95% Confidence Interval, **N/A**= Not Applicable. **ALL** -Acute lymphoblastic leukemia, **CBC**-Complete Blood Count **HL**- Hodgkin's lymphoma, **NHL**-Non-Hodgkin's lymphoma, **UTI**-Urinary tract infection

6. DISCUSSION

The rapid emergence of antibiotic-resistant *Enterococci* and the increasing incidence of colonization with VRE have been emerging and posing a therapeutic challenge to physicians due to the ease of acquiring and transferring antimicrobial-resistant genes with other pathogenic bacteria (56). The prevalence of VRE colonization and infection has been increasing in patients with weakened immune systems, such as cancer patients, who are exposed to healthcare and antibiotics as well as undergoing treatment for certain types of cancer (12).

In the present study, the prevalence of *Enterococci* colonization among patients attending anticancer treatment at oncology ward was found to be 77%. This was consistent with other studies conducted in Spain (78.8%) (57), Egypt (82.8%) (58), and Jimma, Ethiopia, which was reported to be (76%) (26). However, it is higher than the previous reports from Brazil (27%) (29), and Ethiopia in West Amhara (63%) (45), and Dessie (37.3%) (44). The possible explanation for the high colonization of *Enterococci* might be due to predisposing factors, such as prolonged or repeated hospitalization, receipt of broad-spectrum antibiotics, neutropenia, and mucosal damage from high-dose chemotherapeutic agents, which are common among cancer patients attending anticancer treatment at oncology wards (59). Because the present study, showed that 93.8%, of study participants, started Chemotherapy treatment as well as 29.2 % and 23% of the study participant had a history of antibiotics and hospitalization in the last 3 months respectively. Furthermore, this difference could be related to the current study's use of Enterococci selective media, which might increase the chance of isolation.

In addition to that, the difference might be because *enterococci* are normal flora of the gastrointestinal tract and have undergone selective pressure over other normal flora of the intestinal as they are intrinsic and acquired resistance to several antibiotics. As a result at the time of antibiotic treatment, their colonization has increased gradually, allowing for overgrowth of *Enterococci* as well as VRE (60).

Our finding was lower than reports from Algeria (100%) (61) and Gondar, Ethiopia (88.9%) (43). The variation in results might be explained by the different characteristics of the study participants

and the variation in identification methods for *Enterococci.* The method used in Algeria was a molecular technique that has better sensitivity than the conventional one used in this study.

On the other hand, the present study showed that the prevalence of *enterococci* colonization was 80.5% and 77% among apparently healthy clients and patients attending anticancer treatment at oncology wards, respectively; the colonization rate of *enterococci* was similar between apparently healthy clients and patients attending anticancer treatment at oncology wards. This similarity might be explained by the fact that *Enterococci spp* are part of the normal flora of the gastrointestinal tract of both groups. As there is approximately 10^6 to 10^7 *Enterococcus* inhabiting the human intestine with a high proportion colonizing the lower Gastrointestinal tract (GIT) and less than 1% found in the ileum, up to 1% in the colon (62).

In this study, the overall prevalence of VRE among patients attending anticancer treatment at oncology wards was found to be 12.6% (95% CI = 5.7–19.6). This was in line with reports from the United States 9.6% (63), Italy 11.1% (64), Germany 9.9% (35), and Similarly, with the findings reported from Ethiopia: Arba Minch 11.4% (46), and West Amhara, 7.7% (45). However, the prevalence of VRE in our study is lower than the reports from Turkey 39.68% (33), Germany 23.8% (34), Chile 52%(65), Brazil 25% (29), and Egypt 38% (42). The lower prevalence of VRE in this study might be due to the variation in some of the geographical locations, the number of samples examined, the type of sample used, and the methods used for the detection of VRE isolates. In Chile, Germany, and Egypt VRE, colonization was determined by a molecular technique, which is more sensitive than the conventional one that we used in this study. In Brazil anal, nasal and oropharyngeal sample was used, which in increase the chance of recovering the bacteria.

On the other hand, the prevalence of VRE colonization in our study is higher than in studies conducted in the USA (4.7%) (66), Hungary (2.2%) (67), South Korea (4.5%) (68), and Nigeria (4.07%) (69), and Similarly, higher than the following studies conducted in Ethiopia: 5.5%, and 5%, respectively (5,8). This difference could be because Enterococci are normal flora of the intestinal tract, with tremendous genome plasticity, and utilize plasmids, transposons, and insertion sequences to efficiently attain and transfer mobile resistance elements for persistence, transmission, and the dissemination of resistance elements (7). Since VRE have intrinsic resistance to most of the commonly used antibiotics and the ability to acquire resistance to most of the

currently available antibiotics, either by mutation or by receipt of foreign genetic material, they have a selective advantage over other microorganisms in the intestinal flora and pose a major therapeutic challenge. Therefore, the gradual increase of VRE colonization might have contributed to this higher prevalence due to the exposure of immunocompromised patients to antibiotics with activity against gram-negative and gram-positive bacteria, which results in substantial changes in the gut microbiota that facilitate colonization of the GIT by VRE (60).

The isolated VRE in the present study showed various resistances to the tested antibiotics; namely, 46.7% to ampicillin, 46.7% to penicillin, 53.3% to erythromycin, 66.7% to tetracycline, 46.7% to ciprofloxacin, and 13.3% to chloramphenicol. These findings are comparable with studies conducted in Indian 45%,(71), and Ethiopia, 45.5%,(72), for ampicillin; Indian 47%,(73) and Ethiopia, 45.5% (72) for Penicillin ; Ethiopia,49.6% (46), 49.6% (6) for erythromycin; Uganda 69.4% (74), and Ethiopia 64.9% (26), for tetracycline; India,50% (71) and Ethiopia: 45.5% (72) ,50% (26), for ciprofloxacin; Brazil,10.9% (30), and Ethiopia, 12.4% (43) for chloramphenicol. In the case of a MIC, the present study showed compatible results of the disk diffusion test with the results of the E-test method. This is similar to a study conducted in Iran (75).

However, the resistance profiles in our study are lower than previous studies in India 64.9%, (76),and Ethiopia 69.9% (72), for ampicillin; India 75.9% (76), and Ethiopia 66.7%,(77), for penicillin; India 84.5% (76),and Ethiopia 90.9% (72), 77.3% (27),for Tetracycline; India 92.1% (76),Uganda 72% (74),and Ethiopia , 66.7 (72) 63.6% (27) for erythromycin; India 95.5% (73) and Ethiopia 70.8% (77), for ciprofloxacin; India 42.3% (76), and Ethiopia 21.1%(72), 83.7% (46) ,30.9% (45) , for chloramphenicol. These lower drug resistance patterns might be due to variations in sample size, type of sample used, methodology and study participants.

On the other hand, the antibiotic resistance profile in our study is higher than studies conducted in Nepal 39.5% (78) , and Ethiopia 20.9%, (45),34.8% (70), 36% (26) , for ampicillin; Nepal 40.7% (78) and Ethiopia 34.8% (70) for penicillin; Brazil 32.6% (65) and Ethiopia 42.7% (45) for erythromycin; Ethiopia 28.6% (45) , 59.3% (46) for tetracycline; Ethiopia 28.5% (46), 36.4% (43) for ciprofloxacin. The possible reasons might be the gradual change in the multidrug-resistant strains, antibiotic selective pressure in VRE, a significant increment of self-medication, and empirical treatment, which in turn causes the emergence and spread of drug resistance.

Multidrug resistance is defined as resistance to at least one agent of the three antimicrobial classes (79). In the present study, the prevalence of multidrug resistance (MDR) of the isolated VRE was found to be 66.7 %. This finding is of particular concern since the 66.7 % prevalence of colonization with MDR *Enterococci* has left the clinician with no alternative treatment options. In addition to that in case of People with cancer who are treated with chemotherapy are more likely to get infections because of their weakened immune systems. Both Cancer and chemotherapy can damage the immune system, reducing the number of infection-fighting leukocytes and making it harder for the body to fight infections.

Thus cancer patients are particularly affected by multidrug-resistant organisms (MDROs) infections due to immunosuppression related to disease and therapy, which often causes neutropenia and mucositis. The mortality due to infections with MDRO is considerably higher compared to those with non-MDRO and one of the major risk factors to develop MDRO infection is prior MDRO colonization (80). The case is further complicated by the genetic intra-ability of *Enterococci* to exchange resistance determinants and/or transfer to other Gram-positive organisms such as staphylococci and streptococci (56). The situation in the present study warrants the implementation of an efficient infection control program and regular surveillance of antimicrobial resistance of *Enterococci* to establish a rational antibiotic policy for the better management of *Enterococcal* infections.

The present study showed that a patient attending anticancer treatment did not have a statistically significant association ($p = 0.058$) with the colonization of VRE. However, reports are showing that VRE is more common in immunocompromised patients, such as cancer patients undergoing cancer treatment, those who have received multiple courses of antibiotics in the past, and those who have had prolonged healthcare exposures (7,12).The possible reason for not having a statistically significant association might be that the number of samples in our study was too small.

In the present study, concerning the associated factors assessed for VRE colonization, patients attending anticancer treatment at oncology wards who had a history of hospital admission for the last three months were about four times more likely to be colonized with VRE as compared with those who had not had a history of hospitalization for the last three months [AOR = 4.088; 95% CI: (1.083–15.438); P-value = 0.038]. The finding is consistent with previous studies done in Chile (16), Germany (81), South Korea (68), and Ethiopia: Arba Minch (46), West Amhara(45),

Gondar (77), and Dessie (72). The reason might be that VRE has been isolated from virtually every object within patient rooms since they are intrinsically resistant to several commonly used antibiotics in hospitals and can acquire resistance genes. Besides, they are ubiquitous in their presence and have high survivability on dry surfaces, thereby causing high VRE transmission rates within healthcare facilities (32). Longer hospital stays can indicate a greater chance of receiving antibiotics and also a longer exposure time to possible pathogens. Thus, clients who stay in a hospital have the highest odds of getting VRE because bacteria that don't respond to antibiotics spread most easily in places where antibiotics are used most often.

Our study showed that patients attending anticancer treatment at oncology wards who were previously exposed to antibiotics in the last three months were four times more likely to be colonized with VRE as compared with patients attending anticancer treatment at oncology wards who had never been exposed to antibiotics previously [AOR = 4.33; 95% CI: (1.129-16.6); P-value = 0.033]. This result is in agreement with other studies conducted in Brazil (30), South Korea (68), Germany (82), Egypt (83), and Ethiopia: Arba Minch (46), West Amhara (45), and Gondar (43,77)**.** The reason might be that prior exposure to antibiotics for a prolonged period can cause VRE colonization because the antibiotics exert selective pressure on *Enterococci* and alter the competing microbial flora in the Gastrointestinal tract (GIT), allowing VRE to predominate, as evidenced by other studies (18). In other words, exposure to antibiotics with activity against gram-negative and gram-positive bacteria causes changes in the gut microbiota of patients, and these changes cause subsequent alterations in the local immune system. For instance, depletion of the gram-negative microbiota by antibiotics decreases the production of REGIIIγ, which is a C-type lectin with antimicrobial activity against gram-positive bacteria, including VRE, thus facilitating the overgrowth of VRE in the GI tract (4).

7. STRENGTHS AND LIMITATIONS OF THE STUDY

7.1: **Strength**

- ✓ To the best of our understanding, this is the first effort to investigate the prevalence of VRE in patients attending anticancer treatment at oncology wards and comparative groups in the study area as well as in Ethiopia.
- ✓ The Antimicrobial susceptibility pattern of Vancomycin antibiotics was further performed with Minimum inhibition concentration (MIC) using E-test methods, which increase the accuracy and precision of the test.

7.2 **Limitations**

- ✓ Due to resource shortages and budget constraints, the isolated Enterococci were not identified to the species level, although this allows us to access the species-specific antimicrobial resistance properties apart from knowing the epidemiological pattern and their clinical importance in human infections.
- ✓ Sufficient risk factors were not assessed.

8. CONCLUSIONS AND RECOMMENDATIONS

8.1. **Conclusions**

In our study, the prevalence of VRE colonization was 8.4%. Magnitudes of VRE colonization were higher among the patients attending anticancer treatment at oncology wards (12.6%) than in "apparently healthy groups" (4.4%). However, a patient attending anticancer treatment at oncology wards did not have a statistically significant association ($p = 0.058$) with the colonization of VRE. Patients with prior exposure to antibiotics in the past three months and a previous history of hospitalization in the last three months had higher odds of intestinal colonization of VRE than their respective groups. The study also showed that 66.7 % of the isolated VRE was found to be multidrug resistance (MDR). Better susceptibility was observed for chloramphenicol (93.3%), while tetracycline showed lower susceptibility for VRE (66.7%).

8.2 **Recommendation**

Based on the present study the following recommendations were made:-

- ✓ In health care facilities, there should be a need to establish an antimicrobial stewardship program to enhance the rational use of antibiotics.
- ✓ Empiric treatment and management of patients admitted to oncology wards should take into account by giving great concern to VRE.
- ✓ This study also recommends more detailed studies using genotypic methods with a specific focus on molecular characterization of VRE strains carried by patients attending oncology wards and comparative healthy individuals.
- ✓ This study also recommends detecting *Enterococci* at the species level as well as antimicrobial resistance profile against each isolated species level.
- ✓ Furthermore, guidelines should be developed for the prevention and control of hospital-acquired VRE colonization and infection by routine stool screening of patients admitted to oncology wards.

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ANNEXES

Annex I: Participant information sheet:

A. English Version Participant Information Sheet

Title of the Research Project: Magnitude of Intestinal Colonization with Vancomycin-Resistant Enterococci and Associated Risk Factors among Cancer Patients Attending, Oncology units of Jimma University Medical Center (JUMC), Southwest, Ethiopia, 2021.

Name of Investigator: Amanuel Teferi (BSc, MSc candidate)

Name of the Organization: Jimma University, Institute of Health, School of Medical Laboratory Science, Department of Medical Microbiology.

Introduction: My name is Amanuel Teferi. I am an MSc student at Jimma University School of Medical Laboratory Science, Department of Medical Microbiology. You are invited to participate as a study subject in research aimed at determining the Magnitude of Intestinal Colonization with Vancomycin-Resistant Enterococci and Associated Risk Factors among Cancer Patients Attending, Oncology units of Jimma University Medical Center (JUMC), Southwest, Ethiopia, 2021.

Purpose of the study: The purpose of this study is to determine the magnitude of vancomycin-resistant enterococci and associated risk factors among cancer patients attending oncology units of Jimma university medical center (JUMC), southwest, Ethiopia in 2021.

Role of Participant: Study participants are expected to be a volunteer for the specimen collection, give sociodemographic and clinical data. Then stool samples will be collected for the diagnostic purpose by the participant.

How much time does the participant spend participating in this study?

You will spend about 15-20 minutes until the specimen will be collected, the questionnaire will be filled and the consent will be signed.

The risk associated with participation: The study will not cause any anticipated to the participant in any way.

Benefits: You will not receive any payment for your participation in this research study as compensation. However, the result of the study will be used by clinicians, researchers, and concerned stakeholders to be beneficial in the management, prevention, control, and monitoring of the case.

What will be your rights as a participant in this study?

Participation is voluntary and you have the right not to participate in this study. If you are not interested to participate or if you once decide to participate and withdraw at any time, there will be no consequences and you or your child will get all the services provided in the hospital will not be discontinued. You have also welcome if you have any questions for further explanations about the study.

Confidentiality: All the information obtained from you will be kept confidential. It will never be shared with other individuals including identifiers.

Whom to contact: In case if you need any further information about the study please contact:-

Name of investigator Amanuel Teferi

Phone number: +251-917-21-72-38

Email: amanu.ju@gmail.com or gechaman2017@gmail.com

B. Information sheet Amharic version

የተሳታፊዎች ፈቃድና መተማመኛ ቅፅ

የጥናቱ ራእስ: ቫንኮማዩሲን መዳኒቲን የተላመደ ኢኒቲሮኮከሲ ተህዋስያን መጠን ዒና አጋላች መኒሰ በ ካኒሰሪ ታካሚዎችህ ላይ ሚን አህል እንደሚበዛ ማቲናት በጅማ ዩኒቨረሲቲ የእከምና መእከሌ ፤ ጅማ ደቡብ ምዕራብ ኢትዮጵያ 2013፡፡

የጥናቱ ተመራማሪ ስም፡ አማኑኤል ተፈሪ፡፡

የዴርጅቱ ስም ፡- ጅማ ዩኒቨረሲቲ እከምና መእከሌ

መግቢያ፥-አማኑኤል ተፈሪ እባላለሁ፡ በጅማ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የ መድካል ማይኪሮባዮሎጂ ሁለተኛ ድጊር ተማሪ ነኝ። አባክዎን ግዘ ወስደው የመተማመኛ ቅፅ ቫንኮማዩሲን መዳኒቲን የተላመደ ኢኒቲሮኮከሲ ተህዋስያን መጠን ዒና አጋላች መኒሰ በ ካኒሰሪ ታካሚዎችህ ላይ ሚን አህል እንደሚበዛ ለማወቂ የሚሰራ ጥናቲ ሲለ ሆነ እንዲቲሳተፉ በአክቢሮት ኢተዪካለው፡፡

የፕሮጀክቱ አላማ ቫንኮማዩሲን መዳኒቲን የተላመደ ኢኒቲሮኮከሲ ተህዋስያን መጠን ዒና አጋላች መኒሰ በ ካኒሰሪ ታካሚዎችህ ላይ ሚን አህል እንደሚበዛ ማቲናቲ ነው ፡፡

የጥናቱ ተሳታፊዎች ሐላፊነት፡ ከጥናቱ ተሳታፊዎች የሚጠበቀው ለጥናቱ የሚፈለገዉን ናሙና እና መረጃ መስጠት/መፍቀድ ነው፡፡ይህም ናሙና ሠገራ ነዉ፡፡

የሚወሰደዉ ሰኣት፡መረጃና ዒና ናሙና ወሲዶ ለመቸረሲ 15-20 ደቂቃ ዪወሲዳሊ፡፡

ከጥናቱ ጋር የተያያዘ ጉዳት፡ በጥናቱ ላይ በመሳተፍዎ ሊደርስ የሚችል የተለየ ጉዳት የለም፡፡

ከጥናቱ የሚገኝ ጥቅም፡ በዚህ ጥናት ላይ በመሳተፍዎ የሚያገኙት ቀጥተኛ ጥቅም የለም፡፡ ይሁን እንጂ በጥናቱ የሚገኝ የምርመራ ዉጤት ለሂኪምና ባለሞያ ኢና ለ ባለ ዲርሻ አካላት ከፍተኛ ጥቅም አለው በሺታዉን በመከላከሊ ረገዲ፡፡

በጥናቱ ስለመሳተፍና አቋርጦ ስለመውጣት፥-በዚህ ጥናት ላይ መሳተፍ በ ፍቃደኝነት ላይ የተመሰረተ ሲሆን ያለመሳተፍ መብት አንደተጠበቀ ነው። ምንም የሚደርስበት ነገር ሳይኖር ከ ጥናቱ አቋርጦ መውጣትም ይችላል።የላቦራቶሪ ውጤቶን የለምንም ከፍያ ሳይጠየቁ መውሰድ ይችላሉ።

ሚስጢር መጠበቅ፡ የእርስዎ የትኛዉም መረጃ በጥብቅ ሚስጢር ይያዛል፡፡ ለሌላ ሶስተኛ ወገን እርስዎን ማንነት በሚገልጥ መልኩ አይሰጥም፡፡

ጥያቄ ካሎት ለማነጋገር፥- ስለጥናቱ ንም አይነት ጥያቄ ቢኖሮት የሚከተሊትን ኣድራሻ ይጠቀሙ።

ተመራማሪ ሲም አማኑኤል ተፈሪ

ስ.ቁ.፡- +251-917-21-72-38,

ኢሜሌ: amanu.ju@gmail.com or gechaman2017@gmail.com

C. Garagalcha Afaan Oromo (Afan Oromo versions)

Mata duree Qorannichaa: Baaakteeriyaa Intirookokaasi dawaa vaankomaayisiin Kan walbaree yaalamtoota kaansarii irratti haangam akka ta'ee baruufi akkasumas sabaabota kanaf Nama saxillan adda baasufi qorannaa gageefamu Yuuniveersiitii Jimmaatti Muummee fayyaa Kibba Lixa Itoophiyaa, Bara 2021.

Maqaa qorataa: Amaanu'eel Tafarii.

Maqaa dhaabbataa: Yuuniveersiitii Jimmaatti Muummee fayyaa.

Seensa: - Maqaan Koo Amaanu'eel Tafariin Jedhama. Yuuniveersiitii Jimmaa, muumme fayya Kollejjii Saayinsii Fayyaa laaboratorii Keessatti barata Digrii Lammaffaa meedikaala maayikirobaayolojitti. Qaama mata- duree qorannoo "Baaakteeriyaa Intirookokaasi dawaa vaankomaayisiin Kan walbaree yaalamtoota kaansarii irratti haangam akka ta'ee baruufi akkasumas sabaabota kanaf Nama saxillan adda baasufi qorannaa gageefamu Yuuniveersiitii Jimmaatti Muummee fayyaa Kibba Lixa Itoophiyaa, Bara 2021." jedhu ta'uun akka hirmaatan afeeramtanii jirtu. Kanaafuu guca waliigaltee kana of eeggannoon dubbisaa.

Kaayyoo Piroojaktichaa: Qorannoon Kun Yaalamtoota Yuuniveersiitii Jimmaatti Muummee fayyaa irratti Kan gaggeefamu yoo ta'u, kaayyoon qorannichaas Baaakteeriyaa Intirookokaasi dawaa vaankomaayisiin Kan walbaree yaalamtoota kaansarii irratti haangam akka ta'ee baruufi akkasumas sabaabota kanaf Nama saxillan adda baasufi qorannaa gageefamu dha.

Dirqama namoota qorannoo kana keessa jiranii: Jarri qorannoo kanaa hirmaatan qorannoo kanaaf waan barbaachisu iddattoo kennuu qabu. Idaattoon kun boolii guddaa. Dabalataanis af-gaafiin Kan isiniif godhamu ta'a.

Yeroo qo'annichaa: Qorannoon kanaaf yeroo saamuda fudhatamufi Af-gaafii akkasumas waligaltee mallatessufi daqiiqaa 15-20 qofa fudhata.

Miidhaa: qorannoo kana irraatti waan hirmaataniif miidhaan isin irra gahu tokkole hinjiru.

Faaydaa: qorannoo kana irraattii sababa hiraataniif kallatiidhaan faaydaan isin argattan hinjiru. Haata'u malee, firiin qorannoo kanaa ogeessa fayyaa, Abbaa dhimmi ilaalatufi Kan kana fakkattan bu'aa qoraanicha irratti hunda'uni akkata dhukkuba kana itti ittisanif isaan gargaara.

Mirga Qoranicha irratti hirmaachuu fi Addaan kutuu: - Hirmaannaan qoranichaa fedhii irratti Kan hundaa'u waan ta'eef mirgi hirmaachuu fi hirmaachuu dhabuu keessan Kan eegame ta'a. Rakkon tokkoolle oso hin irra gahiin qoranicha addaan kutanii bahuun ni danda'ama. Bu'aa qorannoo Laabraatorii keessan kaffaltii tokko malee fudhachuun ni danda'ama.

Iccitii eeguu: walumaa gala odeefannoon isin nuuf kennitan Kun bu'aan qorannoo kana hundi iccitiin ni qabama. Namni Kan biraa kam iyyuu beekuu hin danda'u.

Odeeffannoo Dabalataaf: - Qoranicha ilaalchisee odeeffannoo dabalataa fi gaafii yoo qabaatan teessoo armaan gadiin kan na argatan ta'a:-

Maqaa: - **Amaanu'eel Tafarii**

Lakk. Bilbilaa: - +251-917-21-72-38

Imeeli: amanu.ju@gmail.com or gechaman2017@gmail.com

Annex II: Informed consent form

ENGLISH VERSION

ID of client _____

I had been informed that the objective of this study. The purpose of this study is to determine the magnitude of vancomycin-resistant enterococci and associated risk factors among cancer patients attending oncology units of Jimma university medical center (JUMC), southwest, Ethiopia in 2021. I had been informed about the confidentiality of this study. The principal investigator requested me to participate in the study which would require my willingness to provide the required data and stool sample, and filling the questionnaire. Therefore, with a full understanding of the importance of the study, I agreed voluntarily to provide the requested samples and my benefit will be only from the free laboratory investigation result/s. I_____ hereby give my consent for providing the requested information and specimens. Signature: _____ Date_____

Parental/guardian consent form English versions

I_____ parent/guardian, after being fully informed about the purpose of this study, titled "Magnitude of Intestinal Colonization with Vancomycin-Resistant Enterococci and Associated Risk Factors among Cancer Patients Attending, Oncology units of Jimma University Medical Center (JUMC), Southwest, Ethiopia, 2021. I, the undersigned, have been told about this research. My child/guardian has to say to choose if I want to be in the study. I have been informed there is no harm during sample collections. I have been informed that other people will not know my child's results. I understand that there may be no benefit to me personally apart from the clinical service I get from these results. I have been encouraged to ask questions and have had my questions answered. I have been told that participation in this study is voluntary and I may refuse to be in the study. I know my participation will also be approved by my child/guardian. By signing below, I agree to let my child participate in this research study. Name of participant_____

Signature _____ Date ____/____/___ Witness (Illiterate) _____

Signature _____ Date ____/____/___ Name of the researcher _____

Signature _____ Date ____/____/___

Informed consent form Amharic version

የተሳታፊዎች ስምምነት ማረጋገጫ

የአማርኛ የስምምነት ቅፅ

የምስጢር ቁጥር ----------

የጥነቱ አላማ በደምብ ተብራርቶልኛል። የጥናቱ አላማ ቫንኮማዩሲን መዳኒቲን የተላመደ ኢኒቲሮኮከሲ ተህዋስያን መጠን ዒና አጋላች መኒሰ በ ካኒሰሪ ታካሚዎችህ ላይ ሚን አህል እንደሚበዛ ማቲናት በጅማ ዩኒቨረሲቲ የእክምና መእከሌ ። ስለ ጥናቱ ሚስጢር ጠባቂነት ተነግሮኛል።የጥናቱ ዋና ተመራማሪ በጥናቱ ላይ ተሳታፊ ሆኜ የሰገራ ናሙና እና ቃለ መጠይቅ በመስጠት አንድሳተፍ ፍላንተን ጠይቆኛል። በመሆኑም የጥናቱ ጥቅም ሙሉ በ ሙሉ በመረዳት ለመሳተፍ ፍቃደኛ መሆነን አረጋግጣለሁ።

አኔ.....

የሚጠበቅብኝን መረጃና ናሙና ለመስጠት ፈቃደኛነቴን አረጋግጣለሁ።

ፊርማ..... ቀን.....

የአማርኛ ግሌባጭ ለ ሊጅ

እኔ----------የሌጂ አስታማሚ ቤተሰብ ወይም የታማሚው አሳዲጊ/ሞግዚት ስሆን የዚህን ጥናት

አሊማ በዉሌ ተረዴቻሁሁ።ቫንኮማዩሲን ቫንኮማዩሲን መዳኒቲን የተላመደ ኢኒቲሮኮከሲ ተህዋስያን መጠን ዒና አጋላች መኒሰ በ ካኒሰሪ ታካሚዎችህ ላይ ሚን አህል እንደሚበዛ ማቲናት በጅማ ዩኒቨረሲቲ የእክምና መእከሌ ።በጥናቱ ሌጂ እንዱሳተፌ ምርጫው የእኔ መሆኑን ነግረውኛሌ።ናሙና ሲወሰዴ ምንም አይነት ጉዲት ሌጂ ሊይ እንደለዴ ተነግሮኛሌ። በጥናቱ ወቅትም የሌጂ መረጀዎች በሚስጥር ስሆሚያዝ በለላ ሰዉ ዘንዲ እንዯማይታወቅ ተረዴቻሁሁ። በውጤቱ ከሚገኘዉ የህክምና አገሌግልት በቀር ለላ ሌጂ በግለ የሚያገኘዉ ጥቅም እንደለዴ ተረዴቻለሁ። ጥያቄ እንዴጠይቅ ዕዴሌ ተሰጥቶኝ ለ ጥያቄዎቼም በቂ ምሊሽ አግኝቻሁሁ። የሌጂ በጥናቱ መሳተፌ በእኔ ፌሊጎት ብቻ እንደሆነ እና በጥናቱም አሆመሳተፌ ምንም አይነት ተፅዕኖ በሌጂ ሊይ እንደማያስከትሌ ተረዴቻሁሁ። ከዚህ ባሻገር የሌጂ በጥናቱ ውስጥ ባመካተት የእኔ የወሊጁ/አሳዲጊ ፍቃዴ እንዯሚያስፈሌግ ተረዴቻሁሁ። በእኔ ፌቃዯኝነት ሌጂ በጥናቱ እንደሚሳተፌ ከዚህ በታች በፉርማዩ አረጋግጣሁሁ።

Afaan oromoo Version

Waliigaltee (qorannoo irratti hirmaachuuf)

Koodii Qoratamaa_____

Kaayyoon qorannichaa sirritti naaf ibsamee hubadheen Jira. Kaayyoon qorannichaas Qorannoon Kun Yaalamtoota Yuuniveersiitii Jimmaatti Muummee fayyaa irratti Kan gaggeefamu yoo ta'u, kaayyoon qorannichaas Baaakteeriyaa Intirookokaasi dawaa vaankomaayisiin Kan walbaree yaalamtoota kaansarii irratti haangam akka ta'ee baruufi akkasumas sabaabota kanaf Nama saxillan adda baasufi qorannaa gageefamu dha. Iciitiin qorannoo dhunfaa kiyya akka naaf eegamu natti himamee Jira. Gaggeessaa dursaan Qoranichaa Boolii guddaa qorannichaaf ta'u fedhii kiyyaan akka kennuu fi Af-gaafiif hayyamamaa ta'uu kiyya na gaafatee jira.Kanaafuu faayidaa qorannichi gutuumaan gutuutti hubachuun qoranicha irratti hirmaachuuf hayyamamaa ta'uu mallatoo koon nan mirkaneessa. Ani Obbo/Adde _____Kan jedhamu odeeffannoo narraa barbaadamuu fi iddattoo/naamunaa boolii guddaa kennuuf hayyamamaa ta'uu Koo Nan mirkaneessa.

Mallattoo: _____

Guyyaa: _____

Garagalcha Afaan Oromo Ijoolledhafi

Ani _____ Kan daa'ima yookin Kan guddise qabadhe Yommuun qorannoo kana irratti hirmaadhu afaan naaf galuun natti himameera yookin naaf ibsameera.Faayidaa qorannoo kanaatis Baaakteeriyaa Intirookokaasi dawaa vaankomaayisiin Kan walbaree yaalamtoota kaansarii irratti haangam akka ta'ee baruufi akkasumas sabaabota kanaf Nama saxillan adda baasufi qorannaa gageefamu Yuuniveersiitii Jimmaatti Muummee fayyaa Kibba Lixa Itoophiyaa, Bara 2021.' Waa'ee qoraano kanaaf saamuda boolii gudda daa'ima yookin Kan guddise irraa akka fudhatamu naaf himameera. Odeeffannoo qorannoo kana irraa argamu hunduu iccitiin akka kaa'amus irratti walii galleerra. Qorannoo kana irratti daaima koo yookin kan guddise hirmaachisuu yoon hin barbaadne yookiin yoon addaan kute, ammas ta'ee fulduraaf fayyadamummaa kiyyarratti rakkoo tokkoollee akka hin uumnee naaf himameera. Ani erga naaf gale booda mallattoo kootin nan mirkaneessa. Maqaa hirmaata_____
Mallattoo _____ Guyyaa ____/____/___ Ragaa (kan hinbaranne) _____ Mallattoo _____ Guyyaa ____/____/___ Maqaa qorata _____ Mallattoo _____ Guyyaa ____/____/___

Annex III: Questionnaire

A. Questionnaire English Version

JIMMA UNIVERSITY

FACULTY OF HEALTH SCIENCES

SCHOOL OF MEDICAL LABORATORY SCIENCE

DEPARTMENT OF MEDICAL MICROBIOLOGY

Questionnaire on Socio-demographic characteristics and clinical features of the study participants under the title "The Magnitude of Intestinal Colonization with Vancomycin-Resistant Enterococci and Associated Risk Factors among Cancer Patients Attending, Oncology units of Jimma University Medical Center (JUMC), Southwest, Ethiopia, 2021".

Sr.no	**I) SOCIO-DEMOGRAPHIC DATA**		
	Questions	**Response**	**Remarks**
01	Patient code	_____	
02	Age	_____	
03	Sex	1. Male 2. Female	
04	Residence	1.Urban 2.Rural	
05	Educational status	1. Illiterate 2. Preschool 3. Elementary school 4. Secondary school 5. Collage and above	
06	Marital status	1. Single 2. Married 3. Separated 4. Divorced 5. Widowed	
07	Occupational status	1. Farmer 2. Civil servant 3. Underage 4. Student 5. Merchant 6. Housewife 6. Others Specify_____	
	II. Clinical data		
08	Have you been taking antimicrobials in the past three months?	1. Yes 2. No	

09	If the answer is 'yes' for question number 08 what are these antibiotics?	**1**.vancomycin **2**.3rd and/or 4th generation cephalosporin's **3**. Aminoglycosides **4**. Carbapenems **5**.ant-anaerobic agents **6**. β-lactamase inhibitors **7**.others,and specify_____	
10	Have you been admitted to the hospital in the last three months?	1. Yes 2. No	
11	If 'yes' for question number 10 in which ward you are admitted?	1. Intensive care units 2. Oncology Units 3. Pediatric ward 4. Surgical ward 5. Emergency ward 6. Medical ward 7. Others, specify_____	
12	If 'yes' to question number 11 for how long have you stayed?	**1**≤7days **2.** 7 to14 days **3**. 15 to 30 days **4**. >30 days	
13	Type of cancer	List it_____	
14	Duration of cancer	List it_____	
15	Did you take anticancer?	**1.** Yes **2.**No	
16	If 'yes' for question number 15, which one?	1. chemotherapy 2. radiotherapy 3. surgery 4. combination of these(mention) 5. others, specify	
17	Duration of treatment	List it_____	

18	Do you have underlying medical conditions?	1. Yes 2. No	
19	If 'yes' for question number 18 ,what is that	1. diabetes mellitus 2. renal failure 3. HIV/AIDS 4. Others ,specify_____	
20	Invasive procedures within the prior 4 weeks	1. Yes 2. No	
21	If 'yes 'for question number 20, what is that	1. Urinary catheter 2. Gastric tube 3. Nasal catheter 4. Central venous catheter 5. others	
22	Result of CBC	_____	

B: Garagalcha Gaaffii Afaan Oromoo

YUNIVERSIITII JIMMA

INSTITIYUTII FAYYAA

KOLLEEJJII BARNOOTAA MEDICAL LABRATOORI

MUUMMEE BARNOOTA MEDICAL MAAYIKIROBAYOLOJII

Gaaffilee waa‟ee odeeffannoo hawwasummaa fi ragaa fayyummaa hirmaataa qorannoo kanaatif mata duree waa'ee "Baakteriyaa Intirookokasii amala dawaan Vaankomayisiin walbaree fi ammalota yookiin wantoota kanafaan nama saxilaan " kan jedhu irratti muummee fayyaa Jimmaa Universititiif irratti kan geggefamudha kibba lixa Itoophiyaa 2021.

Lakk.	**I) Odeeffannoo hawwasummaa**		
	Gaaffii	**Deebii**	**Remarks**
01	Koodii dhukkubsata	_____	
02	Umurii	_____	
03	Saala	1. Dhiira 2. dhalaa	
04	Iddoo jireenyaa	1. Magaalaa 2. Badiyyaa	
05	Sadarkaa barumsaa	1. Kan hinbaranne 2. Kan umuriin hin geenye 3. Sadarkaa tokkoffaa 4. Sadarkaa lamaffaa 5. Kolleegii fi isa ol	
06	Haala fuudhaa fi heerumaa	1. Kan hineerumne ykn hin fuune 2. . Kan heerumte ykn fuute 3. . Kan gargar jiratan 4. . Kan wal hiikan 5 .Kan irra du'e	
07	Haala hojii	1. Qotee bulaa 2. Hojjataa mootummaa	

		3. Umuriin kan hin geenye 4. Barataa 5. Daldalaa 6. Hojattu mana keessa 6. Kan biro _____	
	II. Ragaa fayyaa		
08	Ji'oota darbaan sadii keessatti Qoricha fayyadamtanii beektuu?	1. Eeyyee 2. Lakki	
09	Yoo deebiin keessan 'Eeyyee'ta'ee gaafii lakk 8ttif maqaa isaa ibsaa ykn kaamidha?	**1**.vancomycin **2**.3rd and/or 4th generation cephalosporin's **3**. Aminoglycosides **4**. Carbapenems **5**.ant-anaerobic agents **6**. β-lactamase inhibitors **7**.others,and specify_____	
10	Kana dura mana ji'oota darbaan sadii keessatti mana yaalaa ciistanii beektu:?	1. Eeyyee 2. Lakki	
11	Gaafii lakk 10fi yoo deebiin keessan eeyyee ta'ee, eessa cistaani turtaani?	8. Kutaa yaala addaa (ICU) 9. Kutaa Oncology 10. Kutaa da'iimmani 11. Kutaa baqaaqsani hodhuu 12. Kutaa balaa addaa 13. Kutaa Medicaala 14. Kan biro,ibsii _____	
12	Gaaffii lakk 11ffi yoo deebiin kee eeyyee ta'ee ,guyyaa meqaaf ciiftee?	**1.** ≤7guyyaa **2.** guyyaa 7 hangaa 14 **3**.guyyaa 15 hanga 30 **4**. guyyaa >30 olii	
13	Gosaa kaansarii	Ibsii _____	
14	Turtii kaansarii	ibsii_____	

15	Qoriichaa ykn yaali farraa kaansari eegalteta?	**1.** eeyyee **2.**lakki	
16	Gaaffii lakk 15ttif yoo deebiin kee eyyee ta'ee isaa kaamin hordoofa jirta?	1. chemotherapy 2. radiotherapy 3. surgery 4. combination of these(mention) 5. others, specify	
17	Turtii qoriichaa farraa kaansari yeroo ammamif fudhaacha jirta?	ibsii_____	
18	Dhibee biraa qabdaa?	1. Eeyyee 2. lakki	
19	Yoo gaaffii lakk 18ttiif deebiin kee eeyyee ta'ee ,dhukkubichii maalidha?	5. Dhibee sukaaraa 6. Dhibee kaale 7. HIV/AIDS 8. Others ,specify_____	
20	Jiaa tokkoon darbaan keessatti Invasive procedures sii hojeetame beeka?	3. Eeyyee 2. lakki	
21	Gaaffii lakk 20ttiif, yoo deebbiin kee eeyyee ta'ee maalidha innii?	1. Urinary catheter 2. Gastric tube 3. Nasal catheter 4. Central venous catheter 5. kan bira	
22	Bu'aa qorannoo dhigaa guutuu hirmata(CBC)	_____	

C. የአማርኛ ግሌባጭ

ጅማ ዩኒቨርሲቲ

የጤና ሳይንስ ኢንስቲትዩት

የሕክምና ላብራቶሪ ሳይንስ ኮላጅ

የሜዲካል ማይክሮ ባዮሎጂ ዲፓርትመንት

ይህ መረጃ መሰብሰቢያ ቅፅ የተዘጋጀው በጅማ ዩኒቨርሲቲ ሆስፒታል ኦንኮሎጂ ማኢከሊ ቫንኮማዩሲኒ መድሃኒትን የተላማደ ኢንትሮኮካሲ ባኪተሪያ የስርጭት መጠን ኢና አጋላቹ ባህሪ በሚሊ ርእስ ላዩ የሚደረግ ጥናት ነው 2021፡፡.

ተ.ቁ	I) ማህበራዊ መረጃዎች		
	ጥያቄ	መሌስ	**አስተያየቲ**
01	የህመምተኛዉ መሆያ ቁጥር	_____	
02	እድሜ	_____	
03	ፆታ	1. ወንድ 2. ሴት	
04	የመኖረያ አዴራሻ	1. ገጠር 2. ከተማ	
05	የትምርት ሁኔታ	1. ያሊተማሬ 2. ዕዴሜ ያሌደረሰ 3. አንደኛ ደረጃ 4. ሁለተኛ ደረጃ 5. ኮላጅና ከዘ በሊይ	
06	የትደር ሁኔታ	1. ያሊገባች ወዬም ያላገባ 2. ያገባ ወዬስ ያገባችሂ 3. የተፋቱ 4. የተሇያዩ ቦታ የምኖሩ 5. የሞተባቲ	
07	የሥራ ሁኔታ	1. ገበሬ 2. የመንግስት ሠራተኛ 3. ዕዴሜ ያሌዮረሰ 4. ተማሪ 5. ነጋዳ 6. የቤት እመቤት 6. ለላ (ይገለፅ)____ _____	
	II. የእከምና ማረጃ		
08	ባለፉቲ 3ቲ ወራት ዉስቲ ጸረ ባኪተሪያ መዳኒቲ ወሲዶ ያውካሉ?	1. አዎን 2. አይደለም	
09	ለ ቲያከ ቁቲር 8 መሊሲ አዎ ከሆነ የቱ ነዉ?	**1**.vancomycin **2**.3rd and/or 4th generation cephalosporin’s	

		3. Aminoglycosides **4**. Carbapenems **5**.ant-anaerobic agents **6**. β-lactamase inhibitors **7**.others,and specify_____	
10	ባለፉቲ 3ቲ ወራት ዉሲቲ ሆሲፒታሊ ተኝቶ ያዉቃሉ?	1. አዎን 2. አይደለም	
11	ለ ቲያከ ቁቲር 10 መሊሲ አዎ ከሆነ የቲ ነዉ?	1. Intensive care units 2. Oncology unit 3. Pediatric ward 4. Surgical ward 5. Emergency ward 6. Medical wad 7. ለላ ጊለጽ _____	
12	ለ ቲያከ ቁቲር 10 መሊሲ አዎ ከሆነ ለሲኒቲ ቀኒ?	**1.** ከ ≤7 ቀኒ በታችህ **2.** ከ 7 ቀኒ ኢስከ 14 ቀኒ **3**. ከ 15 ቀኒ ኢስከ 30 ቀኒ **4**. ከ >30 ቀኒ በላዬ	
13	ካኒሰሪ አይናቲ	ጊለፅ_____	
14	የ ካኒሰሩ ቆዪታ	ጊለፅ_____	
15	የ ካኒሰሪ ሂኪሚና ጀሚሃሊ?	**1.** አዎ **2.**አልጀመርኩም	
16	ለ ቲያከ ቁቲር 15 መሊሲ አዎ ከሆነ የቲኛዉኒ ነዉ ?	1. chemotherapy 2. radiotherapy 3. surgery 4. combination of these(mention) 5. others, specify	
17	ለ ቲያከ ቁቲር 16 መሊሲ አዎ ከሆነ ለሲኒቲ ጊዘ??	ጊለፅ_____	
18	ተጉዳኝ በሽታ አሎቲ?	1. አዎ 2. የለኚሚ	

19	ለ ቲያከ ቁቲር 18 መሊሲ አዎ ከሆነ የቲኛዉኒ ነዉ?	9. የስኳር በሽታ 10. የኩላሊት በሽታ 11. ኤችአይቪ / ኤድስ 12. ሌሎች, ይግለጹ _____	
20	ከ 4 ሳምንታት በፊት Invasive procedures ተደርጎሊ ያውቃሊ?	4. አዎ 2. አይ	
21	ለጥያቄ ቁጥር 20 'አዎ' ከሆነ፣ ያ ምንድን ነው?	1. Urinary catheter 2. Gastric tube 3. Nasal catheter 4. Central venous catheter 5. ሌሎች, ይግለጹ	
22	CBC ውጤት	_____	

ANNEX IV: Laboratory Procedures

Laboratory SOP for preparation of culture media, collection, and processing of specimens, Culturing, Identification, and antimicrobials susceptibility testing

A: Preparation of culture media

BILE ESCULIN AZIDE AGAR

Bile Esculin Azide Agar is a solid medium recommended for use in qualitative procedures for selective isolation and presumptive identification of group D streptococci and enterococci.

PRINCIPLE

Group D streptococci and enterococci hydrolyze esculin in the presence of bile to form esculetin and dextrose. Esculetin reacts with the ferric ions supplied by ferric ammonium citrate to form brown-black colonies. Sodium azide and 1% Oxgall (equivalent to 10% bile) are selective agents added to inhibit gram-negative bacilli and most gram-positive bacteria other than group D streptococci and enterococci.

PREPARATION OF DEHYDRATED CULTURE MEDIUM

1. Weigh and Suspend bile esculin azide agar base in distilled water according to manufacturer instructions.
2. Mix thoroughly and heat to boiling to dissolve the medium completely with frequent agitation
3. When cooling adjust the ph. to 7.2
4. Sterilize by autoclaving at 121°C for 15 minutes. Cool the medium at 50°C water bath.
5. Dispense aseptically 20 ml of the solution aseptically into a sterile Petri dish
6. Allow the medium to solidify label with date and store at 2–8°C

QUALITY CONTROL

Each lot number of Bile Esculin Azide Agar has been manufactured, packaged, and processed in accordance with the current Good Manufacturing Practice regulations. All lot numbers have been tested using the following quality control organisms and found to be acceptable. Testing of control organisms should be performed following established laboratory quality control procedures.

CONTROL	RESULTS
Enterococcus faecalis ATCC 29212	Growth, blackening around colonies
Escherichia coli ATCC25922	Inhibition (partial to complete)
Streptococcus pyogenes ATCC19615	Inhibition (partial to complete)

LIMITATIONS

1. Organisms other than streptococci and enterococci can grow on this medium and hydrolyze esculin. Additional biochemical and serological testing is required for definitive identification(84).

BILE ESCULIN AZIDE AGAR WITH 6 µg/ml VANCOMYCIN

Bile Esculin Azide Agar w/ 6 µg/ml Vancomycin is a solid medium recommended for use in qualitative procedures as a screening method for primary isolation and presumptive identification of vancomycin-resistant enterococci (VRE) from surveillance cultures.

PRINCIPLE

Group D streptococci and enterococci hydrolyze esculin in the presence of bile, resulting in the production of esculetin and dextrose. Ferric ammonium citrate supplies ferric ions, which react with esculetin to form a black-brown complex. Sodium azide and 1% oxgall (equivalent to 10% bile) are selective agents inhibitory to gram-negative bacilli and most gram-positive bacteria other than group D streptococci. Vancomycin (6 µg/ml) is added to select for resistant strains of enterococci.

PROCEDURE

1. Inoculate and streak the specimen as soon as possible (within 2 hours) after it is received in the laboratory.

2. Incubate plate aerobically or in 5-10% CO2 at 33-37°C for 24-48 hours.

3. Examine daily for the presence of colonies, which produce a brown to black pigment diffusing into the medium.

4. Verify by Gram stain that esculin-positive colony is gram-positive cocci morphologically characteristic of streptococci or enterococci.

5. Subculture isolate to a nonselective medium, such as blood agar, for additional testing. Definitive identification of group D streptococci and enterococci requires additional biochemical and/or serological testing following established laboratory procedures.

6. Confirm vancomycin resistance using standardized susceptibility methods following established laboratory procedures. Consult appropriate references for further instructions.

INTERPRETATION OF THE TEST

Positive Test - Dark brown to black color around colonies and diffusing into the medium

Negative Test - No blackening of the medium

QUALITY CONTROL

All lot numbers of Bile Esculin Azide Agar w / 6 µg/ml Vancomycin have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed following established laboratory quality control procedures.

CONTROL	INCUBATION	RESULTS
Enterococcus faecalis ATCC®51299	Aerobic, 24 h @ 33-37°C	Growth, blackening of medium
Enterococcus faecalis ATCC®29212	Aerobic, 24 h @ 33-37°C	No growth
Escherichia coli ATCC®25922	Aerobic, 24 h @ 33-37°C	No growth

LIMITATIONS

1. This medium is recommended as a screening method for primary isolation of bile esculin-positive, vancomycin-resistant, gram-positive cocci and is not intended for use as a method of antimicrobial susceptibility testing.

2. Esculin-positive organisms other than enterococci (e.g., Pediococcus, Leuconostoc, and Lactobacillus) may grow on this medium. Further biochemical and serological testing is required for definitive identification**.**

3. Some organisms may overcome the inhibitory effects of this medium on initial isolation.

4. The absence of suspect colonies does not rule out the presence of VRE. Enterococcus casseliflavus and Enterococcus gallinarum are intrinsically resistant to vancomycin due to the presence of the vanC gene which may not be expressed when testing on this medium (84,85).

STANDARD OPERATING PROCEDURE FOR GRAM STAINING

The Gram stain is used to classify bacteria based on their forms, sizes, cellular morphologies, and gram reaction.

Principle

Bacteria stained with a basic dye (crystal or gentian violet or basic fuchsine) in the presence of a mordant (iodine) bind the stain and resist decolorization with ethanol-acetone differently depending upon fundamental differences in the biochemical structure of the bacterial cell wall and cell membrane. Gram-positive bacteria retain basic dye when decolorized, while Gram-negative organisms lose the primary dye and are colorless. They are visualized by the use of a contrasting counterstain (safranin) which is red. The G+ organisms are purple, the G- pink. It is additionally a critical test for the rapid presumptive diagnosis of infectious agents and serves to assess the quality of clinical specimens. Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50-90% of the cell wall), which stains purple while gram-negative bacteria have a thinner layer (10% of the cell wall), which stains pink. Gram-negative bacteria also have an additional outer membrane that contains lipids and is separated from the cell wall by the periplasmic space.

Materials

Reagents

1. Crystal violet,

2. Lugol iodine/Gram’s iodine

3. Alcohol/ Alcohol-Acetone mix/Acetone

4. Safranine

The procedure of Gram's Stain

1. Fix the dried smear with heat by gently passing it over the sprit lamp or Bunsen burner.Note: When the smear is for the detection of gonococci or meningococci, it should be fixed with methanol for 2 minutes.

2. Cover the fixed smear with crystal violet stain for 30 – 60 seconds

3. Rapidly wash off the stain with clean water

4. Tip off all the water, and cover the smear with Gram iodine for 30 – 60 seconds

5. Wash off the iodine with clean water

6. Decolorize rapidly with acetone-alcohol for 30 seconds. wash immediately with clean water. Cover the smear with Neutral red or Safranin for 2 minutes

8. Wash off the stain with clean water.

9. Wipe the back of the slide clean, and place it in a draining rack for the smear to air dry

10. Examine the smear microscopically, first with the 40x objective to check the staining and to see the distribution of material, and then with the oil immersion objective to report the bacteria and cells.

Results

Gram-positive bacteria …..…….. Purple(blue)

Yeast cells ……. Dark purple

Gram-negative bacteria …….…….. Pale to red

Nuclei of pus cell ……..…….…… Red

Epithelial cells ……. Pale red

Quality Control

- Gram-positive S.aureus,25923
- Gram-negative E. Coli,25922

STANDARD OPERATING PROCEDURE FOR CATALASE TEST

The catalase test is a biochemical test for aerobic organisms that detects the production of catalase enzyme in the organism. Catalase is an enzyme, which is produced by microorganisms that live in oxygenated environments to neutralize toxic forms of oxygen metabolites; H_2O_2. The catalase enzyme neutralizes the bactericidal effects of hydrogen peroxide and protects them. Anaerobes generally lack the catalase enzyme. This test demonstrates the presence of catalase, an enzyme that catalyzes the release of oxygen from hydrogen peroxide (H2O2). This test is used to differentiate those bacteria that produce the enzyme catalase such as staphylococci, from non – catalase producing bacteria such as streptococci.

Principle of Catalase Test

The metabolic activity of aerobic and facultative anaerobic microorganisms produces toxic by-products like hydrogen peroxide and superoxide radical (O2—). These products are toxic to the organisms and might even result in cell lysis if not broken down. In the case of pathogenic organisms, different mechanisms are found that break down these products into non-toxic substances. Bacteria capable of synthesizing the enzyme catalase hydrolyze hydrogen peroxide into water and gaseous oxygen, which results in the liberation of gas bubbles.

H2O2 catalase ————> H2O + O2 (gas bubbles)

The production of catalase thus protects the organism against the lethal effect of hydrogen peroxide accumulated at the end of the aerobic metabolism. The presence of the catalase enzyme can be demonstrated by adding hydrogen peroxide to the bacterial inoculum, which results in the rapid liberation of oxygen bubbles. The lack of enzyme is demonstrated by the absence of such bubbles.

Material Required

Hydrogen peroxide (3% H2O2)

Test tubes / Glass slide

Sterile wooden sticks or glass rods or platinum loops

Tube Method

Pour 2-3 ml of the hydrogen peroxide solution into a test tube.

Using a sterile wooden stick or a glass rod, remove several colonies of the test organism and immerse them in the hydrogen peroxide solution.

The procedure of Catalase Test

Slide Method

1. A small amount of organism is collected from a well-isolated 18- to a 24-hour colony with a sterile inoculating loop or wooden applicator stick and placed onto the microscope slide.
2. A drop of 3% H2O2 onto the organism on the microscope slide by using a dropper or Pasteur pipette.
3. The formation of bubbles is observed against a dark background to enhance readability.

Important: Care must be taken when testing an organism cultured on a medium containing blood because catalase is present in red cells. If any of the blood agar is removed with the organism, a false-positive reaction may occur.

Result and Interpretation of Catalase Test

The **positive test** is demonstrated by the immediate appearance of bubbles= Catalase produced

A **negative tes**t is represented by no bubbles or a few bubbles after 20second=No catalase produced

Quality Control

As a form of quality control, the following organisms can be used for positive and negative results:

Positive catalase control – Staphylococcus species

Negative catalase control – Streptococcus species

Salt Tolerance Test – Principle, Procedure, Uses, and Interpretation

To determine the ability of an organism to grow in high concentrations of salt. It is used for the differentiation of Enterococci from Non-Enterococci**.** This test is particularly useful for presumptive identification of the enterococcal group D organisms, which have the specific ability to grow in the presence of 6.5% NaCl incorporated into either a broth or an agar medium.

Principle

The medium is helpful to aid in the differentiation of Enterococcus spp. from streptococci by determining the ability of enterococci to grow in broth or agar containing 6.5% NaCl. Peptone provides nitrogenous and carbonaceous compounds, long-chain amino acids, and vitamins which provide essential nutrients. A high concentration of salt acts as a selective agent and interferes with membrane permeability and osmotic equilibrium. The high salt concentration inhibits a range of bacteria but allows salt-tolerant organisms such as enterococci to grow in the medium. Salt tolerant organisms will produce heavy growth in the broth and on solid agar within 24-48 hours.

The procedure of the Salt Tolerance Test

1. Inoculate one or two colonies from an 18- to 24-hour culture into 6.5% NaCl broth.
2. Incubate the tube at 35°-37°C in ambient air for 48 hours.
3. Examine or observe tubes for turbidity/growth after 24 hours and if negative again at 48 and 72 hours.

Expected Results

Positive test: Visible turbidity in the broth.

Negative test: No turbidity in the broth.

Quality Control

Positive: Enterococcus faecalis (ATCC29212): growth, and turbid

Negative**:** Streptococcus bovis (ATCC9809): inhibition**,** no turbidity in broth

Limitations of Salt Tolerance Test

- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification.
- Some strains of Pediococcus, Leuconostoc, and beta-hemolytic Streptococcus species may grow in Salt Tolerance Broth
- Infusion broth with 6.5% NaCl may produce slow reactions thereby making test interpretation difficult.
- A light inoculum must be used when inoculating broth. Too heavy an inoculum may produce turbidity, thus resulting in a false-positive result.

Pyrrolidonyl Arylamidase (PYR) test

PYR test is a rapid test that is used to determine the ability of an organism to produce L-pyrrolidonyl arylamidase enzyme. Application of this test are as follows: Identification of *Streptococcus pyogenes* (PYR positive)from other beta-hemolytic Streptococci (Negative) Differentiation of *Enterococcus species* (PYR positive) from group D Streptococci (*Streptococcus bovis, Streptococcus equinus*) which are PYR negative. The study showed that 98% of group A streptococci and 96% of group D enterococci hydrolyze PYR. further reported that 98% of group B streptococci, 100% of non-group A, B, and D streptococci, 100% of group D non-enterococci, and 82% of viridans streptococci yield negative PYR test results.

Principle of PYR Test

PYR is a rapid method for presumptive identification of bacteria based on the pyrrolidonyl arylamidase enzyme. The enzyme L-pyrrolidonyl arylamidase hydrolyzes the L-pyrrolidonyl- β-naphthylamide substrate to produce a β-naphthylamine. The β-naphthylamine can be detected in the presence of N,N-methylaminocinnamaldehyde reagent by the production of a bright red precipitate. Following hydrolysis of the substrate by the peptidase, the resulting b-naphthylamide produces a red color upon the addition of 0.01% cinnamaldehyde reagent. When a visible inoculum of microorganism is rubbed onto a small area of a disk impregnated with the substrate, the hydrolysis occurs within 2 min, at which time the cinnamaldehyde reagent is added to detect the reaction by a color change to purple.

Procedure of PYR Test

Disk Method (Rapid)

1. Wet the PYR test disc on the strip with 10 µl of sterile distilled water or deionized water but do not saturate, the disk with sterile water.
2. Put 5-10 colonies of the tested strain from 18-24 hours of culture on the surface of the disc with a loop and smear them lightly on it.
3. Incubate the disc for 1-2 minutes at room temperature.
4. After incubation, add 1 drop of detector reagent N, N-dimethylaminocinnamaldehyde.
5. Observe for red color development within 1-2 minutes.

   Result Interpretation of PYR Test

   Positive: Bright pink or cherry-red color within 1-2 minutes.

   Negative: No color change or a blue color due to a positive indole reaction.

Quality Control for PYR Test

Positive Control: Enterococcus faecalis (ATCC29212), Streptococcus pyogenes (ATCC19615)

Negative Control: Streptococcus agalactiae (ATCC10386)

Limitations of PYR Test

1. PYR may be used in the presumptive separation of group A streptococci and group D enterococci from other streptococci. Additional testing, using a pure culture, is recommended for complete identification.
2. A false-negative test can result if the disk or filter paper is too moist.
3. False-negative tests can result if selective media or tube biochemical agars are used to provide inocula.
4. Escherichia coli and indole-positive Proteus obtained from media containing a high tryptophan content may yield a blue-green color development. This is a negative result.
5. Some less commonly encountered isolates of lactococci and aerococci may be PYRase positive.
6. Non-specific colour reactions may occur if results are read after 20 seconds.

   Limitations:

PYR is only for the presumptive identification of group A Streptococci and group D enterococci from other streptococci thus other tests are recommended for complete identification.

If the disk or filter paper are too moist, a false-negative test can result.

Few isolates of lactococci and aerococci may be PYRase positive.

If reactions are read after 20 seconds, non-specific color reactions may occur.

STANDARD OPERATING PROCEDURE FOR MULLER HINTON AGAR PREPARATION

Mueller Hinton Agar is a standardized solid medium recommended for the study of the susceptibility of bacteria to antimicrobial agents by the method of diffusion (Kirby-Bauer method) or dilution in agar. Mueller Hinton Agar was selected by the CLSI for several reasons:

It demonstrates good batch-to-batch reproducibility for susceptible testing.

It is low in sulfonamide, trimethoprim, and tetracycline inhibitors.

It supports the growth of most non-fastidious bacterial pathogens.

Many data and much experience regarding its performance have been recorded.

Principle of Mueller Hinton Agar (MHA)

Mueller Hinton Agar media contains Beef Extract, Acid Hydrolysate of Casein, Starch, and Agar. Beef Extract and Acid Hydrolysate of Casein provide nitrogen, vitamins, carbon, amino acids, sulfur, and other essential nutrients. Starch acts as a colloid and is added to absorb any toxic metabolites produced. Starch hydrolysis yields dextrose, which serves as a source of energy. Agar is the solidifying agent. The levels of tetracycline and sulfonamide inhibitors, thymidine, thymine, magnesium, and calcium ions, are controlled so as not to interfere with susceptibility testing and to yield good growth. The use of a suitable medium for testing the susceptibility of microorganisms to sulfonamides and trimethoprim is essential. Antagonism to sulfonamide activity is demonstrated by para-aminobenzoic acid (PABA) and its analogs. Reduced activity of trimethoprim, resulting in smaller inhibition zones and inner zonal colonies, is demonstrated on unsuitable Mueller Hinton medium possessing high levels of thymidine. Both the PABA and thymine/thymidine content in

Mueller Hinton Agar are reduced to a minimum, thus markedly reducing the inactivation of sulfonamides and trimethoprim when the media is used for testing the susceptibility of bacterial isolates to these antibiotics.

Procedure

1. Weigh and suspend a commercially available dehydrated Müller-Hinton base/agar according to the manufacturer's instructions.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.
4. Pour cooled MHA into sterile Petri dishes on a level, horizontal surface to give uniform depth. Allow cooling to room temperature.
5. Check prepared MHA to ensure the final pH is 7.3 ± 0.1 at 25 °C.
6. Date the medium and give it a batch number.
7. Store the plates at 2–8 °C in the refrigerator.

Quality Control

When a new lot of media is prepared, do the following checks to ensure the quality of the prepared media. Sterility checks, and Performance checks

Quality Control strain(s) to perform the test for performance test Mueller Hinton Agar

Escherichia coli ATCC 25922

Staphylococcus aureus ATCC 25923

Pseudomonas aeruginosa ATCC 27853

Enterococcus Faecalis ATCC 29212

Streptococcus pneumoniae ATCC 49619 (for Mueller-Hinton agar that contains 5 % sheep blood)

Limitations

1. Numerous factors can affect results: inoculum size, rate of growth, medium formulation, and pH. Strict adherence to protocol is required to ensure reliable results.
2. Inoculum density may affect the zone size. Heavy inoculum may result in smaller zones or too less inoculum may result in bigger zones.
3. Fastidious organisms may not grow on this medium and may require the supplementation of blood.
4. Fastidious anaerobes may not grow on this medium.
5. The disk diffusion method should not be used for obligatory anaerobes, slow-growing organisms, and capnophiles. This method was standardized for facultative organisms or rapid-growing aerobes.
6. Drug inactivation may result from the prolonged incubation times required by slow growers.

Data collection procedures, sample processing, Culture identification, and Antimicrobial susceptibility test.

Data will be collected using a short interview guided by a pre-tested structured questionnaire consisting of the participant information; such as Socio-demographic, clinical, and data on risk factors from each informed and consented study participant, by trained data collectors (nurses). The questionnaires will be translated to the local language Amharic, Afan Oromo, and re-translated back to English to make the reliability of data collection.

Then patient /the legal guardians of patients will be instructed how to collect stool specimens and provided with sterile wide-mouth screw-capped containers to bring about 5–10 g stool specimens. Then label each sample with the unique ID number, date, and time of collection, and immediately, it will be delivered to JUMC Microbiology Laboratory for culture within 30 minutes. Then the collected stool samples will be streaked on sterile Bile esculin agar media and incubated for 24-48 hours at 35-37°C. Plates will be observed for the appearance of characteristic colonies with, black background with gray hallow colonies will be selected randomly for characterization and identification presumptively as Enterococci. The following phenotypic tests will be done: Gram stains; only plates that yield Gram-positive cocci in pairs or short chains will be studied further.

Catalase test; catalase test will be performed and only microbial growth which yields a negative result for catalase production will be considered further. Then similar colonies from each plate will be picked and inoculated into brain heart infusion (BHI) broth containing 6.5% NaCl and incubated at 37°C for 24–48 hours, and growth in the medium indicated by turbidity will be considered and the colonies will be picked, inoculated into BHI broth, and incubated at 45°C for 24 hours, and growth in the medium will be indicated by turbidity. An isolate fulfilling the above criteria will be considered as an Enterococcus species.

VRE will be identified by the Kirby Bauer disk diffusion technique as modified by the CLSI guidelines of 2021 as the following; from the growth on BHI broth, the bacteria will be refreshed onto nutrient agar for 18-24 hours and after growing on nutrient agar 3-5 selected colonies of bacteria will be taken and transferred to a tube containing 5 ml nutrient broth or normal saline and mixed gently to make homogenous suspension and the turbidity of the suspension will be adjusted comparably to a 0.5 McFarland standard. A sterile cotton swab will be used to streak the plates and the excess suspension will be removed by gentle pressing and rotation of the swab against the inside wall surface of the tube. Then the swab will be used to distribute the bacteria evenly over the entire surface of Mueller Hinton agar (MHA). The inoculated plates will be left at room temperature to dry for 3-5 minutes and with the aid of sterile forceps the following concentration of antibiotic discs will be impregnated on the surface of Mueller Hinton agar: vancomycin (30 µg), penicillin (10 IU), ampicillin (10 µg), erythromycin (15 µg), tetracycline (30 µg), ciprofloxacin (5 µg), and chloramphenicol (30 µg) based on 2020 CLSI guidelines, local availability of antibiotic disks and prescription practices. An inhibition zona of less than or equal to 14mm around the vancomycin disk will be considered as VRE. The minimum inhibitory concentration (MIC) of vancomycin was determined by the E-test method (BioMérieux, France) for all the VRE isolates, which were initially determined by the Kirby-Bauer disc diffusion method and grown on VRE screen agar (Bile Esculin Azide Agar with 6µg/ml vancomycin).

Table 1: Culture and antimicrobial susceptibility breakpoint of Kirby disk diffusion data

1. Culture result					
Black hallow colonies	Gram stains	Catalase test	Growth in 6.5% NaCl at 37°C for 24–48 Hrs	Growth in BHI broth at 45°C;	Is the isolate Enterococcus

2. Antimicrobial susceptibility testing					
Antimicrobial disc	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			AST result (mm)	Interpretation
	Sensitive	Intermediate	Resistant		
Penicillin (10 IU)	≥15	-	≤14		
Ampicillin (10 µg)	≥17	-	≤16		
Vancomycin (30 µg)	≥17	15-16	≤14		
Erythromycin (15µg)	≥23	14-22	≤13		
Tetracycline (30 µg)	≥19	15-18	≤14		
Ciprofloxacin (5 µg)	≥21	16-20	≤15		
Chloramphenicol(30 µg)	≥18	13-17	≤12		

Table 2. Interpretive categories and MIC breakpoint of E-test for Vancomycin

Antimicrobial Agent	Methods used	Interpretive Categories and MIC Breakpoints, µg/mL		
Vancomycin	E-test	Sensitive	Intermediate	Resistance
		≤ 4	8–16	≥ 32

Annex V: Declaration Sheet

I, the undersigned, declare that this thesis is my work. I have followed all ethical and technical principles of research in the preparation, data collection, data analysis, and compilation of this thesis. Any scholarly matter that is included in the thesis has been given recognition through citation. This thesis is submitted in partial fulfillment of the requirements for an M.Sc. degree at Jimma University. I confidently declare that this thesis has not been submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

Principal investigator

Amanuel Teferi signature: _____ date_____

Place: Jimma University, Institute of Health, Faculty of Health Science, School of Medical Laboratory Science, Ethiopia

Date of submission_____/_____/_____

Name of advisors:

Dr.Mulualem Tadesse (Phd) Signature _____ Date _____/_____/_____

Mrs. Rahel Tamirat (MSc) Signature _____ Date _____/_____/_____

JIMMA UNIVERSITY

POSTGRADUATE PROGRAM DIRECTORATE

As Thesis Research advisors, we hereby certify that we have read and evaluated this thesis prepared, under our guidance, by Amanuel Teferi, entitled "Intestinal colonization of vancomycin-resistant enterococci and its associated factors among patients attending oncology wards at Jimma medical center, Southwest, Ethiopia: a comparative cross-sectional study ". We recommend that it can be submitted as fulfilling the thesis requirement.

Advisors:

Mulualem Tadesse (PhD) _____ _____

Signature Date

Rahel Tamrat (MSc) _____ _____

Signature Date

As a member of the Board of Examiners of the MSc Thesis-Open Defense Examination, we certify that we have read, evaluated the thesis prepared by Amanuel Teferi, and examined the candidate. We recommended that the thesis be accepted as fulfilling the thesis requirement for the Degree of Master of Science in Medical Microbiology.

_____ _____ _____

Chairperson Signature

Date

__Professor Getinet Beyene_____ _____ _____

Internal Examiner Signature

Date

_____ _____ _____

External Examiner Signature

Date