
አዲስ አበባ ዩኒቨርሲቲ
Addis Ababa University

Addis Ababa University, College of Health Sciences
School of Allied Health Science

Department of Medical Laboratory Sciences

Prevalence and antimicrobial susceptibility of *Entrococcus* species isolated from different clinical samples at Tikur Anbesa Hospital Addis Ababa, Ethiopia

By Zelalem Tena

Department of Medical Laboratory Sciences, School of Allied Health Science, College of Health Sciences, Addis Ababa University.

Approved by the Examining Board

_____	_____
Chairman, Dep. Graduate Committee	Signature
_____	_____
Advisor	Signature
_____	_____
Examiner	Signature
_____	_____
Examiner	Signature
_____	_____

Addis Ababa University Collage of Health Science, school of Allied health science, Department of Medical Laboratory Science

Research Project submission form:

Name of investigator	Zelalem Tena
Full title of the research project	Prevalence and antimicrobial susceptibility of *Entrococcus* species isolated from different clinical samples at Tikur Anbesa Hospital Addis Ababa, Ethiopia
Study Area	Tikur Anbesa Hospital, Addis Ababa, Ethiopia.
Total cost of the project	36,094 ETB
Source of funding	Self
Address of investigator	Tel.: 0912960680 Email: zelalemtena@gmail.com
Name of Advisor	Kassu Desta(BSc., MSc., PhD candidate) Tel.: 0911107099 Email: kassudesta2020@gmail.com

Acknowledgment

I want to acknowledge Addis Ababa University, College of Health Science, School of Allied Health Science, and Department of Medical Laboratory Science for giving me the opportunity.

Sincere thanks and appreciations are forwarded to my advisors Ato Kassu Desta (MSc, PhD Fellow)and Solomon Gizaw (MSc,PhD Fellow) for their valuable support and professional advice for the successful completion of this research from the beginning of proposal to final theses write up.

I would also like to acknowledge Tikur Anbesa Hospital for granting permission to conduct the research.

My deepest gratitude forwarded to all staffs of Tikur Anbesa Hospital bacteriology department especially for Mekuanent Meteku for their assistance during every Laboratory works. Finally I am also indebted to patients who were participated on the study.

Contents

List of Abbreviations

BSI----------Blood Stream Infection

CDC----------Center for Disease Control program

CLSI----------Clinical Laboratory Standard Institute

CNS----------Central Nervous System

GIT----------Gastro Intestinal Tract

HIV----------Human Immuno deficiency Virus

HLAR----------High Level Aminoglycosal Resistance

HLGR----------High Level Gentamaycin Resistance

LAP----------Lucien Amino Peptides

MIC----------Minimum Inhibitory Concentration

MSU----------Mid Stream Urine

PYR----------l-pyrrolidonyl-b-naphthylamide

QC----------Quality Control

RNA----------Ribo Nucleic Acid

SOP----------Standard Operating Procedure

SPP---------- -Species

SPSS----------Statistical soft ware Package

UTI----------Urinary Tract Infection

VRE----------Vancomaycin Resistance Entrococcus

WHO----------World Health Organization

Operational definition

- Nosocomial infection: –infections which acquired in the hospital 72hrs or more after hospital admission.
- Multi drug resistant:-bacteria which resist more than 2 different classes of drugs.
- Minimum inhibitory concentration:-is the lowest concentration of an antimicrobial drug that will inhibit the visible growth of a microorganism after overnight incubation.

List of tables

Abstract

Background: Enterococci which are part of the normal intestinal flora are opportunistic human pathogens. The two most important species, *E.faecalis* and *E.faecium* are among the leading causes of nosocomial infections that may cause severe infections including endocarditis, urinary tract infection, septicemia and wound infections which are often difficult to treat. Their increasing importance is largely due to their resistance to antimicrobials. So the aim this study is to determine the prevalence and antimicrobial pattern of *Entrococcus* spp.

Objective: To determine the prevalence and antimicrobial susceptibility of *Entrococcus* species isolated from different clinical samples at Tikur Anbesa Specialized Hospital Addis Ababa, Ethiopia .

Method: A cross sectional study was conducted from April to May 2016 on patients visiting Tikur Anbesa Specialized Hospital. A total of 422 patients were included in the study. Clinical samples(blood, urine, pus, bodyfluid and csf) was inoculated onto blood agar and mackonky culture media .The isolates were identified by cultural characteristics, Gram's stain, catalase test, growth in 6.5% NaCl broth, bilesculin test, PYR test. Drug susceptibility pattern of the isolates were determined using the Kirby Bauer disc diffusion method. Confirmation of vancomycin susceptibility was done by the Epsilometer test to determine the Minimum Inhibitory Concentration. Results obtained from the study was entered to excel spreadsheet and transport to and analyzed using SPSS version 20 to test for statistical associations. P values less than 0.05 were considered as significant.

Result: From a total of 422 sample processed 15 *Entrococcus* spp were isolated .Among the isolate 53.3% were female and 46.7 %were male patients . In this studyLizolide were the drug of choice for *Entrococcus* spp 100% sensitive followed by Vancomycen 93.3% sensitive in contrast Ampcillen was highly resistance by 80% followed by Doxicyclin 73.3% resistance.

Conclusion: This study showed that the rate of isolated *Enterococci* was 3.5% and had variable degrees of resistance to the antibiotics, but all were sensitive to Linzolide by disc diffusion methods so the presence of VREand multidrug resistant *Enterococci* in our study should be considered as an alarm for serious *Enterococcal* infections .

Key words: ***Entrococcus*, Nosocomial infection, clinical samples , Antibiotic susceptibility.**

1. Introduction

1.1 Background

Enterococcus species were originally classified as enteric Gram-positive cocci and later included in the genus *Streptococcus*(1). In the early 1930s, *Enterococcus* species were classified as group D streptococci and were differentiated from the non-*Enterococcal* group D streptococci by distinctive biochemical characteristics(2). In the late 1930s, Sherman recommended that the term *Enterococcus* species be specifically used for the *Streptococcus* that grow at both 10 and 45oC, at *p*H 9.6, in the presence of 6.5 per cent NaCl, survive at 60oC for 30 min and hydrolyse esculin(3). During the mid 1980s, studies involving fatty acid composition, nucleic acid hybridization and comparative oligonucleotide cataloguing of 16s RNA led to the acceptance that the *Enterococcus* species were sufficiently different from other streptococci to merit their own genus(4).

The members of the genus *Enterococcus* are gram-positive, catalase-negative cocci that occur singly or are arranged in pairs or as short chains. Cells are sometimes coccobacillary when Gram stains are prepared from growth on solid medium but tend to be ovoid and in chains when grown in liquid media, such as thioglycolate broth. After growth on blood agar media for 24 h, colonies are usually between 1 and 2 mm in diameter, although some variants may appear smaller. Some (about one-third) cultures of *E. faecalis* may be b-hemolytic on agar containing rabbit, horse, or human blood but nonhemolytic on agar containing sheep blood. Some cultures of *E. faecalis* and *Enterococcus durans* may be b-hemolytic regardless of the type of blood used. All other species are usually a-hemolytic or nonhemolytic. Enterococci are facultative anaerobes with a homofermentative metabolism that results in the production of l-(1)-lactic acid as the major end product of glucose fermentation. Because of their ability to ferment a wide range of carbohydrates to lactic acid, the enterococci are referred to as typical lactic acid bacteria. Gas is not produced. These microorganisms are usually able to grow at temperatures ranging from 10 to 45°C, with optimum growth at 35 to 37°C. The majority of the species grow in broth containing 6.5% NaCl, and they hydrolyze esculin in the presence of bile salts (bile-esculin test). They also hydrolyze leucine-b-naphthylamide by producing leucine aminopeptidase (LAP).

Most enterococci, apart from *Enterococcus cecorum, Enterococcus columbae, Enterococcus pallens, Enterococcus saccharolyticus,* and some strains of the recently described species *Enterococcus canintestini, Enterococcus devriesei, Enterococcus moraviensis,* and *Enterococcus termitis,* hydrolyze l-pyrrolidonyl-b-naphthylamide (PYR) by producing pyrrolidonyl arylamidase (pyrrolidonase). Results for both LAP and PYR testing, especially for some of the more recently described species, may vary according to the methodology, including the test format and media used to grow the bacterial cell (6).

Enterococcus species are a part of the normal human faecal flora. Sites less often colonized by *Enterococcus* species include the oral cavity, genitourinary tract and skin especially in the perineal area. The main sites of colonization in the hospitalized patients are soft tissue wounds, ulcers and the gastrointestinal tract (GIT). *Enterococcus* species were traditionally regarded as low grade pathogens but have emerged as an increasingly important cause of nosocomial infections in the 1990s(1).

Enterococcus infections may be due to at least 12 species, including *E. avium, E. casseliflavus, E. durans, E. faecalis, E. faecium, E. gallinarum, E. hirae, E. malodoratus, E. mundtii, E. pseudoavium, E. raffinosus,* and *E. solitaries*(6). Additional species such as *E. cecorum, E. columbae, E. saccharolyticus, E. dispar, E. sulfureus, E. seriolicida* and *E. flavescens* have been proposed as additions to this list. Most clinical infections are due to either *E. faecalis* or *E. faecium.* Until recently *E. faecalis* had been the predominant *Enterococcus* species, accounting for 80-90 per cent of all clinical isolates, and *E. faecium* had accounted for 5 to 15 percent(5,6).

1.2 Statement of the problem

Enterococcus species have become problem of the world as emerging nosocomial infection and multi drug resistance bacteria(7). During the past decade, there has been a worldwide trend in increasing occurrence of *Enterococcus* species in the hospitals, a shift in the spectrum of *Enterococcal* infections, and emergence of antimicrobial resistance among such isolates indicates a need for continues surveillance of the bacteria(8). *Enterococcus* species were reported as the second most common cause of nosocomial infections in the US(7). The most frequent infections caused by *Enterococcus* species are urinary tract infections.

The second most frequent *Enterococcal* infections generally have been intra- abdominal and intra - pelvic abscesses or post surgery wound infections(9). In these settings, *Enterococcus* species are usually part of a mixed flora commonly found in the GIT so identifying the bacteria which cause the disease will be critical. Interactions among various bacteria have been demonstrate, and studies suggest that *Enterococcus* species can act synergistically with other bacteria to enhance infection. However, the role played by *Enterococcus* species in these cases has not been fully defined (10), since infections of surgical wounds and of the urinary tract often resolve without specific therapy(10). The third most frequent infection caused by these organisms is blood stream infections (7). Other infections caused with lower frequency are central nervous system and neonatal infections. *Enterococcus* species rarely cause respiratory tract infections, osteomyelitis, or cellulitis(11).

Enterococcus species have become the second or third leading cause of nosocomial urinary tract infections (UTIs), wound infections (mostly surgical, decubitus ulcers, and burn wounds), and bacteremia in the United States (11,13). UTIs are the most common of the *Enterococcal* infections: *Enterococcus* species have been implicated in approximately 10% of all UTIs and in up to approximately 16% of nosocomial UTIs(13). *Enterococcal* bacteremia is frequently associated with metastatic abscesses in multiple organs and high mortality rates. *Enterococcus* have also been considered an important cause of endocarditis; they are estimated to account for about 20% of the cases of native valve bacterial endocarditis and for about 6 to 7% of prosthetic valve endocarditis(14,20). Endocarditis remains among the most difficult to treat *Enterococcal* infections because of limitations on bactericidal antimicrobial therapy for *Enterococcal* infections, especially when caused by vancomycin-resistant *Enterococcus* (VRE).

There is also a growing concern about the role of the *Enterococcus* species in endodontic and implant- and medical device-associated infections (10).

Enterococcus species have become increasingly important not only because of their ability to cause serious infections but also because of their increasing resistance to many antimicrobial agents. The emergence of high level resistance to aminoglycosides (HLAR), β lactam antibiotics and to vancomycin by some strains, together with multi drug resistance has led to failure of synergistic effects of combination therapy(1,2,29).

A major impact on the incidence and epidemiology of *Enterococcal* infections was noted after the first reports on the occurrence and epidemic increase of VRE in hospitals in the United States. By 1993, the rates of VRE had already increased 34-fold in intensive care units of U.S. hospitals (15). The percentage of VRE isolates reported by U.S. hospitals increased from 0.3% in 1989 to over 25% of all isolates in 1999 (13,25,26).

In the 2006–2007 report from the National Healthcare Safety Network at the Centers for Disease Control and Prevention (CDC), the *Enterococcus* species were listed as the third most common antimicrobial-resistant pathogen associated with healthcare-associated infections, accounting for 12% of them (14).

The emerging of nosocomial *Entrococcus* infection and their ability to resist multi drug are also hot issue in Africa as a research in Nigeria, Egypt ,Ethiopia indicates (36,37,38,39,40).

The emerging contest of *Entrococcus* nosocomial infection and resistance rates to the most common prescribed drugs vary considerably in different areas due to local antimicrobial prescribing practices, empirical treatment, and prevalence of circulating resistant *Entrococcus* species in a given area(28). The etiologies and their susceptibility to different antibiotics may have also changed over time. As a result continuous and periodic surveillance of local prevalence of *Entrococcus* species and their susceptibly pattern will have public health significance to promote proper use of the existing antibiotic drugs.

Therefore, the aim of this study was to determined the prevalence of *Entrococcus* species and their antibiotics susceptibility pattern isolated from different clinical samples in Tikur Anbesa Specialized Hospital.

1.3 Significance of the study

The emergence of nosocomial *Entrococcus* infection ,multi drug resistance and VRE in different clinical sample were recorded in different country throughout the worled.

But, to our knowledge there was no previous data or study showing resistance pattern and prevalence of *Entrococcus* spp in the study area.

There for, this study was provided information on to the prevalence of *Entrococcus* species and the susceptibility pattern of antimicrobial agents in different clinical samples, which will have an important public health implication to the community as well as the health workers.

2.Literature reviews

A Sentry study in US(30) indicated that from a total BSI *Enterococcus* accounted 19% in Canada and United States and 3% in Latin America. 16.8% in Canada, 12.5% in US, 11.7% in Europe and 4.2%in Latin America of the UTI were *Enterococcus*. From the *Enterococcus* species *E.faecum* accounted more (19,3%–20%). The study were also included drug susceptibility pattern and the result were (84%–99%) of *Enterococcus* from Latin America and Europe , 76% from US and Canada were susceptible to Ampicillin. US isolates were considerably more resistant to vancomycin than from the rest of the world. . In Latin America all isolates tested in 1997 were susceptible to vancomycin, In the remaining geographic regions, the rates of vancomycin resistance were very low (1%–3%) and did not change appreciably during the reported study period, although the rate of resistance to glycopeptide was 1% in 1998, increasing to 2% a year later.

A study in Serbia by Mihajlović U.M,*et al*.,(31) on the Prevalence of different *Enterococcus* species isolated from blood and their susceptibility to antimicrobial drugs, had shown that, the most common species was *E. faecalis* (55.05%), followed by *E. faecium* (41.57%) from blood sample. *E. faecium were found multi drug* resistance and (54.05%) of *E.faecium* were vancomycin resistance while resistance in *E. faecalis* was not found. (89.18%) isolates of *E. faecium* were high-level gentamycin resistant and (91.4%) were resistant to high concentration of streptomycin, whereas frequency of resistant *E. faecalis* was 61.2 and 63.04%, respectively.

A study condacted in india by Sreeja s *et al*.,(32) on the prevalence and the Characterization of the *Enterococcus* species from Various Clinical Samples in a Tertiary Care Hospital, indicates the prevalence of *Entrococcus* were 2.3%. Among the isolates, those of *E. faecalis* were (76%) and the remaining (24%) were of *E. faecium*. The maximum number of isolates were from pus (43%), followed by the isolates from urine (31%). The sensitivity pattern of these isolates showed an increased resistance to penicillin, ampicillin and ciprofloxacin. A High Level of Gentamicin Resistance (HLGR) was present in (47%) isolates of *Enterococcus* and (27%) isolates were intermediately sensitive to vancomycin by the Kirby Bauer disc diffusion method.

Another study in India by Desai P.J *et al.,* (33) on prevalence identification and distribution of various species of *Entrococcus* species isolated from clinical specimen with special reference to urinary tract infection in catheterized patients indicates the prevalence rate of *Entrococcus* 22.19%. The most frequent source of *Entrococcus* species were found to be foley catheters (48.21%), Burn wounds (29.51) and Ascitic fluid ,abdominal drain fluid and non surgical wound were account (22.28%).Among the isolate those (49.5%) were *E.faecalis* , (35.64%) were *E.faecium* and (14.85%) of the isolate were other *Entrococcus* species.

A Study in Bangladesh by Tashmin A.B.I,*et al.*,(35) on Isolation and species identification of *Enterococcus* species from clinical specimen with their antimicrobial susceptibility pattern in a tertiary care hospital indicates , Out of 16 *Enterococcus*, 10 (62.5%) were *E. faecalis*, 4(25%) were *E. faecium* and 2 (12.5%) were other species .(93.75%) were from urine sample and (6.25%) were from wound swab. All the enterococci (100%) were sensitive to vancomycin and linezolid. Most of the strains were resistant to ciprofloxacin and azithromycin (87.5%), gentamycin (81.25%), ceftriaxone (75%), amoxiclav (31.25%) and imipenem (25%). *E. faecium* was more resistant than *E. faecalis* to azithromycin (100%), ciprofloxacin (100%), amoxiclav (75%) and imipenem (50%). No vancomycin resistant *Enterococcus* were identified.

A study in Kuwait by Edet E.U,*et al.*,(36) on the species prevalence and antibacterial resistance among *Enterococcus* species . They consisted of 415 isolates of which, *E. faecalis* (85·3 %), *E. faecium* (7·7 %), *E. casseliflavus* (4·0 %), *E. avium* (1·2 %), *E. durans* (1·0 %), *E.gallinarium* (0·5 %) and *E. bovis* (0·2 %) isolated from urine (36·6 %), blood (10·4 %), wound swabs (11·0 %), stool samples (12·0 %), high vaginal swabs (9·0 %), endocervical swabs (3·0 %) and miscellaneous sources (18·0 %). All of them were susceptible to linezolid, (12·5 %) isolates were ampicillin resistant but none of them produced B-lactamase. The resistant to erythromycin (63·3 %), tetracycline (60·5 %), ciprofloxacin (40·0 %), chloramphenicol (28·0 %), vancomycin (2·6 %), and teicoplanin (2·6 %). 14%, 19% and 20% of them expressed high-level resistance to gentamicin, kanamycin and streptomycin, respectively.

A study in Nigeria by Ayeni F.A, *et al.*, (37) on the Prevalence and antibiotic susceptibility pattern of *Entrococcus* specious isolated from a Nigeria Hospital indicats (7%) *Entrococcus* prevalence and the source of sample were (9.3%) from urine followed by endocervical swab (8.3%).Among drug suseptability (33.3%) were sensitive to chloramphinicol ,(16.7%) were sensitive to nitrofurantoin ,(66.7%) were resistant to doxycyclin ,(50%) wre resistant to ciprofloxacilin and ampicillin.

Astudy in Egypt by Rania M.K,*et al*.,(38) on Species Identification and Pattern of Antibiotic Resistance of *Enterococcus* Isolated from Cases of nosocomial Infections ,the *Enterococcus* infection rate was 3.3% isolated from urine (65%), pus (30%) and from ascetic fluid (5%). The majority of the isolates were *E.faecalis* (55%) followed by *E. faecium* (30%), *E. durans* (10%) and *E. avium* (5%). The most common source of isolation was the internal medicine departments and ICUs . By disc diffusion method, *E.faecalis* was more resistant than *E.faecium* to tertracyclines and aminoglycosides . On the other hand *E.faecium* was more resistant than *E.faecalis* to penicillins and quinolones All the strains were sensitive to vancomycin by disc diffusion method.

A cross sectional study conducted at Jimma by Alemseged A.,*et al*.,(39) on Antimicrobial resistance profile of *Enterococcus* species isolated from intestinal tracts of hospitalized patients in Jimma university Hospital , shown *Enterococcus* species were isolated from 114 (76%) of the study subjects. The isolates were *E. faecium* (35.1%) followed by *E. faecalis* (29.8%), *E.gallinarum* (17.5%), *E.casseliflavus* (8.8%) and *E. durans* (8.8%). Among 114 tested Enterococci isolates, 41 (36%) were resistant to ampicillin, 62 (54.4%) to streptomycin and 39 (34.2%) to gentamycin. Other alternative antibiotics to treat mixed nosocomial infection caused by *Enterococcus* also showed high rate of resistance in vitro, ciprofloxacin (50%), norfloxacin (49.1%), erythromycin (63.2%), tetracycline (64.9%), chloramphenicol (34.2%), and nitrofrantoin (32.4%). Multiple drug resistance was observed among 89.5% of *E. faecium* and *E. faecalis*. Vancomycin resistant *Enterococcus* were observed in 5% of *E. faecium* isolates.

A cross sectional study conducted at Gondar by Wondwosen A,*et al*.,(40) on the prevalence of vancomaycin resistant *Entrococcus* species and associated risk factors among clients with and without HIV from stool sample indicates that, The overall colonization of *Enterococcus* species was 88.9% of which (5.5%) were VRE. The prevalence of VRE among clients with and without HIV infections were (7.8%) and (3.1%) respectively. 90% of the *Enterococcus* species isolates were resistant to two or more antibiotics tested.

3. Hypothesis

There is difference in prevalence of *Entrococcus* species and pattern of drug susceptibility in different clinical sample.

4 . Objectives of the study

4.1 General Objective

- To determine the prevalence and antimicrobial susceptibility patterns of *Entrococcus* species with in different clinical sample at Black Lion specialized Hospital.

4.2 Specific Objectives

- To determine the prevalence of *Entrococcus* species in different clinical sample.
- To determine antimicrobial susceptibility pattern of *Entrococcus* species in different clinical sample.
- To identify risk factors associated with *Entrococus* species infection in Hospitalized patients.

5. Materials and Methods

5.1 Study area

The study was conducted in Tikur Anbesa specialized hospital, which is a teaching and national referral hospital in Ethiopia. Tikur Anbesa hospital is one of the tertiary referral Hospitals directly under the federal ministry of health. The Hospital gives services to an estimated 730,000 people annually who are referred from all corners of the country. It has a total bed capacity of >500 and on an average 2000 patients visit at the Hospital as outpatient and emergency daily. It gives service under different clinical disciplines which include Surgery, Orthopedics, Obstetrics and Gynecology, Pediatrics, Internal Medicine and ENT.

5.2 Study Design and Period

A hospital based cross sectional study was implemented starting from April to May 2016. Information and clinical sample which were relevant to the study was collected from the study populations.

5.3 Source population

Patients who was seek medical service at Tikur Anbesa specialized Hospital during the study period.

5.4 Study population

Patients visiting Tikur Anbesa Hospital during the study period who were suspected for UTI, Septicemia, Wound infection , Endocarditic and Meningitis .

5.4.1 Inclusion criteria

Voluntary patients who were given different clinical sample for microbiological examination.

5.4.2 Exclusion criteria

- Patient who gives stool and sputum samples, since *Entrococcus* spp is normal flora in intestinal and oral cavity.
- Involuntary patients.

5.5 Study variable

5.5.1 Dependent Variable

- Prevalence of *Entrococcus* species.
- Antimicrobial susceptibility pattern of *Entrococcus* species.

5.5.2 Independent Variable

- Age
- Sex
- Assumed risk factors like previous antibiotic usage , history of catheterization for urine sample, and duration of adimition.

5.6 Sample Size and Sampling Technique

5.6.1 Sample Size

Since there is no previous study in Ethiopia to refer as base line, prevalence of *Enterococcus* species in different clinical sample was calculated by conventional 50% prevalence base line as follows:-

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

Where n= final sample size of the required population,

Z= the standard deviation corresponding to 95% confidence level i.e. 1.96,

P=proportion in the target population to have *Enterococcus* i.e. 50% (0.5).

d= degree of accuracy desired= 0.05

$$n = 1.96^2 \times \frac{0.5 \times (1-0.5)}{0.05^2}$$

n=384.16=384 the 10% non response rate is 38.4

There for the sample size was 422

5.6.2 Sampling Technique

All consecutive *Entrococcal* infection suspected patients were included in the study by conventional sampling technique. Relevant information including; age, sex, source of infection, were gathered on a prepared questionnaires. Clinical samples (Urine, Blood, Bodyfluid, CSF and Pus) were collected according to the micro biological sample collection techniques.

All the samples were analyzed immediately arrived to the laboratory to ensure that the *Entrococcus* species present in the sample were isolated and to avoid over population of the pathogenic organis

5.7.Methods of Data collection

Prior to data collection information how to collect data was given to selected data collectors (two nurses and two laboratory technicians) .The aim of the study was explained to individuals data collector. Individuals who full fill the inclusion criteria and volunteer to take part in study were enrolled as study subjects and a written informed consent was gained. Socio-demographic characters and other risk factors were collected by using a structured questionnaire from the patient, patient card and the responsible physician that was prepared in English and translated to Amharic.

5.7.1 Sample collection and processing

All clinical samples were collected by trained personals with sterile container according to the standard operating procedures (SOP) except urine sample it need special attention as follows:
the first midstream urine passed by the patient at the beginning of the day should be sent for examination. This specimen is the most concentrated and therefore the most suitable for culture and microscopy.

Blood Sample Collection:-

Blood for culture must be collected and dispensed with great care to avoid contaminating the specimen and culture medium. After disinfecting the site of blood collection ,10-15ml of blood

for adult patient and 3-5ml of blood for children were collected. Then all blood samples were cultured in blood culture bottles and incubate at 37°C for 24h to see hemolysis and turbidity. For positive samples gram stainwere done then inoculated onto blood and MacConkey agar plate and was incubated aerobically for 24 h at 37°C.

Each other specimens were cultured on blood and MacConkey agar media immediatelyand incubate at 37ºc for 24h. Then, the suspected colony on the culture was identified primarily as *Enterococcus* spp according to the colony morphology and gram staining.

Biochemical tests:- Different biochemical tests were used to identified *Entrococcus* spp such as PYR test, catalase test, Bile aesculin hydrolysis, Salt tolerance test, and Growth at 10°c and 45°c.

5.7.3 Drug susceptibility Patterns

The antimicrobial susceptibility testing was performed by uesing the Kirby Bauer disc diffusion method according to the National Committee for Clinical Laboratory Standards (NCCLS, 2014) guidelines.

The suspensions of a young culture growth of *Entrococcus* spp were prepared by picking parts of similar colonies with a sterile wire loop. And suspended in sterile broth and incubated up to two hours to allow the bacteria to reach their log-phase in growth. The turbidity of suspension was determined in comparison with 0.5 MacFarland standard (41). A sterile swab was dipped in the broth suspension and excess suspension was removed by pressing the swab against the wall of the tube. The entire surface of MHA plate was uniformly flooded with suspensions and allowed to dry for about 15-30 minutes.

The antimicrobial impregnated disks were placed by using sterile forceps at least 24 mm away from each other to avoid the overlapping zone of inhibition (41).

The susceptibility pattern were performed by using Ampicillin (10 µg), Doxycycline(30µg), ciprofloxacin (5µg), vancomycin (30 µg), gentamicin (120µg), Chloramphinicol (30µg) and linezolid (30µg) and interpreted according to CLSI M100-S24,2014 (41). The disks were placed on agar plates and allowed to stand for 30 minutes to dissolve the antibiotics in the media. The plates were inverted and incubated at 37^{o}c for 24 hours and then inhibition zone were measured

.

Interpretation: Grades of susceptibility pattern was recognized as sensitive, intermediate and resistant by comparison of zone of inhibition as indicated in the manufacturer's guidance (22,41).

Care was taken to view the vancomycin zone of inhibition and vancomaycin resistance. The minimum inhibitory concentration (MIC)of vancomycin was determined by the E test for all the *Enterococci* isolates which showed resistance by the Kirby Bauer disc diffusion method. A lawn culture of *Enterococci,* 0.5 Macfarland's standard was made on 5% Mueller Hinton blood agar. The E –strip was applied with MIC scale facing up by using sterile forceps with the higher concentration facing the edge of the plate. The plates were examined after 24 hours of incubation at37^{o}c. The zone of inhibition was observed in the form of an ellipse. The MIC value is the value at which the zone convenes the comb like projection of the strip (5,6). The antibiotic susceptibility pattern was interpreted as per the manufacturer guide line (23).

5.7.4 Quality Control

Standard Operating Procedures were strictly followed. Verify that media meet expiration date and quality control parameters per CLSI. Visual inspections of cracks in media or plastic petri-dishes, unequal fill, hemolysis, evidence of freezing, bubbles, and contamination were performed. Quality control was performed to check the quality of medium. Each new lot was quality controlled before use by testing the ATCC E.faecalis 29212, as per the Clinical and Laboratory Standards Institute guidelines standard strains (41).

5.8 Statistical analysis and interpretation

All statistical analyses were performed with the SPSS statistical software package (version 20).The descriptive statistics were computed by percentages, arithmetic mean and standard deviation and the bi-variant logistic regression analysis were used to see the relation between dependent variable and independent variables. For tests of whether the distribution of categorical variables differed across study groups the Chi-square test was used. Variables that were showed a significant association were selected for further analysis using multiple logistic regression models. Probability values (P) of < 0.05 were considered as statistically significant. Finally, the study findings were explained in words and tables.

5.9 Dissemination of results

The result of the study was submitted to the Department of Medical Laboratory Sciences, School of Allied Health Sciences, College of Health Sciences, Addis Ababa University, Tikur Anbesa specialized Hospital and other concerned bodies. During the study the finding of other pathogenic bacteria important to the patients were reported to the responsible body. The result of the thesis was submitted to the international or national peer reviewed journal for publication.

5.10 Ethical consideration

Ethical clearance and permissions were obtained from the Department of ethics committee of medical laboratory science in Addis Ababa University; and a letter of support was written to Tikur Anbesa specialized Hospital from the department. Official permission also was obtained from Tikur Anbesa specialized hospital Ethics Review Committee. All study records that identify subjects were kept confidential. All information collected in this study was given code numbers and no names were recorded. The key to this code numbers and paper files were kept in a locked file and computerized files were password-protected and all were only accessible to principal investigator.

All the investigations done for participants of this study was free of charge but hospital care and treatments were paid according to the rule of the hospital. Study participants were not compensated for their participation in this study.

6. Result

6.1 Socio-demographic characteristics of study participants

A total of 422 participants were involved in the study from those 41.71% were females and 58.29% were male participants. The mean age of them wear 23 year (rang 1-87). The highest proportion of the participants were in the age range of 5-15 years (26.3%) followed by the age range 16-26 (21.6%).

Table.6.1. Socio-demographic characteristics of study participants (n =422) among patients attending at Tikur Anbesa Specialized Hospital, Addis Ababa, Ethiopia, 2016.

Characteristics		Frequency No	Percent
Sex	Male	246	58.29
	Female	176	41.71
Residence	Urban	245	58.0
	Rural	177	42.0
Age group	<5	74	17.5
	5-15	111	26.3
	16-26	91	21.6
	27-37	62	14.3
	38-48	31	7.3
	49-59	25	5.9
	>59	28	6.6
Level of education	Illiterate	41	9.7
	Primary school	147	34.8
	Secondary school	58	13.7
	diploma	35	8.3
	>diploma	34	8.1
	Under School age	107	25.4

6.2 *Entrococcus* isolates

Fifteen strains of *Enterococci* were isolated from a total of 422 various clinical specimens. The prevalence rate of *Enterococci* was 3.5 %. The isolation rate of *Enterococci* strains from urine was (46.66%) while it was (40%) from blood, (6.67%) from ascetic fluids and (6.67%) from pus with no statistically significant difference ($p>0.05$). Regarding the relation of isolation rate of *Enterococci* among the different age groups, it was found that 53.3% were (>45 years) with statistical difference ($p=0.0001$). The isolation rate of *Enterococcal* strains was higher in females than males with no statistically significant difference ($p>0.05$). 93.3% of *Entrococcus* spp were isolated from Hospitalized patients from those 10(71.4%) were from medical ward , 3(21.4%) were from pediatrics ward and the rest one was from surgical ward with no statistical significant difference ($p>0.05$) . (Table 6.2)

Table.6.2.Prevalence of *Entrococcus* spp in Relation to different socio-demographic characters among patients attending Tikur Anbesa specialized Hospital, Addis Ababa, Ethiopia, 2016.

Characteristics		Frequency	Negative (%)	Positive (%)	X^2	P.value	OR
Sex	Male	246	239(97.6%)	7(2.4%)	0.4	0.5	0.15
	Female	176	168(95.5%)	8(4.5%)	0.4	0.5	1.6
Age group	<5	71	71(1005)	0(0%)	2.02	0.2	0.00
	5-15	109	105(96.3%)	4(3.7%)	0.05	0.8	1.05
	16-26	90	88(97.8%)	2(2.2%)	0.2	0.7	0.6
	27-37	63	62(98.41%)	1(1.59%)	0.3	0.6	0.4
	38-48	31	31(100%)	0(0%)	0.4	0.5	0.0
	49-59	25	23(92%)	2(8%)	0.5	0.5	2.6
	>59	33	27(81.8%)	6(18.2%)	17.95	0.00002	9.38
Specimen type	urine	116	109(93.9%)	7(6.1%)	2.0	0.2	2.4
	Blood	189	183(96.8%)	6(3.2%)			
	Ascetic fluid	39	38(97.4%)	1(2.6%)			
	Pus	47	46(97.9%)	1(2.1%)			
	Csf	31	31(100%)	0(0%)			
Patient source	Out patient	103	102(99%)	1(1%)		0.2	
	Medical ward	164	154(93.9%)	10(6.1%)			
	Pediatric ward	102	99(97%)	3(3%)			
	Surgical ward	31	30(96.8%)	1(3.3%)			
	Orthopedics	22	22(100%)	0(0%)			
Catheterization from urine sample	catheterized	7	3(42.9%)	4(57.1%)	25.4	0.000002	47
	Non catheterized	109	106(97.2%)	3(2.8%)			

6.3 Associated Risk Factors

Since *Enterococcus* species have now emerged as nosocomial pathogens,In this study from 15 of the *Entrococcus spp* isolated 14 were form Hospitalized patients and the higher prevalence 13 of the 14 *Entrococcus* spp were from patients which were staid in the Hospital from 3 to 5 days with statistical significance difference ($p<0.05$). As we see in table 6.3 the prevalence of *Entrococcus* spp were increase as the number of repeated admission. In our study Catheterized patients were more susceptible to *Entrococcal* UTI than the noncatheterized patient with statistical significance ($p<0.05$).Table 6.3

Table.6.3.Associeted Risk factors for Entrococcal infection at Tikur Anbesa Specialized Hospital Addis Ababa, Ethiopia, 2016.

Characteristics		**Frequency**	**Negative (%)**	**Positive (%)**	**X^2**	**P.value**
Catherization	Catheterized	7	3(42.9%)	4(57.1%)	25.4	0.000002
	Non Cathterized	109	106(97.2%)	3(2.8%)		
Duration of Hospital stay	<72 hours	222	222(100%)	0		
	3-7 days	84	71(84.5%)	13(15.5%)	14.4	0.0001
	>7 days	13	12(92.3%)	1(7.7%)		
Repeated Admission	2 times	10	4(40%)	6(60%)	2.0	0.2
	3 times	1	0	1(100%)		

6.4 Antibiotic susceptibility patterns

The most effective antibiotic for *Entrococcus* spp was Linzolide with 100 % sensitivity (15/15), followed by Vancomaycine 13/15 (86.7%), while high resistance rate was recorded for Ampicilline with resistance patterns of 80 % (12/15isolates) followed by Doxicycline 73.3% (11/15).(Table 6.3)

Table.6.4. Antibiotics resistance patterns of *Entrococcus* spp at Tikur Anbesa specialized hospital, Addis Ababa, Ethiopia, 2016.

Drugs	Resistance(%)	Sensitive(%)	Intermidate(%)
Ampicilline	12(80%)	3(20%)	0
Chloroamphinicol	8 (53.3%)	7(46.7%)	0
Gentamaycin	9(60%)	6(40%)	0
Ciprofloxacilin	8 (53.3%)	7(46.7%)	0
Doxicyclin	11(73.3%)	4(26.7%)	0
Linzolide	0	15(100%)	0
Vancomaycine	1(6.7%)	14(93.3%)	0

Over all only one *Entrococcus* spp was sensitive to all drug ,while the other species were resistance at least for one drug.(Table 6.5)

Table.6.5. Multi drug resistance pattern of *Entrococcus* spp at Tikur Anbesa specialized hospital, Addis Ababa, Ethiopia,2016

Resistance to	R0	R1	R2	R3	R4	R5	R6
Number of *entrococcus* spp	1	1	3	3	3	3	1

Key: R0-Sensive to all,R1- Resistance to one, R2-Resistance to two, R3-Resistance to three, R4-Resistance to four, R5-resistance to five and R6 resistance to six antibiotics.

6.4 Discussion

Nosocomial infections with *Enterococci* are a major concern at many hospitals throughout the world. The epidemiology of *Enterococci* is not fully understood since there are striking differences among different species of resistant isolates obtained from various geographic locations (13).

In our study, the prevalence of *Enterococci* among different clinical samples were 3.5% this prevalence rate correlates with the findings of other authors in Egypt who found that 3.3%, in Bangladish 3.2% ,a study in india indicates 2.3% and 3.6% in Asia pasfic (30,32,35,38).In contrast Ashour et al **(**42**)** reported that the prevalence rate of *Enterococci* is high among AlHussain University hospital and Manshiat El-Bakry hospital in Cairo represents 27.4% of nosocomial infections. In another study in the SENTRY program has shown occurrence rates for 4998 *Enterococci* strains by site of infection (blood, respiratory tract, wound, and urinary tract) indicates with the prevalence of 18.0% in USA and 21.2%in Canada(30).

These differences in our results and the high prevalence of the others may be due to the use of conventional methods for identification of *Enterococci*. This opinion is in agreement with Jui et al.(43) who mentioned that the conventional methods for the identification of microorganisms are based on phenotypic and culture characteristics and may not be able to identify the causative organism correctly when strains with unusual phenotypes, such as non-motile *E. gallinarum* or *E. casseliflavus*, are encountered.

In the present study, *Enterococcus* species were found to be more prevalent in elderly patients>55 year with(p=0.00002) which agrees with study in Egypt 85% were elder patients and also a study in Bangladish support that higher proportions of *Enterococcus* positive patients (68.75%) were > 40 years of age(35,38) . The reason of higher infection rate among elderly people might be due to the fact as age advances people are more exposed to external environment and many of them might have history of taking treatment from different health care

facilities, which might serve as a source of transmitting this infection. Moreover, immunity decreases with the advance of age which may help in colonization of these bacteria.

In the present study, female (53.33%) are more infected than male (46.7%) which is similar with the report of study in Bangladish which shows females were (68.7%) infected (35) .The reason may be anatomically females are more prone to develop UTI than males.

In the present study the majority of *Entrococci* were isolated from urine (46.7%) as *Enterococcus* species were reported to be the most common gram positive bacteria causingn22% to 24% of all UTIs in hospitalized adults as CDC reports (12). Our study is in accordance with Rania M.K etal 65% *Entrococcus* were isolated from urine(38).A study in Nigeria also agree with our study which states that the dominant sample for *Entrococcus* isolation were urine sample (37).

In the present study catheterized patients were more susceptible for *Entrococcal* UTI than the uncatheterized patients with ($p<0.05$) as instrumentation has been found to be the major cause of *Entrococcal* urinary tract infection .The result agrees with a study conducted in India to compare the foley catheter colonization and urinary tract infection due to *Entrococci* in catheterized patients, (84%) of *Entrococcus* isolated from catheterized urine sample (33) .

Resistance to several commonly used antimicrobial agents is a remarkable characteristic of most *Enterococcal* species. Moreover, most of the information about this is based on studies with *E. faecalis* and *E. faecium,* the two species that are more frequently associated with human infections(3). In the present study, *Enterococcus* isolates showed high level of resistance to Ampicillin (80%) and Doxycycline (73.3%) which agrees with the previous studies in Gondar, Ethiopia, Ampicillin (79.60%) resistance isolated from stool sample (40).

In this study 73.3% resistance of Doxycycline was comparable with study in Nigeria 66.7% (37), these little increments in current study may be due to emergence of drug resistant bacteria. 53.3% resistance of ciprofloxacin were comparable with many studies 50% resistance in Jimma,

51% resistance in Nigeria, 50% resistance in India, but lower percent of ciprofloxacin resistant recorded in Gondar 33.8% and 40% in Kuwait (32,36,37,39,40).

When we see high level gentamycin resistance in current study 60% of *Entrococcus* spp were resistance to gentamycin ,higher percent of gentamycin resistance recorded in Gondar 71.6% and lower percent gentamycin resistance were seen in study from Jimma 35.1%, India 47% and Kuwait 13.9% (32,36,39,40).

In current study high level chloraphnicol resistance *entrococcus* spp were also seen 53.3% which was higher than a study in Gondar 12.4%, in Jimma 34.2%, in Nigeria 33.3% and in Kuwait 40%.The difference may be due to the repeated use of CAF after the last different study .

The emergence of VRE is a cause for concern because of the limited therapeutic options for treating serious infections and because of their potential to transfer vancomycin-resistance genes to other organisms, such as methicillin-resistant *Staphylococcus aureus*. Conjugative in vitro transfers of resistance genes to *S. aureus, Listeria monocytogenes,* and group A and viridans streptococci have been reported (12,30).

In our study three vancomycin resistance *Entrococcus* spp were isolated by discdifussion methods . Those were further tested by E.test for conformation of their minimum inhibitory concentration and only one of the three was >32ug/ml and the other two were in the sensitive range that was 1-4ug/ml . This difference agrees with Sergaa S,etal study which says the Kirby Bauer disc diffusion method is not an accurate method for detecting the Vancomycin Resistant *Enterococci* (32). so only one of the three *Entrococcus* were VRE (vancomycin resistance *Entrococcus)* spp (6.7%) ,our finding were in line with A study done in jimma(5%), Gondar (5.5%) ,India(8.3%) and (4.2%) in Europe (28,30,39,40). The incidence of VRE infections is high in the United States (17%) in comparison with that in current study, probably because the rate of use of vancomycin in United States hospitals has increased dramatically in recent years(30).

But the incidence of VRE infection is high in current study in comparison with study conducted in Egypt (0%), Nigeria (0%) and LatineAmerica (0%) VRE (30,37,38).This increment of VRE in current study may be due to the increment of multi drug resistance bacteria with time as the SENTRY program in US indicated the VRE among US *Enterococcal* isolates ranged from 14% in 1997 to 17% in 1999 (30).

In our study the VRE was found in old Hospitalized female patients 65 years old these may be due to the use multiple antibiotic and the low immune resistance due to her age(8).

In the present study, all *Enterococcus* spp isolates were sensitive to Linzolide. This result agrees with India and Bangladesh study(34,35) who reported that none of the *Enterococci* isolates were resistant to Linzolide. But in contrast to our study 2% lizolide resistance *Entrococcus* were found in Tehran (44).

In general only one *Entrococcus* spp were sensitive to all drugs used in our study but the other isolates were resistance to at least one drug . 66.7% of the isolated *Entrococci* were multi drug resistance which were resistance to three and above drugs. Thise results were similar to study in Kuwait ,Egypt and Nigeria 55%,61% and 63% respectively .Our result was lower when we compare to a study in Gondar which were 90%, this difference may be due to the patient type they use HIV positive patients in stool sample.

6.5 Limitation of the study

The isolated *Enterococccci* were not indentified to species level.

6.6.1 Conclusion

This study showed that the rate of isolated *Enterococci* was 3.5% and had variable degrees of resistance to the antibiotics, but all were sensitive to Linzolide by disc diffusion methods so the presence of VRE and multidrug resistant *Enterococci* in our study should be considered as danger alarm for serious *Enterococcal* infections and further study in large scale is needed.

6.6.2 Recommendations

- Antimicrobial susceptibility test should be encouraged on routine basis so as to figure out the best antibiotic for treatment of *Entrococcus* infections.
- Since *Entrococcal* nosocomial infection is vary in different geographical regions and even over time in the same location and population, routine surveillance of *Entroccus* prevalence is important.
- Large scale studies are needed by considering the presence of VRE and multi drug resistance *Entrococcus* to explore the type and burden of *Entrococcus* in the Hospitalized patients in Addis Ababa.

7. Reference

1. Sood S, Malhotra M, Das B.K , Kapil A. *Enterococcal* infections & antimicrobial resistance. *Indian J Med Res 2008 :128 : 111-121*

2. Mundy LM, Sahm DF, Gilmore MS: Relationship between Enterococcal virulence and antimicrobial resistance. *Clin Microbiol Rev* 2000; 13:513–522.

3. Wright GD. Mechanisms of resistance to antibiotics. *Curr. Opin. Chem. Biol* 2003; **7**: 563-569.

4.Edwards D.D. *Enterococci* attract attention of concerned microbiologists. *ASM News* 2000; *66* : 540-550.

5 Bhat K.G, Paul C, Ananthakrishna N.C. Drug resistant *Enterococci* in a south Indian hospital. *Trop Doct* 1998; *28*:106-7.

6. Facklam R.R, Teixeira L.M, Lollier L, Balows A, Sussman M, editors. *Topley & Wilson's microbiology and microbial infections*. 9th ed. New York: Oxford University Press; 2004; 669-682.

7. Silverman J, Thal L.A, Perri M.B, Bostic G, Zervos M.J . Epidemiologic evaluation of antimicrobial resistance in community-acquired *Enterococci*. *J Clin Microbiol 1999:36:830–882.*

8. Murray B.E. Diversity among multidrug-resistant *Enterococci*. *Emerg Infect Dis* 1998; *4*: 37-47.

9. Mohanty S, Jose S, Singhal R, Sood S, Dhawan B, Das BK, *et al.* Species prevalence and antimicrobial susceptibility of *Enterococci* isolated in a tertiary care hospital of north India. *Southeast Asian J Trop Med Public Health* 2005; *36* : 962-965.

10. Baldassarri, L, Creti R, Montanaro L, Orefici G, Arciola C**.** Pathogenesis of implant infections by *Enterococci*. *Int. J. Artif. Organs 2005;* 28**:**1101–1109.

11. Bearman, G. M. L., Wenzel R.P**.** Bacteraemias: a leading cause of death. *Arch. Med. Res* 2005; 36**:**646–659

12. Centers for Disease Control and Prevention**.** Nosocomial *Enterococci* resistant to vancomycin—United States, 1989–1993. *MMWR Morb. Mortal. Wkly. Rep.1993;* 42:597–599.

13. National Nosocomial Infections Surveillance System. National Nosocomial Infections Surveillance (NNIS) system report, data summary from January 1992–April 2000, issued June 2000. *Am. J. Infect. Control* 2000; 28:429–448.

14. Dargere S, Vergnaud M, Verdon R, Saloux E . *Enterococcus gallinarum* endocarditis occurring on native heart valves. *J Clin Microbiol 2002: 40:2308–2310.*

15. Werner G, . Coque T.M, Hammerum A.M, Hope R, Hryniewicz W, Johnson A. I. etal. Emergence and spread of vancomycin resistance among *Enterococci* in Europe. *Euro Surveill*2008; 13:1–11.

16. . Zehnder M., Guggenheim B. 2009. The mysterious appearance of *Enterococci* in filled root canals. *Int. Endod. J.* 42:277–287.

17. . Werner G, . Coque T.M, Hammerum A.M, Hope R, Hryniewicz W, Johnson A. I. etal. Emergence and spread of vancomycin resistance among *Enterococci* in Europe. *Euro Surveill*2008; 13:1–11.

18. . Narayanaswamy A, Rajalakshmi K, Varadharajan M . Speciation and antimicrobial susceptibility pattern of *Enterococci* from a tertiary health care center of south India. *J Pharm Res 2011: 4:989–990*

19. Shah L, Mulla S, Patel KG, Rewadiwala S . Prevalence of *Enterococci* with higher resistance level in a tertiary care hospital: a matter of concern. *Natl J Med Res 2012: 2:25–27.*

20. . Facklam RR, Collins MD. Identification of *Enterococcus* species isolated from human infections by a conventional test scheme. *J Clin Microbiol 1989: 27:731–734.*

21. Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, Pollock DA. NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. *Infect Control Hosp Epidemio 2008: 29:996–1011.*

22. Adhikari L. High-level aminoglycoside resistance and reduced susceptibility to vancomycin in nosocomial enterococci . *J Global InfectDis 2010: 2:231–235*

.23. Arias C.A, Murray B.E. Emergence and management of drug-resistant *Enterococcal* infections. *Expert Rev Anti Infect 2008: 6:637–655.*

24. Treitman A.N, Yarnold P.R, Warren J, Noskin G.A. Emerging incidence of *Enterococcus faecium* among hospital isolates from 1993 to 2002. *J Clin Microbiol 2005 43:462–463*

25. **.** Ricardo T.-de-A, Maurício R. F ,Celeste A. N. S, Isabela P. R, João E. F, Rejane S. etal. Molecular Epidemiology and Antimicrobial Susceptibility of *Enterococci* Recovered from Brazilian Intensive Care Units. *The Brazilian Journal of Infectious Diseases* 2004;8(3):197-205.

26. Wei J, Gang L, Wen W. Prevalence and Antimicrobial Resistance of *Enterococcus* Species. *Int. J. Environ. Res. Public Health* ***2014****, 11, 3424-3442*

27. Adeleh H. Z , Mana S, Raziyeh N, Majid A, Masumeh S, Hamid A. Genotyping of Vancomycin Resistant *Enterococci* in Arak Hospitals. *JundishapurJMicrobiol.2015;8(4):162-187*

28. Nita G , Manisha M , Sunita P. Prevalence of Multidrug Resistant Enterococci in a Tertiary Care Hospital in India. http://www.scirp.org/journal/ojmm http://dx.doi.org/10.4236/ojmm.2014.41002

29. Manero A, Blanch A.R . Identification of *Enterococcus* species with a biochemical key. *Appl Environ Microbiol 1999: 65:4425–4430*

3o. . Low D.E, Keller N, Barth A, Jones R.N. Clinical prevalence, antimicrobial susceptibility, and geographic resistance patterns of *Enterococci*: results from the SENTRY Antimicrobial Surveillance Program, 1997-1999. *Clin Infect Dis* 2001; *32* : 133-145.

31. Mihajlović U. M, Medić D , Jelesić Z, Gusman V , Milosavljević B, Radosavljević B. Prevalence of different *Enterococcal* species isolated from blood and their susceptibility to antimicrobial drugs. *Afr. J. Microbiol. Res 2014:8(8):819-824.*

32. Sreeja s, Sreenivasa B. P.R., Prathab A.G.The Prevalence and the Characterization of the *Enterococcus* Species from Various Clinical Samples in a Tertiary Care Hospital. *J of Clinical and Diagnostic Research 2012 :6(9): 1486-1488.*

33. Desai P.J, Pandit D, Mathur M, Gogate A. Prevalence identification and distribution of various species of *Entrococci* isolated from clinical specimen with special reference to urinary tract infection in catheterized patients. *India J Med Microbiology 2001:19(3):132-137.*

34. Uma C, Madhu S, Aparana Y **.** Antimicrobial Susceptibility Patterns of Common and Unusual *Enterococcus* species Isolated from Clinical Specimens. *J Infect Dis Antimicrob Agents 2007;24:55-62.*

35. .Tashmin A.B.I, Shamsuzzaman S.M. Isolation and species identification of *Enterococci* from clinical specimen with their antimicrobial susceptibility pattern in a tertiary care hospital Bangladesh. *Coastal life J med* 2015; 3(10): 787-790.

36. Edet E.U, Noura A , Oludotun A. P, Tulsi D. C. Species prevalence and antibacterial resistance of *Enterococci* isolated in Kuwait hospitals. *Journal of Medical Microbiology 2003: 52, 163–168.*

*37.*Ayeni F.A, Bello A.E, Olley M. Prevalence and antibiotic susptability pattern of *Entrococcus* species isolated from a Nigarian Hospital.

African Journal of Pharmaceutical Research & Development 2015:7(1):24-31.

38. Rania M.K, Said H. A, Gehan S.E.H, Nahed I. G. Species Identification and Pattern of Antibiotic Resistance of *Enterococci* Isolated from Cases of Nosocomial Infections. *Egyptian Journal of Medical Microbiology 2007:10(2):235-240.*

39. Abdulhakim A, Beyene W, Alemseged A. Antimicrobial resistance profile of *Enterococcus* species isolated from intestinal tracts of hospitalized patients in Jimma, Ethiopia. *BMC Res Notes 2015: 8:213*

40. Wondwoson A, Mengistu E, Moges T, Feleke M. Prevalence of vancomycin resistant *Enterococci* and associated risk factors among clients with and without HIV. [cited 2014]; Available from: http://www.biomedcentral.com/1471-2458/14/185.

41. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk Susceptibility Tests; *Twenty-fourth Informational Supplement. CLIS 2014; 32(3): M100 S24*

42. Ashour MS, Helal S, Salem M . Prevalence of antibiotic resistant *Enterococci* in some Egyptian hospitals. The 12th international conference of the Egyptian society for medical microbiology (ESMM), Cairo University 2004: 12: 34-80

43. Jui CT, Hsueh RP, Lin HM, Chang HJ, Shen WH . Identification of Clinically Relevant *Enterococcus* Species by Direct Sequencing of groES and Spacer Region. *J. Clini. Microbiol.*2005: 43(1):235-41

44. Yasline S, Mohabti mobarez R**,** Hosseini doust M, Satari O ,Teymornejad S.

Linezolid Vancomycin resistant *Enterococcus* isolated from clinical samples in Tehran Hospitals. *Indian J Med Sci*.2009:63(7):297-302

8. Annexes

8.1 Annex I- English version of participant information sheet, consent form and data collection sheet

8.1.1 participant information sheet

Name of the organization: Department of Medical Laboratory Science, School of Allied Health Sciences, Collage of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia.
Title of the Research Project: . prevalence and antimicrobial susceptibility of *Entrococcus* species isolated from different clinical samples in Black Lion Hospital of Addis Ababa Ethiopia .
Name of Investigator: Zelalem Tena

First of all I would like to thank you in advance for your cooperation and consent in participation in this study. Please take as much time as you need to read or listen the information sheet. If you have any question regarding the study please ask freely.

Background information

Entrococci form part of the normal flora of the intestine, the oral cavity and the vagina, but in recent time they have become emerging nosocomial pathogens. Their increasing importance is largely due to their resistance to antimicrobials. Treatment of *Entrococci* is often started empirically and a large proportion of unrestrained antibiotic usage results in upsurge of resistance among the *Entrococcus* species in both community and health care settings. Therefore knowledge the periodic local distribution of the *Entrococcus* species and their drug susceptibility test is important to provide the best empirical therapy, the rational selection of antimicrobial agents and to the development of appropriate prescribing polices.

Purpose of the Research Project

We are asking you to take part in this study because we are trying to learn more about *Entrococcus* species prevalence and antimicrobial susceptibility patterns with associated risk factors in different clinical sample at Black Lion Specialized Hospital.

Potential benefits to subjects and/or to the society

You will not have any financial incentives or other inducements as the compensation from participating in the study. However, results will be given to their physician for treatment or to get counseling. Most importantly, the result of the study will be beneficial to provide information or data for future and further nationwide study and to develop health programs for health policy makers. Hence, you are indirectly benefiting other patients and the society in this respect.

Risks and complications

There is no risk to the participants in participating in the study other than benefiting from the research.

Confidentiality

In order to keep the confidentiality of the participants the sample will be labeled with code instead of giving name. No personal information will be disclosed to third party or will not appear in any report from this study. You can choose whether to be a part of this study or not and you have a right to get a laboratory diagnosis result for free.

Assurance of Principal Investigator

I put my signature below to confirm you that I take over the responsibility for the scientific ethical and technical conduct of the research project and for provision of progress reports for all stakeholders of the research project. If you have any question you can contact and ask at any time you want.

Zelalem Tena: Department of Medical Laboratory Sciences, Collage of health Sciences, Addis Ababa University, Addis Ababa, Ethiopia

Cell phone: +251-9 12960680 E-mail: zelalemtena@gmail.com

8.1.2 Informed consent

Card No---------- I have been informed about the study which plans to determine the pravalence and antimicrobial susceptibility pattern of *Entrococcus* species in different clinical sample at Tikur Anbesa specialized Hospital, Addis Ababa, Ethiopia. The objective and the application of the study were briefly explained to me which is importance to treat me and patients. Moreover, I have been well informed of my right to refuse information, decline to cooperate and drop out of the study if I want and none of my actions will have any bearing at all on my overall health care. The principal investigator requested me to participate in the study that would require my willingness to respond to an interview . I agreed that the specimen would be tested for *Entrococcus* species . I have had the opportunity to ask questions about the project and received clarification to my satisfaction in a language I understand. Therefore, with 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 giving of the requested information and specimen for this study.

8.1.3 Data Collection Sheet

Date: _____
Code No.: _____
Address: Sub-city_____Woreda_____Kebele_____Tele:_____

Section I: Socio Demographic Characteristics

1. Sex: M ☐ F ☐
2. Age: _____ Years
3. Permanent residence place: Rural ☐ Urban ☐
4. Current visiting status: In patient ☐ Out patient ☐
For in patient, Ward: Medical ☐ Surgical ☐ Gyn& OB ☐ Others:….
5. Marital status: Married ☐ Unmarried ☐ Widowed Divorced ☐
6. The highest level of education you have achieved: Illiterate ☐ Writing & reading ☐
Primary school ☐ Secondary school ☐ College/TVET diploma ☐

University 1st degree ☐ 2nd degree ☐ 3rd degree ☐

7. Occupation: _____

Section II. Risk factors associated with entrococci infection

1. Have you taken any antibiotic in the past 14 days? Yes ☐ No ☐

History of previous antibiotic treatment: _____

2. Duration of admission to the hospital?

for how long? <72 hrs ☐ 7 to 15 days ☐ 1 Month ☐ > 1 Month ☐

which ward have you admitte? Medical ward ☐ Surgical ward ☐

Pediatric ward ☐ Gyn & ObS ☐ Others: _____

3. History of previous repeated hospital admission? Yes ☐ No ☐

If yes how many times? 2 times ☐ 3 times ☐ 4 time ☐ more ☐

4. History of previous catheterization? Yes ☐ No ☐

If yes, for how long? 3 days ☐ week ☐ week ☐ lore than two weeks ☐

5. Have you ever had urinary tract surgical procedure? Yes ☐ No ☐

6.Type of wound infection

Surgical wound ☐ Burn ☐ Others----------

7.Do you have previous multiple invasive surgical intervention? Yes ☐ No ☐

If yes, how many times? 2 times ☐ 3 times ☐ more ☐

Section III: Clinical data

Fever _____ Frequency_____

Dysuria _____ Flank pain_____

Urgency _____ Suprapubic pain_____

Skin lesion _____ New heart murmur _____

Section IV: Laboratory results

Date of sample collection: _____

Cultures and Identification:

Isolation of *Entrococcus* species : Yes ☐ No ☐

Type of *Entrococcus* _____

Antimicrobial susceptibility testing:

Doxicycine/DOX: _____ Linzolide/LIN: _____
Gentamycin/GM: _____ Ampicillin/AMP _____
Chloramphinicol/C: _____ Sulfa/Trime/STX: _____
Vancomycin/Va: _____ Ciprofloxacin/CIP: _____

TELLUR : _____

8.2 Annex II Amharic version of participant information sheet, consent form and data collection sheet (**የተሳታፊዎች መረጃ ቅጽ ፤ የፈቃደኝነት ማረጋጋጫና መረጃ መሰብሰቢያ ቅጽ**)

8.2.1 **የተሳታፊዎች መረጃ ቅጽ**

አዲስ አበባ ዩንቨርስቲ ጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ሳይንስ ዲፓርትመንት

የጥናቱ ርዕስ፡-በአዲስ አበባ ከተማ በጥቁር አንበሳ ሆስፒታል ከሚታከሙ ህሙማን የኢንትሮኮከስ ባክቴሪያ ስርጭትና ለመድሀኒት ያለዉ ግትርነት ከተለያዩ ናሙናወች አንፃር ማሳየት፡፡

አጠቃላይ መረጃ፡-

በጥናቱ በመሳተፈዎ ከልብ እያመሰገን ከመወሰንዎ በፊት ይህንን ቅፅ በትክክል ያንብቡ ወይም ሲነበብልዎ በትክክል ያድምጡ እንዲሁም ግልፅ ያልሆነለዎትን ነገር በሙሉ ነፃነት ይጠይቁ፡፡

መግቢያ

ኢንትሮኮከስ የተባልው ባክቴሪያ በተፈጥሮ ያለምንም ጉዳት በአንጀት፣በአፍ አካባቢ፣በሴቶች ሀፍረተ ስጋ እና አንዳንዴ በሰውነት ቆዳ ላይ ይገኛል፡፡ነገር ግን አሁን አሁን ባክቴሪያው ሆስፒታል በሚቆዩ በሺተኞች ላይ የተለያየ ህመም ያመጣል ከነዚህ ህመሞች መካከል የሺንት ቧንቧ ህመም ፣ ቁስል ላይ ተጨማሪ ህመም እና የደም ውስጥ ባክቴሪያ ህመም ዋናዋናዎቹ ናቸው፡፡በዚህም ምክንያት ሆስፒታል ውስጥ ለሚቆዩ ህሙማን የባክቴሪያውን ስርጭትና ለመድሃኒት ያለውን ግትርነት ማወቅ ባክቴሪያው የሚያስከትለውን ጉዳት ለመቀነስ ለህብረተሰቡ በጣም አስፈላጊ ነው፡፡

የጥናቱ ዓላማ

በአዲስ አበባ ከተማ በጥቁር አንበሳ ሆስፒታል ውስጥ ከሚታከሙ ህሙማን የኢንትሮኮከስ ባክቴሪያ ስርጭት ፣ባክቴሪያው ለመድሃኒት ያልውን ግትርነትና የባክቴሪያውን መንስኤወችን ማሳየት፡፡

ለጥናቱ ተሳታፊዎች ያለው ልዩ ጥቅም

በጥናቱ ለሚሳተፉ ፈቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለውም፡፡ ነገር ግን በጥናቱ መሰረት ባክቴሪያው ለተገኘባቸዉ ተሳታፊዎች ከሚመለከታቸው የሆስፒታሉ ባለሙያዎች ጋር በመነጋገር አስፈላጊ የሆነ ህክምና እንዲደርግላቸዉ ይደረጋል፡፡

በጥናቱ ተሳታፊዎች ላይ ያለው ጉዳትና ተዛማጅ ችግር

በዚህ ጥናት በመሳተፈዎ ሊደርስብዎ የሚችል አንድም ጉዳት አይኖርም ፡፡ ለጥናቱ የሚያስፈልገዎን ናሙና ከመስጠት በስተቀር፡፡

የመረጃ ሚስጥራዊ አጠባበቅ

የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዛም በኋላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘው በስም ሳይሆን በመለያ ቁጥር ይሆናል፡፡ በጥናቱ ላይ እያሉ በፈለጉት ጊዜ የማቆም ወይም የማቋረጥ መብት አለዎት፡፡ ይህ መረጃ በጥንቃቄ የሚያዝና መረጃውን በፈለጉ ጊዜ ሊያገኙ የሚችሉ ይሆናል፡፡ በመጨረሻም የጥናቱ ውጤት ለሚመለከተው አካል ለጥናቱ አላማ ብቻ የሚገለጽ ይሆናል ፡፡

ያስታውሱ፤ ስለዚህ ጥናት ማንኛውንም ጥያቄ ካለዎት በማንኛውም ጊዜ ከዚህ በታች በተጠቀሱት አድራሻዎች መጠየቅ ይችላሉ፡፡

የዋናው ተመራማሪ አድራሻ

-ዘላለም ጤና፡- የህክምና ላቦራቶሪ ቴክኖሎጂ ዲፓርትመንት ፣ የጤና ሳይንስ ኮሌጅ፣ አዲስ አበባ ዩንቨርስቲ ፣ አዲስ አበባ ፣ ኢትዮጵያ ::
-ኢ-ሜይል፡-zelalemtena@gmail.com
-ስልክ፡- +251912960680

8.2.2 የፈቃደኝነት ማረጋገጫ ቅፅ

በአዲስ አበባ ከተማ በጥቁር አንበሳ ሆስፒታል ከሚታከሙ ህሙማን የኢንትሮኮከስ ባክቴሪያ ስርጭትና ለመድሃኒት ያነዉ ግትርነት ከተለያዩ ናሙናዎች አንፃር ሲታይ እና መንስኤዎቹን ማሳየት በሚል ረዕስ ላይ ለማጥናት በተመለከተ በሚደረገው ጥናት ላይ ለመሳተፍ መሆኑ የጥናቱ አላማ እና ጥቅም ተገልፆልኛል፡፡
በተጨማሪም ጥናቱ ውስጥ አለመሳተፍ መብቴ እንደሆነና በማንኛውም ጊዜ ከጥናቱ በራሴ ውሳኔ መውጣት እንደምችልና በዚህም ምክንያት ምንም አይነት መጉላላት እንደማይደርስብኝ በሚገባ ተረድቻለሁ፡፡ ስለሆነም ሁኔታውን በሚገባ በማጤን በፈቃደኝነት በምርምሩ ላይ ለመሳተፍ ለተመራማሪው ፈቃደኝነቴን ሰጥቻለሁ፡፡ በተጨማሪም የምሰጠው ናሙና ለኢንትሮኮከስ ባክቴሪያ ምርመራ ብቻ እንደሚውል ተነግሮኝ ተስማምቻለሁ፡፡ ማንኛውንም ያልገባኝን ነገር የመጠየቅ ዕድል ተሰጥቶኝ በሚገባኝ ቋንቋ መልስ አግኝቻለሁ፡፡ በተጨማሪም የሁሉም የላብራቶሪ የምርመራ ውጤቶች ለተቋሞች እንደሚሰጥና ውጤቱን ማወቅ ከፈለኩ ማግኘት እንደምችል ተነግሮኛል፡፡ እኔ ---------- የተባልኩ ግለሰብ ይህን ሁሉ በማገናዘብ ምርምሩ ላይ ስለኔ መረጃ እና ለምርምሩ ናሙና ለመስጠት ተስማምቻለሁ፡፡

ፊርማ---------- ቀን ----------

መረጃውን ያስረዳው አካል ፊርማ ---------- ቀን ----------

8.2.3 መረጃ መሰብሰቢያ ቅጽ

በአዲስ አበባ ዩንቨርስቲ የጤና ሳይንስ ኮሌጅ የሕክምና ላብራቴሪ ዲፓርትመንት
ይህ የመረጃ መሰብሰቢያ ቅጽ ስለተሳታፊዎች አጠቃላይ ለኢንትሮኮከስ ባክቴሪያ ሊያጋልጣቸው የሚችሉትን ምክንያቶች ለማወቅ የሚረዳ መረጃ መሰብሰቢያ ቅጽ ነው፡፡ለጥናቱ መሳካት እና ትክክለኛነት ያግዘን ዘንድ ጥያቄዎችን በጥንቃቄ አንብበው ወይም ተረድተው በታማኝነት ያለምንም ፍራቻ እንዲሞሉልን በትህትና እንጠይቃለን፡፡

የተቋሙ ስም ---------- ዓ/ም----------

የተሳታፊው መለያ ቁጥር ----------

አድራሻ፡ ክልል ---------- ክፍለ ከተማ---------- ዞን ----------

ወረዳ ---------- ቀበሌ----------

የመረጃው ሰብሳቢ ስም ---------- ቀን ---------- ፊርማ ----------

ሀ. ስለበሽተኛው አጠቃላይ መረጃ

ቀን ----------

የሚስጥር ቁጥር መለያ----------

1. ጾታ፡ ወንድ ☐ ሴት ☐

2. እድሜ፡ ----------

3. ቋሚ የመኖሪያ ቦታ፡ ከተማ ☐ ገጠር ☐

4. የህክምናው ክፍል፡ ተኝቶ ታካሚ ☐ ተመላላሽ ታካሚ ☐

ለተኝቶ ታካሚ የተኙበት ክፍል ፡ የውስጥ ደዌ ክፍል ☐ የማዋለጃ ክፍል ☐ የቀዶ ጥገና ክፍል ☐ ሌላ------

5. የጋብቻ ሁኔታ፡ አግብቻለሁ ☐ አላገባሁም ☐ በሞት ተለያይተናል ☐ በፍቺ ተለያይተናል ☐

6. አሁን ያሉበት ከፍተኛ የትምህርት ደረጃ፡

መፃፍና ማንበብ የማይችል ☐ መፃፍና ማንበብ የሚችል ☐ የመጀመሪያ ደረጃ ☐ ሁለተኛ ደረጃ ☐ የኮሌጅ ወይም የሙያ ዲፕሎማ ☐ የመጀመሪ ዲግሪ ☐ ሁለተኛ ዲግሪ ☐ ሶስተኛ ዲግሪ ☐

7. የስራ ሁኔታ፡ ----------

ለ. ለኢንትሮኮከስ ባክቴሪያ ህመም ኢጋላጭ መንስኤዎችን ለማወቅ

ነ. ከአስራ አራት(14) ቀን ወዲህ የወሰዱት መድሃኒት አለ? አዎ ☐ የለም ☐

አዎ ካሉ ምን ዓይነት መድሃኒት? ----------

2. ሆስፒታል ተኝተው የቆዩበት ጊዜ

ከሰባሁለት ሰአት ያነሰ ☐ ከ7-15 ቀን ያህል ☐ ለአንድ ወር ☐ ከአንድ ወር በላይ ☐

በየትኛው አስተኝቶ ማከም ክፍል? በውስጥ ደዌ ☐ በቀዶ ጥገና ☐ በህፃናት ክፍል ☐ በማዋለጃ ክፍል ☐

ሌላ ክፍል ----------

3.ከዚህ በፊት በተደጋጋሚ ሆስፒታል ተኝተዉ ያውቃሉ? አዎ ☐ የለም ☐

አዎ ከሆነ መልስዎ ለምን ያህል ጊዜ? 2 ጊዜ ☐ 3 ጊዜ ☐ 4 ጊዜ ☐ ከዚያ በላይ ☐

4. ካቴተር ተደረጎሎዎት ያውቃል? አዎ ☐ የለም ☐

አዎ ካሉ ለመን ያህል ጊዜ? ለሦስት ቀን ☐ ለአንድ ሳምንት ☐ ለሁለት ሳምንት ☐ ከሁለት ሳምንት በላይ ☐

5. የሽንት ቧንቧ ቀዶ ህክምና ተደርጎሎት ያውቃል? አዎ ☐ የለም ☐

6.የቁስል ህመምዎ መነሻ ምንድን ነው

የቀዶ ጥገና ☐ ቃጠሎ ☐ ሌላ----------

7.ከዚህ በፊት የቀዶ ጥገና ህክምና ተደርጎለዎት ያዉቃል? አዎ ☐ የለም ☐

አዎ ከሆነ መልስዎ ስንት ጊዜ? 2 ጊዜ ☐ 3 ጊዜ ☐ ከዚህ በላይ ☐

8.3 Annex III - Laboratory procedures (CLIS guidelines)

1 .Sample collection

All clinical samples will be collected by trained personals with sterile container according to the standard operating procedures (SOP) except urine sample it need special attention as follows:

The first urine passed by the patient at the beginning of the day should be sent for examination. This specimen is the most concentrated and therefore the most suitable for culture, microscopy, and biochemical analysis. Midstream urine (MSU) for microbiological examination is collected as follows:

1. Give the patient a sterile, dry, wide-necked, leak proof container and request a 10–20 ml specimen.

- **Important:** Explain to the patient the need to collect the urine with as little contamination as possible, i.e. a 'clean-catch' specimen.

A. Female patients: Wash the hands. Cleanse the area around the urethral opening with clean water, dry the area with a sterile gauze pad, and collect the urine with the labia held apart.

B. Male patients: Wash the hands before collecting a specimen (middle of the urine flow).

Note: When a patient is in renal failure, it may not be possible to obtain more than a few milliliters of urine.

2. Label the container with the date, the name and number of the patient, and the time of collection.

3. **As soon as possible**, deliver the specimen with a request form to the laboratory. When immediate delivery to the laboratory is not possible, refrigerate the urine at 4–6 °C. When a delay in delivery of more than 2 hours is anticipated, add boric acid preservative to the urine.

A. Culture and identification for bacteria

1. Suspected growth from blood culture.

Positive/present ☐ Negative/absent ☐

2 . Suspected growth of bacteria from blood agar

Positive/present ☐ Negative/absent ☐

Identification steps for suspected colonies

- colony morphology----------Colonies are larger than those of streptococci, usually 1–2mm, with a wet appearance. Haemolysis is variable.
- Gram stain ----------are Gram positive, round or ovoid cells occurring in pairs, short or long chains or sometimes in clusters.

4.Catales test

Positive/present ☐ Negative/absent ☐

5.PYR

Positive/present ☐ Negative/absent ☐

6. Salt tolerance test

Positive/present ☐ Negative/absent ☐

7 .bile aesculine test

Positive/present ☐ Negative/absent ☐

8. Species identification test (API 20 STREPT)

Item	MAN	SOR	ARG	ARA	SBL	RAF	TEL	MOT	PIG	SUC	PYU	MGP	TRE	XYL	GALL
POS															
NEG															

Abbreviations and symbols: MAN, mannitol; SOR, sorbose; ARG, arginine; ARA, arabinose; SBL, sorbitol; RAF, raffinose; TEL, 0.04% tellurite; MOT, motility;
PIG, pigment; SUC, sucrose; PYU, pyruvate; MGP, methyl-a-d-glucopyranoside; TRE, trehalose; XYL, xylose; GAL, 2-naphthyl-b-d-galactopyranoside.

Table shows phenotypic characteristics used for identification of some of the common *Entrococcus* specious.

Specious	MAN	SOR	ARG	ARA	SBL	RAF	TEL	MOT	PIG	SUC	PYU	MGP	TRX	XYL	GA
E.faecum	+	-	+	+	v	v	-	-	-	+	-	-	+	-	v
E.facalis	+	-	+	-	+	-	+	-	-	+	+	-	+	-	-
E.gallinarem	+	-	+	+	-	+	-	+	-	+	-	+	+	+	+
E.durans	-	-	+	-	-	-	-	-	-	-	-	-	-	-	v
E.dispar	-	-	+	-	-	+	-	-	-	+	+	+	+	-	+
E.hirae	-	-	+	-	-	+	-	-	-	+	-	-	+	-	v
E.mundtii	+	-	+	+	v	+	-	-	+	+	-	-	+	+	v

Symbols:- (+) indicates positive, (-) indicates negative and (v) indicates variable which means sometimes positive and some times negative.

B. BIOCHEMICAL TESTS

Identification of *Entrococcus* species involves the use of biochemical screening Medias, which is Catalase test, Bile aesculin test and PYR test.

1. CATALASE TEST

Principle

Detects the presence of the enzyme catalase which hydrolyzes H_2O_2 to produce H_2O and O_2. This test is used to differentiate Staphylococci (catalase positive) from Streptococci (catalase negative).

Reagents

Hydrogen peroxide (H_2O_2), 3%

Store in a dark bottle and avoid any undue exposure to light.

Keep refrigerated at all times when not in use.

Other Materials

Clean glass microscope slides

Plastic culture loop or wooden applicator stick

Procedure

A. Pick a colony from an 18-24 hr culture and place it on a clean glass slide. Avoid carry over of blood agar which can cause false positives.

B. Put one drop of 3% H_2O_2 over the organism on the slide. Do not reverse the order of the procedure as false positive results may occur. Do not mix.

C. Observe for immediate bubbling (gas liberation) and record the result.

D. Discard the slide into a discard container.

Interpretation

Positive test: Immediate bubbling, easily observed (0_2 formed)

Negative test: No bubbling

Note:-*Entrococcus* species are catalase negative but rarely they are pseudo positive need characterization of the colony.

2.BILE ESCULIN TEST

Principle

This test determines the ability of an organism to grow in the presence of bile and to hydrolyze the glycoside esculin to esculetin and glucose. The test is used to presumptively identify Group D Streptococci.

Materials

Bile esculin agar slant / plate

Culture loop

Procedure

A. Heavily inoculate a bile esculin slant / plate with the suspect organism.

B. Incubate in O_2 at 35^oC for 18-24 hr.

Interpretation

Positive: Presence of a dark brown to black colour on the slant.

Negative: No blackening of the medium. Growth may occur, but this does not indicate esculin splitting.

Note:-*Entrococcus* species are bile aesculin test positive.

3.PYR TEST

Principle

PYR (L-pyrrolidonyl-β-naphthylamide) impregnated disks serve as a substrate for the detection of pyrrolidonyl peptidase. Following the hydrolysis of the substrate by the enzyme the resulting β-naphthylamine produces a red colour upon the addition of cinnamaldehyde reagent. This test is used, in conjunction with others, for the identification of catalase negative, gram positive cocci including *Enterococci* and Group A Streptococci.

Reagents

PYR discs

Cinnamaldehyde reagent (0.01% p-dimethylamino-cinnamaldehyde)

(Disks and reagents are both in PYR kit)

Glass slide

Inoculating loop

Forceps

Sterile distilled water

Procedure

A. Place a PYR disk onto a glass slide and moisten it with one drop of sterile distilled water.

B. Rub a loopful of the culture onto the moistened disk holding it in place with sterile forceps.

C. Leave at room temperature for 2 minutes.

D. After 2 minutes, add 1 drop of cinnamaldehyde reagent.

Interpretation

Positive: Pink or cherry red colour within one minute

Negative: No colour change or slight yellow colour

Note:- *Entrococcus* species are PYR positive.

Antibiotics susceptibility result for Bacteria isolates

	Isolated Bacteria	Antibiotics		AMP	C	GM	LIN	CIP	VAN	DOX	SULF	TEL
		Susceptibility pattern	S									
			I									
			R									

NOTE: Chloramphericol(C), Linzoide(LIN), Vancomaycin(VAN), Gentamicine(GM), Ciprofloxacin (CIP), DOxacillin(DOX), and Ampicilin(AMP), Sulfa(SULF), Tellur(TEL)

- The last two drug which are SULFA and TELLUR used as a diagnostic tool for entroccocus species .*Entrococcus* speciesare SULFA resistance and *E. faecalis* for TELLUR zone <12mm and black colonies close to the disk.

Procedure for Performing the Disc Diffusion Test

Inoculums Preparation

- At least three to five well-isolated pure colonies of the same morphological type will be selected from Blood agar plate. The top of each colony is touched with a loop, and the growth is transferred into a tube containing 4 to 5 ml of tryptone soy broth.
- The turbidity of the broth culture will be adjusted with that of the 0.5 McFarland standards.

Inoculation of Test Plates

- Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab is dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level.

- The dried surface of a Mueller-Hinton agar plate is inoculated by streaking the swab over zthe entire sterile agar surface.
- The lid may be left ajar for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

NOTE: Extremes in inoculum density must be avoided. Never use undiluted overnight broth cultures or other unstandardized inocula for streaking plates.

Application of Discs to Inoculated Agar Plates

- The predetermined battery of antimicrobial discs is dispensed onto the surface of the inoculated agar plate. Each disc must be pressed down to ensure complete contact with the agar surface.

- The plates are inverted and placed in an incubator set to 37°C within 15 minutes after the discs are applied.

Reading Plates and Interpreting Results

- After 16 to 18 hours of incubation, each plate is examined. The diameters of the zones of complete inhibition (as judged by the unaided eye) are measure, including the diameter of the disc. Zones are measure to the nearest whole millimeter, using sliding calipers which is held on the back of the inverted plate.

- The MIC of vancomycin will determine by the E test for all the *Enterococci* isolates which showes intermediate sensitivity by the Kirby Bauer disc diffusion method. A lawn

culture of *Enterococci*, 0.5 Macfarland's standard wiil made on 5% Mueller Hinton blood agar. The E –strip will apply with an MIC scale, facing up, by using sterile forceps, with the higher concentration facing the edge of the plate. The plates will examines after 24 hours of incubation at 37^0C. The zone of inhibition will be observed in the form of an ellipse. The MIC value is the value at which the zone convenes the comb like projection of the strip.

SIGN DECLARATION SHEET

I, the undersigned, assert that this MSc thesis is my original work; the work has not been submitted previously, in whole or in part, to qualify for any other academic award. Any editorial work carried out by a third party is acknowledged and ethics procedures and guidelines have been followed.

Investigator: Zelalem Tena Signature: _____ Date_____

Advisor: Kassu Desta (MSc, PhD fellow) Signature: _____ Date_____

Solomon Gizaw (MSc,PhD fellow) Signature: _____ Date_____