ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Prevalence of Multi Drug Resistant Gram Positive Cocci Isolated From Different Clinical Samples Collected From Patients Refereed To Arsho Advanced Medical Laboratory, Addis Ababa, Ethiopia.

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A Research Thesis Submitted to the Department of Medical Laboratory Science, College of Health Science, Addis Ababa University in partial fulfillment of the requirements for the degree of masters in Clinical Laboratory science (diagnostic and public health microbiology specialty track).

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This is to certify that the thesis prepared by **Nuhamin Melaku Gebremariam**, entitled:

Prevalence of Multi drug Resistant Gram Positive Cocci isolated from different Clinical samples collected from patients Refereed to Arsho advanced Medical laboratory, Addis Ababa, Ethiopia and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (diagnostic and public health microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abbreviations

AAML	Arsho Advanced Medical Laboratory
AMR	Antimicrobial Resistance
AST	Antimicrobial Susceptibility Test
CLSI	Clinical Laboratory Standard Institute
CoNS	Coagulase Negative Staphylococcus
MDR	Multi-Drug Resistant
MIC	Minimum Inhibitory Concentration
MRCNS	Methicillin Resistant Coagulase Negative Staphylococcus
MRSA	Methicillin Resistant *Staphylococcus Aureus*
PI	Principal Investigator
PLC	Private limited Company
SOP	Standard Operational Procedure
V2C	Vitek 2 Compact
VRE	Vancomycin Resistant Enterococci

Abstract

Background: *Gram-positive bacteria are common causes of both community acquired and nosocomial infections. This is compounded by the development of multi-drug resistance in this group of bacteria. The aim of this study is to characterize and evaluate drug susceptibility profile of Gram-positive cocci isolated from different clinical samples in the study area.*

Objective: *The main objective of this study was to determine the prevalence of gram positive cocci and multidrug resistance pattern of Gram positive cocci isolated from different clinical samples.*

Methods: *A laboratory based cross-sectional study was conducted* ***t****o determine the prevalence of multidrug resistant strains of Gram-positive bacteria isolated from 792 patients referred to Arsho Advanced Medical Laboratory, from January to April 2018.Different clinical samples were collected and inoculated onto primary isolation culture media, blood culture bottles and blood agar. Preliminary identification was carried on based on number, type, morphology appearance of colonies and Gram reaction after growth on culture media. Identification and drug susceptibility testing also carried out by GP and AST-GP71 cards of the VITEK 2 compact systems.*

Result*: A total of 792 patients were participated on the study. The mean (std. deviation) ages of patients was 34.8 (20.2).The overall prevalence of gram positive cocci were 12.6 % (n=100/792) of which the most frequent one is S. aureus 54% (n=54/100) followed by CoNs 42% (n=42/100), S.agalactiae 1% (n=1/100) and E.faecalis3 % (n=3/100). The overall prevalence of multi drug resistant gram positive cocci (resistant for≥ two different classes of antibiotics) was (75%).Penicillin showed the highest resistance rate (85%), followed by Sulphamethazole /trimethoprim (47%), and Oxacillin (38%) for gram positive cocci bacteria*.

Conclusion*: High antimicrobial resistance and Multi drug resistance were demonstrated over the Study period, such as Penicillin was the most resistant followed by trimethoprim/ sulfamethoxazole and oxacillin. To prevent further emergence and spread of MDR bacterial pathogens, rational use of antibiotics and regular monitoring of antimicrobial resistance patterns are essential and mandatory.*

Keywords: *Antimicrobial resistance, Multidrug resistance and Antimicrobial susceptibility*

1. Introduction

1.1. Background

Gram-positive organisms (including bacteria of the genera *Staphylococcus, Streptococcus* and *Enterococcus*) are among the most common bacterial causes of clinical infection. This is primarily due to their association with a diverse spectrum of pathology, ranging from mild skin and soft tissue infections (SSTIs) to life-threatening systemic sepsis and meningitis [1].

Among in the genus *Staphylococcus, S.aureus* is a type of bacteria about 30% of people carry in their noses. Most of the time, *Staphylococcus* do not cause any harm; however, sometimes it may causes infections. In healthcare settings, these infections can be serious or fatal, including: Bacteremia or sepsis when bacteria spread to the bloodstream, Pneumonia, Endocarditis, which can lead to heart failure or stroke, Osteomyelitis, which can be caused by staph bacteria traveling in the bloodstream or put there by direct contact such as following trauma (puncture wound of foot or intravenous drug abuse. *S.saprophyticus* is a major cause of urinary tract infections, particularly in sexually active young women ranking second to *E. coli* while *S. epidermidis* is a major cause of peritonitis in patients with renal failure who are undergoing peritoneal dialysis through indwelling catheter [2, 3].

Genus *Streptococcus* can be classified into three categories based on their lytic activity on blood agar mediumi.e. Alpha hemolytic (*S.pneumoniae* and *S.viridians*), Beta hemolytic (*S.pyogen* and *S.agalactiae*) and Gama hemolytic (*Enterococcus* species).*S.pyogen* is a major cause of pharyngitis and scarlet fever as well as skin infections. It is also causes severe invasive infections like bacteremia. Of major concern is post streptococcal infection such as rheumatic fever and acute glomerulonephritis. *S.pneumoniae* and *S.agalactiae* are major causes of pneumonia, meningitis, and sepsis. *Enterococci* are cause of range of different disorders, such as urinary tract infections, intra-abdominal abscesses, wound infections, endocarditis and bacteremia [4].

Although a number of antimicrobial agents already exist for the treatment of such diseases, emerging issues such as antimicrobial resistance has become one of the most serious public health concerns. The global increase in both community and hospital acquired antimicrobial-resistant bacteria is endangering the ability to effectively treat patients, underlining the need for continued

surveillance, more appropriate antimicrobial prescribing, careful infection control, and new treatment alternatives [5-11].

Methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* species (VRE), and penicillin resistant *Streptococcus pneumoniae* are of particular concern among the gram positive bacteria [12-14].An important Consequence of antimicrobial resistance is treatment failure for serious infections in hospitalized patients. This is often associated with higher rates of morbidity, mortality, longer hospital stays, and increased costs of medical care [15-18].

In Ethiopia, like other developing countries, diagnostic microbiology laboratories are poorly organized. Diagnostic laboratories that isolate and characterize bacteria by using even few routine biochemical tests are rare. Furthermore, drug susceptibility testing of bacterial isolates has also been determined by using agar diffusion technique with all its limitations. In addition, agreement with respect to the distribution of bacterial species associated with infections and their drug susceptibility pattern among different local studies is lacking. In view of this, application of fully automated systems for bacterial characterization and for the assessment of their antimicrobial susceptibility profile has become important.

The VITEK 2 compact (bioMe'rieux, France) is a machine capable of running bacterial identification and drug susceptibility simultaneously. Reduced turnaround times, better specimen management, enhanced quality control, reproducibility, precision, and the ability to track results are other benefits of the VITEK 2 compact system over conventional methods. With regards to Identification, the machine characterize a total of 115 Gram-positive cocci and non-spore-forming bacilli to the species level by using 64 biochemical tests and substrates. Identification of bacterial isolates to species level provides indispensable information on its pathogenic potential and is of greatest importance for the correct explanation of antibiotic susceptibility testing [19].

Against this background, the aim of this study is to characterize and evaluate drug susceptibility profile of Gram-positive cocci isolated from different clinical samples collected from patients referred to Arsho Advanced Medical Laboratory by employing the fully automated VITEK 2 compact system.

1.2. Statement of the problem

The emergence of AMR is a complex process often involving the interplay of human, environmental and pathogen-related factors. In sub-Saharan Africa, the endemicity of acute respiratory infections, diarrheal diseases, HIV/AIDs, tuberculosis, malaria and helminthic infections has increased the demand for antimicrobial therapies both for prophylaxis and treatment. Further, short falls in the healthcare environment ranging from limited diagnostic capacity and resources, unregulated access to antibiotics, constrained access to health facilities and poor training with respect to antibiotic use have increasingly stoked the demand for antibiotics. The current lack of new antimicrobials on the horizon to replace those that become ineffective brings added urgency to the need to protect the efficacy of existing drugs [19-22].

Drug resistant strains of Gram-positive cocci have attracted increasing attention around the globe. Indeed, infections caused by methicillin-resistant *S.aureus*, vancomycin-resistant Enterococci, and penicillin-resistant *S. pneumonia* have been reported to be continuously rising in different countries. Understanding of the pattern of antibiotic resistance among isolates is very important both clinically and epidemiologically [20].

A systematic review made by Sylvia Omulo and his friends from 2,155 probable articles from Kenya, Tanzania, Uganda, Ethiopia, Rwanda and Burundi, published between 1974 and 2013 the gradual increase in publications from the mid-70s to date suggests that antimicrobial resistance (AMR) research is increasing attention within eastern Africa. Most of the reported AMR research was conducted in Kenya and Ethiopia [21].

In Ethiopia, it is widely practiced that antibiotics can be purchased without prescription and without real etiological agent identification. This leads to misuse of antibiotics by the public thus contributing to increase spread of antimicrobial resistance of gram positive cocci collected from various clinical samples [23].Also there are only a few studies in Ethiopia, which have studied on automated identification to the species level. In line with this conducting a research regarding MDR against gram positive cocci have vital medical importance in addition, it is timely and one of the priorities in Ethiopia.

1.3. Significance of the study

The antimicrobial susceptibility patterns of common pathogenic bacteria are essential to guide empirical and pathogen specific therapy; therefore, empiric antibiotic treatment is not effective in elimination of these pathogens much time in clinical practice. So identifying the various bacteria belonging gram positive cocci and also studying their antibiotic susceptibility patterns in our study area could be one indicator in the appropriate treatment of patients

The results of this study could help to give appropriate information about the alternative drug of choice. It is also an insight into the present situation regarding the etiology and antimicrobial susceptibility of major gram positive cocci bacteria isolated from different anatomical sites. In addition it helps to initiate further large scale epidemiological study on MDR gram positive cocci.

2. Literature Review

In many countries, oxacillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus* and penicillin-resistant *Streptococcus pneumoniae* are already prevalent and resistance continues to increase [24].In 2013, the Centers for Disease Control (CDC) in the USA asserted that the human race is now in the"post-antibiotic"era. In May 2014, the World Health Organization (WHO) stated that the AMR crisis is becoming terrible [25].

According to authors, very high prevalence rates of beta-lactam and macrolide resistance in *S.pneumoniae* have been found in Asian countries. Particularly, erythromycin resistance has increased dramatically, where > 70% of clinical isolates were fully resistant and a very high rates (> 50%) of MRSA, which is the most important cause of hospital-acquired infections such as pneumonia, surgical site infections (SSI), and bloodstream infections. MRSA kills more than 19,000 patients annually in the U.S. alone. The prevalence of VRE, which emerged in the late 1980s, has risen rapidly in many countries. Its prevalence among clinical isolates has been estimated to range from 12% to 21% in Korea, and similar estimates have been made in Taiwan. The prevalence of non-duplicated blood VRE isolates in a Taiwanese hospital increased significantly from 3.9% in 2003 to 18.9% in 2010. In Chinese hospitals, the prevalence of VRE increased from 0 in 2005 to 4.9% in 2010 [26].

A study conducted by Ruiqin Z. et al in Chinese hospital to assess prevalence of multidrug resistant gram positive cocci shows that 7789 Gram-positive bacterial strains were isolated including Coagulase negative *Staphylococci*(33%), *S.aureus* (19%), *E.faecium* (17%), and *E.faecalis* (15%).When we see antimicrobial resistance among staphylococcus species methicillin-resistant Coagulase negative *Staphylococcus* (MRCNS) isolates were 61.6% whereas MRSA is 31.5%, the study also tried to show the Antimicrobial resistance rate of MRSA throughout the years and it was generally decreased. Among these drugs, the sulfamethoxazole/trimethoprim combination was the most effective regimen against MRSA throughout the 8 years of study (resistance rates decreasing from 37.5% to 18.4% between 2006 and 2013).MRSA showed the highest resistance to azithromycin (from 100% in 2006 to 98.2% in 2013).The other antibiotics studied (erythromycin, Levofloxacin, clindamycin, and gentamicin) had intermediate effects and showed higher activity during the last 2 years. There was no resistance observed toward vancomycin from

both *Enterococci* species isolated from the finding it is concluded that continuous monitoring of antibiotic sensitivity and rationalizing the use of antibiotics remain an important and effective strategy to minimize the emergence of multiple resistance strains [20].

Another study from Bangladesh on Prevalence of Antimicrobial Sensitivity and resistant Pattern of Gram Positive Cluster Forming cocci from different Clinical Samples shows that; Out of 776 clinical specimen, 363 showed bacterial growth, out of which 271were cluster forming gram positive cocci and 28 chain forming gram positive cocci. Most of gram positive cocci were isolated from pus sample which is (221), followed by Sputum (46), Throat swab 19, Vaginal discharge (5) and wound swab (6).The drug sensitivity test was carried out by using isolates of gram positive cluster forming cocci with 10 antibiotics by disc diffusion technique. Cefuroxime and ceftriaxone showed highest sensitivity. The resistance pattern of the antibiotics was comparably high for ampicillin (34%), amoxicillin (26%) and co-trimoxazole (19%) whereas for other antibiotics such as cephalexin (7%), cefradine (6%), cefixime (3%) and doxycycline (2%) were comparably low. From this finding they concluded that it is very important to reduce frequent misuse; inadequate dosages and easy availability of antimicrobials to keep away human generation from emerging antibiotic resistance [27].

A study which is conducted in Saudi Arabia on Frequency and antimicrobial susceptibility Patterns of bacterial pathogens isolated from septicemic patients in Makkah hospitals from April 2004 to March 2005 the finding shows that the most often pathogens isolated from blood cultures were *Staphylococci*, (43.3%) of all isolates. The two most common bacterial organisms were Coagulase negative *Staphylococci* (24.7%) and *S. aureus (*18.6%). The results of drug susceptibility pattern of Coagulase negative staphylococci (61%) were resistant to Oxacillin, 53% were also resistant to gentamycin. However, all these (oxacillin-resistant) isolates remained susceptible to vancomycin. More than (80%) of the oxacillin-susceptible Coagulase-negative *Staphylococci* were resistant to penicillin, co-trimoxazole (55%), erythromycin (42%), and ampicillin (38%).Oxacillin resistance was seen in (53%) of *S. aureus* isolates. Oxacillin resistant *S. aureus* showed a high prevalence of resistance to penicillin (97.5%) and ampicillin (95.5%) but none to vancomycin. A total 93 out of161 (58%) methicillin-resistant *S.aureus* (MRSA) isolates were multidrug resistance (MDR) i.e.

resistant to penicillin and oxacillin and for three or more of the following agents: erythromycin, clindamycin, gentamicin and oxy tetracycline. Finally they concluded that the rates of antibiotic resistance among pathogens in this study are much higher than what has been reported elsewhere in the Kingdom as well as in many of international studies [28].

A study from Iran on antimicrobial profiles of bacterial strains isolated from patients with hospital acquired blood stream and urinary tract infections shows that the most prevalent blood stream infection(BSI) pathogen was CoNS 34.8% with highest resistance rate against penicillin (91.1%) followed by ampicillin (75.6%), and the lowest rate was against vancomycin (4.4%) whereas *S. aureus* is found to *be* (3.9%) with high resistance against penicillin, ampicillin and cotrimoxazole which is 100% and susceptible to vancomycin. On the other side CoNS were found to be the second most prevalent UTI pathogen(11.7%) next to E.coli (66.7%) followed by S.aureus (6.7%). CoNS showed highest resistance to Penicillin (100 %) and ampicillin (97.1%) with lowest rate of resistance to naldixic acid (20%) and *S.aureus* showed higher resistance to Ampicillin and Penicillin (90%) and with lower resistance to Ciprofloxacin (15%) Ceftazidime (20%) Amikacin (25%) and Imipenem (25%) finally they proposed that to reduce the incidence of nosocomial infections, the appropriate use of antibiotics according to the standard antimicrobial susceptibility tests has to be done [29]

A study conducted by Ana C. and his colleagues carried out on Antimicrobial Susceptibility of Gram-Positive Bacteria Isolated in Brazilian Hospitals Participating in the sentry Program from 2005 to 2008 shows 31.0% of *S. aureus* strains were resistant to oxacillin (MRSA) and the vast majority of MRSA strains were also resistant to clindamycin, ciprofloxacin and Levofloxacin. Furthermore,68.1% of MRSA strains were resistant to trimethoprim/sulfamethoxazole, Daptomycin and vancomycin were active against all *S. aureus* strains tested (100% susceptible).Almost 80% of CoNS strains were resistant to oxacillin. This organism showed high rates of resistance to most antimicrobial agents tested (Erythromycin, Clindamycin, Ciprofloxacin, Levofloxacin Tetracycline TMP/SMX Linezolid).Vancomycin was active against all CoNS strain. In conclusion, daptomycin and linezolid showed excellent *in vitro* activity against contemporary Gram-positive organisms (3,907) collected in Brazilian hospitals monitored by the sentry Program, including MRSA, vancomycin-resistant *enterococci* (VRE) and other multi drug resistant

organisms. Although vancomycin resistance rates in Brazil appears to be relatively low compared to those reported in the USA, VRE has emerged and rapidly disseminated in some Brazilian medical centers [3].

A study from Egypt carried out on an emerging antimicrobial resistance in early and late onset neonatal sepsis, among the isolates gram positive cocci showed highest resistance to ampicillins(amoxicillinsulbactam100%andamoxicillinclavulanate75%),cephalosporins(Ceftazidime94%,cefoperazone100%,cefepime86%,ceftriaxone100%,cefuroxime100%,cefoxitin80%,carbapenems(Imipenem84%,meropenem86%,piperacillin-tazobactum 100%,and erythromycin 86%. Less resistance was evident to amino glycosides (Amikacin, 49%, gentamicin, 57%), quinolones (ciprofloxacin 77%, Levofloxacin 75%), clindamycin (53%), and rifampicin (49%). Least resistance among gram positive bacteria was found to vancomycin (18%) finally they concluded that there shall be global regulations to restrict the use of antimicrobials in the community as well as in the hospital setting [30].

A study conducted in Rwanda on prevalence of antimicrobial resistance among common bacterial isolates in tertiary health care from different clinical samples 82 % of S.aureus isolated were oxacillin resistant and 6% were vancomycin resistant. The isolates were resistant to commonly used oral antibiotics penicillin (88%),sulfamethoxazole/trimethoprim (70%), tetracycline (62%), and erythromycin (33%).100% of CoNS were resistant to oxacillin but susceptible 50% to erythromycin and29% to tetracycline [6].

Mohammedaman and his colleagues in Jimma town, Ethiopia conducted a study on Antimicrobial susceptibility pattern of bacterial isolates from wound infection and their sensitivity to alternative topical agents. A total of 145 bacterial isolates were obtained, (53%) were gram negative while (47%) were gram positive. *S. aureus* was the predominant organism isolated (32.4%) and Coagulase negative Staphylococci (14.5%).The drug susceptibility pattern for the Gram positive bacteria against selected 14 antibiotics showed that the organisms varied in their susceptibility to all the antimicrobials used. Majority of them showed multi-drug resistances. Rate of isolates resistant to ampicillin was 94%, followed by penicillin G, 86.8%. All isolates were 100% susceptible to vancomycin and Amikacin, and showed low resistance to norfloxacin (10%), ciprofloxacin (10%),sulphamethoxazole/trimethoprim (8.8%) and gentamicin (8.8%).Their

conclusion was ampicillin, penicillin, cephalothin and tetracycline were the least effective. Gentamicin, norfloxacin, ciprofloxacin, vancomycin and Amikacin were the most effective antibiotics [23].

Another study conducted at dessie regional laboratory, North east Ethiopia on Bacteriology and antibiogram of pathogens from wound infections reveals that Out of 599 wound swab samples analyzed, 422 (70.5%) were culture positives. *S.aureus* was the most frequently isolated pathogen which accounted for 208 (41.6%) of isolates whereas Coagulate negative staphylococcus (1.8%).Analysis of species specific resistance rates indicated that most of *S. aureus* was mostly resistant to amoxicillin (79.1%), tetracycline (71.0%) and show low resistance to norfloxacin (8.0%), ciprofloxacin (8.8%), cloxacillin(10.3%) and gentamicin (12.4%).In this study, the overall multiple antimicrobial resistances rate was 65.2% and only 13% of the isolates were sensitive to eleven antimicrobial agents tested [22].

A study conducted on Prevalence and antimicrobial susceptibility pattern of methicillin resistant Staphylococcus aureus isolated from different clinical samples at Yekatit 12 Hospital Medical College in Addis Ababa, Ethiopia. Among 1360 participants 194(14.3%) S. aureus was isolated mainly from pus/abscess, ear discharge, blood, nasal swab and throat swab which together, accounted for 175(90.2%) of all isolates. The rest of the isolates were from urine, vaginal discharge, eye swab, body fluid, stool and sputum, accounting less than 10% of the total. Out of 194 S. aureus recovered 34(17.5%) were found to be MRSA and the remaining 160(82.5%) were MSSA.S. aureus isolated in this study were highly resistant to penicillin 187(96.4%), trimethoprim sulphamethoxazole 103(53.1%), erythromycin 103(53.1%) and ciprofloxacin 61(31.4%). On the contrary, lower resistant was manifested by amoxicillin-clavulanate 36(18.5%), gentamicin 26(13.4%), clindamycin 23(11.9%) and vancomycin 10(5.1 %).All isolates were resistant to at least one antimicrobial agent. MRSA isolates were 100% resistant for penicillin, Erythromycin, trimethoprim/sulphamethoxazole, amoxicillin clavulanate, cefuroxime, cephalothin and least resistant for vancomycin 10 (29.4%). In this study they concluded that S. aureus isolates exhibited very high degree of resistance to different antibiotics. The isolates were also multidrug resistant to several combinations of the tested antibiotics. The emergence of vancomycin resistant S. aureus highlights the value of prudent prescribing of antibiotics and avoiding their irrational use [31].

A Study conducted by Sosina on Bacterial profile and drug resistance pattern of pathogens isolated from wound infection at Armed Force Referral and Teaching Hospital, Addis Ababa, Ethiopia shows that out of 300 wound samples from the study population 205 (68.3 %) samples were found culture positive while 95 (31.7 %) showed no growth.102 (42.9 %) were Gram positive while the rest 136 (57.1 %) were Gram negative. Among the gram positive isolates S. aureus 91 (38.2 %) was the most frequently isolated and CoNS were found 4% whereas *Streptococcus spps* was the least prevalent etiologic agent (0.4 %).The antimicrobial resistance pattern among Gram positive bacteria showed high level of resistance against Penicillin G (86.3 %), and ampicillin (67.6 %). *S. aureus* was mostly resistant to Penicillin G (91.2 %), Ampicillin (73.6 %) and tetracycline (67%).Whereas it was susceptible to Ceftriaxone (7.7 %), norfloxacin (7.7 %), and Cefotaxime (9.9 %). Similarly, (50 %) of CoNS were resistance to penicillin G. Fortunately, *Streptococcus spp.* was (100 %) sensitive to many of the antimicrobial drugs tested. Her conclusion is that Knowledge of the microbial flora of wound and the resistance pattern are important tools in the management of wound and are also useful in formulating rational antibiotic policy [32].

2.1. Conceptual Framework

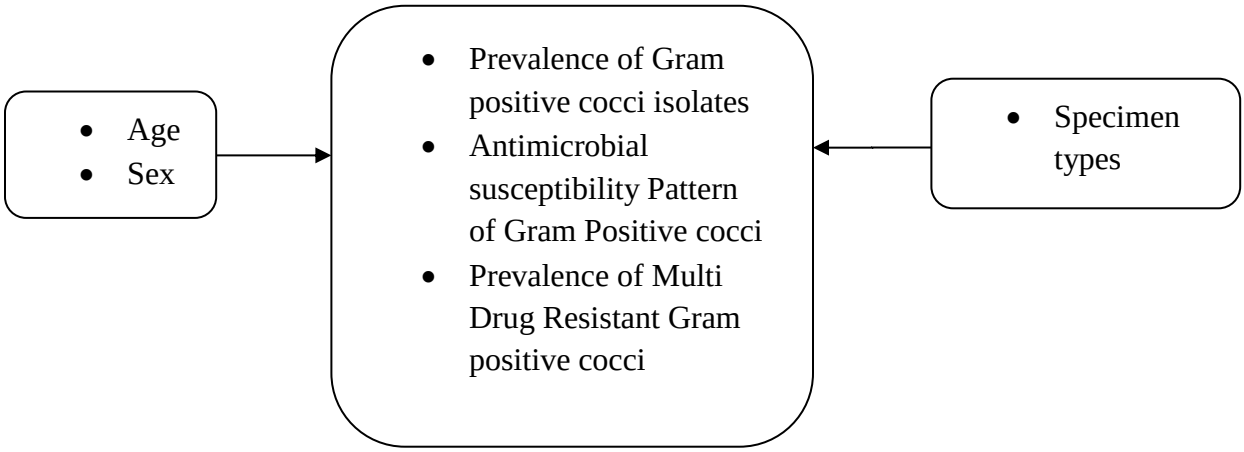

Figure 1: Conceptual frame work for prevalence of multi drug resistant gram positive cocci

3. Objectives

3.1. General objective:

- To determine the prevalence of gram positive cocci and multidrug resistance pattern of Gram positive cocci isolated from different clinical samples.

3.2. Specific objectives:

- To determine the prevalence of isolated Gram positive cocci
- To determine the overall antimicrobial susceptibility profile and multi drug resistance pattern of Gram- positive Cocci.
- To analyze the distribution of gram positive bacteria on sex and different age group

4. Hypothesis

There is no difference in the Prevalence gram positive cocci bacteria and multi drug resistant gram positive cocci isolated with the previous studies conducted in Ethiopia.

5. Materials and Method

5.1. Study Area

The study was conducted on patients referred to Arsho Advanced Medical laboratory (AAML) from different health facilities (hospitals, health centers, clinics, institution) and Arsho branch laboratories. AAML is a private diagnostic laboratory found in Addis Ababa Ethiopia with seven branches located different part of the city. On average about 40 patients per day were referred for culture and drug sensitivity testing.

5.2. Study design and period

A descriptive cross sectional study was conducted from January to April 2018

5.3. Population

5.3.1. Source population

All patients referred to Arsho Advanced medical laboratory for bacteriological culture test were the study subjects.

5.3.2. Study population

Patients referred to Arsho Advanced medical laboratory for bacteriological culture test that fulfill the inclusion criteria during the study period.

5.4. Inclusion and Exclusion criteria

5.4.1. Inclusion criteria

All patients referred to Arsho Advanced medical laboratory for bacteriological culture test.

5.4.2. Exclusion criteria

Patients that received antibiotic treatment for the last one week (7 days) prior to data collection time and unable to consent to participate in this study.

5.5. Study variables

5.5.1. Dependent variable

Prevalence of gram positive cocci

Prevalence of muli drug resistant gram positive cocci

5.5.2. Independent variable

Age, sex and specimen type

5.6. Measurement and Data collection

5.6.1. Sample size calculation

The sample size was calculated based on single population proportion. Since there is no published data available on prevalence and antimicrobial susceptibility pattern of gram positive cocci from different clinical samples by employing vitek 2 compact system in Ethiopia. The value of *p* taken as 50% (0.50).Considering 95% confidence interval, 5% margin of error, and the sample size is calculated using the following standard formula.

The sample size $n = z\ (\alpha/2)^2\ p\ (1-p)/d^2$

Where

n = Sample size

α = level of significance

z = at 95% confidence interval Z value (a = 0.05) =>Z α/2=1.96

p = Proportion of occurrence of the event to be studied 9% (0.09)

d = Margin of error at (5%) (0.05)

$n = (1.96)^2 0.5(1-0.5)/ (0.05)^2$

$n \approx 384$

To minimize errors arising from the likelihood of noncompliance, ten percent of the sample size was added to the normal sample. Accordingly the required sample size was 422.But during analysis we found limited isolates of gram positive cocci, therefore to increase the representativeness of our result 792 clinical samples were collected and processed.

5.6.2. Sampling method

Convenient sampling techniques were used. All volunteer patients referred to microbiology culture test during the study period were included in the sampling procedure.

5.6.3. Data collection procedure

Data was collected using structured data collection form to obtain information on socio demographic status previous antibiotic usage. Informed consent was taken from each patient and verbal informed consent was taken on behalf of children from their parents or guardians (see more on annex 1-8).

5.7. Laboratory analysis

5.7.1. Specimen collection and processing

Different clinical samples (Wound, Blood, Urine, Ear swab, Nasal swab, Body fluid, Eye swab, CSF, semen, Urogenital) were collected from study subjects consented to participate in this study and transported to microbiology laboratory of AAML aseptically and processed following standard operational procedures. Specimens collected from each patient were inoculated onto primary isolation culture media such as blood culture bottles and blood Agar base (Oxoid, Basingstoke, and Hampaire, UK) to which 10% sheep blood is incorporated. Blood culture bottles were incubated at 37^0C in a 5% CO_2 incubator for seven days where as blood agar base plates were incubated at 37^0C in 5% CO_2 incubator for 24 hours. Culture media with no bacterial growth in the above incubation conditions were re incubated for another 24 hours. Pure isolates of bacterial pathogen were characterized by colony morphology, hemolysis and gram stain. Bacteria identification and AST and were performed by VITEK 2 Compact (bioMe´rieux, France).

5.7.2 Principle of VITEK 2 compact system

The VITEK 2 compact system is an automated microbiology bacterial identification and antimicrobial susceptibility system. Uses advanced colorimetry technology to determine individual biochemical reactions contained in a variety of microbe identification cards. After inoculation with a standardized suspension of the unknown organism, each self-contained cards is incubated and read

by the instrument's internal optics. Comparison of results to known species specific reactions in the VITEK 2 database yields organism identifications. A transmittance optical system allows interpretation of test reactions using different wavelengths in the visible spectrum. During incubation, each test reaction is read every 15 minutes to measure either turbidity or colored products of substrate metabolism. In addition, a special algorithm is used to eliminate false readings due to small bubbles that may be present [19].

5.7.3 Inoculation

Identification cards were inoculated or filled with microorganism suspensions using an integrated vacuum apparatus. Each card has a pre-inserted transfer tube used for inoculation and has bar codes that contain information on product type, lot number, expiration date, and unique identifier that can be linked to the sample. A test tube containing the microorganism suspension was placed into a special rack (cassette) and the identification card were placed in the neighboring slot while inserting the transfer tube into the corresponding suspension tube. The cassettes can accommodate up to 10 tests (VITEK 2 Compact). The filled cassette were placed manually (VITEK 2 compact) into a vacuum chamber station. After the vacuum applied and air was re-introduced into the station, the organism suspension was forced through the transfer tube into micro-channels that fill all the test wells [19].

5.7.4. Card sealing, Loading and Incubation

Inoculated cards are passed by a mechanism, which cuts off the transfer tube and seals the card prior to loading into the carousel incubator. The carousel incubator can accommodate up to 30cards. All card types were incubated on-line at 35.5 + 1.0ºC. Each card removed from the carousel incubator once every 15 minutes, transported to the optical system for reaction readings based on their wave length, and then returned to the incubator until the next read time. Then data collected at 15-minuteintervals during the entire incubation period [19].

5.7.5. Bacterial Identification

Species identification and antimicrobial susceptibility testing of Gram-positive bacteria were determined with automated VITEK 2 compact system using bacterial isolation and identification

cards based on manufacturer's instruction. The VITEK 2 compact system is an integrated modular system that Consists of a filling-sealer unit, a reader-incubator, a computer control module, a data terminal, and a multi copy printer. The system detects bacterial growth and metabolic changes in the micro-wells of thin plastic cards by using a fluorescence-based technology.

The reagent cards have 64 wells that can each contain an individual test substrate. Substrates measure various metabolic activities such as acidification, alkalinization, enzyme hydrolysis, and growth in the presence of inhibitory substances. An optically clear film present on both sides of the card allows for the appropriate level of oxygen transmission while maintaining a sealed vessel that prevents contact with the organism-substrate admixtures.

The GP identification card is based on established biochemical methods and newly developed substrates. There are 43 biochemical tests measuring carbon source utilization, enzymatic activities and resistance. Final identification results are available in approximately eight hours or less [19].

5.7.6. Drug susceptibility testing

Antimicrobial Susceptibility testing with the VITEK-2 compact system was performed using an AST GP 71 card. It is intended for use with the VITEK-2 systems in clinical laboratories as an in-vitro test to determine the susceptibility of clinically significant aerobic gram positive cocci to antimicrobial agents. The cards were filled with inoculums in filling chambers. The VITEK-2 System automatically processes the antimicrobial susceptibility cards until MIC's are obtained. The VITEK-2 compact system subsequently corrects, where necessary for MIC's or clinical category in accordance with the internal database of possible phenotypes for microorganism antimicrobial agent combinations. Preparation of inoculums was done by transferring 280µL of culture suspension from the 0.5 McFarland culture suspension used for filling the identification cards into a fresh 3mL(0.45% of)sterile saline solution obtaining a final turbidity of $8x10^6$ CFU/mL) in the filling chamber according to the instruction of the insert kit of the AST card.

Antibiotics used for determination of drug susceptibility profile of Gram-positive bacteria in this investigationwere;Ciprofloxacin(1,2,4),Clindamycin(0.5,1,2),Daptomycin(0.5,1,2,4,16),Erythromycin(0.25,0.5,2),Gentamicin(8,16,64),Levofloxacin(0.25,2,8),Linezolid(0.5,2,8),Minocycline(0.12,0.5,1),Moxifloxacin(0.25,2,8),Nitrofurantoin(16,32,64),Quinupristin/Dalfopristin(0.25,0.5,2),Rifam

picin(0.25,0.5,2),Tetrcycline(0.5,1,2),Tigecycline(0.25,0.5,1),Trimethoprim/Sulfamethoxazole(2/38,8/152,16/304),Vancomycin (1,2,4,8,16).In addition CLSI guideline, 2017 were strictly followed [19, 33].

5.8. Data quality assurance

To maintain the quality of data the data collection form was pre-tested and collected data was checked carefully on spot and daily basis for their completeness, accuracy, and clarity. To assure the quality of laboratory results Standard operating procedures (SOPs) of the microbiology laboratory of AAML were strictly followed in all steps of the Pre-analytical, analytical and post-analytical.

5.8.1. Pre analytical phase

Socio-demographic characteristics of patients were collected using structured data collection sheets after getting informed Consent. All clinical specimens were collected by well-trained laboratory professionals following standard operational Procedure. When specimens reach the laboratory, it was checked to ensure that the correct specimen had been sent and the name on the specimen is the same as that on data collection form. To avoid sample contamination leak proof sample container was used.

5.8.2. Analytical phase

All materials, equipment and Procedures were adequately controlled. All stains and reagents were clearly labeled, dated, and stored correctly. The preparations, fixation, staining and reporting of smears as detailed in the SOPs of the microbiology laboratory of AAML were strictly followed. At regular intervals and whenever a new batch of gram stain is prepared, control smears of appropriate organisms were stained to ensure correct staining reactions. For each item of equipment there is clear operating and cleaning instructions, and service sheets. The operating temperature of a refrigerator, incubator, and water-bath was monitored and documented. Culture media was tested for Performance and sterility. To standardize the inoculums density of bacterial suspension for the susceptibility test, a 0.5 McFarland standard was used and standard reference strain tests S.*aureus* ATCC 25923, S.*agalactiae* ATCC 12386 and S.*pyogen* ATCC 19645 was used as Control bacteria strains.

5.8.3. Post Analytical Phase

Post-analytical phase the results were recorded with the patients' identification number. The terminology and format used in reporting was standardized. All reports were concise and clearly presented. Before leaving the microbiology laboratory, all reports were double checked for correctness. Purified bacterial culture isolates were stored in nutrient broth with 20% glycerol at -80°C for further need if required.

5.9. Data analysis and interpretation

Data entry and analysis will be done using SPSS (Statistical Package for social sciences statistical software version 20).The descriptive statistics was used to calculate and to see the relation between dependent variable and independent variables using frequencies and crosstabs. Finally, the results were presented on words, charts.

5.10. Ethical Consideration

All ethical Considerations and obligations were duly addressed, and the study was conducted after the approval of Departmental Ethics and Research committee" of the Department of Medical Laboratory Sciences, Collage of Health Science, and School of Allied Health Sciences of Addis Ababa University and Internal Review Board (IRB) of Arsho Advanced Medical Laboratory private limited company. Written Informed Consent was obtained from the participants before data collection. Each respondent was given the right to refuse to take part in the study and to withdraw at any time during the study period. All the information obtained from the study subjects were coded to maintain confidentially. When the participant is found to be positive for bacterial pathogen, they were informed and received proper treatment. An assent form was completed and signed by a family member and/or adult guardian for participants under the age of 18 years

5.11. Dissemination of results

The findings of this study will be presented to the Department of Medical Laboratory Sciences, School of Allied Health Sciences, and Addis Ababa University. And an attempt will be made to present the findings in different scientific conferences and will be sent for peer-review journals for publication.

5.12. Operational Definition

Antimicrobial resistance: Antimicrobial resistance is the ability of microbes to resist the effects of drugs that is, the germs are not killed, and their growth is not stopped. It happens when microorganisms such as bacteria change when they are exposed to antimicrobial drugs [34].

Multi Drug Resistance: MDR is defined as non-susceptibility to at least one agent in two or more antimicrobial categories [35, 36].

6. Results

6.1. Socio demographic characteristics

Seven hundred ninety-two (n=792) eligible study participants were investigated during the study period. Of these patents 63 % (n=499/792) of them were females and 37 % (n=293/792) were males. The majority of patients 44.4 % (n=352/792) and 15.5 % (n=123/792) were between 25-44 and 1-14 years of age respectively as shown below in Table 1, and the mean (std. deviation) ages of patients was 34.8(20.2).Among the total clinical samples processed 87.4% (n=692/792) showed no growth for gram positive bacterial and growth for gram positive bacterial were observed in 12.6 % (n=100/792) samples; of which females had a higher isolation rate than males 51% (n=51/100) versus 49 % (n=49/100).Rate of isolation of gram positive cocci was highest in 25-44 years 37% (n=37/100) as shown in Table 1.

Table 1: Frequency of gram-positive cocci isolates in relation to sex and age group at AAML from January to April 2018, Addis Ababa, Ethiopia (n=792).

Variables	Category	Total processed samples	No growth for gram positive bacteria N (%)	Growth for Gram positive cocci N (%)
Sex	Female	499 (63.0)	448(64.7)	51 (51)
	Male	293 (36.9)	244(35.3)	49(49)
	Total	792(100)	692(87.4)	100(12.6)
Age group[1]	<1	8(1.01)	8(1.2)	0
	1-14	123(15.5)	102(14.7)	21(21)
	15-24	82(10.3)	65(9.4)	17(17)
	25-44	352(44.4)	315(45.5)	37(37)
	45-64	147 (18.6)	135(19.5)	12(12)
	>65	80(10.1)	67(9.7)	13(13)
	Total	792	692(87.4)	100(12.6)

[1]WHO age classification for health [37]

6.3. Prevalence of gram positive cocci from clinical samples

Among gram positive cocci bacterial isolates the most frequent one is *S. aureus* 54% (n=54/100) followed by CoNs 42% (n=42/100), *S.agalactiae* 1% (n=1/100) and E.*faecalis*3 % (n=3/100) (Figure 1). The isolated gram positive cocci organism found from a wide range of clinical specimens such as in wound 48%(n=48/100), in blood 18% (n=18/100), in urine 17% (n=17/100), in ear swab 7% (n=7/100), in Nasal 3% (n=3/100), in Body fluid and eye swab 2%(n=2/100) in semen, CSF and in urogenital swab 1%(n=1/100) (Table 2).

Table 2: Frequency distribution of Gram positive cocci isolated from different clinical specimens at AAML from January to April 2018 (N=100).

Specimen	***E.faecalis***	***S.agalactiae***	***S.aureus***	***CoNs***							**Total**
				S.epidermidis	***S.haemolyticus***	***S.hominis***	***S.intermedius***	***S.saprophyticu***	***S.simulans***	***S.warneri***	
Wound	0	0	42	1	1	0	3	0	0	1	48
Blood	1	0	1	3	4	1	0	1	2	5	18
Urine	2	1	1	2	0	0	1	5	3	2	17
Ear swab	0	0	4	0	0	0	3	0	0	0	7
Nasal	0	0	2	0	0	0	0	0	0	1	3
Body fluid	0	0	1	0	0	0	0	0	0	1	2
Eye swab	0	0	2	0	0	0	0	0	0	0	2
CSF	0	0	0	0	0	0	0	0	0	1	1
Semen	0	0	0	0	0	0	0	0	0	1	1
Urogenital	0	0	1	0	0	0	0	0	0	0	1
Total	3	1	54	6	5	1	7	6	5	12	100

6.4. Antimicrobial susceptibility pattern of gram positive cocci isolates.

The overall drug susceptibility profile of Gram positive cocci bacteria against eighteen antibacterial drugs tested is summarized under Table 3. Penicillin showed the highest resistance rate (85%), followed by Sulphamethazole /trimethoprim (47%), and Oxacillin (38%). Gram positive bacteria showed highest sensitivity towards Linezolid (97%), vancomycin (96%), minocycline (95%), daptomycin (95%) and Quinopristin/Dalfopristin (95%) followed by Gentamycin and Tigecycline (92%) respectively.

As far as species specific antimicrobial resistance rates are concerned, *S.aureus* showed high resistance rates for penicillin (81.5%), trimethoprim/sulfamethoxazole (51.8%), tetracycline (22.2%) and oxacillin (18.5%) which is MRSA. Whereas gentamycin, Quinopristin /Dalfopristin, Linezolid minocycline, Nitrofurantoin, and Tigecycline showed no resistance. The least resistance was seen for vancomycin, daptomycin and Rifampicin (1.9 %) respectively.

The second commonly isolated gram positive cocci organisms *CoNs* exhibited high resistance for Penicillin (90.4%), oxacillin (64.2%) and Tetracycline (47.6%).The least resistance was observed in Quinopristin /Dalfopristin, Linezolid, Minocycline and Nitrofurantoin (4.7%) each followed by vancomycin and daptomycin (7.1%) respectively.

Similarly, three of the isolated *E.faecalis* showed 100% resistance for penicillin, Minocycline, Tetracycline, clindamycin and Quinopristin/Dalfopristin. The least resistance was observed in the case of Oxacillin, gentamycin, Linezolid, daptomycin, Vancomycin, Tigecycline, Nitrofurantoin, rifampicin, and trimethoprim/sulfamethoxazole (33.3%) respectively. One isolate of *E.faecalis* were found vancomycin resistant.

S.agalactiae which the least isolated gram positive cocci organism was susceptible to most of the drugs but it showed 100% resistance to Ciprofloxacin, Levofloxacin, Moxifloxacin and Nitrofurantoin

Table 3: Percentage of antibacterial susceptibility pattern of gram positive cocci bacteria isolated from different clinical specimens at AAML from January to April 2018 (N=100).

Bacteria	P	PEN	OXA	GEN	CIP	LEV	MXF	ERY	CL	QDA	LNZ	DAP	VAN	MNO	TET	TGC	NIT	RIF	TMP
S.aureus (n=54)	I	0	0	0	3.7	1.9	0	0	0	0	0	0	0	0	1.9	0	5.6	0	0
	R	82	18.5	0	3.7	1.9	1.9	9.3	5.6	0	0	1.9	1.9	0	22.2	0	0	1.9	51.9
	S	18	81.5	100	92.6	96.2	98.1	90.7	94.4	100	100	98.1	98.1	100	75.9	100	94.4	98.1	48.1
E.faecalis (n=3)	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	100	33.3	33.3	67	66.7	66.7	67	100	100	33	33	33.3	100	100	33	33.3	33.3	33.3
	S	0	66.7	66.7	33	33.3	33.3	33	0	0	67	67	66.7	0	0	67	66.7	66.7	66.7
S.agalactiae (n=1)	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	0	0	0	100	100	100	0	0	0	0	0	0	0	0	0	100	0	0
	S	100	100	100	0	0	0	100	100	100	100	100	100	100	100	100	0	100	100
CoNS (n=42)	I	0	0	4.7	4.7	2.3	9.5	0	0	0	0	0	2.4	0	2.4	0	2.4	0	0
	R	90.5	64.3	11.9	28.5	28.5	14.3	50	36	4.7	4.7	7.1	7.1	4.7	47.6	9.5	4.7	21.4	42.8
	S	9.5	35.7	83.3	66.6	69	76.1	50	64	95.2	95.2	92.8	90.4	95.2	50	90.4	90.4	78.6	57.1
Total (n=100)	I	0	0	2	4	2	4	0	0	0	0	0	0	0	2	3	3	0	0
	R	85	38	6	17	16	6	28	21	5	3	5	4	5	35	5	11	20	47
	S	15	62	92	79	82	86	72	79	95	97	95	96	95	63	92	86	80	53

PEN:Penicillin,**OXA**:Oxaciline,**GEN**:Gentamicin,**CIP**:Ciprofloxacin,**LEV**:Levofloxacin,**MXF**:Moxifloxacin,**ERY**:Erythromycin,**CL**:Clindamycin,**QDA**:Quino pristin/Dalfopristin,**LNZ**:Linezolid,**DAP**:Daptomycin,**VAN**:Vancomycin,**MNO**:Minocycline,**TET**:Tetracycline,**TGC**:Tigecycline, **NIT**: Nitrofurantoin, **RIF**:Rifampicin, **TMP**:Sulphamethazole/Trimethoprim**P**:Pattern,**I:**Intermediate,**R**:Resistant, **S**: Susceptible

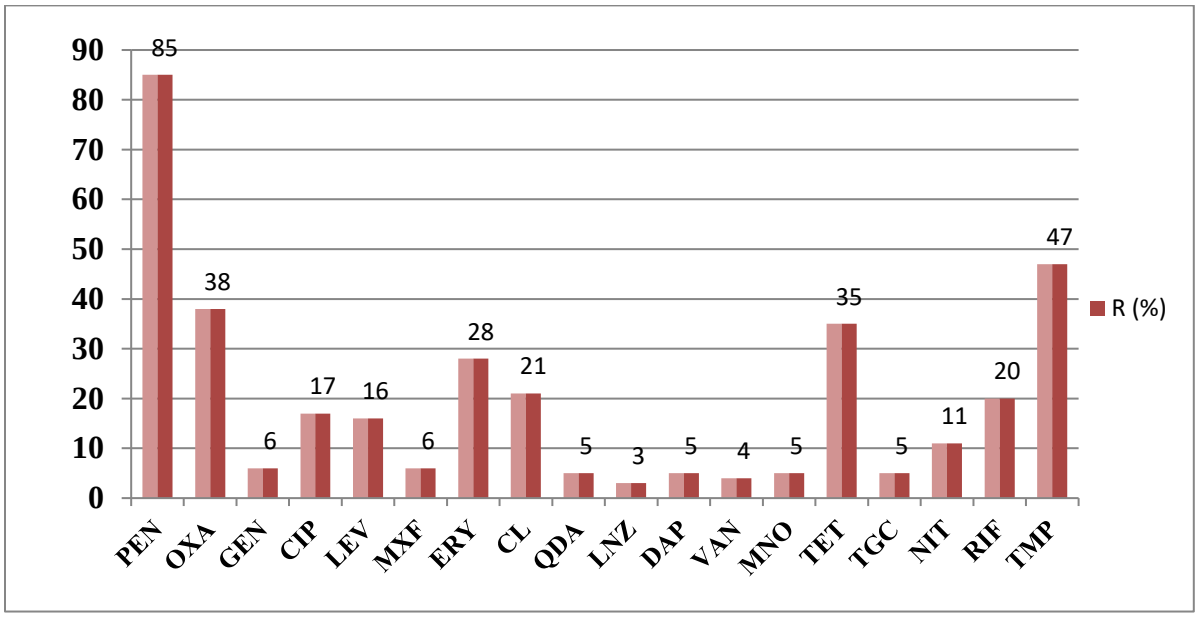

Figure 2: Antimicrobial resistance Pattern of Gram Positive Cocci isolated from different clinical samples at AAML (n=100)

6.4. Multidrug resistance patterns for the isolated gram positive cocci bacteria

Based on the finding of the present study as shown that in Table 4 below, the overall prevalence of multi drug resistant gram positive cocci (resistant for≥ two different classes of antibiotics) was (75%).However, 17%(n=17/100) resistance only for one class of antibiotic, only 8%(n=8/100) bacterial isolate were sensitive for all antibiotics tested this raveled that wide range of antibiotics becomes resistant. Among the isolated Gram positive bacteria, *S.aureus* showed 66.7 % (n=36/54), *CoNS 83.3* % (n=35/42) and *E.faecalis* 100% (n=3/3) were MDR.

Table 4: Multidrug resistance pattern of gram positive cocci bacterial isolates from various clinical patient samples at Arsho Advanced medical laboratory from January to April 2018.

Isolated Gram Positive cocci Bacteria	**Resistant antibiotics N (%)**						
	R0	R1	R2	R3	R4	>R4	MDR(≥R2)
S.aureus (n=54)	5(9.3)	12(22.2)	23(42.6)	7(12.9)	5(9.3)	1(1.8)	36(66.7%)
CoNS (n=42)	3(7.1)	4(9.5)	7(16.7)	5(11.9)	7(16.7)	16(38)	35(83.3%)
E.faecalis(n=3)	0	0	0	0	0	3	3(100%)
S.agalactiae (n=1)	0	0	1(100)	0	0	0	1(100%)
Total (n=100)	8(8)	17(17)	31(31)	12(12)	12(12)	20(20)	75(75%)

R0- no resistant for any antibiotic, **R1**-resistant for 1 class of antibiotic, **R2**- resistant for 2 class of antibiotics, **R3**- resistant for 3 classes of antibiotics,**R4**- resistant for 4 classes of antibiotics,**>R4**- resistant for more than 4 class of antibiotics.

NB: Class of antibiotics is made based on CLSI 2017 category [33]

7. Discussion

Infection with the antibiotic-resistant bacteria has made the therapeutic options more difficult. Antibiotic use and misuse in humans, animals and agriculture, clustering, overcrowding, and poor infection control were reason for emerging antibiotic- resistance bacterial strain. Multidrug-resistant bacterial infection becomes a real threat in developing countries including Ethiopia. In the study area the majority of pathogenic bacteria isolated from various clinical specimens were drug resistant [38, 39].

The present study showed that females had a higher isolation rate of gram positive cocci than males this is in agreement with a study done in Addis Ababa by Dessie W. et al [40] but disagrees with the finding of Shivani K et al from India which reported higher frequency of gram positive cocci among Male patients [41].Rate of isolation of gram positive cocci was also the highest in 25-44 years of age group.

In this study among gram positive bacterial isolates the most common one is *S.aureus* 54% (n=54/100) which is similar to the finding of previous works conducted in Addis Ababa36.8%, Jimma 28.4%, Gondar 21.1%, Mekelle 37.5%, Cameron 20.9%, India 47.7%, and Nepal 65% [42-48] even if there is variation in their prevalence and dissimilar to the other studies that report *CoNs* as major isolate. This variation is due to the difference in study settings and specimen types [49, 50].In most studies *CoNs* were considered contaminant, but now they are potentially important pathogens and their increasing incidence has been recognized. In recent years, *CoNs* have become the major cause of nosocomial bloodstream infections to some extent as results of the increasing use of intravascular devices and increased number of hospitalized immune compromised patients [44, 51].

The most effective drugs for gram positive isolates in our study were Linezolid (97%), vancomycin (96%), minocycline (95%), Quinopristin/Dalfopristin (95%), daptomycin (95%) which is similar with the finding of Bitew A et al. [52].Whereas Penicillin showed the highest resistance rate (85%), followed by Sulphamethazole/trimethoprim (47%), and Oxacillin (38%)which was consistent with a study done in Jimma, Gondar and Addis Ababa [23, 53,54].

However, *S.aureus* isolated in this study showed 1.9% vancomycin resistance pattern which is higher than previous studies in Gondar, Nigeria and Dessie [55-57] which showed no vancomycin resistance but it is lower than (6%) a study done by*Cyprien.Net al*.at Rwanda, (5.1%) reported by Dilnessa T and Bitew A in Addis Ababa, Ethiopia [58, 31].The difference could be due to the difference in prescribing the antibiotic for treatment from hospital to hospital. It also showed high level of resistance to penicillin (81.5%) which is consistent with the finding of Eshetu S (86.7%) but lower than (91.5 %) reported by Mohammedaman M et al from Jimma and (93.8 %) by Wondemagegn M et al from Debremarkos [54,23, 59].On the other hand lower finding were reported by Asdesach T (66.7%)in Addis Ababa, Ethiopia[60]. High penicillin resistance was seen in many part of the word, probably due to indiscriminate use of antibiotics.

In this study about 18.5 % of *S.aureus* were found oxacillin/Methicillin resistant (MRSA)which is consistent with (17.5%) reported by Dilnessa T et al and 18.5 % by Derese H et al [31,61].on the other hand it is higher than (10.6%) reported by Legese MH et al [62] and yet (18.5%) was lower than the finding of Amare et al, JR Anguzu in Uganda and Giacometti A in Italy where 34.6%, 25% and 54.4% MRSA were reported respectively [53,63,64],the difference could be due to the difference in prescribing this antibiotic for treatment from hospital to hospital.

The second commonly isolated gram positive cocci *CoNs* exhibited high resistance for Penicillin (90.4%), oxacillin (64.2%) which is similar to the study done by, Hamed G et al and Eshetu S [29, 54]. On the other hand, it showed 7.1% resistance for vancomycin which is higher than the study done by in Ethiopia by Amare et al which is 4.5% and 4.4 % by Hamed G et al [53, 29] and lower than the finding of ten hove et a1 which is 13.4 % [65].The difference could be due to the difference in prescribing this antibiotic for treatment from hospital to hospital.

In the study area the majority of pathogenic bacteria isolated from various clinical specimens were multi drug resistant. The overall multidrug resistance level of gram positive bacterial isolates was 75 % (n=75/100). This finding was in line with a study done in Addis Ababa, Ethiopia by Ayalew S. 73.6% and Teklehymanot F. et al 72% [32, 66]. On the other hand, this finding was higher than a study done by Derese H et al where the MDR level was 54.3%and Azene MK et al 65.2% [61, 67].Our finding was lower as compared to what has been recorded in South West Ethiopia which was 85.2% [23]. This high rate of antibiotic resistance might reflect in appropriate use of antibiotic,

lack of laboratory diagnostic tests for appropriate antibiotic selection, unavailability of guideline for the selection of antibiotics, unskilled practitioners, expired antibiotics, self-medication, counterfeit drugs, or inadequate hospital control measures [64, 68].

The most frequently isolated pathogens, *S.aureus* showed 66.7% (n=36/54) of an MDR level which is lower than the previous studies done Sosina A and Girma G et al where they reported 79.2 % and 86.2 % respectively [32,69]. But higher than the finding of Eshetu S.46.6 %and Derese H et al 48% [54,61].This difference might be due to the difference in study setting, previous antibiotic usage and definition for MDR.

The second commonly isolated gram positive cocci, *CoNs* showed 83.3% (n=35/42) of an MDR level which is consistent with the study done at Tikur Anbesa specialized hospital 84.2%[62].on contrary higher rates (100%) and lower rate (66.7%)were reported by Teklehymanot F et al and Legese MH et al respectively [66,70].

8. Strength and limitation

8.1. Strength

Used the advanced VITEK 2 Compact system which is an automated microbial identification and antimicrobial susceptibility system that provides highly accurate and reproducible results.

8.2. Limitation of the study

- Lack of patient's clinical history in their request paper which could have been a good variable for this study.
- Being the study as a single laboratory based it may lack representativeness

9. Conclusion

In this study high antimicrobial resistance and multi drug resistance was demonstrated over the study period, such as Penicillin was the most resistant followed by trimethoprim/sulfamethoxazole and Oxacillin were the least active agent against gram positive bacteria while Linezolid and vancomycin were exhibited the most susceptible drug. To prevent further emergence and spread of MDR bacterial pathogens, rational use of antibiotics and regular monitoring of antimicrobial resistance patterns are essential and mandatory.

10. Recommendations

- A periodically surveillance of antimicrobial resistance pattern record and report is essential to develop treatment guideline.
- Establish antibiogram based on the susceptibility pattern for empiric therapy at national level is very crucial.
- Establishing health laboratories with modern methods such as VITEK 2 compact system for accurate identification of bacteria pathogens to the species level and determining drug susceptibility pattern of the etiologic agents for efficient management of bacterial infections should be considered for routine laboratory diagnosis.

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12. Annexs

Annex 1: Participants information sheet [English version].

Principal Investigator: Nuhamin Melaku, Addis Ababa University school of Allied Health Sciences.

Purpose: The purpose of this study is to assess the prevalence of multi drug resistant gram positive cocci in our laboratory.

Procedures to be carried on: you are invited to participate in the study after giving your Consent and by giving the requested sample for investigation.

Risks associated with the study: There is no risk and serious invasive procedure at the beginning as well as at the end of the study and there is no additional time required from you to stay during study.

Benefits of the study: There will be no financial benefit to you. But the result of the study will be used for to develop antibiogram helps patients avoiding empirical treatment.

Confidentiality of your information: The results of the laboratory findings will be kept confidential and could only be accessed by the researcher and the responsible physician. There will be no personal information to be attached to your data.

Termination of the study: We will respect your decision if you later on change your mind and you can refuse to participate or withdraw from the study at any time. Refusal to participate will not result in loss of medical care provided or any other benefits. You can get your results of the analysis.

Annex 2. Informed Consent [English version]

For adult patients who are able to respond:

I, the undersigned individual, am oriented about the objective of the study. I have informed that all of my information will be kept confidential and used only for this study. Your signature below indicates that you have read /or listened, and understand the information provided for you about the study.

Before you sign, please understand purpose of the study, procedure, risks and benefits of participation, right to refuse or withdraw, confidentiality and privacy, and who to contact if you have any question.

I have read /or listened to the description of the study and I understand what procedures are and what will happen to me in the study. Moreover, I have also been well informed of my right to keep hold of information, decline to cooperate and drop out of the study if I want and that none of my actions will have any bearing at all on my overall health care and the laboratory's service access.

Based on the above information I agree to participate in the research

Study participant Signature: _____ Date: _____

Name of Data collector _____ Signature _____ if you have any question you can ask the principal investigator

Principal investigator Mrs. Nuhamin Melaku [MSc candidate]

Mobile 0911989298

E-mail: namdan433@gmail.com

Annex 3: Participant's information sheet [Amharic version]

ጥናቱን የምታጠናው፤ኑሃሚን መላኩ በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ሳይንስ ዲፓርትመንት

የጥናቱ አላማ፤

የጥናቱ ዓላማ መድሃኒት የተለማመዱ ግራም ፖዝቲቭ ኮክሳይ ባክቴሪያዎችን ስርጭት በአርሾ አድቫንስድ ሚዲካል ላቦራቶሪ ለተለያየ የማይክሮባዮሎጂ ካልቸር ምርመራ ከተላኩ ናሙና ውስጥ በመለየት የፀረ ባክቴሪያ መድሃኒት የመቋቋም አቅማቸውን ማወቅ አሁን ያሉበትን ደረጃ ማሳየት እና የመፍትሄ አቅጥጫ ማስቀመጥ፡፡

ለጥናቱ ተሳታፊዎች ያለው ልዩ ጥቅም፤በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለውም ነገር ግን ከጥናቱ የሚገኘው ውጤት ለርስዎ ህክምና ተጨማሪ መረጃ ለማግኘት በተመሰሳይ ለመድሃኒት ልምምድ ያደረጉትን ካላደረጉት በመለየት ውጤታማ የሆኑትን መድሃኒቶች ይጠቁማል፡፡

በጥናቱ ተሳታፊዎች ላይ ያለው ጉዳት፤በጥናቱ መጀመሪያም ይሁን መጨረሻ በዚህ ጥናት ላይ በመሳተፍዎ ሊደርስብዎ የሚችል አንድም ጉዳት አይኖርም፡፡በጥናቱ ምክንያት የሚያባክኑት ተጨማሪ ጊዜም አይኖርም፡፡

የመረጃ ሚስጥራዊ አጠባበቅ፤የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዚያ በኋላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘዉ በስም ሳይሆን በመለያ ቁጥር ይሆናል፡፡በጥናቱ ላይ ያለመሳተፍ መብት አለዎት፡፡ይህ መረጃ በጥንቃቄ የሚያዝ ይሆናል፡፡ በመጨረሻም የጥናቱ ውጤት ለሚመለከተዉ አካል ለጥናቱ አላማና ለህክምና ባለሙያዎች ብቻ የሚገለፅ ይሆናል፡፡

ስለዚህ ጥናት ማንኛውም ጥያቄ ካለዎት በማንኛውም ጊዜ ከዚህ በታች በተጠቀሱት አድራሻዎች መጠየቅ ይችላሉ፡፡

እኔም የጥናቱ ተሳታፊ ይህንን በመገንዘብ ጥናቱ ላይ ለመሳተፍ ተስማምቼያለሁ፡፡

ፊርማ ----------

መረጃውን የሰበሰበው ግለሰብ ስም---------- ፊርማ ----------

የዋና ተመራማሪዋ አድራሻ

ኑሃሚን መላኩ

አዲስ አበባ ዩኒቨርሲቲ፣የጤና ሳይንስ ኮሌጅ፣የሕክምና ላቦራቶሪ ቴክኖሎጂ ዲፓርትመንት

አዲስ አበባ፣ኢትዮጵያ

ኢ-ሜይል፣namdan433@gmail.com

ስ.ቁ +251-911-989298

Annex 4. Informed Consent [Amharic version]

በዚህ ጥናት ለሚዳሰሱ ሀሳባቸዉን መግለጽ ለሚችሉ የስስምምነት መጠየቂያ ቅጽ

እኔ ፊርማዬ ከዚህ በታች የተቀመጠው ግለሰብ ከእኔ የሚወሰደዉ ናሙና ለጥናቱ አላማ ብቻ እንደሚዉል ተረድቻለሁ፡፡ ሁሉም መረጃዎች እና የናሙና ዉጤቱ ምስጢራዊ መሆኑን ተገንዝቤአለሁ፡፡ በጥናቱ ላይ በመሳተፌ ምንም የገንዘብ ክፍያ እንደማላገኝ ተረድቻለሁ፡፡ ከምርመሩ መሳተፍ ወይም አለመሳተፍ መብቴ የተጠበቀ መሆኑን እና ላለመሳተፍ ብወስን በላቦራቶሪው በሚደረግበልኝ ምርመራ ላይ ምንም ተፅዕኖ እንደማይኖረዉ ተረድቻለሁ፡፡ስለዚህ የጥናቱን ጠቃሚነት አምኜበት የስምምነት ቃሌን የሰጠሁት በፍፁም ፈቃደኝነት ነዉ፡፡

ስለዚህ ጥናት ማንኛውም ጥያቄ ካለዎት በማንኛውም ጊዜ ከዚህ በታች በተጠቀሱት አድራሻዎች መጠየቅ ይችላሉ፡፡
እኔም የጥናቱ ተሳታፊ ይህንን በመገንዘብ ጥናቱ ላይ ለመሳተፍ ተስማምቼያለሁ፡፡

የጥናቱ ተሳታፊ ፊርማ ----------ቀን----------
መረጃውን የሰበሰበው ግለሰብ ስም----------
ፊርማ ----------
የዋና ተመራማሪዋ አድራሻ
ኑሃሚን መላኩ
አዲስ አበባ ዩኒቨርሲቲ ፣የጤና ሳይንስ ኮሌጅ፣ የሕክምና ላቦራቶሪ ቴክኖሎጂ ዲፓርትመንት
አዲስ አበባ፣ ኢትዮጵያ
ኢ-ሜይል፣ namdan433@gmail.com
ስ.ቁ +251-911-989298

Annex 5. Parental/Guardian consent form in English

I, the undersigned, have been told about this research. My child has to say to choose if I want to be in the study. I have been informed that other people will not know my child results as it coded with number rather than writing name. I understand that there may be no benefit to me personally apart from 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. By signing below I agree to let my child to participate in this research study.

Parent/guardian Signature: _____ Date: _____

Name of Data collector _____ Signature _____ if you have any question you can ask the principal investigator

Principal investigator Mrs. Nuhamin Melaku [Msc candidate]

Mobile 0911989298

E-mail: namdan433@gmail.com

Annex 6.Guardian /parental consent form in Amharic

የወላጅ /የአሳዳጊ /የሞግዚት የስስምምነት መጠየቂያ ቅጽ

እኔ ፊርማዬ ከዚህ በታች የተቀመጠው -የታማሚው ወላጅ /አሳዳጊ/ ሞግዚት ስሆን የዚህን ጥናት አላማ በዉል ተረድቻለሁ፡፡

በጥናቱ ወቅትም ታማሚው መረጀዎች በሚስጥር ስለሚያዝ በሌላ ሰዉ ዘንድ እንደማይታወቅ ተረድቻለሁ፡፡ በውጤቱ ከሚገኘዉ የህክምና አገልግሎት በቀር ሌላ ታማሚው በግሉ የሚያገኘዉ ጥቅም እንደሌለ ተረድቻለሁ፡፡ ጥያቄ እንድጠይቅ ዕድል ተሰጥቶኝለ ጥያቄዎቼም በቂ ምላሽ አግኝቻለሁ፡፡ የልጀ በጥናቱ መሳተፍ በእኔ ፍላጎት ብቻ እደሆነ እና በጥናቱም አለመሳተፍ ምንም አይነት ተፅዕኖ ታማሚው ላይ እንደማያስከትል ተረድቻለሁ፡፡ ከዚህ ባሻገር ታማሚው በጥናቱ ውስጥ ለመካተት የእኔ ወላጅ አሳዳጊ /ሞግዚት ፈቃድ እንደሚያስፈልግ ተረድቻለሁ፡፡ በእኔ ፍቃደኝነት ታማሚው በጥናቱ እንደሚሳተፍ ከዚህ በታች በፊርማዬ አረጋግጣለሁ፡፡

የጥናቱ ተሳታፊ ወላጅ /አሳዳጊ /ሞግዚት ፊርማ _____

መረጃውን የሰበሰበው ግለሰብ ስም----------

ፊርማ ----------

የዋና ተመራማሪዋ አድራሻ

ኑሃሚን መላኩ

አዲስ አበባ ዩኒቨርሲቲ ፣የጤና ሳይንስ ኮሌጅ፣ የሕክምና ላቦራቶሪ ቴክኖሎጂ ዲፓርትመንት

አዲስ አበባ፣ ኢትዮጵያ

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ስ.ቁ +251-911-989298

Annex7: Assent form for adolescent (12 -17 years old) study participants (English version)

I, the undersigned, have been told about this research. My parents or guardian have to say to choose if I want to be in the study. I have been informed that other people will not know my results as it coded with number rather than writing my name if I am in this study. I understand that there may be no benefit to me personally apart from 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 parents/guardian. By signing below I agree to participate in this research study.

Study participant Signature: _____ Date: _____

Name of Data collector _____ Signature _____ if you have any question you can ask the principal investigator

Principal investigator Mrs. Nuhamin Melaku [Msc candidate]

Mobile 0911989298

E-mail: namdan433@gmail.com

Annex 8: Assent form for adolescent (12-17 years old) study participants (Amharic version)

በአማርኛ የተዘጋጀ ዕድሜያቸዉ ከ12 እስከ 17ዓመት ለሆኑ ታዳጊ ወጣት የጥናት ተሳታፊዎች የተሳትፎ ማራጋጋጫ ቅጽ

ከዚህ በታች ስሜ የተገለፀው በዚህ ጥናት ውስጥ እንድሳተፍ ፍቃደኝነቴን ተጠይቂያለሁ። ወላጆቼም/አሳዳጊዎቼም በጥናቱ እንድሳተፍ ወይም እንዳልሳተፍ ምርጫው የእኔ መሆኑን ነግረውኛል።በጥናቱ ወቅትም የእኔ መረጀዎች በሚስጥር ስለሚያዝ በሌላ ሰዉ ዘንድ እንደማይታወቅ ተረድቻለሁ። በውጤቱ ከሚገኘዉ የህክምና አገልግሎት በቀር ሌላ በግሌ የማገኘዉ ጥቅም እንደሌለ ተረድቻለሁ። ጥያቄ እንድጠይቅ ዕድል ተሰጥቶኝ ለጥያቄዎቼም በቂ ምላሽ አግኝቻለሁ። በጥናቱ መሳተፍ በእኔ ፍላጎት ብቻ እንደሆነ እና በጥናቱም አለመሳተፍ ምንም አይነት ተፅዕኖ በእኔ ላይ እንደማያስከትል ተረድቻለሁ። ከዚህ ባሻገር የኔ በጥናቱ ውስጥ ለመካተት የወላጆችም ወይም የአሳዳጊዎቸ ፈቃድ እንደሚያስፈልግ ተረድቻለሁ። በፍቃደኝነቴ በጥናቱ እንደምሳተፍም ከዚህ በታች በፊርማዬ አረጋግጣለሁ።

የጥናቱ ተሳታፊ ፊርማ ----------ቀን----------

መረጃውን የሰበሰበው ግለሰብ ስም----------

ፊርማ ----------

የዋና ተመራማሪዋ አድራሻ

ኑሃሚን መላኩ

አዲስ አበባ ዩኒቨርሲቲ ፣የጤና ሳይንስ ኮሌጅ፣ የሕክምና ላቦራቶሪ ቴክኖሎጂ ዲፓርትመንት

አዲስ አበባ፣ ኢትዮጵያ

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Annex 9: Data collection form

1. Patient identification

Sample ID._____

Age (years) _____

Gender Male ☐ Female ☐

Antibiotic intake before 7 days yes ☐ No ☐

II. Data Collection Sheet

1. Date of specimen collection_____

2. Specimen type: _____

3. Media used _____

4. Gram stains result _____

5. Biochemical test_____

6. Organism isolated _____

7. Drug susceptibility pattern

7.1. Sensitive to _____

7.2. Intermediate to _____

7.3 Resistance to _____

III. Comments_____

Name of principal investigator _____

Signature _____ Date _____

Annex 10. Procedure for specimen collection and processing

A. Laboratory procedure for collection and transportation of wound swab

1. Cleansing the wound with normal saline prior to obtaining swab specimens
2. Rotate sterile cotton-tipped applicator 1cm square area for 5 seconds with sufficient pressure to express fluid and bacteria to surface
3. Placing the swabs in to sterile test tubes having 0.5 ml of sterile normal saline solution
4. Label the sample as soon as possible with the patient code number
5. Transport the specimen to the laboratory at room temperature within 30 minutes of collection

B. Laboratory procedure for collection and transportation of blood culture

1. Palpate and identify appropriate vein
2. Disinfect the puncture site with iodine or alcohol (70%) beginning in the center and rubbing vigorously outward in concentric circles (approximately 50mm diameter).
3. Iodine should remain in contact with skin for about 1 minute or until dry to ensure disinfection.
4. Blood is obtained by inserting a needle into a vein in the arm.
5. **1-3 ml** of blood from a child is added to **25 ml** of blood culture broth and **5-7 ml** of blood from an adult is added to **50 ml** of blood culture broth. It is important to use appropriate ratios of blood to culture broth for optimal bacterial growth.
6. Put the first collected blood in one bottle and repeat collecting of another blood from different site (example; left and right hand) with the same volume for the second bottle. These two bottles constitute one blood culture set.
7. Gently rotate the bottles to mix the blood & the broth (do not shake vigorously).
8. Label both bottles with, Patient name, unique identifier, Time of collection, and Initial of collector as well as site of collection.
9. Safely dispose of all contaminated materials.

C. Laboratory procedure for collection and transportation of Ear swab

1. Clean external surface of the ear using 70% alcohol.
2. If there is make up and ointment gently remove with sterile swab and normal saline(if there is any chemical including skin lotion or hair treatment, we have to clean)

3. Collect fluid via syringe or take two swabs of uncontaminated infection for ruptured ear drum rotate the swab several times until wet. (Aspirate is preferable specially for for internal ear infection)
4. Put the swabs in sterile test tube or insert to transport media (Amies transport medium for swab, but can use sterile tube or container for aspirates)
5. Label the Patient's name, ID, Date, Time of collection, Collector initial and the culture Site on the specimen tube.
6. Send specimen to the laboratory immediately

D. Laboratory procedure for collection and transportation of Eye swab

1. If there is make up and ointment gently remove with sterile swab and normal saline
2. Collect specimen using the cotton swab from the affected parts of the eye and rotate the swab several times until wet (Take care of contamination of skin flora from skin outside the eye)
3. Put /Insert swab into sterile test tube or into the transport media. Push the cap to bring the swab into contact with the transport medium
4. Medium may be inoculated at time of collection

E. Laboratory procedure for collection and transportation of Throat swab

1. Let the patient tilt the head back and open the mouth wide
2. Hold the tongue down with the depressor. Use a strong light source wipe area of inflammation and exudates in the posterior pharynx and the tonsillar region of the throat behind the uvula.
3. The swab is applied to any area that appears either very red or discharging pus.
4. Label the Patient's name, ID, Date, Collection name or initials, Time of collection and the culture Site on the specimen tube.
5. Send specimen to the laboratory immediately unless we use transport media

F. Laboratory procedure for collection and transportation of Nasal swab

1. Using cotton swabs take materials from the affected area of nose
2. Insert swabs into sterile test tube
3. Add physiological saline

4. Label the Patient's name, Time of collection, Date, Collector's initial or name, specific site for collected specimen
5. Transport the specimen to laboratory. Take care of using the specimen in to loosely closed container (it will contaminate the transporter and other specimens transported together)

G. Laboratory procedure for collection and transportation of urine

1 Provide a sterile container to a patient

2 Instruct the patient to void several milliliters to the toilet, collect appropriate volume of midstream urine (5-10ml) without stopping or squeezing the genital area (will cause contamination with skin flora), finally void the remaining urine in the toilet

3 For catheterized specimens, a urine sample is taken by inserting a thin rubber tube or catheter through the urethra into the bladder. The urine is collected in a sterile container at the other end of the tube. Never try to collect urine sample from catheter bags

4 Label the Patient's Name, Time of collection, Collectors name or initial and the culture Site on the specimen tube.

5 Transport specimen to laboratory within 1hr. If not possible, refrigerate the sample to 2-8oC (will maintain up to 18hrs)

H. Laboratory procedure for collection and transportation of semen

The man should:

- Pass urine.
- Wash hands and penis with soap, to reduce the risk of contamination of the specimen with commensal organisms from the skin.
- Rinse away the soap.
- Dry hands and penis with a fresh disposable towel.
- Ejaculate into a sterile container.

Note: The time between collection of the semen sample and the start of the investigation by the microbiological laboratory should not exceed 3 hours.

Collection of semen at home

- A sample may be collected at home in exceptional circumstances, such as a demonstrated inability to produce a sample by masturbation in the clinic or the lack of adequate facilities near the laboratory.
- The man should be given clear written and spoken instructions concerning the collection and transport of the semen sample. These should emphasize that the semen sample needs to be complete, i.e. all the ejaculate is collected, including the first, sperm-rich portion, and that the man should report any loss of any fraction of the sample. It should be noted in the report if the sample is incomplete.
- The man should be given a pre-weighed container, labeled with his name and identification number.
- The man should record the time of semen production and deliver the sample to the laboratory within 1 hour of collection.
- During transport to the laboratory, the sample should be kept between 20 °C and 37 °C.
- The report should note that the sample was collected at home or another location outside the laboratory.

Collection of semen by condom

- A sample may be collected in a condom during sexual intercourse only in exceptional circumstances, such as a demonstrated inability to produce a sample by masturbation.
- Only special non-toxic condoms designed for semen collection should be used; such condoms are available commercially.
- The man should be given information from the manufacturer on how to use the condom, close it, and send or transport it to the laboratory.
- The man should record the time of semen production and deliver the sample to the laboratory within 1 hour of collection.
- During transport to the laboratory, the sample should be kept between 20 °C and 37 °C
- The report should note that the sample was collected by means of a special condom during sexual intercourse at home or another location outside the laboratory.

Comment 1: Coitus interrupts is not a reliable means of semen collection, because the first portion of the ejaculate, which contains the highest number of spermatozoa, may be lost. Moreover, there

may be cellular and bacteriological contamination of the sample and the low pH of the vaginal fluid could adversely affect sperm motility.

Comment 2: If a man cannot provide a semen sample, the postictal test.

I. Collection and transport of CSF sample

The collection of CSF is an invasive procedure and should only be performed by experienced personnel under aseptic conditions.

1 ml CSF specimens (free of blood from the lumbar puncture) should be transported to a microbiology laboratory as soon as possible. Specimens for culture should not be refrigerated or exposed to **extreme cold, excessive heat, or sunlight**. They should be transported at temperatures between 20°C and 35°C. For proper culture results, CSF specimens must be plated **within 2hour**. If a delay of several hours in processing CSF specimens is anticipated and Amies with charcoal transport media.

J. Collection and Transport of Body Fluids

The collection of Body fluids (Synovial fluid, pleural fluid, peritoneal, Ascetic fluid, pericardial fluid etc.) should only be performed by experienced personnel under aseptic conditions. >5 ml of body fluid specimen should be transported to a microbiology laboratory as soon as possible (not > 2 hrs.).Specimens for culture should not be refrigerated or exposed to **extreme cold, excessive heat, or sunlight**. They should be transported at temperatures between 20°C and 35°C. For proper culture results, specimens must be plated **within 2hour**. If a delay of several hours in processing body fluid specimens is anticipated and Amies with charcoal transport media.

K .Collection and transport of urogenital specimen

Collection Procedure of (Cervical Secretion)

1. No, cleaning of vagina for overnight.
2. Insert the speculum into the vagina canal to open the cervix
3. Remove mucus and secretions from the cervical area with swab, and discard the swab.
4. Firmly yet gently sample the endo-cervical canal with a new sterile swab.
5. Insert swabs into sterile test tube

6. Add physiological saline
7. Label the Patient's Name, Time of collection, Collectors name or initial and the culture Site on the specimen tube.
8. Transport specimen to the laboratory immediately unless we used transport media

Collection Procedure (Vaginal Secretion)

1. Instruct the patient to open her genital area fully
2. Wipe away old secretion and discharge.
3. Obtain secretion from the mucosal membrane of the vaginal wall with a sterile swabs
4. Insert the swab in to sterile container such as tube
5. Label the Patient's Name, Time of collection, Collectors name or initial and the culture Site on the specimen tube.
6. Transport to laboratory immediately

Collection procedure (Urethral Discharge) (Both male and female)

1. Instruct the patient not to wash their urethra prior to sample collection
2. Wipe away old secretion and discharge
3. Obtain secretion from the tip of urethra
4. Insert the swab in to sterile container such as tube (preferably to transport media)
5. Label the Patient's Name, Time of collection, Collectors name or initial and the culture Site on the specimen tube.
6. Add sterile normal saline and transport to laboratory immediately unless we used transport media

Annex 11. Laboratory procedure for Gram staining technique

1. Labeling the slides clearly with patient code number.

2. Making of smears by spread evenly covering an area about 15-20mm diameter on a slide.

3. Drying of smears after making smears, the slide should be left in a safe place to air-dry, protected from flies and dust.

4. Fix the dried smear by using heat or chemicals (methanol).

5. Cover the fixed smear with crystal violet stain for 30-60 seconds.

6. Rapidly wash off the stain with clean water. If the tap water is not clean, use filtered water or clean boiled rainwater.

7. Tip off all the water, and cover the smear with lugol's iodine for 30-60 seconds.

8. Wash off the iodine with clean water.

9. Decolorize rapidly (few seconds) with acetone alcohol. Wash immediately with clean water.

10. Cover the smear with neutral red or safranine stain for 2 minutes.

11. Wash off the stain with clean water.

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

13. Examine the smear microscopically, first with the 40 X objective to check the staining and to see the distribution of materials and then with the oil-immersion objective to look for bacteria and cells.

Result

• Gram positive bacteria ----------dark purple

• Gram -negative bacteria ----------pale to dark red

Annex 12. Laboratory procedure for Media Preparation

A. SOP for preparation of Blood agar plate (BAP)

AIM of Blood agar plate: A non-selective medium for the isolation and cultivation of many pathogenic and non-pathogenic microorganisms. The medium is often used to investigate the forms of hemolysis from pathogenic microorganisms from clinical specimen. Blood Agar Base formulation has been used as a base for preparation of blood agar and to support good growth of a wide variety of fastidious microorganisms. Because it is a highly nutritious medium it can also be used as a general purpose growth media without adding blood. Blood Agar Base is suitable to isolate and cultivate a wide range of microorganisms with difficult growth.

Procedure for Preparation to make about 30-35agar plates

- Measure 500ml of distilled water using a measuring cylinder.
- Transfer the distilled water into a 1litre capacity conical flask.
- Weigh 20g of Blood Agar Base II powder using a weighing balance.
- And then add into the 500ml of distilled water and mix thoroughly.
- Boil until completely dissolved
- Autoclave at 121°C for 15 minutes.
- Allow to cool to 45-50°C in a water bath.
- Once the medium has been melted and cooled to 45-50 ºC
- Add 5-10% of defibrinated sterile sheep blood. Be careful to avoid bubble formation when adding the blood to the cooled medium and rotate the flask or bottle slowly to create a homogeneous solution.
- Aseptically add 25 ml of sterile defibrinated sheep blood with constant shaking.
- When mixing, avoiding froth formation.
- Arrange the petri-dishes onto the clean safety hood.
- Gently pour 15-20 ml of the ready media on to the plates by using dispenser and allow setting.
- If air bubbles occurred, using a Bunsen burner gently invert and pass the flame over the poured blood agar in the plate to remove air bubbles. Leave standing for thirty minutes to solidify.
- Label on the bottom top of the blood agar plates the batch number & date prepared.

- Store the culture media plates upside down at 2-8^{0}C sealed in plastic bags to reduce chances of contamination.

Annex 13. SOP of Vitek 2 compact analyzer

Purpose

To describe the procedures for the preparation and identification of test microorganisms (test microbes and Quality Control Organisms) using the VITEK 2 Compact Instrument.

Procedure and Analysis

Follow the operational instructions below strictly for the proper use and required quality control activities on VITEK 2 Compact analyzer.

1. Initiation of the V2C System

- The V2C Instrument is always “on”; the instrument will say “Ready” or “Not Ready” on the digital screen. Once the computer is initialized, the instrument will say “Ready.”The V2C will not run if it is not on ready mode.

- Select VITEK 2 Compact to initiate the system from the upper left side of the screen.

- After the system is initiated, log onto the system using the appropriate user name and password.

- The system is now initialized and ready for data entry.

2. Preparation of Organisms

A. QC organisms

- If starting from a frozen stock culture, remove the 0.5 mL cryovials from the -80°C freezer. Avoid repeated thawing and freezing of the frozen culture by aseptically removing a small portion (or loopful) of the frozen inoculum, then immediately return cryovials to -80°C freezer.
- Streak isolates the inoculum from a frozen stock culture or other source onto agar plate appropriate for the QC organism.

- Following this streak isolation, a second streak isolation on the appropriate media is recommended.

B. Non-QC organisms
   - Use growth on tubes or plates to perform streak isolation on BAP or NA warmed to room temperature. A second streak isolation step is not required unless there is evidence of a mixed culture.

C. For cultures used on BCL and GN cards, incubate cultures for 18-24 h at 36±1°C. For cultures used on GP cards, incubate cultures for 12-48 h at 36±1°C. For cultures used on ANC cards, incubate cultures under anaerobic conditions for 18-24 h (or until sufficient growth is obtained) at 36±1°C. All organisms to be identified must be pure cultures.

3. Perform Gram stain using an isolated colony from a pure culture plate and document the Gram stain reaction.

4. Preparation of Inoculums

- Aseptically 3.0 ml of sterile saline (0.45% to 0.5% NaCl, pH 4.5 to 7.0) were transferred into two clear plastic (polystyrene) test tubes (12mm x 75mm).
- By using a sterile stick or swab a sufficient number of morphologically similar colonies (pure culture) were transferred into to the first saline tube.
- Pure cultures of bacterial isolates were suspended to achieve a turbidity equivalent to that of a McFarland 0.50 standard (range, 0.50 to 0.63), as measured by the Densi Check (bioMe´rieux) turbidity meter (see Table 5).
- 280 µl of the suspension prepared in the first step were transferred to the second tube containing 3 ml of saline. Then place this tube in the cassette with a susceptibility card (AST-GP71 cards).
- Fill in a cassette worksheet with the test card and specimen information for the cassette. Bar Code Scanner was used for data entry. Test cards and specimens were placed in their appropriate slots.
- The cassettes were loaded in to the filler station

.

Table 5: Suspension Turbidities Used for Card Inoculation

Card	McF Range
GN	0.5-0.63
GP	0.5-0.63
ANC	2.7-3.3
BCL	1.8-2.2

Place the prepared suspensions in the cassette (see section 15, Instrument User Manual).

To use the Densi CHEK plus Meter to read samples:

i. Ensure the instrument is ON and set to the PLASTIC tube setting.

ii. Blank the Densi CHEK Plus by filling a test tube with sterile saline and inserting the tube into the instrument. Press the "0" key and slowly rotate the test tube. Ensure one full rotation is completed before the reading is displayed. The instrument will display a series of dashes followed by 0.00.

iii. To measure a sample, place a well-mixed organism suspension into the instrument and slowly rotate the test tube. Ensure one full rotation has completed before the reading is displayed. The instrument will display a series of dashes followed by a reading.

iv. Remove the test tube after completion of a reading. The instrument will automatically shut off when test tubes are not inserted after one minute.

NOTE: If the instrument flashes 0.00 or 4.00, the suspension is either below 0.0 McF or above4.0 McF and is not within the reading range. Ensure suspensions are within the appropriate reading range to avoid compromised card results. If necessary, re-calibrate the Densi CHEK Plus instrument after processing each cassette.

5. Insert the straw (in the V2C card) into the inoculated suspension tube in the cassette.
NOTE: The age of the suspension must not exceed 30 minutes before inoculating the cards.

6. Proceed to data entry.

7. Filling the Cards

- Place the cassette in the Filler box on the left side of the V2C unit and hit Start Fill button on the instrument. Filling the cards takes approximately 70 seconds for a cassette regardless

of the number of cards in the cassette holder. The V2C instrument will beep when the filling cycle is complete.

- Discard individual cards that may have been exposed to multiple fill cycles.

NOTE: The cassette must be placed inside the Loader Door within 10 minutes from the end of the filling cycle to avoid the cards being rejected.

- When the cards are finished filling, the Load Door is automatically unlocked. Place the cassette in the Load Door. The V2C Instrument will verify the scanned barcodes against the Virtual Cassette (the information scanned in by the analyst). Cards are sealed; straws are cut and the cards are loaded automatically into the carousel. The V2C will beep once all cards are loaded into the cassette.

- When the cards are loaded, remove the cassette and dispose of the tubes and straws in biohazard container.

- The V2C automatically processes the cards once all the cards are loaded.

NOTE: Review the Navigation Tree. If the cassette status description in the Navigation Tree is red, the cassette needs more information to completely process the tests cards. Open up the red colored file and make sure all fields are defined.

8. Results

The VITEK system analyses the data results and determines the identity of the test microbes/QC organism based on colorimetric tests (biochemical reactions).Results are concurrently printed and the data sent to the Results View folder on the left side of the screen also called the Navigation Tree where the information is archived. A red cassette in the Navigation Tree is indicative of an error. If an error occurs during processing, refer to the Software User Manual.

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Nuhamin Melaku (B.Sc.)

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: Dr.Adane Bitew (PhD, Associate Professor)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.