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COLLEGE OF HEALTH SCIENCES
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Profiling and Antimicrobial Susceptibility Pattern of Non-Fermentative Gram- Negative Bacilli Isolated from Patients Referred to Arsho Medical Laboratory Addis Ababa, Ethiopia.

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This is to certify that the thesis prepared by M**ihiret Tatek**, entitled: **Profiling and Antimicrobial Susceptible Pattern of Non-Fermentative Gram- Negative Bacilli Isolated from Patients Referred to Arsho 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.

Signed by the Examining Committee:

External Examiner__________________ Signature ___________Date ____________

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Abbreviations

AST	Antimicrobial Susceptibility Tests
AMIC$_S$	Antibiogram and minimal inhibitory concentrations
AAML	Arsho Advanced Medical Laboratory
BCC	*Burkholderia cepacia* complex
CDC	Centers for Disease Control
ESBL	Extended Spectrum Beta Lactamases
GNB	Gram-Negative Bacteria
ICU	Intensive care unit
MAD	Medical Aid Unit
MDR	Multidrug resistant
McF	Mac Fernand
NFGNB	Non-Fermenting Gram-Negative Bacilli
QC	Quality Control
VAP	Ventilator-associated pneumonia

Abstract:

Background: Non-Fermenting Gram-Negative Bacilli are defined as strictly aerobic and non-spore forming group of bacteria. They are intrinsically resistant to many antibiotics. Globally the burden of multidrug resistance in Gram-negative bacilli represents a daily issue for the management of antimicrobial therapy in patients.

Objective: The aim of this study was profiling and antimicrobial susceptible pattern of non-fermentative Gram- negative bacilli isolated from patients referred to Arsho Advanced Medical laboratory Addis Ababa, Ethiopia.

Methods: A cross sectional study were conducted in Arsho advanced medical laboratory Addis Ababa, Ethiopia from January 2020 to August 2021 on 705 clinical samples. From this, isolates were detected from different clinical samples such as urine, body fluid, blood, CSF and other samples which was collected from patients. All the samples received in the microbiology laboratory were inoculated on blood agar, MacConkey agar and chocolate agar and incubated at 37°C for 48hr. Non fermentative Gram-negative bacilli was isolated and identified from clinical specimens by standard procedure and antibiotic sensitivity test was performed by VITEC 2. Gram reaction of the organisms was determined by Gram stain.

Results: From the total of 705 various specimens, 137 (34.5%) were culture positive for Non-fermentative Gram-negative bacteria, Among the isolated bacteria, the highest number was *Acinetobacter baumannii* 26/137(3.7%). While *Pseudomonas aeruginosa* accounts 20/137 (2.8) followed by *Proteus mirabilis* 16/137 (2.3%). Among NFGNB *P. aeruginosa* (100%), *and A. baumanii* (100%) were multi drug resistance.

Conclusion: Antimicrobial resistance and multi drug resistance was performed during the study period was high, it is due to ordering antibiotic without knowing the right pathogen and also unlimited use of antibiotics.

KEY WORD Non-fermentative, Gram- negative bacilli, antimicrobial susceptible, antimicrobial resistantAddisAbaba,Ethiopia.

1. Introduction:

1.1 Background

Aerobic Non-Fermenting Gram-Negative Bacilli (NFGNB) are a taxonomically diverse group of organisms**.** NFGNB are defined as strictly aerobic and non-spore forming group of bacteria that do not ferment carbohydrates but generate energy required for its metabolic activities by oxidative pathway. NFGNB bacteria have emerged as important healthcare-associated pathogens as they exhibit intrinsic multidrug resistance (1).

NFGNB, like *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Stenotrophomonas maltophilia*, are important nosocomial pathogens contributing significantly to morbidity and mortality, while others are being increasingly recognized as clinical pathogens (2) (3).

These organisms are ubiquitous in nature particularly in soil and water. Within the hospital environment, they'll be isolated from instruments like ventilator machine humidifiers, mattresses, and other equipment's likewise as from the skin of healthcare workers (4).

These organisms are generally saprophytic, but cause opportunistic infections in seriously ill, hospitalized and immunocompromised patients. They're particularly related to tract infections, ventilator pneumonia, surgical site infections and bacteremia (5).

Antibiotics are medicines that used to prevent and treat infections caused by bacteria. Antibiotic resistance develops when bacteria evolve in response to the utilization of those medicines. Antibiotic resistance finally leads to higher medical costs, prolonged hospital stays, and higher morbidity and mortality rate (6). The burden of antimicrobial resistance in Gram-negative bacilli (GNB) becomes an everyday challenge to face for medical aid unit (MAU) physicians. Indeed, GNB are responsible for 45–70% of ventilator-associated pneumonia (7)**.**

Isolations in some countries of multi-drug-resistant (resistant to some or more classes of antimicrobials), extensively-drug-resistant (resistant to any or about one or two classes) or perhaps pan-drug resistant (resistant to all or any available classes) Gram-negative pathogens are causing therapeutic problems and within an equivalent time are posing infection control issues in many hospitals (8).

Antimicrobial resistance among GNB is increasing worldwide. This may be often a significant public pathological status and a cause for both substantial morbidity and mortality (9).

Several of the clinically important non-fermenters are multi resistant organisms and coverage for infections caused by non-fermenters are somewhat different from those for infections caused by fermenters (10) (11).

Centers for Disease Control and Prevention (CDC) reports as microorganisms with a threat level of serious: multidrug resistant (MDR) *Pseudomonas aeruginosa* and *Acinetobacter baumannii.* Two other groups of non-fermenting GNB didn't make the list, probably due to their relatively lower incidence of infection: *Stenotrophomonas maltophilia* and *Burkholderia cepacia* complex (Bcc). Despite their lower incidence, these two groups of organisms also represent growing concerns, particularly to vulnerable hospitalized and immunosuppressed patients (12).

Among non-fermenting Gram-negative bacilli, the foremost clinically important species are *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Stenotrophomonas maltophilia*, which are frequently multi resistant. *P. aeruginosa* resistance to beta-lactams depends on the assembly of chromosomal and plasmid-mediated beta-lactamases, altered permeability and active efflux system (13).

Enterobacteriaceae are a neighborhood of the environmental microflora and include common animals' commensals. *Enterobacteriaceae* strains ingested through food may contain Extended Spectrum Beta Lactamases (ESBL) and plasmidic AmpC (pAmpC) genes found on mobile genetic elements. These isolates can then colonize humans or their genes are transferred to other bacteria during transit within the intestinal tract (14).

Treatment of infections caused by Gram-negative non-fermenters is challenging and expensive due to multidrug resistance in these bacteria. When dealing with these organisms, better susceptibility testing, antibiotic stewardship, and infection-control measures are required to prevent or slow the emergence and spread of multidrug-resistant, nonfermenting gram-negative bacteria in health care unit (15).

In Ethiopia, NFGNB are poorly known due to poor laboratory organization, laboratory equipment and supplies. The lack of clinical microbiology laboratories to identify the specific etiologic agents and their antimicrobial susceptibility testing has increased empirical therapy which in turn leads to emergence of antimicrobial resistance. Moreover, self-antibiotic prescription, lack of access to local antibiogram data and poor awareness of prescriber about

antimicrobial resistance were the leading local factors for antimicrobial resistance development in Ethiopia.

1.2 Statement of the Problem:

Nosocomial infections are one in all the foremost common complications of hospitalization and lead to increased morbidity and mortality. Infection with multidrug-resistant pathogens may also complicate treatment (16).

Antibiotic resistance remains a serious threat to public and animal health globally. However, antimicrobial consequences in developing countries are worsened by lack of molecular diagnostics, expensive treatments, inadequate numbers of qualified clinicians and scientists, and unsanitary environments (17).

Resistance to antimicrobial agents could be an increasing clinical problem and is a recognized public health threat (18). *P. aeruginosa* is one of the most frequent and severe causes of acute nosocomial infections, particularly affecting immunocompromised patients or those admitted to the intensive care unit (19).

Risk factors for the development of infections caused by NFGNB like *Pseudomonas* include neutropenia, cystic fibrosis, severe burns, and foreign device installations. The general human population is refractory against infections caused by *Pseudomonas* species. *Pseudomonas* species are physiologically highly flexible and able to act as opportunistic pathogens in humans with weakened immune system. *Pseudomonas aeruginosa* is a notoriously difficult organism to manage with antibiotics or disinfectants (20) (21).

And *Acinetobacter baumannii* is a non-fermentative Gram- negative coccobacillus that is emerged as opportunistic nosocomial pathogen. *Acinetobacter baumannii* bacteremia are invasive procedures such as central venous catheterization, mechanical ventilation and surgical procedures. Other risks include previous antimicrobial treatment, number of antimicrobial agents prescribed, treatment with carbapenems, cephalosporins or aminoglycosides, prolonged hospital stay, previous ICU stay, enteral nutrition (22).

Due to the lack of comprehensive studies about the identification of the microbiological cause of a case and especially important for preservation of the sensitivity of bacteria to antibiotics and for regulation of drug therapy this study aimed to evaluate profiling and antimicrobial susceptible pattern of non-fermentative gram- negative bacilli isolated from patients referred to Arsho Medical laboratory Addis Ababa, Ethiopia during Jan 2020- August 2021.

1.3 Significance of the study

The findings of this study have a significant impact on the development and implementation of antimicrobial susceptibility control programs, as well as the ability to detect and manage antibiotic prescriptions, as well as monitor trends in drug resistance transmission. In this study, we also characterize the occurrence of drug resistant of NFGNB isolates**.**

The findings of this study will also be used to identify NFGNB and monitor their susceptibility patterns, which may help clinicians provide better patient care and treatment. This study can also be used to conduct epidemiological investigations of non-fermentative gram-negative bacilli antimicrobial susceptibility patterns in the country, as well as to detect trends in prevalence and drug resistance patterns.

Furthermore, it may persuade policy-makers, program managers, and scientific communities to take necessary steps and measures for the well-being of patients.

2. Literature Review:

A study conducted in United States total of 74,394 isolates were characterized between the organisms most often isolated were *P. aeruginosa* (22.2%), *E. coli* (18.8%), *K. pneumoniae* (14.2%), *Enterobacter cloacae* (9.1%), *Acinetobacter* spp. (6.2%), *Serratia marcescens* (5.5%), *Enterobacter aerogenes* (4.4%), *Stenotrophomonas maltophilia* (4.3%), *Proteus mirabilis* (4.0%), *Klebsiella oxytoca* (2.7%) and *Citrobacter freundii* (2.0%). These 11 species accounted for 93.4% of the whole number of isolates. The urinary tract (52.1%), urine (17.3%), and blood cultures (14.2%) were the sources of ca. 84% of isolates. *P. aeruginosa* was the organism most often isolated within the urinary tract (26.9%), while *E. coli* was most often isolated from both urine (42.4%) and blood (23.9%) (23).

A cross-sectional study was conducted in Saudi Arabia, out of the 8908 non-fermenters, most were *Pseudomonas aeruginosa (72.9%),* followed by *Acinetobacter baumannii (25.3%)* and *Stenotrophomonas maltophilia* (1.8%). Resistance rates among *P. aeruginosa* were*:* polymyxin B, 2.2%; imipenem, 15.9%; ciprofloxacin, 22.0%; amikacin, 22.9%; and gentamicin, 31.2%. Resistance rates among *A. baumannii* were: imipenem, 5.4%; polymyxin B, 13.2%; ciprofloxacin, 64.0%; trimethoprim/sulfamethoxazole, 73.8%; amikacin, 76.9%; and gentamicin, 77.8%. Resistance rates among *S.* maltophilia were: polymyxin B, 6.9%; trimethoprim/sulfamethoxazole, 20.5%; and ciprofloxacin, 38.9%. There was major variation in resistance rates between nations. Resistance rates among non-fermenters were high in Asian nations and were variable among regions (24).

A study conducted in Pakistan reported that the prevalence of MDRs was reported 100% among *A. baumannii*. The antibiotic susceptibility profile showed that minocycline and tigecycline were the foremost effective drugs against *A. baumannii*. Most of *A. baumannii* isolates were carbapenemase and metallo β-lactamase producers. AmpC prevalence was observed in 41.76%, while none of the isolates was ESBL producer. Antibiogram and minimal inhibitory concentrations (MICs) indicated tetracycline is comparatively effective against Highest prevalence of *A. baumannii* was found in neo-natal intensive care unit NICU (85%), followed by Medical (ICU) (78%) and therefor the least in out-patient department (89%). Among 91 patients infected with *A. baumannii*, the higher percentage belonged to the age-group between 0-29 days

(85%) followed by age groups between 40-60 years (68%) and age groups between 1-20 years (14, 15.38%) (25).

A prospective study was conducted from January to December 2012 in India showed that of 122 *Acinetobacter* spp. was isolated. 110 (90.16 per cent) were from inpatients, and 12 (9.83 per cent) were from outpatients. Out of 122 isolates, 44 (36.06 per cent) were from the ICU. Most of the isolates, 47 (38.52 per cent), were from pus samples followed by 25 (20.49 per cent) from endotracheal tube aspirate. Out of 122 isolates, 87 (71.31 per cent) were multi-drug resistant from which 15 (12.29 per cent) were resistant to all drugs tested (26).

Similarly, a cross-sectional study conducted in a multi-specialty hospital of Uttarakhand, India, during October 2015 to March 2016 out of 366 samples received 99 NFGNB were isolated *Acinetobacter baumannii* was the foremost common NFGNB isolated 63 (63.63%) followed by *Pseudomonas aeruginosa* 25 (25.25%), *Elizabethkingia meningoseptica* seven (7.07%) and *Stenotrophomonas maltophilia* four (4.04%). We observed that 90.5% *Acinetobacter baumannii* were immune to imipenem and 95.2% resistant to meropenem, *Pseudomonas aeruginosa* came resolute be 52% resistance to imipenem and 56% resistance to meropenem while *Stenotrophomonas maltophilia* and *Elizabethkingia meningoseptica* were 100% resistance to carbapenems as they're intrinsically resistant to carbapenems (27).

Another cohort study conducted in China shows that *A. baumannii* isolates were mainly distributed in respiratory department, ICU, and emergency department, accounting for 21.7%, 16.7% and 11.7%, respectively. while multidrug-resistant strains, were mainly in ICU and emergency department, accounting for 29.7% and 20.3%, respectively. Among 120 drug resistant strains, 64 were multidrug resistant (28).

Another prospective study conducted from India shows that of 2793 samples were received for culture and sensitivity during the study period, among which 100 NFGNB were isolated accounting for an isolation rate of 58%. Age of our cases ranged from day 4 to 80 years, males were 68 and females were 32 with male to female ratio of two.125. Maximum number of cases were observed within the cohort of 21-30 years and 41-50 years. Among the NFGNB isolated *P. aeruginosa* (60%) was the foremost common followed by *Acinetobacter baumannii* (22%), *Acinetobacter lwoffii* (12%), *Sphingobacterium* spp. (3%) and *Pseudomonas stutzeri,*

Flavobacterium spp. and *Stenotrophomonas maltophila* (1%) each. Most of the isolates of *P. aeruginosa* showed highest sensitivity to amikacin (83.3%) and least sensitivity to piperacillin (38.3%). *A. lwoffii* were also sensitive to most of the drugs. *Flavobacterium*, *S. maltophila* and *P. stutzeri* showed 100% sensitivity to all or any the antibiotics. However, *A. baumannii* and *Sphingobacterium* were resistance to most of the drugs (29).

A descriptive cross-sectional study was conducted in North of Iran, out of 83 patients who were microbiologically diagnosed as Ventilator-associated pneumonia [VAP] within the ICU wards of the 18 hospitals of Mazandaran province, 52 non-duplicated NFGNBs (*24 P. aeruginosa and 28 A. baumannii)* were causative of VAP, among which MDR NFGNBs were chargeable for 48 (57.83%) cases. The most frequently found MDR NFGNBs were*: Acinetobacter baumannii* 27 (56.25%) and *Pseudomonas aeruginosa* 21 (43.75%). Other agents of VAP belonged to the Enterobacteriaceae family. The demographic features of patients with MDR *P. aeruginosa* were 21 patients (11, 52.38%, male and 10, 47.61%, female) with a mean age of 41.01±24.05 years and a mean duration of hospitalization within the ICU of 17.69±14.51 days; 27 patients (17, 62.96% male and 10, 37.03% female) with a mean age of 62.17±18.31 years and a mean duration of hospitalization within the ICU of 12.43±4.23 days (30).

Another analytical cross-sectional study was applied in South Benin (West Africa), There have been a total of 111 Gram-negative strains. *Enterobacteriaceae* (62.16%), NFGNB (33.33%) and 4.50% of other GNB were identified. *Acinetobacter baumannii* (6.79%), *Bacillus* spp (22.62%), *Escherichia coli* (5.86%) and *Staphylococcus aureus* (18.55%) were present in all samples together. The NFGNB strains consisted of 18 species. *Acinetobacter baumannii* (40.54%) and *Pseudomonas oryzihabitans* (27.02%) are the foremost represented. *Acinetobacter baumannii* is resistance to several antibiotics. It can cause high mortality, up to 35% (31).

A cross-sectional study was conducted in Egypt, out of 516 cultivated clinical samples, 97 isolates (18.7%) were identified as being NFGNB. Among them, *P. aeruginosa* (44/97) had higher isolation frequency (45.4%) followed by *Acinetobacter baumannii* (36/97, 37.1%) then Bcc (16/97, 16.5%) and eventually *Burkholderia pseudomallei* (B. *pseudomallei)* which had the lower isolation frequency (only one isolate, 1.03%). Tigecycline antibiotic was the foremost effective antibiotic against Bcc isolates (68.8% susceptibility) in disc diffusion method (32).

A retrospective descriptive study conducted in South Africa shows that among adults, 740 non-duplicate pathogens were isolated. Nearly three-quarters of infections were healthcare acquired. Enterobacteriaceae and non-fermentative Gram-negative bacilli were predominant among healthcare-acquired pathogens (39.2% and 28.5%, respectively), while *Enterobacteriaceae* and Gram-positive organisms were the foremost common among community-acquired pathogens (39.2% and 54.3%, respectively). The bulk of community-acquired *Enterobacteriaceae* were highly at risk of antibiotics (gentamicin 95.6%, ceftriaxone 96.1% and ciprofloxacin 92.2%), whereas 64.6% of healthcare-associated isolates were prone to gentamicin, 58.5% to ceftriaxone and 70% to ciprofloxacin. All community-acquired *Staphylococcus aureus* isolates 52.4% of healthcare-acquired isolates were prone to cloxacillin. The susceptibility of healthcare-acquired *Pseudomonas aeruginosa* and *Acinetobacter baumanii* complex isolates was <80% to all or any antibiotics with the exception of colistin. *Klebsiella* spp, *S. aureus* and *Escherichia coli* were the most typical causes of healthcare acquired infections in all areas outside of the ICUs, whereas *Acinetobacter* was common in the ICUs and rare in all other areas (33).

A study conducted in Ethiopia showed that of 996 clinical samples processed, NFGNB were recovered in 135 samples. Among the isolates, 78 (57.8%) were *Pseudomonas* species and 47 (34.8%) were *Acinetobacter* species. The remaining 10 (7.4%) isolates were represented by bacteria belonging to six different genera of NFGNB. *Pseudomonas aeruginosa* (45.2%) and *A. baumannii* (20.7%) were the 2 most often isolated NFGNB. The general resistance rates of NFGNB were above 80% against ampicillin (89.6%), cefuroximeaxetil (88.9%), nitrofurantoin (85.9%), cephalothin (84.4%), cefoxitin (83.7%), cefazolin (83.0%), and cefuroxime (83.0%). Tobramycin with a resistance rate of 19.3%, gentamicin with a resistance rate of 23.7%, piperacillin/tazobactam combination with a resistance rate of 25.9%, and cefepime with a resistance rate of 25.9% were better active antimicrobial agents. *Pseudomonas aeruginosa* showed less resistance rates against tobramycin (6.6%), gentamicin (13.1%), piperacillin/tazobactam combination (16.4%), cefepime (19.7%), ciprofloxacin (19.7%), levofloxacin (23.0%), and ceftazidime (27.9%). The resistance rates of the pathogen toward another antimicrobial agents tested were over 85% (34).

3. Objectives:

3.1. General objective

To determine profiling and antimicrobial susceptible pattern of non-fermentative Gram- negative bacilli isolated from patients referred to Arsho Medical laboratory Addis Ababa Ethiopia.

3.2. Specific objectives:

To isolate and to identify NFGNB in all the clinical samples

To determine antibiotic susceptibility pattern of the isolated NFGNB

4. Materials and methods

4.1. Study area:

The study was conducted in Arsho Advanced Medical Laboratory. I t is locate in Addis Ababa Bole sub city. Arsho is a brand name of private diagnostic Laboratory practices in Ethiopia. It originated in 1972 in a small individual practice and grows up to internationally accredited big service and name. The Laboratory qualifications comprise a wide variety of areas, including, Clinical Chemistry, Hematology, Histopathology & Cyto-pathology, Microbiology/Virology, Serology and Molecular Diagnosis. The laboratory is always functioning, changing, and growing providing best quality laboratory service to medical practitioners and their patients for many decades. AML is focused on delivering quality diagnostic testing in the most cost-effective way.

4.2. Study design and period:

A cross sectional study was conducted from January 2020 to Aug 2021

4.3. Population:

4.3.1. Source population

All patient specimens which were submitted for routine culture and susceptibility testing to Arsho advanced medical laboratory

4.3.2. Study Population

Samples which were referred for bacterial culturing at AML.

4.4. Inclusion and exclusion criteria

4.4.1. Inclusion criteria:

All samples referred for culturing to Arsho Advanced medical laboratory microbiology department that are clinically suspected of anti-microbial susceptible were included in the specified period of the study.

No history of antibacterial drug therapy for not less than 15 days prior to their attendance was also include in the study.

4.4.2. Exclusion criteria:

Patient request format that lacking full clinical data was excluded.

4.5. Study variables

4.5.1. Dependent variables:

- Bacterial profiling of NFGNB and
- Antimicrobial susceptible pattern of NFGNB isolated from patients were used as dependent variable.

4.5.2. Independent variables:

The age, the sex, and type of clinical specimen.

4.6. Measurement and Data collection

4.6.1. Sample size calculation:

The sample size was determined based on the prevalence data from previous publication of 'High Prevalence of Multi-Drug Resistance and Extended Spectrum Beta Lactamase Production in Non-Fermenting Gram-Negative Bacilli in Ethiopia ' using the formula for single proportion and with a desired 5% margin of error and 95% confidence interval plus 10% non-response rate.

n= (Zα/2).2P(1-P)/d2 Where;

n = the sample size

$(Z\alpha/2).^2$ = at 95% confidence interval Z value (α = 0.05). = 1.96

P- High Prevalence of Multi-Drug Resistance and Extended Spectrum Beta Lactamase Production in Non-Fermenting Gram-Negative Bacilli in Ethiopia 50% (0.5).

d = margin of error at 5% (0.05).

$n= (1.96).^2pq/d^2$

$(1.96)^2*0.1355*0.8645/ (0.05)^2$

3.8416*0.1355*0.8645/0.0025

180+10%

n=198

The minimum Sample size is therefore=198

Our sample size was as we explained above but in Arsho Advanced medical laboratory runes an average of 40 to 50 microbiology test per day so we decide to increase our sample size so we collect and processed 705 various clinical sample that we gate during the study period.

4.6.2. Sampling Method:

Convenient sampling method were applied.

4.6.3. Data collection procedure:

Demographic and clinical data of each patient was obtained using standardized work sheet.

4.6.4. Laboratory analysis and principle

4.6.4.1 Specimen collection

Different non-repeated clinical samples such as urine, blood, pus and CSF was collected from patients according to the site of infection, using standard microbiologic methods. Different clinical specimens received in bacteriology section of laboratory were inoculated in to appropriate culture medium by inoculating loop. Such as for urine specimen morning midstream were collected from patients then inoculated onto Blood Agar and MacConkey agar medium by using a calibrated loop with a capacity of 1μl, for blood culture analysis blood culture bottles containing brain heart infusion with eight up to ten ml patient blood were incubated at 37 ^{0} C for 24 hours aerobically in 5 % CO2 incubator. Pure isolates of bacterial pathogen were characterized by Vitek compact 2 analyzer and characterization of colony morphology, Gram-stain, before being reported as No growth.

4.6.4.2 Inoculation from primary isolation

We select the appropriate card based on the Gram stain reaction and the organism's microscopic appearance. And wait for cards to come to room temperature before opening the package liner. Aseptically transfer at least 3 mL of sterile saline into a clear polystyrene 12×75 mm test tube, prepare a homogenous organism suspension by transferring three up to five pure isolated colonies from the plates to the saline tube. Adjust the suspension to the McFarland standard required by the ID reagent using a calibrated V2C DensiCHEK Plus Meter. The age of the suspension must not exceed 30 minutes before inoculating the cards. Then Proceed to data entry, filling the card, when the cards are finished filling, the load door is automatically unlocked, then we put the cassette in the load door. When the cards are loaded, the V2C

automatically processes the cards once all the cards are loaded When the cards are processed and results obtained, cards will be automatically ejected into the waste collection bin (37).

4.6.4.3 Identification and drug susceptibility testing

4.6.4.3.1 Identification

Identification was achieved by VITEK-2 compact system (uses a fluorogenic method) using a Gram Negative (GN72) cards according to the Manufacturer's instructions. GN identification card has 64 wells, containing 41 testing substrates such as Ala-Phe-Pro-Arylamidase, Adonitol, L-Pyrrolydonyl-Arylamidase, L-Arabitol, D-Cellobiose, Beta-Galactosidase, H2S production and the like including;18 testing wells for sugar assimilation, 2 decarboxylase tests and 3 miscellaneous tests (for utilization of malonate, urease and tryptophan deaminase).

Inside the filling chamber the culture suspension will be inoculated into the GN Card by the help of a vacuum device then transferred in to loading chamber to be sealed and incubated in a rotating carousel at 37°C. Every 15 minutes the cards will be remove and transported to the optical system for reaction readings and returned again to the carousel incubator until the next read time. Data was captured at 15-minute intervals during the whole incubation period (38).

4.6.4.3.2 Drug susceptibility testing

Antimicrobial Susceptibility testing was also done simultaneously performed by VITEK-2 compact system (a turbidimetric method) using AST GN72 cards according to the Manufacturer's instructions. This card is intended for use with the VITEK-2 systems to determine the susceptibility of clinically relevant aerobic Gram-negative rod to antimicrobial agents. This includes; Ampicillin, Amoxicillin/Clavulanic Acid, Cefalotin, Cefazolin, Cefepime, Cefoxitin, Cefpodoxime, Ceftazidime, Ceftriaxone, Cefuroxime, Ciprofloxacin, Gentamicin, Levofloxacin, Nitrofurantoin Piperacillin/Tazobactam, Tetracycline Tobramycin Trimethoprim/sulfamethoxazole (38).

The cards were filled with an inoculum (Prepare by transferring 185µL of culture suspension from the 0.5 McFarland culture suspensions, which was prepared for identification card in the filling chamber. 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 Minimum Inhibitory Concentrations (MICs) or clinical category in accordance with the internal database (referred from CLSI guideline) which were prepared for possible phenotypes for microorganism antimicrobial agent combination (38).

4.6.3.4 Principle

The Vitek 2 Compact (30 card capacity) system uses a fluorogenic methodology for organism identification and a turbidimetric method for susceptibility testing using a 64 well card that is barcoded with information on card type, expiration date, lot number and unique card identification number. Test kits available include ID-GN (Gram negative bacillus identification), ID-GP (Gram positive cocci identification), AST-GN (Gram negative susceptibility) and AST-GP (Gram positive susceptibility). The Vitek 2 ID-GN card identifies 154 species of *Enterobacteriaceae* and a select group of glucose nonfermenting Gram negative organisms within 10 hours. The Vitek 2 ID-GP card identifies 124 species of *staphylococci*, *streptococci*, *enterococci* and a select group of Gram-positive organisms within 8 hours or less. The Vitek 2 antimicrobial susceptibility tests are for most clinically significant aerobic Gram-negative bacilli, *Staphylococcus* spp., *Enterococcus* spp., and *Streptococcus agalactiae*. Susceptibility results are available for bacteria in less than 18 hours (38).

4.7. Quality Assurance

Pre-analytic phase; Demographic and clinical data was collected from patients request paper were obtained. All the information obtained was coded to maintain confidentiality. All bacteriological samples will be cheeked for the fulfillments of all specimen acceptance criteria's such as; having full patient information, type of specimen and test order, proper labeling, date and time of specimen collection, amount and quality of the specimen and make sure that all information on the request forms was fulfilled before submitting and processing the specimen. All culture media`s was prepared according to the manufacturer instruction then after the sterility of prepared media`s was verified by overnight incubation at 35-37°c and checked the absence of visible microbial colonies then the performance of culture media`s was checked again using ATCC *P. aeruginosa* 27853 and stored properly at 2-8 ^{o}c.

Analytic phase; The Vitek-2 Compact machine was validated using standard strains *P. aeruginosa* ATCC 27853, *Acinetobacter baumannii* ATCC BAA-747, *Escherichia coli* ATCC

25922 and *Klebsiella pneumonia* ATCC 700603 as per the manufacturer's instructions. Before inoculation of the specimen sterility and performance of the culture media was checked by inoculation of those known strain. All shipments of new lots for ID and sensitivity cards must be entered in the VITEK 2 Computer prior to use, if not the QC cards may terminate. All QC results must be reviewed and any unexpected results were investigated and susceptibility results are available for bacteria in less than 22 hours.

Post analytic phase; All captured laboratory results including dependent variable, independent variables and epidemiological data was checked at a time of enrolment for its completeness, during data entry and during result analysis double entry was used to check the correctness of the data.

4.8. Data analysis and interpretation:

Data entry, cleaning and analyses was done using SPSS software version 25. A double data entry was also implemented to avoid data entry errors.

4.9. Operational definitions:

- **Nosocomial infections-** A nosocomial infection is specifically one that was not present or incubating prior to the patient's being admitted to the hospital, but occurring within 72 hours after admittance to the hospital (35) (36).
- **Antimicrobial resistance**- the resistance pattern of bacteria for the given concentration of antibiotics based on CLSI guideline (30).
- **MDR** microbials that acquired non-susceptibility to at least one agent in three or more antimicrobial categories (39).

4.10. Ethical considerations

All relevant documents were submitted to AAU for necessary ethical approval, after approval by scientific and ethical review committee of AAU. Official letter of co-operation from the school was written to Arsho Advanced medical laboratory. The confidentiality of sampled client information was kept.

4.11. Dissemination of the result

The final printed finding of this study was reported to AAU. And also, it may present in conferences and scientific meetings and disseminate and publish in different journal.

5. Results

In this chapter of our study, presents various data obtained from the various clinical samples analyzed though SPSS version 25. It consists of the socio demographic information of the research participants (demographic variables) in terms of their sex and age. Besides, it contains the results of data analysis of type of clinical specimen, isolated bacteria and the analysis of antimicrobial test.

5.1 Socio-demographic characteristics

In our study we used 705 clinical samples. out of 705 clinical samples 475(67.38%) were taken from female and the remaining 230(32.62%) were from males.

Majority of the clinical sample was taken from age group of 25-44 which is 39.15% of the total clinical samples. On the other hand, 25.25% of the clinical samples were taken from age group of 45-64. And 106(15.04%) clinical samples were taken from age group of greater than 65. The remaining clinical samples comprise of 9.5% and 1.42% which are taken from clinical samples with the age range of 1-14 and less than 1 respectively. (Table 1)

The association of the isolated bacterial pathogen from the taken samples with gender was statistically insignificant (p = 0.983).

Table 1. Geographical distribution of Sex and Age with percent.

Age	**Sex**		Total number percent
	Female	**Male**	
Less than 1	5	5	10(1.4%)
1-14	54	13	67(9.5%)
15-24	53	15	68(9.6%)
25-44	204	72	276(39.1%)
45-64	102	76	178(25.2%)
>65	57	49	106(15%)
Total	475	230	705(100%)

WHO age classification (40)

Majority of the NFGNB isolates were identified from urine 343 (48.7%) sample followed by blood 158 (22.4%), pus 76 (10.8%), CSF 54 (7.7%), semen 13 (1.8%), eye 4 (0.6), ear 25 (3.5%), and body fluid 25 (3.5%) was cultured for NFGNB. (Table 2)

Table 2. Frequency of bacterial isolate from the clinical specimen during the study period.

Specimen's type	Frequency	Percent
Urine	343	48.7
Blood	158	22.4
Pus	76	10.8
CSF	54	7.7
Semen	13	1.8
Eye swab	4	0.6
Ear swab	25	3.5
Body fluid	25	3.5
Nasal swab	7	1.0
Total	705	100.0

5.2 Prevalence of bacterial isolates from the processed clinical samples

In our study out of 705 total clinical specimens 137 Gram negative bacterial was isolated with 21 different types of bacteria. Among the isolated bacteria the highest number was *A. baumannii* 26 (37%). While *P. aeruginosa* accounts 20 (28%) followed by Proteus mirabilis 16 (23%), *P. pseudoalcaligerus* 10 (14%), *Acinetobacter Iwoffii* 10 (14%) *P. Putida* 7 (1.0%). On the other hand, *C. freundii*, *Citrobacter koseri* and *Provendentia rettgeri* accounts for 5(0.7%) of each. The details of the remaining isolated bacteria are presented in the upcoming table. (Table 3)

Table 3: Frequency distribution of NFGNB bacterial isolated from different clinical specimens (n=137)

Isolates	**Types of clinical specimens**									**Total**
	Urine	**Blood**	**Pus**	**CSF**	**Semen**	**Eye swab**	**Ear swab**	**Body fluid**	**Nasal swab**	
P. aeruginosa	11(55%)	0 (0%)	7 (35%)	2 (%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	20
P. fulorescens	0 (0.0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (100%)	0 (0%)	4
A. calcoaceticus	4 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4
C.freundii	5 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5
A. baumanii	10 (38%)	9 (35%)	0 (0%)	7 (27%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	26
S.maltophilia	0 (0.0%)	0 (0%)	3 (33%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3
Burkholderia cepacia	4 (80%)	0 (0%)	1 (20%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5
P.stuarti	4 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4
Proteus mirabilis	7 (43%)	0 (0%)	5 (31%)	0 (0%)	2 (12%)	0 (0%)	2 (12%)	0 (0%)	0 (0%)	16
E. coli (AD)	2 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2
Citrobacter koseri	5 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5
Serratia marcescery	0 (0%)	3(100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3
Provendentia rettgeri	4 (80%)	0 (0%)	1 (20%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5
Oligella Ureolytica	0 (0%)	0 (0%)	1 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (50%)	2
Morganella Morganii	0 (0%)	0 (0%)	1 (100%	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1
P. pseudoalcaligerus	0 (0%)	0 (0%)	0 (0%)	6 (60%)	0 (0%)	0 (0%)	4 (40%)	0 (0%)	0 (0%)	10
Proteus Vulgaris	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (33%)	0 (0%)	2 (67%)	3
Aeromonas hydrophilla	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (100%	0 (0%)	0 (0%)	2
Acinetobacter Iwoffii	0 (0%)	7 (70%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (30%)	0 (0%)	0 (0%)	10
P. putida	7(100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	7
Total	63/343	19/158	19/76	15/54	2/13	0/4	12/25	4/25	3/7	137/705

5.3. Antibacterial Resistance pattern of NFGNB isolates

As it is depicted on the table below the species-specific antimicrobial resistance rates are presented and *A. baumanni,* was the most frequently isolated bacterium, with resistance rates of 100% for ampicillin and amoxicillin. On the other hand, 73.1% resistance and 26.9% sensitive for the antimicrobial Augmentin, Ceftazidime, Tetracycline, Tobramycin, and Meropinum. While this frequently isolated bacterium was 26.9% resistance and 73.1% sensitive for Cefazoline, cefuroximeAxetile and Gentamycin. *A. baumanii* was also 100% sensitive for the anti-microbial cefepime, cefpodoxime, cefuroxime, cotrimoxazole, levofloxacin, nitrofurantoine, and piperacilline. The second highly isolated bacteria were *P. aeruginosa* with 100% resistance rate to ampicillin, amoxicillin, augmentin and tobramycin. (Table 4)

Table-4 Antibacterial Resistance pattern of NFGNB isolates in Percent

Species	**Antimicrobial Agents**																	
	AMX	AMP	AGM	CFZ	CFP	CFX	CFD	CAZ	CRX	CFRO	CFROA	CPL	CIP	COM	GM	LEV	NFT	PIP
P.aeruginosa (20)	100	100	100	30	30	30	30	30	30	30	30	30	30	80	50	30	30	30
P.pseudoalcaligerus (10)	100	100	100	100	100	100	100	100	100	100	12.3	100	100	100	100	100	100	100
P.putida (7)	100	100	100	100	0	0	100	0	100	100	100	0	100	100	100	100	100	100
P.fulorescens (4)	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0	0.0	0.0	0.0	0.0
A.baumanii (26)	100	100	73.1	26.9	0	100	0	73.1	100	0	26.9	100	0	0	26.9	0.0	0.0	0.0
Acinetobacter Iwoffii (10)	70	70	0	30	70	0	100	70	0	0	70	30	0.0	0	0.0	0.0	0.0	70
A.calcoacticus (4)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Proteus mirabilis (16)	100	100	0	93.8	0	0	0	0	93.8	0	93.8	0	0	93.8	0.0	0.0	93.8	0.0
Proteus Vulgaris (3)	100	100	66.7	66.7	66.7	66.7	66.7	66.7	66.7	66.7	66.7	0	0	0	0.0	66.7	66.7	66.7
Provendentia rettgeri (5)	100	100	100	20	20	20	21.7	20	20	20	20	20	100	20	0.0	0.0	20	20
Burkholderia cepacia (5)	100	100	100	100	100	100	100	100	100	100	100	100	100	40	80	100	100	100
P.stuarti (4)	100	100	100	0	0	0	0	0	0	0	0	0	100	0	0.0	100	0.0	0.0
S.maltophilia (3)	33.3	33.3	33.3	100	33.3	100	33.3	33.3	33.3	33.3	100	100	100	0	33.3	0.0	33.3	33.3
E.coli(AD) (2)	50	50	50	50	50	0	50	50	50	50	100	100	50	100	0.0	50	0.0	100
Citrobacter koseri (5)	100	0.0	0.0	0	0	0	0	0	0	100	100	0	0	0	0.0	0.0	0.0	0.0
Serratia marcescery (3)	0	0	100	100	0	100	0	0	0	100	100	100	0	0	0.0	0.0	0.0	0.0
Oligella Ureolytica (2)	100	100	50	100	100	100	100	100	100	100	100	100	100	50	50	0.0	3.6	100
Morganella Morganii (1)	100	100	100	100	0	100	0	0	100	100	100	100	0	100	0.0	0.0	0.0	0.0
Aeromonas hydrophilla (2)	100	100	0.0	100	0	0	0	100	100	0	100	0	0	100	100	0.0	100	100
C.freundii (5)	100	100	100	100	0	0	100	0	0	100	0	100	0	100	0.0	0.0	100	100

AMX=Amoxacilline, AMP=Ampicilline, AGM=Agumentine, CFZ=Cefazoline, CFP=Cefepime, CFX=Cefoxitin, CFD=Cefpodoxime, CAZ=Ceftazidime, CRX=Ceftriaxone, CFRO=Cefuroxime, CFROA=CefuroximeAxetile, CPL=Cephalothine, CIP=Ciprofloxacin, COM=Cotrimoxazole, GM=Gentamycin, LEV= Levofloxacin, NFT= Nitrofurantoine, PIP= Piperacilline, TEC= Tetracyclines, TMB= Tobramycine, MRP=Meropinum

Table 5: Percentage of antibacterial susceptibility

Percentage of antibacterial susceptibility pattern of the non-fermentative Gram-negative bacteria (n=137).																					
P	AMX	AMP	AGM	CFZ	CFP	CFX	CFD	CAZ	CRX	CFRO	CFROA	CPL	CIP	COM	GM	LEV	NFT	PIP	TEC	TMB	MRP
I	0.0	0.0	3.00	0.00	0.00	2.25	2.25	0.00	1	0.0	1	0.0	0.0	0.0	4	0.0	0.0	0.0	5	0.0	0.0
R	74.5	85.7	60.14	10.4	29.32	45.1	35.3	45.1	60.9	36.8	60.9	51.8	32.3	46.6	31.57	29.3	42.1	36.0	61.65	35.33	43.6
S	25.5	14.3	36.86	8.5	72.68	52.63	63.9	54.9	38.1	63.2	38.1	48.2	67.7	53.4	64.43	70.7	57.9	64	34.35	64.67	56.4

AMX=Amoxacilline, AMP=Ampicilline, AGM=Agumentine, CFZ=Cefazoline, CFP=Cefepime, CFX=Cefoxitin, CFD=Cefpodoxime, CAZ=Ceftazidime, CRX=Ceftriaxone, CFRO=Cefuroxime, CFROA=CefuroximeAxetile, CPL=Cephalothine, CIP=Ciprofloxacin, COM=Cotrimoxazole, GM=Gentamycin, LEV= Levofloxacin, NFT= Nitrofurantoine, PIP= Piperacilline, TEC= Tetracyclines, TMB= Tobramycine, MRP=Meropinum P =pattern I= intermediate R= resistanc S=sensitive.

Percentage of antibacterial susceptibility pattern of non-fermentative Gram-Negative bacteria

	R_0	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	$R_{>8}$	N0 of MDR

against the twenty-one anti-bacterial drugs tested is summarized in the table above. Ampicillin resulted with the highest overall resistance rate of (85.7%) against non-fermentative Gram-negative bacteria, followed by amoxicillin (74.5%), and tetracycline (61.65%). The non-fermentative Gram-negative bacteria showed highest sensitivity towards cefepime (72.68%), followed by levofloxacin, ciprofloxacin, and to tobramycin with the overall sensitivity rates 70.7%, 67.7%, and 64.67%, respectively. (Table 5)

5.4 Prevalence of multi drug resistance in NFGNB against different classes of antibiotic

Multi drug resistance pattern of NFGNB bacterial isolates from various clinical specimens were recovered, out of 137 isolates, 91 were MDR. Among 45 isolates of Pseudomonas species, 37 were MDR, out of 39 isolates of Acinetobacter species, 36 isolates were MDR, followed by, 26 isolates of A. baumannii, 25 were MDR, whereas out of 4 isolates of *A.calcoacticus* 4 of them were MDR. Then out of 10 isolate of *Acinetobacter Iwoffii* 7 of them is MDR. Out of 10 spec ices isolates of other NFGNB, 36 isolates were MDR. (Table 6)

P. aeruginosa (20)	3	0	1	1	8	0	1	0	0	6	16
P.fulorescens (4)	0	4	0	0	0	0	0	0	0	0	0
A.calcoacticus (4)	0	0	0	0	0	0	0	0	0	4	4
C.freundii (5)	2	0	0	3	2	0	0	0	0	1	6
A.baumanii (26)	0	0	1	1	1	0	1	0	5	17	25
S.maltophilia (3)	0	0	0	0	0	0	2	0	0	1	3
Burkholderia cepacia (5)	0	0	0	0	0	0	0	0	0	5	5
P. stuarti (4)	0	0	0	0	0	0	4	0	0	0	4
Proteus mirabilis (16)	0	1	0	0	0	0	0	15	0	0	15
E. coli (AD) (2)	0	0	1	0	0	0	0	0	0	1	1
Citrobacter koseri (5)	0	0	5	0	0	0	0	0	0	0	0
Serratia marcescery(3)	0	0	0	0	0	0	3	0	0	0	3
Provendentia rettgeri (5)	0	0	4	0	0	0	0	0	0	1	1
Oligella Ureolytica (2)	0	0	0	0	0	0	0	0	0	2	2
Morganella Morganii (1)	0	0	0	0	0	0	0	0	0	1	1
P. pseudoalcaligerus (10)	0	0	0	0	0	0	0	0	0	10	10
Proteus Vulgaris (3)	0	0	1	0	0	0	0	0	0	2	2
Aeromonas hydrophilla (2)	0	0	0	0	0	0	0	0	0	2	2
Acinetobacter Iwoffii (10)	0	0	3	0	0	0	0	0	0	7	7
P. putida (7)	0	0	0	0	0	0	0	0	0	7	7

Table 6: Multi drug resistance pattern of NFGNB isolates from various clinical specimens

R_o- no resistant for any antibiotic, R_1-resistant for 1 class of antibiotic, R_2- resistant for 2 different class of antibiotics, R_3- resistant for $_3$ classes of antibiotics, R_4-resistant for 4 different class of antibiotics, R_5- resistant for 5 different classes of antibiotics, R_6 - resistant for 6 different class of antibiotics R_{7-}resistant for 7 class of different antibiotics R_8 resistant for 8 classes of different antibiotics and >R_8 resistant for greater than 8 different class of antibiotics .

NB: Class of antibiotics made based on CLSI category.

6. Discussion

The resistance pattern of non-fermentative Gram-Negative bacteria towards antimicrobial resistance is an essential method for treating infectious disease mainly when the isolation continues to show widespread resistance to different antimicrobials currently available. Nabi SG *et al* (41) Hence, the selection of the appropriate anti-microbial treatment is mandatory.

The association of the isolated bacterial pathogen from the taken samples with gender was statistically insignificant (p = 0.983). Highest cases of 39.1% (276) isolated bacteria were recorded among young and middle-age patients with an age group of 25-44 years followed by age groups of 45-64(25.6%, 178). By this our study agree with the study conducted by Benachinmardi KK et al, in India (29).

NFGNB, mainly considered as contaminants, and it is critical opportunistic pathogens. The isolation rates of non-fermentative Gram-negative bacteria are not uniform. The positivity rate of NFGNB in this study was 19.4%. Lower positivity rates of NFGNB than this study have been shown in a research carried out in Brazil (2.2%), Bruno D, *et al* (42) But higher positivity rates of NFGNB have been shown in a research conducted in Saudi Arabia (16%). Eltahawy AT, et al. (43)

The isolation of frequently observed NFGNB from different clinical specimens were much similar with local study conducted by Anteneh A*, et al* (44) and another Cross-sectional study conducted by Asres GS, *et al* 2017(45) shows more frequently found isolation with another local similar study conducted by E Seneshat, 2017(46) and international researches conducted by Rudrajit Paulin et al in India (47) are also similar.

Out of 705 total clinical specimens 137 Gram negative bacterial was isolated with 21 different types of species. Among the isolated bacteria the highest number was observed by *A. baumannii* 26 (37%) the next one is *P. aeruginosa* 20 (20.0%). This finding correlates with the study conducted by Shah Set *et al*, in India show that *Acinetobacter baumannii* was the foremost common NFGNB isolated 63 (63%) followed by *Pseudomonas aeruginosa* 25 (25%) (27).

The percentage of antibacterial resistance pattern of non-fermentative Gram-negative bacteria against the twenty-one anti-bacterial drugs were studied. The result showed that the highest overall resistance rate was (85.7%) towards ampicillin, followed by (74.4%) towards

Amoxacilline, this is similar with local study conducted by Mamuye Y. (48) and also our study agrees with a similar study conducted locally by (49) Tsige E, that_high level of drug resistance to ampicillin and amoxicillin was exhibited and internationally in Pakistan by Ali A *et al* (50) also with similar finding observed.

In this research we also try to investigate the multi drug resistance (MDR) of microbials. Based on the result *A. baumanii* accounts the highest rate of MDR (96.6%). This shows out of 26 *A. baumanii* isolated microbials 25 of them was multi drug resistance. Our study agreed with a study conducted in Pakistan reported that the prevalence of MDRs was reported 100% among *A. baumannii (25). P. aeruginosa* was the second highest MDR (80%), out of 20 *P. aeruginosa* isolated microbials 16 of them shows multi drug resistance. This study differs from the study conducted by Khan MI *et al in 2020,* (51) such variation may be due to the Geographical variations and drug resistance properties**.** We also identified, out of 16 *Proteus mirabilis* microbials 15 was multi drug resistance. The least drug resistance was observed from *P. fulorescens* and *Citrobacter koseri* microbials in which they are not MDR at all but it is similar with the study conducted in Europe by Soul M *et al* (52).

The ability of microbials to resist different antibiotics may be due to quickly adapt to selective changes in environmental pressures, upregulation of the intrinsic resistance mechanisms, and acquisition and transferring of drug resistance genes through mobile genetic elements such as plasmids and transposons (30). Therefore, in Ethiopia, a few availabilities of recent drug generations, treatment of infection caused by NFGNB is difficult to manage, that's why we observed high prevalence of MDR strains of *Acinetobacter baumanni* and *Psedumonas aeruginosa*, and other NFGNB in this observation (34).

Therefore, appropriate identification of microbials to a species level by implementing appropriate mechanism is very crucial this is because of different microbial species have different antibiotic susceptibility pattern, serious bacterial infections prompted by means of genuine pathogen have reduced in current years in proportion to these triggered with the aid of opportunistic bacteria that as soon as were viewed to be of low virulence. Such infections cannot be traced epidemiologically or documented without identification of micro-organism to a species stage (48).

The main objective of this research was to profiling and study antimicrobial resistance pattern of non-fermentative Gram- Negative bacilli. As the result of the study, it presented and discussed above, we believe that we addressed the intended objective.

7. Strength and limitation

7.1 Strength of the study

- Accurate bacterial identification and antimicrobials susceptible testing was employed by automated techniques.
- Good enough sample size used

7.2 Limitation of the study

Due to time and financial constraints conventional method not utilized side by side

8. Conclusion and Recommendation

8.1 conclusion

In our study, NFGNB shows high antimicrobial resistance and multi drug resistance rate was studied. Such as Ampicillin resulted with the highest overall resistance rate against non-fermentative Gram-negative bacteria, followed by amoxicillin. The non-fermentative Gram-negative bacteria showed highest sensitivity towards cefepime, levofloxacin, ciprofloxacin, and to tobramycin. This is may be due to ordering antibiotic without knowing the right pathogen and also unlimited use of antibiotics makes treatment of infections caused by these pathogens both difficult and expensive.

8.2 Recommendation

Since the isolation of NFGNB was shows highly resistant and multi drug resistance rate, so we recommend screening of patients before prescribing any antibiotic will help patients to get the appropriate diagnosis and treatment.

We also recommended for routine laboratory organizing of microbiological laboratories with modern machinery like VITEK compact 2analyzers for accurate identification of bacteria's and determination of drug susceptibility pattern for effective treatment.

As our study provides baseline information in terms of NFGNB are highly resistant in many antibiotics it indicating that the great increasing rate time to time hence, we recommend further study to be done.

9. References

1. Juyal D, Prakash R, Shanakarnarayan SA, Sharma M, Negi V, Sharma N. Prevalence of non-fermenting gram negative bacilli and their in vitro susceptibility pattern in a tertiary care hospital of Uttarakhand: A study from foothills of Himalayas. Prevalence. 2013 1;2(2):108-12.
2. Chiu CW, Li MC, Ko WC, Li CW, Chen PL, Chang CM, Lee NY, Lee CC. Clinical impact of Gram-negative non-fermenters on adults with community-onset bacteremia in the emergency department. Journal of Microbiology, Immunology and Infection. 2015 1;48(1):92-100.
3. Rattanaumpawan P, Ussavasodhi P, Kiratisin P, Aswapokee N. Epidemiology of bacteremia caused by uncommon non-fermentative gram-negative bacteria. BMC infectious diseases. 2013 (1):1-8.
4. Luna CM, Aruj PK. Nosocomial acinetobacter pneumonia. Respirology. 2007 (6):787-91.
5. Mellmann A, Bimet F, Bizet C, Borovskaya AD, Drake RR, Eigner U, Fahr AM, He Y, Ilina EN, Kostrzewa M, Maier T. High interlaboratory reproducibility of matrix-assisted laser desorption ionization-time of flight mass spectrometry-based species identification of nonfermenting bacteria. Journal of clinical microbiology. 2009 1;47(11):3732-4.
6. Memish ZA, Shibl AM, Kambal AM, Ohaly YA, Ishaq A, Livermore DM. Antimicrobial resistance among non-fermenting Gram-negative bacteria in Saudi Arabia. Journal of antimicrobial chemotherapy. 2012 1;67(7):1701-5.
7. Ruppé É, Woerther PL, Barbier F. Mechanisms of antimicrobial resistance in Gram-negative bacilli. Annals of intensive care. 2015 1;5(1):21.
8. Meletis G, Bagkeri M. *Pseudomonas aeruginosa*: Multi-drug-resistance development and treatment options. Infection Control. 2013 29:33-56.
9. Lockhart SR, Abramson MA, Beekmann SE, Gallagher G, Riedel S, Diekema DJ, Quinn JP, Doern GV. Antimicrobial resistance among Gram-negative bacilli causing infections in intensive care unit patients in the United States between 1993 and 2004. Journal of clinical microbiology. 2007 1;45(10):3352-9.

10. Gatell JM, Trilla A, Latorre X, Almela M, Mensa J, Moreno A, Miro JM, Martinez JA, Jimenez de Anta MT, Soriano E, Garcia San Miguel J. Nosocomial bacteremia in a large Spanish teaching hospital: analysis of factors influencing prognosis. Reviews of infectious diseases. 1988 1;10(1):203-10.
11. Hancock RE. Resistance mechanisms in *Pseudomonas aeruginosa* and other non-fermentative gram-negative bacteria. Clinical Infectious Diseases. 1998 1;27(Supplement_1): S93-9.
12. El Chakhtoura, N.G., Saade, E., Iovleva, A., Yasmin, M., Wilson, B., Perez, F. and Bonomo, R.A. Therapies for multidrug resistant and extensively drug-resistant non-fermenting gram-negative bacteria causing nosocomial infections: a perilous journey toward 'molecularly targeted'therapy. *Expert review of anti-infective therapy*,2018 *16*(2), 89-110.
13. Vila J, Marco F. Interpretive reading of the non-fermenting gram-negative bacilli antibiogram. Enfermedades Infecciosas y Microbiología Clínica. 2010 26;28(10):726-36.
14. Mesbah Zekar F, Granier SA, Marault M, Yaici L, Gassilloud B, Manceau C, Touati A, Millemann Y. From farms to markets: gram-negative bacteria resistant to third-generation cephalosporins in fruits and vegetables in a region of north Africa. Frontiers in microbiology. 2017 24; 8:1569
15. McGowan Jr JE. Resistance in nonfermenting gram-negative bacteria: multidrug resistance to the maximum. American journal of infection control. 2006 1;34(5): S29-37.
16. De Francesco MA, Ravizzola G, Peroni L, Bonfanti C, Manca N. Prevalence of multidrug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in an Italian hospital. Journal of infection and public health. 2013 1;6(3):179-85.
17. Sekyere JO, Reta MA. Genomic and Resistance Epidemiology of Gram-Negative Bacteria in Africa: a Systematic Review and Phylogenomic Analyses from a One Health Perspective. Msystems. 2020 22;5(6).
18. Carmeli Y, Troillet N, Eliopoulos GM, Samore MH. Emergence of antibiotic-resistant Pseudomonas aeruginosa: comparison of risks associated with different antipseudomonal agents. Antimicrobial agents and chemotherapy. 1999 1;43(6):1379-82

19. Cabot G, Zamorano L, Moyà B, Juan C, Navas A, Blázquez J, Oliver A. Evolution of *Pseudomonas aeruginosa* antimicrobial resistance and fitness under low and high mutation rates. Antimicrobial agents and chemotherapy. 2016 1;60(3):1767-78.
20. Yayan J, Ghebremedhin B, Rasche K. Antibiotic resistance of *Pseudomonas aeruginosa* in pneumonia at a single university hospital center in Germany over a 10-year period. Plos one. 2015 2;10(10): e0139836.
21. Lambert PA. Mechanisms of antibiotic resistance in *Pseudomonas aeruginosa*. Journal of the royal society of medicine. 2002;95(Suppl 41):22
22. Granov D, Ljubovic AD, Zec SL, Granov N, Hukic M. The Impact of Antibiotic Consumption on Development of *Acinetobacter baumannii* Resistance. Materia socio-medica. 2016;28(6):449.
23. Lockhart SR, Abramson MA, Beekmann SE, Gallagher G, Riedel S, Diekema DJ, Quinn JP, Doern GV. Antimicrobial resistance among Gram-negative bacilli causing infections in intensive care unit patients in the United States between 1993 and 2004. Journal of clinical microbiology. 2007 1;45(10):3352-9.
24. Memish ZA, Shibl AM, Kambal AM, Ohaly YA, Ishaq A, Livermore DM. Antimicrobial resistance among non-fermenting Gram-negative bacteria in Saudi Arabia. Journal of antimicrobial chemotherapy. 2012 1;67(7):1701-5.
25. Begum S, Hasan F, Hussain S, Shah AA. Prevalence of multi drug resistant *Acinetobacter baumannii* in the clinical samples from Tertiary Care Hospital in Islamabad, Pakistan. Pakistan journal of medical sciences. 2013;29(5):1253.
26. Sivaranjani V, Umadevi S, Srirangaraj S, Kali A, Seetha KS. Multi-drug resistant Acinetobacter species from various clinical samples in a tertiary care hospital from South India. The Australasian medical journal. 2013;6(12):697.
27. Shah S, Singhal T, Naik R, Thakkar P. Predominance of multidrug-resistant Gram-negative organisms as cause of surgical site infections at a private tertiary care hospital in Mumbai, India. Indian Journal of Medical Microbiology. 2020 1;38(3-4):344-50.
28. Carmeli Y, Troillet N, Eliopoulos GM, Samore MH. Emergence of antibiotic-resistant *Pseudomonas aeruginosa*: comparison of risks associated with different antipseudomonal agents. Antimicrobial agents and chemotherapy. 1999 1;43(6):1379-82.

29. Benachinmardi KK, Padmavathy M, Malini J, Naveneeth BV. Prevalence of non-fermenting Gram-negative bacilli and their in vitro susceptibility pattern at a tertiary care teaching hospital. Journal of the scientific society. 2014 1;41(3):162.
30. Bagheri-Nesami M, Rezai MS, Ahangarkani F, Rafiei A, Nikkhah A, Eslami G, Shafahi K, Hajalibeig A, Khajavi R. Multidrug and co-resistance patterns of non-fermenting Gram-negative bacilli involved in ventilator-associated pneumonia carrying class 1 integron in the North of Iran. Germs. 2017 ;7(3):123.
31. Afle FC, Agbankpe AJ, Johnson RC, Houngbegnon O, Houssou SC, Bankole HS. Hospital Acquired Infection: Bacteriological Profile of Species from Environmental Surfaces of Cotonou 5 Hospital in South Benin (West Africa). Int. J. Curr. Microbiol. App. Sci. 2018;7(4):1503-.
32. EL-Behedy EM, Hend M, EL-Arini MM, Gerges MA, Mohamed NA, Gamil NM. Occurrence of unusual non-fermentative gram- negative bacilli in intensive care units of a university hospital, Egypt. African Journal of Clinical and Experimental Microbiology. 2016 21;17(4):256-66.
33. McKay R, Bamford C. Community-versus healthcare-acquired bloodstream infections at groote schuur hospital, cape town, South Africa. South African Medical Journal. 2015 8;105(5):363-9.
34. Bitew A. High Prevalence of Multi-Drug Resistance and Extended Spectrum Beta Lactamase Production in Non-Fermenting Gram-Negative Bacilli in Ethiopia. Infectious Diseases: Research and Treatment. 2019; 12:1178633719884951.
35. Larson E, Horan T, Cooper B, Kotilainen H, Landry S, Terry B. Study of the definition of nosocomial infections (SDNI). American journal of infection control. 1991 1;19(6):259-67.
36. Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM. CDC definitions for nosocomial infections, 1988. American journal of infection control. 1988 1;16(3):128-40.
37. Microbial identification using the biomérieux vitek® 2 system david h. pincus biomérieux, inc. hazelwood, mo, usa
38. Biomeriux VITEK-2 Compact ref Manual.; Available from: https://store.pda.org/tableofcontents/ermm_v2_ch01.pdf.
39. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance

40. Series M. World Health Organization. Provisional Guidelines on Standard International Age Classifications: Statistical Papers, WHO, United Nations, New York 1982; 74: 4-11.

41. Nabi SG, Zaffar G, Mumtaz S. Antimicrobial resistance pattern of gram- negative bacilli Isolated from Clinical samples. JIMDC. 2014;3(1):25-8.

42. Bruno D, Nishino MK, Priore WN, Prevalence of Gram-negative non-fermenters patients in Porto Alegre-RS. J Brasil Patol Med Laborator. 2011;47: 529-534

43. Eltahawy AT, Khalaf RM. Antibiotic resistance among Gram-negative non-fermentative bacteria at a teaching hospital in Saudi Arabia. J Chemother. 2001;13: 260-264.

44. Anteneh A, Zeleke G, Demissie A and Setegn E. Antimicrobial resistance pattern of bacterial isolates from different clinical specimens in Southern Ethiopia Hawasa.African Journal of Bacteriology Research; 2017: 9(1): 1-8.

45. Asres G, Legese M, Woldearegay G. Prevalence of multidrug resistant Bacteria in postoperative wound infections at Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia. Arch Med. 2017;9(4):12.

46. Seneshat Eshetu. bacterial profile and antimicrobial susceptibility pattern of blood culture isolates at tikuranbessa specialized hospital.Addis Ababa University, 2017

47. Rudrajit Paulin India. Daxboeck F.,Assadian O., A. Blacky. W. Koller. A. M.Hirschl .Resistance of gramnegative non-fermentative bacilli causing bloodstream infection, Vienna, 1996–2003.Eur J ClinMicrobiol Infect Dis .2004 :23: 415–416

48. Mamuye Y. Antibiotic resistance patterns of common Gram-negative uropathogens in St. Paul's Hospital Millennium Medical College. Ethiopian Journal of Health Sciences. 2016 ;26(2):93-100.

49. Tsige E. Antibiotic Resistance Patterns of Gram Negative Fermentative and Non-Fermentative Bacilli Isolated from Patients Referred to Arsho Advanced Medical Laboratory, Addis Ababa, Ethiopia (Doctoral dissertation, Addis Ababa University).

50. Ali A, Liaqat S, Tariq H, Abbas S, Arshad M, Li WJ, Ahmed I. Neonatal calf diarrhea: A potent reservoir of multi-drug resistant bacteria, environmental contamination and public health hazard in Pakistan. Science of The Total Environment. 2021; 799:1494

51. Khan MI, Xu S, Ali MM, Ali R, Kazmi A, Akhtar N, Bilal M, Hu Y, Li F. Assessment of multidrug resistance in bacterial isolates from urinary tract-infected patients. Journal of Radiation Research and Applied Sciences. 2020;13(1):267-75

52. Souli M, Galani I, Giamarellou H. Emergence of extensively drug-resistant and pan drug-resistant Gram-negative bacilli in Europe. Eurosurveillance. 2008;13(47):19045.

10. Annex

Annex-I Participant information sheet

Name of the organization: Department of Medical Laboratory Science, Collage of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia.

Title of the Research: Profiling and antimicrobial susceptible pattern of non-fermentative gram-negative bacilli isolated from patients referred to Arsho Advanced Medical laboratory Addis Ababa Ethiopia.

Name of Investigator: Mihiret Tatek (BSc)

Background information: Arsho is a brand name of private diagnostic Laboratory practices in Ethiopia. It originated in 1972 in a small individual practice and grows up to internationally accredited big service and name. The Laboratory qualifications comprise a wide variety of areas, including, Clinical Chemistry, Hematology, Histology & cyto-pathology, Microbiology/Virology, Serology and Molecular Diagnosis. The laboratory is always functioning, changing, and growing providing best quality laboratory service to medical practitioners and their patients for many decades. AML is focused on delivering quality diagnostic testing in the most cost-effective way.

We are asking you to take part in this study because we are trying to learn more about Profiling and antimicrobial susceptible pattern of non-fermentative gram- negative bacilli isolated from patients referred to Arsho Advanced Medical laboratory Addis Ababa Ethiopia. Potential benefits to subjects and/or to the society You will not have any financial incentives or other as inducements the compensation from participating in the study. However, results will be given to their physician for treatment. Most importantly, the result of the study will be beneficial to provide information or data for future and 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 in participating in the study other than benefiting from the research.

Confidentiality

In order to keep the confidentiality, the information was disclosed to third party or was not appear in any report from this study.

Annex-II Laboratory procedure **for identification and AST testing using vitek-2 Compact instrument**

A. Using the Densi Check Plus

1. Place the tube of saline in the Densi Check Plus and rotate one full turn. The reading should be 0.0. If the reading falls out of the acceptable range, press the ZERO button and try again.
2. The Densi Check Plus hand held will be calibrated and standards run monthly.
3. Fill polystyrene tube with 3 ml of 0.45% sterile saline.
4. Make a homogeneous suspension of testing organism in the saline.
5. Insert tube into the optical block and check the display for density reading. Acceptable readings: 0.5 – 0.63 for gram negative

B. Test Card Setup Procedure

1. Prepare inoculums from a pure culture, according to good laboratory practices. In case of a mixed culture, an isolation step is required.
2. Aseptically transfer 3.0 ml of sterile saline (0.45% to 0.5% NaCl, pH 4.5 to 7.0) into a clear plastic (polystyrene) test tube (12mm x 75mm).
3. Use a sterile stick or swab to transfer a sufficient number of morphologically similar colonies to the saline tube prepared in step 2.

   NOTE: the age of the suspension before loading the instrument for AST testing must be less than 30 minutes.
4. In a second tube containing 3.0ml of saline, transfer 145ul of the suspension prepared in step 3 for AST-GN cards. Then place this tube in the cassette with a susceptibility card. The tube with the initial bacterial suspension can also be used for inoculation of an identification card.

C. Processing Test Cards

1. Fill in a cassette worksheet with the test card and specimen information for the cassette.
2. Place the test cards and specimen test tubes in their appropriate slots.

3. Enter the information from the cassette worksheet into the Maintain Virtual Cassette window on the workstation.
4. Load the cassette into the Filler Station.
5. Transfer the cassette to the Vitek 2 Compact cassette loading station within 10 minutes.
6. If the cassette bar code was not read and performed on the user interface screen access the Manage Cassette view function on the workstation. Enter a cassette number.
7. If using the Load and Go Method enter the information from the cassette worksheet into the Setup Tests Post Entry.
8. Set up a purity plate for both patient isolates and quality control isolates after removal of cassette from the loading station.

Quality control for Vitek 2 Compact:

1. Each new lot number of ID cards is tested with stock culture organisms to ensure proper performance.
2. Susceptibility cards are tested weekly against stock culture organisms to ensure proper performance.
3. All shipments of new lots for ID and sensitivity cards must be entered in the VITEK 2 PC prior to use, or the QC cards will TERMINATE.
   a) Click on QC file Icon (graph and arrow)>Truck icon.
   b) Enter the lot number of the card by scanning the barcode on the box.
   c) Enter Quantity of the shipment.
   d) The expiration dates will auto-fill from the lot number.
   e) Click OK to save.
4. All QC results must be reviewed and any unexpected results will be investigated. Add documentation on the VITEK PC for each out-of-range QC (be sure to document which ATCC strain failed QC).
5. Unacceptable results with Quality Control strains can be categorized into 1) random, 2) identifiable, and 3) system related errors. If the reason for an unacceptable result can be identified and corrected (wrong organism used, organism viability, contamination, etc.), document the reason for the failure, and retest the day the failure is observed. No further action is required if the repeat results are expected. If the reason for the unacceptable result cannot be identified, perform corrective action to determine if the error is random or system related.

Annex -III Laboratory SOPs for Bacteriology Laboratory Procedures

Blood agar plate

Blood agar plate is A non-selective media it is used for investigate the forms of hemolysis Blood Agar Base has been used as a base for preparation of blood agar and to support good growth of a wide variety of fastidious microorganisms.

Procedure

- ✓ Measure 1000ml of distilled water using a measuring cylinder.
- ✓ Transfer the distilled water into a 1litre conical flask.
- ✓ Weigh 51g of Blood Agar Base powder using a weighing balance.
- ✓ Add into the 1000ml 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
- ✓ Add 5-10% of defibrinated sterile sheep blood.
- ✓ Rotate the flask or bottle slowly to create a homogeneous solution.
- ✓ Gently pour 15-20 ml of the ready media on to the plates by using dispenser and allow to set.
- ✓ 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 2-80 C sealed in plastic bags to reduce chances of contamination.

Expired date: up to four months provided if there is no change.

Mac Conkey Agar

Mac Conkey Agar is preferable for the isolation and differentiation of clinically important gram-negative bacilli by inhibiting gram positive cocci.

Principle MacConkey agar is selective and differential medium to distinguish gram negative Enterobacteriaceae and lactose fermenting bacteria from non- lactose fermenters. MacConkey Agar is a selective and differential medium. It is only slightly selective since the concentration of bile salts, which inhibit gram-positive microorganisms, is low in comparison with other enteric

plating media. Crystal violet also is included in the medium to inhibit the growth of gram-positive bacteria, especially Enterococci and staphylococci. Differentiation of enteric microorganisms is achieved by the combination of lactose and the neutral red indicator. Colorless or pink to red colonies are produced depending upon the ability of the isolate to ferment the carbohydrate.

Procedure

Prepare as instructed by the manufacturer.

- ✓ Suspend 51.1g of powder in 1 liter of distilled or deionized water.
- ✓ Heat and boil until completely dissolved with frequent agitation.
- ✓ Sterilize in autoclave at 121^{0} C for 15 minutes.
- ✓ Cool to 45-50 ^{0}C.
- ✓ Mix well and dispense by dispenser (15-20 ml) aseptically into sterile petri dishes.
- ✓ Leave standing for thirty minutes to solidify.
- ✓ Perform sterility testing.
- ✓ Label the bottom of each plate with date of preparation and batch number.
- ✓ Store the culture media plates upside down at $2\text{-}8^{0}$ C sealed in plastic bags to reduce chances of contamination.

Chocolate agar

Chocolate agar; a non-selective media supplies the factors X and V required for the proper growth of H. influenza. When Blood agar is heated, the red cells are lysed and the medium becomes brown in color; it is referred to as chocolate agar. It is appropriate for isolating pathogenic bacteria in sputum, throat swabs, eye swabs, ear swabs, urogenital swabs, cerebrospinal fluid.

Procedure:

Prepare as described for Blood agar except after adding blood,

- ✓ heat the medium in a 70^{0} C water bath until it becomes brown in color. for about 10-15 minutes during which time the medium should be
- ✓ mixed gently several times.

- ✓ Allow the medium to cool to about 45^0 C, remix and
- ✓ dispense in sterile petri dishes using a dispenser as described for blood agar.
- ✓ Leave standing for thirty minutes to solidify.
- ✓ Perform sterility testing as described for blood agar plate.
- ✓ Label the bottom of each plate with date of preparation and batch number.
- ✓ Store the culture media plates upside down at 2-8^0 C sealed in plastic bags to reduce chances of contamination.

Expire date: Up to four months if kept it properly and if there is no change.

Nutrient agar

Nutrient agar is used for the cultivation of a wide variety of non-fastidious bacteria. It was originally developed in recognition of the need for a standardized medium for use in the examination of water and waste water, dairy products and various foods. Currently it is used as a maintenance medium for S. aureus, P. mirabilis, and E. coli. Tube slants are used primarily for the cultivation and maintenance of pure cultures.

Procedure:

- ✓ Suspend 23 g of the powder in 1 liter of purified water.
- ✓ Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
- ✓ Autoclave at 121˚C for 15 minutes.
- ✓ Cool to 45-500 C.
- ✓ Pour 15-20 ml of the ready media into sterile 20ml glass universal tubes.
- ✓ Leave standing for thirty minutes to solidify,
- ✓ leaning the tubes at 30˚- 60˚ to produce the slope effects in the tubes.
- ✓ Perform sterility testing
- ✓ Label the side of each tube with date of preparation and batch number.
- ✓ Store the tubes at 2-8^0 C sealed in plastic bags to reduce chances of contamination.

Expired date: up to eighteen months provided there is no change

Annex -IIII Data extraction format

SID	Age	Sex	Sample type	Finding			Antibiogram																					Comment
				Positive	Negative		Amoxacilline	Ampicilline	Agumentine	Cefazoline	Cefepime	Cefoxitin	Cefpodoxime	Ceftazidime	Ceftriaxone	Cefuroxime	Cefuroxime-	Cephalothine	Ciprofloxacin	Co-	Gentamycin	Levofloxacin	Nitrofurantoi	Piperacilline-	Tetracyclines	Tobramycine	Meropinum	
				Isolate	No growth	No pathogen																						

11. 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: **Mihiret Tatek (B.Sc.)**

Signature: ____________________

Date of submission: ____________________

This thesis has been submitted with our approval as advisors.

Advisor: **Adane Bitew (MSc, PhD)**

Signature: ______________________

Date: ______________________

Place: Addis Ababa, Ethiopia.