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SCHOOL OF MEDICINE, COLLEGE OF HEALTH SCIENCES

Assessing the Prevalence of Multidrug Resistant Gram-Negative Bacteria and Associated Factors among Gram-negative Blood Culture Isolates at Tikur Anbessa Specialized Hospital: A Retrospective Study

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Abstract

Background

The rate of Multidrug resistant gram-negative bacteria causing bloodstream infection is increasing. The lack of control of access to antibiotics and restriction and weak antimicrobial stewardship in Sub-Saharan Africa makes antimicrobial resistance one of the current deadliest health care agendas. Continuous surveillance of the prevalence of AMR, identifying risk factors and implementing infection prevention is a priority.

Objectives

The aim of this study was to determine prevalence, bacteriologic profile, and associated risk factors of MDR-gram negative isolates and Carbapenem resistant gram-negative BSI admitted patients of Tikur Anbesa Specialized comprehensive Hospital, Addis Ababa, Ethiopia.

Methods

A single-center cross sectional retrospective study was conducted in medical wards of Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia from September1,2021 until September 1, 2023. Gram-negative blood isolates of 137 patients were assessed. The clinical profiles with demographic information, underlying comorbidities, sites of admission(wards), sepsis or septic shock during admission use of instrumentations, surgical procedures, use of prior antibiotics and type, length of hospital stay, use of immunosuppressive therapy are compared to the gram-negative blood stream infection as risk factors. Furthermore, rates of multidrug resistance and carbapenem resistance are determined with related risk factors. The microbiology data for the specimens collected for culture was retrieved from the electronic record system LIS while clinical parameters were entered both from the electronic medical database and the medical cards of patients. The data was entered, and bivariate and multivariable analysis was done using SPSS Ver 26.

Results

A total of 137 gram-negative blood isolates from patients in the medical wards were assessed during the study period. The mean age of the patients with gram-negative blood culture and SD Age of 43.1±18.17 years. Most of the blood cultures were collected from the patients at the Emergency room (29.2%). Almost 25% percent of the study participants had prior antibiotics exposure in the preceding three months before the blood culture. One-third (33%) of the Enterobacterales isolated from the blood cultures were Escherichia coli (33%) followed by Klebsiella pneumoniae (30.1%). The Antimicrobial susceptibility testing revealed that the Enterobacterales isolates showed a higher resistance towards with *E. coli* resistance to third and fourth generation Cephalosporins with 96.8% (Ceftazidime), and 87.1%(Cefepime) respectively. Resistance towards Carbapenems was an alarming 58.1% (79 of 136 blood isolates tested) in our study. Enterobacterales were 52.4% resistant to Carbapenems. The overall MDR rate among the gram-negative blood isolates in our study was 81%. Most of the MDR isolates are Enterobacterales with 83.5% being MDR. The *K. pneumoniae* blood isolates had Carbapenem resistance rate of 77.4%, while *E. coli* blood isolates had 47.8%. Risk factors associated with MDR gram-negative bloodstream infection were hematologic malignancy (AOR=1.4, 95%CI=1.12, 4.27), Enterobacterales isolated from the blood (AOR 4.4, 95%CI=1.57, 34.18), blood isolates from the ICU (AOR=2.1, 95%CI=1.24, 16.56). Risk factors associated with carbapenem resistance in gram-negative BSI were immunosuppressive treatment (AOR=2.1, 95%CI=1.24, 3.78), patients having history of prior Cefepime(AOR=4.9, 95%CI=1.27, 41.24), CNS shunt device(AOR=12.4, 95%CI=4.26, 22.56) and having *Acinetobacter Spp.* (AOR=4.2, 95%CI=2.19, 14.63).

Conclusion

The study highlighted the growing prevalence of MDR and Carbapenem gram-negative bloodstream infections in a hospital with limited resources. Our findings emphasize the urgent need for effective antimicrobial surveillance and infection control measures throughout the country.

Key words: Multidrug resistance, Carbapenem resistant, Gram-negative bacteria, bloodstream infections

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Abbreviations

AAU- Addis Ababa University

ACB- *A. baumannii–A. calcoaceticus* complex

AMR- Antimicrobial resistance

AMS- Antimicrobial stewardship

AWaRe- Access, Watch, And Reserve groups of Antibiotics

CDC- Center of diseases and control

CPE- Carbapenemase producing Enterobacterales.

CRAB- Carbapenem resistant *Acinetobacter Baumannii*

CRE- Carbapenem resistant Enterobacterales

CRE-BSI- Carbapenem resistant Enterobacterales blood stream infection

CRPA- Carbapenem resistant *P. aeruginosa*

CSE- Carbapenem susceptible Enterobacterales

ECCMID- European Society of Clinical Microbiology and Infectious Diseases

eCIM- EDTA-modified Carbapenemase inactivation method

IPC- Infection prevention and control

KPC-*K. pneumoniae* Carbapenemases

MBL- metallo ß-lactamases

mCIM- Modified Carbapenemase inactivation method

MDR- Multidrug resistant

MEWS-Modified Early Warning Score

NDM -New Delhi ß-lactamases

NEWS- National Early Warning Score

QI- Quality Improvement

qSOFA- quick Sequential Organ Failure criteria

TASH- Tikur Anbessa Specialized Hospital

VIM-Verona Integron-Mediated metallo ß lactamases

WHO- World health organization

Introduction

Background

The order 'Enterobacterales' is a large and diverse group of Gram-negative, facultatively anaerobic, non-spore-forming, rod-shaped bacteria within the class Gammaproteobacteria. The type of genus of this order is *Enterobacter*. Enterobacterales contains seven validly published families (*Budviciaceae, Enterobacteriaceae, Erwiniaceae, Hafniaceae, Morganellaceae, Pectobacteriaceae* and *Yersiniaceae*). Enterobacteriaceae are a family of bacteria that are gram-negative rods with over thirty genera and one hundred species. They are a family of facultative anaerobes that are non-spore forming and mostly reduce nitrate to nitrite. The family includes notable human pathogens such as *Salmonella, Escherichia coli, Klebsiella, Shigella, Enterobacter* and *Citrobacter*. (1)

Acinetobacter baumannii is a gram-negative coccobacillus from the class Gammaproteobacteria and the family of Moraxellacea. It is a part of the *A. calcoaceticus–A. baumannii* (ACB) complex or *the A. baumannii–A. calcoaceticus* (ABC) complex with *A. calcoaceticus*, Acinetobacter genomic species 13TU and Acinetobacter genomic species 3. The ability of *A. baumannii* to develop resistance to several classes of antibiotics coupled with its presence in MICUs and High dependency units makes it one of the deadliest bacteria causing nosocomial infections. Multidrug-resistant isolates that have resistance to broad-spectrum penicillin and cephalosporins, carbapenems, most aminoglycosides, fluoroquinolones, chloramphenicol and tetracyclines are found worldwide.(2)

Pseudomonas Aeruginosa is a gram-negative bacillus that facilitative anaerobe. It is part of the class Gammaproteobacteria and the family Pseudomonadacaea. This ubiquitous bacterium causes infection as an opportunistic pathogen and nosocomial infection. *Pseudomonas aeruginosa* has different mechanisms of resistance such as intrinsic antibiotic resistance, efflux systems, and antibiotic-inactivating enzymes. In addition, the ability of the bacteria to form biofilms makes infections via invasive devices such as indwelling catheters or endotracheal tubes difficult to prevent.(3)

The definition of non-susceptibility of bacteria to antibiotics as either resistant, intermediate, or non-susceptible results obtained from in vitro susceptibility testing. The definition of multidrug resistant bacteria is non-susceptible to at least 1 agent in ≥3 Antimicrobial categories.(4) Carbapenem resistant Enterobacterales as defined by the United States Centers for Disease Control and Prevention (CDC) are bacteria within the Enterobacterales order that are resistant to at least one Carbapenem (meropenem, ertapenem, doripenem or imipenem) or that produce a Carbapenemase enzyme. Enterobacterales of the Morganellacaeae family (*Proteus* spp, *Morganella* spp, and *Providencia* spp) have intrinsic resistance to Imipenem hence Antimicrobial Susceptibility Test (AST) results for Meropenem, Doripenem and Ertapenem are used to define as CRE. Carbapenem resistant *A. baumannii* and Carbapenem resistant *P.*

aeruginosa are defined by the United States Centers for Disease Control and Prevention (CDC) as resistant to at least one Carbapenem (meropenem, ertapenem, doripenem or imipenem) or that produce a Carbapenemase enzyme.(5) Isolates of *P. aeruginosa* that are carbapenem resistant are mostly non-Carbapenemase producing.(6)

The Global action on Antimicrobial Resistance (AMR) was adopted during the 2016 meeting on AMR, committed by heads of state at United nations at the general Assembly in New York. Subsequently, the World Health Organization (WHO) issued the first Bacterial Priority Pathogen List (BPPL) in 2017, a watchlist for multidrug-resistant bacterial pathogens that are emergent and of global importance. The priority or critical pathogens are Carbapenem-resistant *Acinetobacter baumannii*, Carbapenem-resistant *Pseudomonas aeruginosa*, Carbapenem-resistant Enterobacteriaceae and ESBL-producing Enterobacteriaceae.(7) At the European congress of Clinical Microbiology and Infectious Diseases (ECCMID) conference in 2023, the WHO presented its methodology for revision of its 2022-2023 Bacterial Priority Pathogen List (BPPL) which will be published soon. Since the first BPPL there have been few new antimicrobials developed. Center of disease control also lists CRE and CRAB infections as urgent threats and MDR *Pseudomonas aeruginosa* as serious threat to national and global health.(5)Furthermore, the WHO has come up with a categorization of antibiotics called AWaRe(access, watch and reserve) for effective use and safety. The first group is called "Access" that is constituted of antibiotics that are first- or second-line treatment of choices which minimize risk of AMR when used. The second group is the "Watch" group that is composed of antibiotics indicated for specific infectious diseases syndromes and are more prone to developing AMR. The group includes Azithromycin, Ceftriaxone, Ceftazidime, Cefepime, Cefixime, Cefotaxime, Cefuroxime, Cefazoline, Cloxacillin, Chloramphenicol, Ofloxacin, Ciprofloxacin, Delafloxacin, Erythromycin, Gatifloxacin, Kanamycin, Meropenem, Imipinem-cilastatin, Piperacillin-tazobactam Vancomycin, Moxifloxacin, Rifabutin, Rifampicin and Streptomycin. The "Reserve" group is the last resort with antibiotics used for severe infections which are monitored and regulated. Antimicrobial stewardship programs should focus on the "Watch" and the "Reserve" group of antibiotics.(8)

Systematic analysis on the global burden of antimicrobial resistance in 2019 revealed an estimated 4.95 million deaths were associated with bacterial AMR. The six leading pathogens for deaths associated with resistance were *Escherichia coli*, followed by *Staphylococcus aureus*, *Klebsiella pneumoniae, Streptococcus pneumoniae, Acinetobacter baumannii*, and *Pseudomonas aeruginosa.* They were responsible for 929,000 (660,000–1,270, 000) deaths attributable to AMR and 3·57 million (2·62–4·78) deaths associated with AMR in 2019. The regional level data shows that the estimated all-age death rate attributable to resistance to be highest in western sub-Saharan Africa at 27.3 deaths per 100,000. Although sub-Saharan Africa had the highest all-age death rate attributable to and associated with AMR, the percentage of all infectious deaths attributable to AMR was the lowest in this region. (9)

The burden of carbapenem resistant *A. baumannii* has increased due to the inadvertent use of carbapenems. (10)A study done to evaluate the burden of illness in patients infected with CRAB versus Carbapenem susceptible *A. baumannii* isolates over a five-year period between 2014 and 2019. It showed that *A. baumannii* isolated from the tracheal aspirate were carbapenem resistant (CR 40.7% and CS 27.0%, $P < 0.01$). But carbapenem susceptible isolates were more common from blood cultures (CS 16.7% and CR 8.6%, $P < 0.01$).(11)The epidemiological trend of CRAB from 30 European countries via European Antimicrobial Resistance Surveillance Network (EARS-Net) showed population-weighted mean proportion is 35.6% (95% confidence interval [CI] 29.7–42.0%). (12)

Significance of the Study

This study highlights the in-hospital rates of MDR gram-negative BSI of patients with risk factors related to acquiring MDR priority pathogens. Hospital based studies from Ethiopia assessing the rate of mortality from blood stream infections and related factors showed that infection with gram-negative bacteria that were resistant towards Cephalosporins were related to mortality. (13,14) The growing concern of MDR gram negative infections in the country needs such an input to come up with better prevention of their spread. The awareness raised with findings is QI projects in the IPC and establishing antimicrobial stewardship programs.

Literature Review

Enterobacterales are gram-negative rods that are found in the gut of humans and animals. Carbapenem-resistant Enterobacterales are now the common cause of bloodstream infections worldwide. There is a growing number of resistances among Enterobacterales with mechanisms related to the production of enzymes, alteration of porins and binding sites or efflux mechanisms.(1) Their main resistance mechanism is by the production of extended spectrum of beta-lactamase (ESBL) production. To combat the rising ESBL-producing Enterobacterales, Carbapenems are widely used for several infections especially those with hospital-onset. (15)The increasing use of Carbapenems is in turn responsible for the alarming CRE rates worldwide.(16)

A hospital based retrospective study from China assessing epidemiology of gram-negative blood stream infections showed that there was an increasing trend in gram-negative BSI. Most patients that had gram-negative BSI were from the hematology ward (23.7%) and ICU (8.74%). It also showed similar rates of gram-negative BSI in male and females. The age group with the most gram-negative BSI was the group of 41 to 60 years old. The most common gram-negative isolates were *E. coli* (49%) followed by *Klebsiella pneumoniae* (6.29%.) and *Acinetobacter baumannii* (3.73%). *E coli* showed higher rates of drug resistance towards 1st and 2nd generation cephalosporins (82.28%), and to quinolones (56.3%.) Similarly, *K. pneumoniae* high resistance toward first and second generation cephalosporins (73.75%). Significant risk factors independently associated with MDR gram-negative BSI were, having *Enterobacteriaceae*

isolates, central venous catheters, and treatment with first and second generations cephalosporins in the 30 days prior to BSI.(17)

A similar study from a hospital in Türkiye showed that the commonest blood isolates were gram-negative bacteria (59.4%). Among the blood isolates *E. coli* were 22.8%, *K. pneumoniae* 11.2% and *Pseudomonas spp* were 7.3%. The ESBL positivity rate was 60.3% for *E. coli* isolates and 26.9% for *K. pneumoniae.* Most of the MDR BSI were from the ICU. Among the risk factors assessed in relation to MDR BSI, sepsis, history of surgery, history of broad-spectrum antibiotic use within 3 months or longer than 5 days, health care associated infections, hospitalization within 3 months and urinary catheter insertions showed significant correlation. It also showed rate high rates of carbapenem resistance for *Acinetobacter spp* with 82.3%, K. pneumoniae 19.2% and Pseudomonas spp 17.6%. (18)Gram-negative bloodstream infections in hematology wards from Italy showed that the most common gram-negative bacteria were *E. coli* (52.7%), *K. pneumoniae* (19.2%) and *P.* a*eruginosa* (14.6%). From the AST, 31.5% of BSI had at least one MDR gram-negative bacteria. Fluoroquinolone use as prophylaxis was independently associated with MDR gram- negative BSI. Other risk factors associated with MDR infections were previous colonization with MDR gram-negative bacteria and previous exposure to carbapenems and aminoglycosides.(19)

In multi-center surveillance study from Ghana, gram-negative bacteria were found to be the majority of the blood isolates. The common gram-negative isolates were *E. coli* (26.4%), *K. pneumoniae* (25.5%) and P. *aeruginosa* (7.5%). The rate of MDR was 88% from all isolates and ESBL production was found in 44.6%. There was no difference in age or gender when compared to MDR BSI. The highest rate of resistance was towards Amoxicillin (89.3%). Alarming rates of resistance to third generation cephalosporins were also reported (Cefotaxime 77.8% and ceftriaxone 73.7%). The rate of resistance towards ciprofloxacin was 55.3%. Carbapenem resistance rates were higher in *P. aeruginosa* isolates (52-84%) and *A. baumannii* isolates (30.8-90%).(20)A hospital-based study from Zambia with a focus on BSI by drug resistant gram-negative bacteria showed similar results. Most of the gram-negative isolates (77%) were Enterobacterales with *E. coli* isolates of 42% and *K. pneumoniae* were 30%. *E. coli* isolates were prevalent in Internal medicine ward (56%) and *A. baumannii* isolates were from surgical/burn unit (30%) and *P. aeruginosa* from the renal unit (71%). The rate of MDR was 42% for *E. coli* isolates and 31% for *K. pneumoniae*.(21)

In Ethiopia, there were several studies exploring the bacterial profile and antibiotic resistance pattern of BSI. The rate of blood stream infections rate was estimated to be 25. 8 % from a systematic analysis in 2020.The pooled prevalence of gram-positive bacterial isolates was 15.50 % and 10.48% for gram-negative bacteria. The five common gram-negative blood culture isolates were Klebsiella species 7.04 % (95% CI: 5.37–8.72%?) followed by E. coli 1.69% (95% CI: 1.21–2.16%), Salmonella species 1.09% (95% CI: 0.79–1.38%), S. pyogenes 0.88% (95% CI:0.54–1.22%), and Pseudomonas species 0.39% (95% CI: 0.08–0.70%). (22) A hospital-based study from Arbaminch showed that gram-positive isolates were higher (59.1%) than gram-

negative bacteria. *E. coli* (18.1%) and *K. pneumoniae* (18.1%) were more common gram-negative isolates. The highest blood culture positive rates were from Internal Medicine ward (40%), ICU (22.7%) and surgical wards (22.7%). The MDR rate from the gram-negative bacteria from the blood the 66.7%. Additionally, the risk factors associated with positive blood culture were having a peripheral IV device with odds of having a BSI 4.82 times more than those that didn't have a peripheral IV device.(23) A multi-center study from Addis Ababa assessing blood cultures showed that *K. pneumoniae* were the most common isolates (26.1%). The other frequent isolates were *K. varicola* (18.1%) and *E. coli* (12.4%). The most frequent blood culture isolates from Tikur Anbessa specialized hospital were K. pneumoniae (25%.), *E. coli* (20.4%) and *A. baumannii* (10.9%). There was a high level of resistance to Ampicillin (96.2%), and Ceftriaxone (78.7%) among the *Enterobacteriaceae* blood isolates. The lowest resistance of the *Enterobacteriaceae* blood isolates were to Piperacillin-Tazobactam (14.8%), Meropenem (9.4%) and Amikacin (4.1%). The overall MDR rate among the *Enterobacteriaceae* was 83.2% and in Tikur Anbessa specialized hospital the MDR rate rose to 93.2%.(24)A laboratory-based study assessing blood culture isolates from Addis Ababa showed that gram negative bacteria were 50.6%. *K. pneumoniae* with prevalence of 14.1% and *E. coli* with 9% were the common gram-negative isolates. The antimicrobial resistance of gram-negative blood isolates was highest to Ampicillin (80.8%) and lowest to Imipenem (5.2%). The MDR prevalence rate for the gram-negative bacteria from the blood was 41.6 %. *A. baumannii* isolates had 85.8%, *K. pneumoniae* had 77.3%, E. *coli* and *Pseudomonas spp* isolates each had 28.6 % MDR rates.(25)

In a recent institutional-based cross-sectional study in Addis Ababa, the three common blood culture gram-negative isolates from blood were *K. pneumoniae, Acinetobacter* spp and *E. coli.* The male to female ratio for blood culture positivity rate was 1.3:1. The highest rate of pathogen isolated from blood cultures was from the surgical ICU (59%). While assessing risk factors related to blood culture positivity, ICU admission, use of instrument, NGT, being febrile, having a chronic illness, having wound, urinary tract or respiratory infections should significant relation. From this study, Ampicillin was the most resisted drug (94.6%), and Amikacin was the least resisted drug (10.8%). The MDR rate among the gram-negative blood isolates was 95.3%. (26)The follow-up study from the same group showed that 54 % of the gram-negative bacteria were ESBL and 25.7% produced Carbapenemases. The predominant isolates were *Klebsiella pneumoniae* (32.5%), *Acinetobacter Spp.* (20.4%), and *Escherichia coli* (16.5%). Most *Klebsiella pneumoniae* (77.3%) and *Klebsiella oxytoca* (77.8%) were ESBL producers likewise, most *Acinetobacter spp* (53.2%) were Carbapenemase producers. Patients admitted to general surgery ($p = 0.001$), surgical intensive care unit (SICU), and intensive care unit (ICU) were sites where patients will acquire almost five times and two times more likely to acquire Carbapenemase and MBLs producer bacteria than those who were admitted in other wards. The significant association to acquire infection with Carbapenemase and MBLs producing GNB were with care that involves the insertion of medical instruments, chronic wound infection on the skin, and urinary tract infection (UTI). (27)

Assessment of risk factors related to Carbapenem-resistant gram-negative infections was explored by a systematic review of 116 studies from 2018-2019. The risk factor most frequently and significantly associated with CR infection was antibiotic use, in 91.1% of studies (72/79) examining this factor. Of specific antibiotic classes examined, Carbapenem use was found to be associated with CR infection in 82.6% of studies (57/69) and was significantly related in multivariate analysis (42.2% of studies; 30/71). The individual risk factors most frequently reported as significantly associated with CR infection on univariate analysis were Pitt Score (100% of studies; 8/8); previous colonization with relevant bacteria, including CR isolates (72.7%; 8/11); non-specific device use (75.0%; 6/8); mechanical ventilation (66.7%; 36/54); previous intensive care unit (ICU) stay (64.4%; 38/59); dialysis (61.1%; 11/18); catheter placement of any type (58.0%; 40/69); length of stay in hospital (54.5%; 30/55); the presence of comorbidity (52.7%; 39/74); Acute Physiology and Chronic Health Evaluation II Score (51.7%; 15/29); and intubation (gastric/nasogastric/tracheal tube) (51.4%; 18/35).(28)

The European Prospective Cohort Study on Enterobacteriaceae showing resistance to Carbapenems (EURECA), a multinational study performed in 50 European hospitals from 2016 to 2018, explored risk factors associated with CRE infections. The study was an internationally matched case-control study where Carbapenem susceptible Enterobacterales (CSE) infections were compared to CRE infections. When exposures were compared between patients with CRE and CSE infection, these exposures were significantly more frequent among CRE patients in univariable comparison: hospitalization in the last three months, previous colonization/infection by CRE, chronic heart failure, dementia, chronic renal failure, central venous and urinary catheter, dialysis, and previous use of antibiotics. In the final multivariate analysis, CRE infections were independently related to being admitted from home (protective), previous colonization or infection by CRE, chronic renal failure, urinary catheter, and exposure to broad-spectrum anti-gram-negative antibiotics. (29) Admission with renal disease, agranulocytosis, invasive procedures, and the time of hospital stay to positive CRE were the risk factors for CRE infection in the retrospective multicentered study from Beijing, China.(30)

Bloodstream infections caused by CRE are one of the important types of CRE infections causing significant morbidity and mortality. Studies from acute care units and long-term care facilities of the Campania region in Italy showed that the mortality of patients with BSI by carbapenem-resistant *K. pneumoniae* was high: 32.5% at day 7 and 41.9% at day 90 from the index culture. The presence of at least one risk factor for CRE infection including previous rectal colonization, recent hospitalization, and admission to high-risk units, such as ICUs were associated with mortality. (31)

Bacteremia secondary to *A. baumannii* is one of the two most common clinical manifestations. It usually occurs with the presence of central venous catheters or spread from extensive pneumonia. In the past, bacteremia was seen in patients that are from the military and confined mostly to intensive care units. It has now spread to inpatient and outpatient settings with its unique ability to withstand harsh conditions and acquire resistance to most classes of antibiotics.

(32,33)One of the major risk factors for bacteremia caused by CRAB is colonization. Most patients admitted to intensive care units will have respiratory and gastrointestinal colonization. In a prospective, observational study in adult patients admitted to the ICUs in Italy, CRAB blood stream infection rates and factors were assessed in patients with relation to colonization with CRAB. Around 44% of CRAB-colonized patients admitted to an ICU subsequently developed a CRAB BSI during hospitalization, more frequently in the first 7 days from colonization. The burden of comorbidities as seen by Charlson Comorbidity index, multisite colonization, COVID-19 infection, and mechanical ventilation were associated with CRAB BSI.(34) In a retrospective study from a Chinese hospital showed that the risks for MDR *A. baumannii* bacteremia to be previous exposure to carbapenems and penicillin+β-lactamase inhibitors.(35) A multicentered study from China based on the Chinese Antimicrobial Resistance Surveillance of Nosocomial Infections Network compared carbapenem susceptible *A. baumannii* bacteremia to CRAB BSI. It showed that male gender, prior carbapenems exposure and presence of endotracheal intubation were independent risk factors for acquiring CRAB BSI.(36)

Pseudomonas aeruginosa is a gram-negative, aerobic, non-spore forming rod commonly found in freshwater. It can cause infection in patients with immunocompromised state such as cystic fibrosis, bronchiectasis, neutropenia, burns, cancer, AIDS, organ transplant, uncontrolled diabetes mellitus, and ICU admissions.(37) According to the CDC, the overall incidence of P. aeruginosa infections in US hospitals averages about 0.4 percent (4 per 1000 discharges). It is the fourth commonly isolated nosocomial pathogen accounting for 10.1 percent of all hospital-acquired infections. Bloodstream infections with *P. aeruginosa* are a major threat for inpatient care. A population-based study in the USA identified rate of BSI from *P. aeruginosa* bacteremia was 10.8 (in males and 3.7 in females; and for monomicrobial P. aeruginosa bacteremia was 8.4 in males and 2.5 in females.(38)In the 2019 antibiotic resistance threat report, MDR *P. aeruginosa* infection in hospitals across the US was 32,600. Most of the isolates were carbapenem-resistant and 3% of CRPA carry a mobile genetic element that makes a Carbapenemase enzyme.(5) European Centre for Disease Prevention and Control (ECDC) and the WHO published 2020 antimicrobial resistance surveillance reported 18% carbapenem resistance rate in *P. aeruginosa* isolates. (39).The major risk factors for patients acquiring CRPA BSI were assessed in a retrospective multicenter cohort study in China. It showed that prior ICU hospitalization, immunosuppressive therapy, and exposure to carbapenems within 90 days (including meropenem, imipenem, biapenem) were independent risk factors associated with development of CRPA bacteremia. (40) A retrospective study included data from Ethiopian Public Health Institute from 2017 to 2021 reviewing the prevalence of CRAB and CRPA among the *A. baumannii* and *P. aeruginosa* isolates from different body samples. It showed a prevalence of 61% of CRAB and 22% of CRPA. The prevalence of CRAB species was 50% in 2017 and the prevalence of CRPA isolates was 30.3% in 2017. Around 14.2 % of both CRAB and CRPA isolates were from the blood.(41)

Objectives

General objective

- To assess the prevalence of multidrug resistant Gram-negative bacteria and associated factors among blood culture isolates at Tikur Anbessa Specialized Hospital

Specific objective

- To determine the prevalence of multidrug resistant Gram-negative bacteria among blood culture isolates at Tikur Anbessa Specialized Hospital.
- To examine factors associated with multidrug resistant Gram-negative bacteria among blood culture isolates at Tikur Anbessa Specialized Hospital.

Method

Study area and Period.

Study area.

The study area was Internal medicine wards at Tikur Anbessa Specialized Hospital (TASH), Addis Ababa, Ethiopia. It is the country's largest teaching and referral hospital opened in 1972 and incorporated to Addis Ababa University in 1998. It serves the 120 million Ethiopians with over seven hundred beds.

Study period.

The study period was from September1,2021 until September 1, 2023.

Study design.

A retrospective study was conducted to determine the bacteriologic profile of bloodstream infections among adult patients admitted to the hospital, associated risk factors and in-hospital mortality will be reviewed for MDR gram-negative BSI and Carbapenem resistant gram-negative BSI.

Source population and study population.

Source population.

All adult patients that were admitted to the various wards during the study period with suspected bloodstream infections.

Study population.

All adult patients with blood stream infection who were admitted to the hospital with positive blood cultures for Enterobacterales, *A. baumannii*, *P. aeruginosa* during the study period.

Inclusion and exclusion criteria

Inclusion criteria

Patients aged 18 and above who are admitted to Tikur Anbessa specialized Hospital from September 1, 2021, to September 1, 2023, who had yield from blood culture.

Patients with complete registry notes on I-care, and/or medical charts were included in the study.

All patients with growth on blood culture as determined by the TASH microbiology unit and for which AST was performed (CLSI-2023 breakpoints for disc diffusion method were used to determine antimicrobial susceptibility pattern for all growths)

Patients' clinical information was included only once in the study and bacteriological profile of isolates from patients was recorded only once during the study period. The first blood culture isolation in the study period was used.

Exclusion Criteria

Patients with incomplete information on their medical charts.

Patients with Polymicrobial growth reported on from a single blood culture.

Sample size determination and Sampling technique.

Variables

Independent variables

- Age
- Sex
- Comorbidities
- Charlson Comorbidity Index
- Admitted ward- medical, surgical, Obstetrics, Gynecology, MICU, CCU
- Instrumentation or insertion of medical devices
- Surgical procedures done.
- Use of prior Antibiotics
- Type of prior Antibiotics
- Antibiotics used after blood culture result.
- Antimicrobial susceptibility testing.
- Sepsis

- Septic shock

Dependent variable

- Multidrug resistant gram-negative bacteria from the blood (Enterobacterales, *A. baumannii* and *P. aeruginosa*)
- Carbapenem resistant gram-negative bacteria from the blood (Enterobacterales, *A. baumannii* and *P. aeruginosa*)

Data Collection Procedure

The research questionnaire wad developed using ODK. Data collectors (medical inters) identified patients with blood-stream infection who have yield from blood culture in the study period from the microbiology unit registry. The electronic medical number or I-care number was extracted from the electronic registration at the microbiology lab. Patients 'medical record was enrolled in the study only once. The data collection process was supervised by the primary investigator.

Data Analysis and Presentation

The collected data was cleaned, checked for completeness, compiled, and analyzed. SPSS Version 26 was used to compete standard descriptive methods (means/percentage and standard deviations). Bivariate analysis was used to compare the independent variables, and further multivariate analysis was conducted among independent variables with significance. The results were presented in tables, figures, and statements. Significance of the association between dependent and independent variables was determined using chi-square test where necessary, and differences will be considered significant at $p < 0.05$.

Ethical Considerations

The study was conducted after approval of the proposal by the ethical review committee of Internal Medicine Department and was approved by the ethical institutional review board of Addis Ababa University, College of Health Sciences.

OPERATIONAL DEFINITIONS

MDR- non-susceptible to ≥1 agent in >3 antimicrobial categories. (42)

In the microbiology laboratory, bacterial inoculums were tested for antimicrobial susceptibility using the current CLSI guidelines (2023- M100 PERFORMANCE STANDARDS FOR ANTIMICROBIAL SUSCEPTIBILITY TESTING, 33RD EDITION,M100ED33) for Disc diffusion method

Charlson-Deyo Comorbidity Index

The Charlson Comorbidity Index is a method of categorizing comorbidities of patients based on the International Classification of Diseases (ICD) diagnosis codes found in administrative data, such as hospital abstracts data. Each comorbidity category has an associated weight (from 1 to 6), based on the adjusted risk of mortality or resource use, and the sum of all the weights results in a single comorbidity score for a patient. A score of zero indicates that no comorbidities were found. The higher the score, the more likely the predicted outcome will result in mortality or higher resource use.(43)

1each: Myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular disease, dementia, chronic pulmonary disease, rheumatologic disease, peptic ulcer disease, liver disease (if mild, or 3 if moderate/severe), diabetes (if controlled, or 2 if uncontrolled)

2 each: Hemiplegia or paraplegia, renal disease, malignancy (if localized, or 6 if metastatic tumor), leukemia, lymphoma.

6 each: AIDS

Patients who are 50 years old or more get additional points:

- 50-59 years old: +1 point
- 60-69 years old: +2 points
- 70-79 years old: +3 points
- 80 years old or more: +4 points(43)

Prior Antibiotics

Antibiotics used in the last three months prior to the bloodstream infection.

Lack of source control

The presence of site of infection that needed to be incised and drained or surgically managed, or removal of catheters, drainage tubes that were diagnosed to be source of infection by the treating physician and team. Source control included drainage of an abscess, debriding infected necrotic tissue, removal of a potentially infected device, or definitive control of a source of ongoing microbial contamination.(44)

Sepsis and septic shock

Sepsis was defined **as being diagnosed by the treating physician and team** with documentation on medical records or combined - quick Sequential Organ Failure criteria(qSOFA) with either Modified Early Warning Score (MEWS) or National Early Warning Score (NEWS). (45)

5. Result

5.1 Sociodemographic characteristics of the study participants

During the study period, a total of 179 patients with blood cultures growing gram-negative bacteria were identified, and 40 were excluded for missing medical records and eight for mixed infection. A total of 137 patients with gram-negative blood culture were included in the final analysis. Fifty-one percent of the study participants were female. The mean age of the patients with gram-negative blood culture and SD Age of **43.1±18.17 years** with the majority age group being between 19-44 years of age (48.2%). Most of the blood cultures were collected from the patients at the Emergency room **(29.2%).** The second most common site were the Intensive care units (Medical ICU, Surgical ICU, and Isolation ICU) with 27% and the mean length of stay in the ICU prior to BSI, duration of patient on mechanical ventilation, duration on vasoactive medication and length of ICU stay were **9, 10.11, 3.38** and **9.18** days respectively as shown in Table 1.

Table 1.The sociodemographic characteristics of the study participants among Gram-negative Blood Culture Isolates

Sociodemographic characteristics	Frequency	Percent
Sex		
Male	67	48.9
Female	70	51.1
Age of the patient in years (mean +SD) = **43.1±18.17**		
14-18	10	7.3
19-44	**66**	**48.2**
45-64	38	27.7
≥65	23	16.8
Ward of blood sample taken		
Emergency room	**40**	**29.2**

ICU	37	27.0
Hematology ward	32	23.4
Medical ward	16	11.7
Surgical ward	6	4.4
Neurosurgery ward	4	2.9
Urology ward	2	1.5
Length of stay in the ICU prior to BSI		
Mean (SD)=**9.0±7.23**		
Duration of patients on Mechanical ventilation		
Mean (SD)=**10.11±12.32**		
Duration on Vasoactive medication		
Mean (SD)=**3.38±3.61**		
Length of ICU stay with BSI		
Mean (SD)=**9.18±8.29**		

5.2 Comorbidities and underlying medical conditions

Twenty-nine percent of the participants had hematologic malignancy, ALL accounted for 48.7% followed by AML with 30.8%. Around twenty-two percent of the study participants had solid organ tumor and a total of 12 patients (8.7%) were on immunosuppressive agents. A total of 29 patients (21.2%) had recent surgical procedures. Around twelve percent of the participants had stroke. Ten percent of the participants had CKD and half required dialysis and 21.2% had recent surgeries. The findings of the study also showed that 9.5% of the study participants had Diabetes Miletus of which 76.9% (n=10) had Type II Diabetes Mellitus as seen in Table 2. The underlying comorbidities were assessed using the Charleson comorbidity index depicted in Table 3.

Table 2.Comorbidity and underlying disease characteristics of the study participants having Gram-negative Blood Culture Isolates

Comorbidities and underlying disease	Frequency (N=137)	Percent

Diabetes Mellitus	13	9.5
Types of DM (n=13)		
Type 1	3	23.1
Type 2	10	76.9
Stroke	17	12.4
Hemiplegia or paraplegia	8	5.8
HIV	11	8
CKD	14	10.2
CKD require dialysis (n=14)		
Yes	7	50
No	7	50
CHF	15	10.9
IHD	4	2.9
Peripheral vascular disease	2	1.5
CLD	3	2.2
Hematologic malignancy	39	28.5
Types of hematologic malignancy		
ALL	19	48.7
AML	12	30.8
Aplastic anemia	2	5.1
CLL	2	5.1
CML	1	2.6
High grade NHL	2	5.1
Primary CNS Lymphoma	1	2.6

Solid organ tumor	30	21.9
Immunosuppressive treatment or history of prior immunosuppressive treatment	12	8.7
Recent surgical procedures	29	21.2

Table 3. The index of Charleson comorbidity among gram negative bacterial infection patient

Calculate the Charlson Comorbidity Index (CCI).	Frequency	Percent
0.00	5	3.6
1.00	6	4.4
2.00	38	27.7
3.00	25	18.2
4.00	18	13.1
5.00	22	16.1
6.00	13	9.5
7.00	2	1.5
8.00	3	2.2
9.00	2	1.5
10.00	1	.7
11.00	1	.7
16.00	1	.7
Total	137	100.0

5.3 Hospital admission related characteristics of the patient having Gram-negative blood stream infection.

More than half **(52.6%)** of the study participants were referred from other health facilities and **65.7%** of the patients had **Sepsis** and **16.1%** had **Septic shock** on admission. Twenty-nine

percent of the study participants had a history of prior hospital admission within 3 months of the current BSI. The length of stay in the hospital prior to the Gram-negative BSI was 17±19.38 days. Almost 25% percent of the study participants had prior antibiotics exposure in the preceding three months before the blood culture. Among those who took antibiotics, **Ceftriaxone** was taken by **76.5%** followed by Vancomycin (52.9), Cefepime (35.3%) and Metronidazole (23.5%). The details of admission characteristics can be seen in Table 4.

Table 4. Hospital admission related characteristics of the patient having Gram-negative bloodstream infection.

Hospital Admission characteristics	Frequency	Percent
Place patient during admission		
Referral from other health facilities.	72	52.6
Emergency room	57	41.6
OPD	8	5.8
History of prior hospital admission within 3 months of the current BSI		
Yes	40	29.2
No	54	39.4
Unknown	34	31.4
Admission diagnosis		
Does patient have Sepsis on Admission?		
Yes	90	65.7
No	47	34.3
Does patient have Septic shock on Admission?		

Yes	22	16.1
No	115	83.9
Length of hospital stay prior to blood stream infection in days		
≤2	**17**	**12.4**
3-7	**45**	**32.8**
8-14	**22**	**16.1**
15-30	**33**	**24.1**
>30	**20**	**14.6**
History of ICU admission prior to bacteremia		
Yes	35	25.5
No	102	74.5
History of prior antibiotics exposure in the preceding 3 months		
Yes	34	24.8
No	50	36.5
Unknown	53	38.7
Types of antibiotics used in the last three months (n=34)		
Ceftriaxone	26	76.5
Ceftazidime	6	17.6
Cefepime	12	35.3
Ciprofloxacin	5	14.7
Meropenem	5	14.7
Trimethoprim-Sulfamethoxazole	3	8.8
Amikacin	1	2.9
Vancomycin	18	52.9

Metronidazole	8	23.5
Other antibiotics	4	11.8

5.4 Hospital stay related characteristics the patients with gram-negative BSI.

Of the common devices used, 78.3% had transurethral urinary catheter, 63% used Nasogastric tube and 17.4% had CNS shunting device. Five percent of the study participants had a lack of infection source control as seen in Table 5.

Table 5. Hospital stay related characteristics of patients with gram-negative BSI.

Risk factors	Frequency	Percent
Invasive device used		
Yes	46	33.6
No	91	66.4
Types of invasive device (n=46)		
Central venous catheter	1	2.2
Transurethral urinary catheter	36	78.3
CNS shunting device	8	17.4
Peritoneal drainage tube	2	4.3
Chest tube	4	8.7
Nasogastric tube	29	63
Other invasive devices	9	19.6
Lack of source control in the management of the patients		
Yes	7	5.1
No	130	94.6
Types of sources for non-control infection (n=7)		

Bed sore	2	28.6
Brain abscess	1	14.3
Gluteal abscess	1	14.3
Peritoneal collection	1	14.3
Unspecified wound	1	14.3

5.5 Characteristics of blood culture Isolated gram-negative bacteria

Three-fourths of the study participants had blood cultures of Enterobacterales followed by *Acinetobacter baumannii* (19.7%) and *Pseudomonas aeruginosa* (5.1%). One-third (33%) of the Enterobacterales isolated from the blood cultures were ***Escherichia coli*** (33%) followed by ***Klebsiella pneumoniae*** (30.1%) as shown in Table 6. The distribution of gram-negative isolates to specific medical wards showed *K. pneumoniae* was the most prevalent isolate from Emergency room (27.5%) as *E. coli* came second (25%). *E. coli* was the most prevalent blood isolate in the Hematology wards (32.3%). *Acinetobacter Spp*. and *P. aeruginosa* were more prevalent in the Emergency room when compared to other wards with 33.3% isolates and 42.9% (3/7) of the respectively. Details of the distribution of the gram-negative blood isolates in relation to the medical wards can be found in Figure 1.

Table 6. The blood culture Isolated gram-negative bacteria

Variable	Frequency	Percent
Blood culture Isolated		
P. aeruginosa	7	5.1
Acinetobacter Spp	27	19.7
Enterobacterales	103	75.2
Types of Enterobacterales (n=103)		
E. coli	34	33
K. pneumoniae	31	30.1

K. oxytoca	8	7.8
K. ozaenae	2	1.9
K. rhinoscleromatis	2	1.9
E. cloacae	4	3.9
S. typhi	3	2.9
P. stuartii	3	2.9
C. diversus	9	8.7
P. mirabilis	1	0.8
Ent. aeruginosa/Hafnia	5	4.9
M. morganii	1	0.8

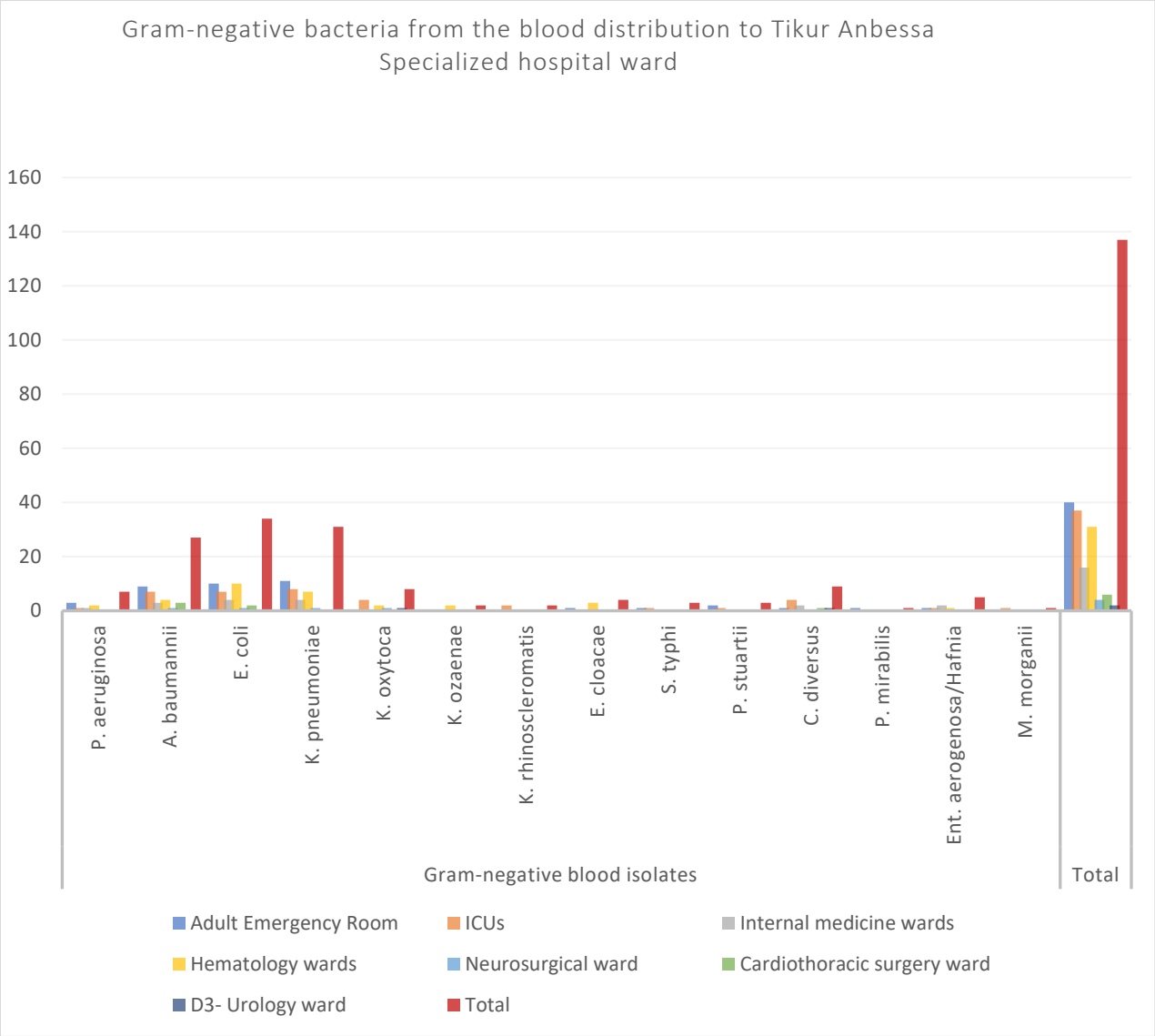

Figure 1.Gram-negative bacteria from the blood distribution to specific medical wards

5.6 The characteristics of the susceptibility for specific antibiotics for gram-negative bacterial culture

The finding of the study showed that most gram-negative blood culture isolates were resistant to Ceftazidime (92.5%) Ceftriaxone (91.8%) Cefepime (87.3%), and Ciprofloxacin (86.4%). The most resistance rate was seen for Amoxicillin-clavulanic acid were all the 14 isolates tested for AST showed resistance. Our study revealed a high rate of resistance to Sulfamethoxazole with 89.2% of the gram-negative blood isolates tested being resistant. The least resistance rate for antibiotic was seen for Amikacin where 52.9% of the 121 isolates tested were found to be

susceptible. Of the 116 gram-negative blood isolates tested 56% were resistant to Meropenem as seen in Table 7.

Table 7. The characteristics of the specific antibiotic sensitivity for gram-negative bacterial cultures

Antibiotic	Antibiotic susceptibility test by Disc diffusion test				
	Not available	Susceptible	Intermediate	Resistant	Non-susceptible rate from tested (isolates tested for)
Ceftazidime	**4**	**10**	**6**	**117**	**92.5%(133)**
Ceftriaxone	15	10	4	108	91.8%(122)
Cefepime	19	15	2	101	87.3%(118)
Ciprofloxacin	19	16	13	89	86.4%(118)
Amoxicillin-Clavulanic acid	123	0	0	14	**100%(14)**
Amikacin	16	64	7	50	**47.1%(121)**
Gentamicin	43	28	2	64	70.2%(94)
Piperacillin-Tazobactam	88	14	5	30	71.4(49)
Ertapenem	77	29	4	27	51.7%(60)
Meropenem	21	51	2	63	56%(116)
Imipenem	136	0	0	1	100%(1)
Chloramphenicol	124	6	0	7	53.8%(13)
Sulfamethoxazole	35	11	1	90	89.2% (102)

5.11 The relation between the isolated gram-negative bacteria from blood and AST test compared with classes of antibiotics.

Antibiotic susceptibility test results for the gram-negative bacteria blood isolates are shown in Table 8. *K. pneumoniae* showed higher drug resistance rates to third **(Ceftazidime 96.8%, Ceftriaxone 93.5%,)**, fourth generation cephalosporins **(Cefepime 93.3%)** and fluroquinolones **(Ciprofloxacin 96.4%).** *E. coli* had higher resistance rates to third generation cephalosporins **(Ceftriaxone 91.2%, Ceftazidime 88.2%)**, fourth generation cephalosporins **(Cefepime 87.1%)** and Fluroquinolones **(Ciprofloxacin 90%).** *Pseudomonas aeruginosa* isolates from the blood isolates were all resistant to Ceftazidime and Ceftriaxone. The resistance rates of *Acinetobacter baumannii* to Meropenem was **76.9%** as shown in Table 8.

Table 8.Frequency of gram-negative bacterial species and antibiotic susceptibility pattern from blood culture

Bacteria identified		**P. aeruginosa**	**Acinetobacter Spp.**	**E. coli**	**K. pneumoniae**	**K. oxytoca**	**K. ozaenae**	**K. rhinoscleromatis**	**E. cloacae**	**S. typhi**	**P. stuartii**	**C. diversus**	**P. mirabilis**	**Ent. aerogenosa/Hafnia**	**M. morganii**	**Total gram-negative isoaltes tresistant**
	Total isolates	7	27	34	31	8	2	2	4	3	3	9	1	5	1	137
Ceftriaxone	R(%,number tested)	**2(100%,2)**	19(95%,20)	**31(91.2%, 34)**	**29(93.5%,31)**	5(71.4%,7)	**2(100%,2)**	1(100%,1)	3(100%,3)	2(66.6%,3)	2(66.6%,3)	**9(100%,9)**	**1(100%,1)**	4(80%,5)	1(100%,1)	**112(91.8%,122)**
Ceftazidime	R(%,number tested)	**7(100%.7)**	23(88.5%,26)	**30(88.2%,34)**	**30(96.8%,31)**	8(100%,8)	2(100%)	2(100%,2)	**4(100%,4)**	No AST test	2(66.7%,3)	9(100%,9)	1(100%,1)	4(80%,5)	1(100%,1)	**123(92.5%,133)**
Cefepime	R(%,number tested)	3(60%,5)	19(82.5%,23)	**27(87.1%,31)**	**28(93.3%,30)**	4(66.7%,6)	2(100%,2)	2(100%,2)	**4(100%,4)**	0(0%,1)	2(66.6%,3)	6(100%,6)	1(100%,1)	3(100%,3)	1(100%,1)	**103(87.3%,118)**

Imipenem		Meropenem		Ertapenem		Piperacillin-Tazobactam		Gentamicin		Amikacin		Amoxicillin Clavulanic acid		Ciprofloxacin	
R(%,number tested)		R(%,number tested)		R(%,number tested)		R(%,number tested)		R(%,number tested)		R(%,number tested)		R(%,number tested)		R(%,number tested)	
1(100%,1)		4(57.1%,7)		No AST		3(60%,5)		3(42.9%,7)		**1(14.3%,7)**		No AST		**2(40%,5)**	
No AST		**20(76.9%,26)**		1(100%,1)		**4(66.7%,6)**		19(95%,20)		**16(66.7%,24)**		No AST		**17(70.8%,24)**	
No AST		**7(23.3%,30)**		6(19.35%,31)		**8(57.1%,14)**		7(35%,20)		**7(23.3%,30)**		3(100%,3)		**27(90%,30)**	
No AST		17(73.9%,23)		10(66.7%,15)		**8(72.7%,11)**		18(81.8%,22)		**16(53.3%,30)**		7(100%,7)		**27(96.4%,28)**	
No AST		5(62.5%,8)		3(60%,5)		2(100%,2)		4(80%,5)		5(71.4%,7)		No AST		7(87.5%,8)	
No AST		0(0%,1)		1(50%,2)		No AST		2(100%,2)		1(50%,2)		1(100%,1)		2(100%,2)	
No AST		1(50%,2)		1(50%,2)		1(100%,1)		2(100%,2)		1(50%,2)		1(100%,1)		2(100%,2)	
No AST		3(75%,4)		3(100%,3)		2(100%,2)		3(100%,3)		2(66.7%,3)		1(100%,1)		2(66.7%,3)	
No AST		No AST		No AST		No AST		No AST		No AST		No AST		2(100%,2)	
No AST		1(33.3%,3)		1(50%,2)		No AST		1(50%,1)		0(0%,2)		No AST		3(100%,3)	
No AST		4(66.7%,6)		3(50%,6)		4(80%,5)		3(60%,5)		5(62.5%,8)		1(100%,1)		5(100%,5)	
No AST		0(0%,1)		No AST		No AST		No AST		0(0%,1)		No AST		1(100%,1)	
No AST		2(50%,4)		2(66.7%,3)		3(100%,3)		3(60%,5)		2(50%,4)		No AST		4(100%,4)	
No AST		1(100%,1)		No AST		No AST		1(100%,1)		1(100%,1)		No AST		1(100%,1)	
1(100%,1)		**65(56%,116)**		**31(51.6%,60)**		**35(71.4%,49)**		**66(70.2%,94%)**		**57(47.1%,121)**		**14(100%,14)**		**102(86.4%,118)**	

Chloramphenicol	R(%,number tested)	No AST	No AST	0(0%,3)	3(75%,4)	0(0%,1)	No AST	1(50%,2)	1(100%,1)	1(100%,1)	No AST	No AST	No AST	1(100%,1)	No AST	**7(5.3.8,13)**
Sulfamethoxazole	R(%,number tested)	No AST	15(93.8%,16))	22(81.5%,27)	26(92.9%,28)	4(80%,5)	2(100%,2)	1(100%,1)	2(100%,2)	2(66.7%,3)	3(100%,3)	8(88.9%,9)	1(100%,1)	4(100%,4)	1(100%,1)	**91(81.3%,112)**

5.9 Gram-negative blood isolates and Probable ESBL production

The ESBL production was predicted from the pattern of resistance towards third and fourth generation cephalosporins tested for antimicrobial susceptibility. Our findings show that 86.1% of the gram- negative blood isolates were probable ESBL producers.

Table 9.Gram-negative blood isolates and Probable ESBL production

Gram- negative blood isolate	Probable ESBL		Total
	Yes	No	
P. aeruginosa	3(42.9%)	4(57.1%)	7
A. baumannii	**23(85.2%)**	4(14.8%	27
E. coli	**30(88.2%)**	4(11.8%)	34
K. pneumoniae	**29(93.5%)**	2(6.5%)	31
K. oxytoca	7(87.5%)	1(12.5%)	8
K. ozaenae	2(100%)	0	2
K. rhinoscleromatis	2(100%)	0	2
E. cloacae	4(100%)	0	4

S. typhi	1(33.3%)	2(66.7%)	3
P. stuartii	2(66.7%)	19(33.3%)	3
C. diversus	9(100%)	0	9
P. mirabilis	1(100%)	0	1
Ent. aerogenosa/Hafnia	4(80%)	1(20%)	5
M. morganii	1(100%)	0	1
Total	118**(86.1%)**	19(13.9%)	137

5.6 The prevalence of Multidrug Resistant Gram-Negative BSI

The overall prevalence of MDR in the gram-negative blood isolates was 81%(Figure 2) The highest rate of MDR was among the Enterobacterales with 83.5% and *K. pneumoniae* (93.5%) isolates had more MDR rates than *E. coli* isolates (73.5%). Specific details can be found in Table 10.

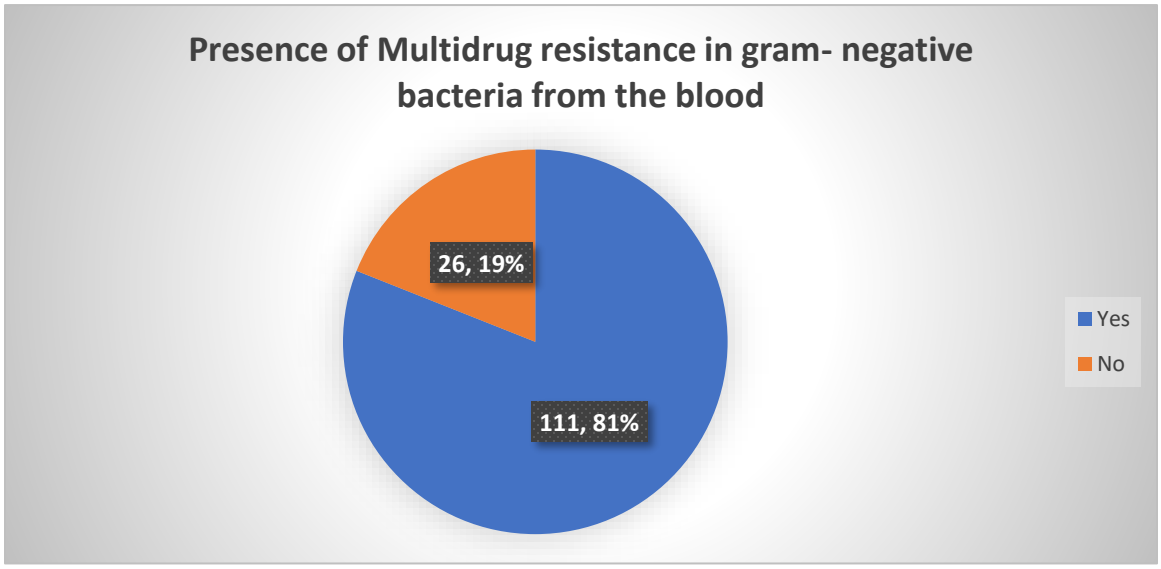

Figure 2. The prevalence of multidrug resistance in gram-negative BSI

Table 10. Multidrug resistance in gram-negative blood isolates

		Multidrug resistant		Total
		No	Yes	
Gram-negative blood isolate	*P. aeruginosa*	4	3	7
	A. baumannii	5	22	27
	E. coli	9	25	34
	K. pneumoniae	2	29	31
	K. oxytoca	1	7	8
	K. ozaenae	0	2	2
	K. rhinoscleromatis	0	2	2
	E. cloacae	1	3	4
	S. typhi	2	1	3
	P. stuartii	1	2	3
	C. diversus	0	9	9
	P. mirabilis	0	1	1
	Ent. aerogenosa/Hafnia	1	4	5
	M. morganii	0	1	1
Total		26	111	137

5.12 The Pearson correlation between number of drug resistance of gram-negative bacteria

The Pearson correlation test showed that duration of patient on Mechanical ventilation and number of classes of antibiotic groups that the gram-negative isolate was resistant to have a significant positive relation. This means that as duration of patient on mechanical ventilation increases the number of antibiotics the gram-negative blood isolate is resistant to also increases.

The Charleson comorbidity index (CCI) showed a significant association with the number of antibiotics the gram-negative blood isolate was resistant to. The number of days the patient on vasoactive medications had a negative significant relation with number of antibiotic resistances by gram negative bacteria as shown in Table 9.

Table 11. The Pearson correlation between number of drug resistance of gram-negative bacteria

The Pearson correlation test between antibiotic group vs independent variables		Number of resistance antibiotic group
Age in years	Pearson Correlation	-.016
	Sig. (2-tailed)	.849
	N	137
The length of ICU stays with BSI-days	Pearson Correlation	.007
	Sig. (2-tailed)	.968
	N	34
the patient on mechanical ventilation	Pearson Correlation	.856
	Sig. (2-tailed)	**.032**
	N	34
Length of stay in the ICU prior to BSI (in days)	Pearson Correlation	.017
	Sig. (2-tailed)	.923
	N	34
Number of days the patient on vasoactive medications	Pearson Correlation	-.424*
	Sig. (2-tailed)	**.012**
	N	34
Calculate the Charlson Comorbidity Index (CCI).	Pearson Correlation	.564
	Sig. (2-tailed)	**.049**

	N	137
Length of in-hospital stay prior to blood stream infection (in days)	Pearson Correlation	-.094
	Sig. (2-tailed)	.272
	N	137
**. Correlation is significant at the 0.01 level (2-tailed).		
*. Correlation is significant at the 0.05 level (2-tailed).		

5.13 Associated factors of MDR for gram-negative bacteria BSI

The multivariate logistic regression revealed that study participant having a hematologic malignancy had **1.4** folds increase in having MDR gram-negative BSI (AOR=1.4, 95%CI=1.12, 4.27) and Enterobacterales isolated from the blood culture had **4.4** folds increase of being MDR as compared to the *Pseudomonas aeruginosa* blood culture isolates (AOR 4.4, 95%CI=1.57, 34.18). Blood cultures received from **ICU** that grew gram-negative bacteria were **2.1** likely to be MDR compared to those received from Emergency room (AOR=2.1, 95%CI=1.24, 16.56) as seen in Table 12.

Table 12. The bivariate and multivariate logistic regression of association between multidrug resistance and risk factors.

Variable	Presence of Multi drug resistance		P-value	COR with 95%CI	P-value	AOR with 95%CI
	Yes	No				
Age in year						
14-18	9	1	1		1	
19-44	51	15	0.137	0.38 (0.04, 3.23)	0.125	0.25(0.04, 1.46)
45-64	32	6	0.647	0.59(0.06, 5.58)	0.963	0.93(0.33, 14.13)
≥65	19	4	0.591	0.53(0.05, 5.43)	0.412	0.78(0.041, 4.28)

Place of patient at bacterial test						
Emergency	31	9	1		1	
ICU	34	3	0.094	3.3(0.82, 13.27)	**0.049**	**2.1 (1.24, 16.56)**
Medical ward	15	1	0.181	4.4(0.50, 37.61)	0.272	2.1(0.43, 9.63)
Hematology ward	22	10	0.404	0.64(0.22, 1.83)	0.444	0.48(0.14, 12.34)
Surgical ward	3	3	0.169	0.29(0.05, 1.69)	0.517	0.71(0.21, 11.36)
Neurosurgery ward	4	0	*			
Urology Ward	2	0	*			
Diabetes Miletus						
No	99	25	1		1	
Yes	12	1	0.248	3.1(0.38, 24.42)	0.589	1.9(0.19, 18.47)
Hemiplegia or paraplegia						
No	106	23	1		1	
Yes	5	3	0.184	0.36(0.08, 1.62)	0.140	0.22(0.03, 1.63)
HIV						
CHF						
No	101	21	1		1	
Yes	10	5	0.142	0.42(0.13, 1.34)	0.095	0.27(0.06, 1.25)
Hematologic malignancy						
No	83	15	1		1	
Yes	**28**	**11**	**0.087**	**2.2(0.89, 5.28)**	**0.018**	**1.4(1.12, 4.27)**
Immunosuppressant treatment history						
No	102	22	1		1	
Yes	8	4	0.263	2.1(0.58, 7.29)	0.587	0.64(0.13, 3.21)

Blood culture isolated						
Pseudomonas aeruginosa	3	4	1		1	
Acinetobacter Spp.	22	5	0.052	5.9(0.98, 34.94)	0.362	2.8(0.30, 27.0)
Enterobacterales	86	17	0.018	6.7(1.38, 32.91)	0.014	**4.4(1.57, 34.18**)

5.14 The determinant factors of Carbapenem resistant Gram-negative blood cultures

The bivariate logistic regression revealed that participants having history of prior Cefepime use, CNS shunt device and *Acinetobacter Spp.* isolated from the blood culture had an association with Carbapenem resistance. The multivariate logistic regression revealed that participant who took immunosuppressive treatment were **2.1** more likely to have gram-negative bacteria form blood culture resistant to carbapenems (AOR=2.1, 95%CI=1.24, 3.78) and patients having history of prior Cefepime use had **4.9** folds increase in having Carbapenem resistant gram-negative bacteria from the blood culture (AOR=4.9, 95%CI=1.27, 41.24).Patients that had CNS shunt device were **12.4** folds increase in having carbapenem resistant gram-negative blood stream infections (AOR=12.4, 95%CI=4.26, 22.56). *Acinetobacter Spp* blood isolates were **4.2** folds increase its carbapenem resistance compared to those of Enterobacterales (AOR=4.2, 95%CI=2.19, 14.63). See table 13 below.

Table 13.The bivariate and multivariate logistic regression of association between independent variable and carbapenem resistance of gram-negative bacteria.

Variable	Presence of carbapenem resistance		P-value	COR with 95%CI	P-value	AOR with 95%CI
	yes	No				
HIV status						
Negative	72	54	1		1	
Positive	8	3	0.322	2.0(0.51, 7.89)	0.121	3.1(0.12, 7.84)
Taking Immunosuppressive treatment						

No	71	53	1		1	
Yes	8	4	0.109	1.7(0.49, 5.75)	0.045	**2.1(1.24, 3.78)**
Prior Ceftazidime use						
No	13	15	1		1	
Yes	4	2	0.176	2.3(0.36, 14.72)	0.051	1.9(0.12, 18.47
Prior Cefepime use						
No	8	14	1		1	
Yes	9	3	0.038	5.3(1.09,25.21	0.012	**4.9(1.27, 41.24)**
CNS shunting device						
No	20	18	1		1	
Yes	7	1	0.099	6.3(0.71, 56.29)	0.044	**12.4(4.26, 22.56)**
Types of gram-negative blood culture isolates						
Pseudomonas aeruginosa	4	3	0.848	1.2(0.25, 5.46)	0.489	0.49(0.28, 12.33)
Acinetobacter spp.	21	6	0.027	3.1(1.14, 8.19)	0.044	**4.2(2.19, 14.63)**
Enterobacterales	55	48	1		1	

Discussion

This study aimed at exploring the current antimicrobial resistance in blood cultures in a hospital with a low-resource setting. A total of 137 gram-negative blood isolates were assessed during the study period. The number of isolates from female patients were slightly higher than male patients. The mean age group of the patients with gram-negative blood culture was 43.1±18 years of age. This is like the study from Arbaminch General Hospital.(23) The common site of gram-negative BSI from the current study was the Emergency room followed by the ICUs across the hospital. This is unlike the hospital-based study from China that showed the hematology ward had the most BSI with the ICU in second place(17). Our findings are also different form studies in the country as the study from Arbaminch revealed, the Internal medicine ward had the

most yield for blood cultures. In a recent study from Addis Ababa, the ICUs were the most common sites of gram-negative BSI.(26)

The most common gram-negative blood isolates form the study were Enterobacterales. It showed that the prevalent species was *E. coli*(33%), followed by *K. pneumoniae*(30.1%) and *Acinetobacter spp*. (19.7%).Our findings conquer with reports from China, Türkiye, Zambia and Arbaminch.(17,18,21,23) However, in studies from Addis Ababa that were both hospital-based, and lab based, *K. pneumoniae* was the predominant gram-negative blood isolate.(22,24–26) While assessing the distribution of gram-negative blood isolates to the different adult medical wards, we found *K .pneumoniae* was the most prevalent isolate from Emergency room (27.5%) as *E. coli* came second(25%). *E. coli* was the most prevalent blood isolate in the Hematology wards (32.3%). and hematology wards (28.6%). The non-fermenting bacteria also seemed to have a comparable distribution with 33.3% of Acinetobacter *Spp*. blood isolates and 42.9% (3/7) of all *P. aeruginosa* blood isolates from the Emergency room. These findings are unlike Yamba et al that described *E. coli* isolates more prevalent in the Internal medicine wards (56%), A. baumannii blood isolates more prevalent in the surgical/burn unit (30%) and *P. aeruginosa* from the renal unit(71%).(21) The disparity in our findings may be explained by the fact that patients in our study at the Emergency room are mostly referrals from other health care facilities(52.9%),with a history of hospital admission or outpatient clinic visit from referring center.

The AST that was done on the gram-negative blood isolates showed an alarming rate of resistance to Cephalosporins: Ceftazidime (92.5%) Ceftriaxone (91.8%) Cefepime (87.3%). All these Cephalosporins are part of the Watch group from the WHO AWaRE classification of essential medication groups which indicates the need of Antimicrobial stewardship programs.(8) This was unlike all the previous reports coming from China, Ghana, Arbaminch and Addis Ababa where the rates of resistance to Cephalosporins were ≤85%.(17,20,23–26) All of the blood isolates tested for Amoxicillin-clavulanic acid were resistant to the antibiotic from our study. The least level of resistance was seen towards Amikacin with more than half (52.9%) of the gram-negative blood isolates tested being susceptible. This was in line with the findings of Beshah *et al.* that showed Amikacin had the lowest rate of resistance.(26)Resistance to sulfamethoxazole and fluroquinolones were also high in our findings with 89.2% and 86.4% of the gram-negative blood isolates having resistance respectively. The high Ciprofloxacin resistance is concerning as Ethiopia has a high burden for Tuberculosis and treatment for MDR TB is by respiratory quinolones that have cross resistance. The findings from Ghana showed a different antibiotic resistance profile as the resistance to Amoxicillin was 89.3%, and the level of trimethoprim-sulfamethoxazole and fluroquinolone resistance was relatively lower than our study with 76.1% and 55.3% resistance seen respectively.(20)These findings also differed from studies done in Addis Ababa. A laboratory-based assessment done from a private center in Addis Ababa showed that gram-negative blood isolates had the highest resistance towards Ampicillin (80.8%) and the lowest was to Imipenem (5.2%).(25)Similarly, the findings from Beshah *et al.*

showed that the highest level of resistance from gram-negative blood isolates were towards Ampicillin (94.6%).(26)

In the current study the AST revealed that the Enterobacterales isolates showed a higher resistance towards Cephalosporins. The most frequent isolate, *E. coli* had higher resistance rate to third and fourth generation Cephalosporins with 96.8% resistance to Ceftazidime, 91.2% to Ceftriaxone and 87.1% resistant to Cefepime. Findings from different parts of the world showed *E. coli* blood isolates having higher resistance towards first and second generation of cephalosporins with varying rates of resistance to third and fourth generation cephalosporins. Shin *et al* reported *E. coli* blood isolates having 55.9% resistance to third generation cephalosporins, Donkor et al reporting resistance rates for Ceftriaxone 61.4% and 67% to Ceftazidime. (17,20)Similarly, Yamba *et al* reported *E. coli* blood isolates that showed 68% resistance to Ceftriaxone.(21) Our results were also higher than a recent report from Beshah et al were *E. coli* blood isolates showed 69.8% resistance to cephalosporins.(26) *K. pneumoniae* blood isolates from our study showed similar antibiotic resistance rates as *E. coli*, with resistance to third generation cephalosporins with resistance to Ceftazidime 96.8% and Ceftriaxone 93.5%. The level of resistance to the fourth-generation cephalosporin Cefepime was 93.3%. These also showed an increase from reports from China, Ghana, and Zambia.(17,20,21)Furthermore Bitew et al reported >82% of resistance for all Cephalosporins except cefoxitin from their *K. pneumoniae* blood isolates.(25) But our findings conquer with Beshah et al where *K. pneumoniae* from the blood had 93% resistance to all cephalosporins.(26)The rate of resistance towards cephalosporins showed by non-fermenting gram-negative bacteria was also concerning. The AST showed that *Acinetobacter Spp.* blood isolates had high resistance to third generation cephalosporins (Ceftriaxone 95% and Ceftazidime 88.5%) and fourth generation Cephalosporins (Cefepime 82.5%). Our findings also showed that all the *P. aeruginosa* blood isolates were resistant to Ceftazidime. These findings are like Bitew et al where *A. baumannii* showed resistance towards Cephalosporins ranging from 71.4% to 100% and the *P. aeruginosa* isolates showing 100 % resistance towards Cephalosporins except Ceftazidime (42.9%) and Cefepime (28.6%).(25) Resistance towards Cephalosporins are important predictors of mortality from bloodstream infections. (13,14)

The rates of resistance towards Fluoroquinolones by Enterobacterales in the current study was 80.5%. *E. coli* blood isolates showed 90% resistance to Ciprofloxacin while *K. pneumoniae* blood isolates showed 96.4% resistance. The reports from Yamba et al showed *E. coli* with 57% and *K. pneumoniae* isolates with 62% resistance.(21) Shi et al also had lower rates of resistance to fluoroquinolones with 56.3% of *E. coli* blood isolates resistant and an even lower rate with 15.63% of *K. pneumoniae* blood isolates showing resistance to fluroquinolones. (17) Similarly, Bitew et al reported 50% of the *E. coli* blood isolates showed resistance to both Ciprofloxacin and Levofloxacin.(25)The high rates of resistance from our study can be explained by the fact that Ciprofloxacin can be accessed by the community over the counter due to lack of control and regulation of antibiotics.

In this study, the least resisted drug was Amikacin (47.1%). Compared to reports from Legese et al (4.1%) and Beshah et al (10.8%) that reported the least resisted drug as Amikacin our findings showed still showed a higher resistance. When assessing the resistance rates of Enterobacterales towards Aminoglycosides, our study showed. *pneumoniae* blood isolates were more resistant to Amikacin than *E. coli* blood isolates (53.3% Vs 23.3% respectively). *K. pneumoniae* blood isolates also had higher resistance towards Gentamicin with 81.8% resistance as compared to *E. coli* blood isolates with 35% resistance. Bitew et al also that showed K. pneumoniae blood isolates had 72.7% resistance to Gentamicin. (25)The non-fermenting bacteria in the current study showed varied resistance towards Aminoglycosides. *Acinetobacter Spp*. had 95% resistance to Gentamicin and 66.7% to Amikacin. *P. aeruginosa* blood isolates had lower resistance towards Amikacin1/7(14.3%) as compared to Gentamicin with 3/7(42.9%) of the isolates showing resistance. The low rate of resistance of P. aeruginosa to Aminoglycosides was also demonstrated by Bitew et al.(25)

The overall MDR rate among the gram-negative blood isolates in our study was 81%. Most of the MDR isolates are Enterobacterales with 83.5% being MDR. Reports from Donkor et al also showed an overall MDR rate of 88%.(20) On the other hand Birru et al (66.7%) and Trecarichi et al (31.5%) had reports lower than our findings.(19,23) The MDR rate reported from recent studies by Beshah et al showed overall rate of 95.3% while Legese et al noted that 93.2% of Enterobacterales were MDR.(24,26)In exploring risk factors that are associated with resistance to antibiotics by gram-negative bacteria, we found that duration of patient on mechanical ventilation and number of classes of antibiotics that the gram-negative isolate was resistant to had significant positive relation. This means that as duration of patient on mechanical ventilation increased the number of antibiotics the gram-negative blood isolate is resistant to also increased. The Charleson comorbidity index (CCI) also showed a significant association with the number of antibiotics the gram-negative blood isolate was resistant to. Oddly, the number of days the patient on vasoactive medications had a negative significant relation with number of antibiotics a gram-negative blood isolate is resistant to. The high rate of MDR gram-negative bacteria prompted assessing risk factors related to MDR gram-negative BSI. We found that those patients with underlying hematologic malignancy had **1.4** folds increase in having MDR gram-negative BSI (AOR=1.4, 95%CI=1.12, 4.27) and Enterobacterales isolated from the blood culture had **4.4** folds increase of being MDR as compared to the *P. aeruginosa* blood culture isolates (AOR 4.4, 95%CI=1.57, 34.18).This was similar to Shi et al that reported *Enterobacteriaceae* blood isolates had a correlation with MDR. They also found that the use of central venous catheter and prior treatment with cephalosporins were related to acquiring MDR gram-negative BSI. (17) In our study, the use of invasive devices prior to the gram-negative BSI were explored but none of the devices showed a correlation. In addition, antibiotic use, or exposure 3months prior to the BSI showed no correlation with the MDR gram-negative BSI in the study. Blood cultures received from **ICU** that grew gram-negative bacteria were **2.1** likely to be MDR compared to those received from Emergency room (AOR=2.1, 95%CI=1.24, 16.56). Other risk factors related to MDR gram negative BSI like sepsis, surgery, urinary catheter was reported by Kalayci et

al.(18)Studies focused on hematology ward have also seen a correlation between fluroquinolone use, prior MDR gram-negative bacteria colonization and exposure to Carbapenems with developing MDR gram-negative BSI.(19) These factors showed no correlation in our findings.

In addition, the rate of resistance towards Carbapenems was an alarming 58.1% (79 of 136 blood isolates tested) in our study. Enterobacterales were 52.4% resistant to Carbapenems. The *K. pneumoniae* blood isolates had Carbapenem resistance rate of 77.4%, while *E. coli* blood isolates had 47.8%. These rates are higher than previous studies in the country that showed rate. Bitew et al reported a resistance level of 6.5% to Meropenem vs 5.2% to Imipenem by gram-negative blood isolates.(25) Beshah et al reported prevalence of Carbapenem-resistant *Acinetobacter Spp.* as 30 (63.8%), Carbapenem-resistant *Pseudomonas spp.* were 40% (2/5), Carbapenem-resistant *K. pneumoniae* were about 20 (26.7%) and Carbapenem-resistant *E. coli* were 7 (18.4%).(26)As compared to previous studies from the country the rate of resistance of *Acinetobacter Spp*. and *P. aeruginosa* towards Carbapenems was also higher in our study with 76.9% and 57.1% (4/7) of resistance to Meropenem respectively. Abdeta et al reported prevalence of CRAB and CRPA among the *A. baumannii* and *P. aeruginosa* isolates from different body samples as 61% and 22% respectively.(41)

The current growing rate of Carbapenem resistance and Carbapenemase production is the most concerning aspect of AMR worldwide. The risk factors associated with carbapenem resistant gram-negative blood stream infections were explored in our study. In the bivariate logistic regression participants having history of prior Cefepime use, CNS shunt device and *Acinetobacter Spp.* isolated from the blood culture had an association with Carbapenem resistance. The multivariate logistic regression revealed that patients having history of prior Cefepime use had **4.9** folds increase in having Carbapenem resistant gram-negative bacteria from the blood culture (AOR=4.9, 95%CI=1.27, 41.24). Our finding is comparable with a systematic review exploring carbapenem resistance in bacteria that showed the most frequently reported risk factor associated with CR infection was antibiotic use, in 91.1% of studies (72/79). But they found out that in multivariate analysis the antibiotics with a significant association were Carbapenems with (42.2% of studies; 30/71).(28) In a retrospective study exploring the risk factors for acquiring CRAB bacteremia showed previous use of cefepime (OR: 2.60; 95% CI 1.11–6.08; p = 0.028) was one of the independent risk factors. Additional the study showed showed hematological malignancy [odds ratio (OR): 4.04; 95% confidence interval (CI): 1.29–12.70; p = 0.017]and use of total parenteral nutrition (OR: 3.06; 95% CI 1.12–8.39; p = 0.029) had correlation to CRAB BSI.(46) Similarly the European Prospective Cohort Study on Enterobacteriaceae showing resistance to Carbapenems (EURECA) showed exposure to broad-spectrum anti-gram-negative antibiotics was related in carbapenem resistant Enterobacteriaceae. Other risk factors that showed correlation to carbapenem resistant Enterobacteriaceae in the cohort were chronic renal failure and use of urinary catheter.(29)

In the current study patients on immunosuppressive treatment were **2.1** more likely to have gram-negative bacteria form blood culture resistant to Carbapenems (AOR=2.1, 95%CI=1.24, 3.78). Immunosuppressive therapy has similarly been highlighted as one of the independent risk factors associated with CRPA BSI in a multicenter study from China.(40)Patients that had CNS shunt device were **12.4** folds increase in having carbapenem resistant gram-negative blood stream infections (AOR=12.4, 95%CI=4.26, 22.56). This may be explained as the lack of standard CNS shunting devices used (NGT used as CNS shunt) in the hospital with prolonged exposure to the environment. When comparing the gram-negative blood isolates in this study, *Acinetobacter Spp.* were **4.2** folds increase its carbapenem resistance compared to those of Enterobacterales (AOR=4.2, 95%CI=2.19, 14.63). Although we explored additional risk factors in relation to Carbapenem resistance in gram-negative blood isolates, they didn't show significant relation from our study. The major risk factors reported from other studies were male gender, renal disease, Charlson Comorbidity index, multisite colonization, invasive procedures, length of hospital stay, ICU hospitalization, exposure of carbapenems and tracheal intubation.(30,34,36,40)

Conclusion and recommendations

The study highlighted the growing prevalence of MDR and Carbapenem gram-negative bloodstream infections in a hospital with limited resources. Our findings emphasize the urgent need for effective antimicrobial surveillance and infection control measures throughout the country. Building microbiology capacity of health centers to provide molecular testing in the country. Further research is needed to address the underlying factors contributing to the spread of antimicrobial resistance.

Limitations

This study was a single centered retrospective study that tried to assess the antimicrobial resistance and factors associated to gram-negative blood stream infection. Tests for enzyme productions and molecular analysis for resistance were not done. Prospective research should be conducted in line with antimicrobial surveillance in the hospital.

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