

**ADDIS ABABA UNIVERSITY,
COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES,
DEPARTMENT OF MICROBIAL CELLULAR AND MOLECULAR BIOLOGY
(INFECTION BIOLOGY)**



EPIDEMIOLOGICAL AND MOLECULAR CHARACTERIZATION OF MULTI-DRUG
RESISTANT GRAM-NEGATIVE BACTERIAL ISOLATES FROM BLOODSTREAM
INFECTIONS AMONG PATIENTS ADMITTED AT TIKUR ANBESSA SPECIALIZED
HOSPITAL, ADDIS ABABA, ETHIOPIA

A DISSERTATION SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES COLLEGE
OF NATURAL AND COMPUTATIONAL SCIENCES ADDIS ABABA UNIVERSITY,

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**ADDIS ABABA UNIVERSITY,
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COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES,
DEPARTMENT OF MICROBIAL CELLULAR MOLECULAR BIOLOGY**

I hereby certify that I have read the revised version of this Ph.D. dissertation prepared under my supervision by Daniel Beshah Haile titled: “Epidemiological and Molecular Characterization of Multi-Drug Resistant Gram-Negative Bacteria Clinical Isolates Among Bloodstream Infections Patients Admitted at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia” and recommend to be accepted as fulfilling the Dissertation requirement for the Degree of Philosophy in microbial cellular molecular biology infection biology stream.

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Declarations

I, Daniel Beshah Haile declare that the thesis hereby submitted for the Degree of Doctor of Philosophy in Microbial Cellular and Molecular Biology (infection biology) to the School of Graduate Studies of Addis Ababa University is my independent original work, and has not previously been submitted by me or anybody else at another university. The materials obtained from other sources have been duly acknowledged in the thesis.

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DEDICATION

This thesis is dedicated to my beloved wife Hasset T/Michael and my families, who have always supported me morally and financially in what I aspire to achieve my goals.

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LIST OF ABBREVIATIONS

AmpC-BL	AmpC beta-lactamase
AMR	Antimicrobial resistance
AST	Antimicrobial susceptibility tests
CDC	Centers for Disease Control
<i>CoNS</i>	<i>Coagulase-negative staphylococcus</i>
CPs	Carbapenemase
CVC	Central vein catheter
DNA	Deoxyribonucleic acid
DRB	Drug resistance bacteria
DRC	Drug resistance category
ESBL	Extended-spectrum beta-lactamases
GII	Gastrointestinal tract infection
HAI	Hospital-acquired infection
ICU	Intensive care unit
IPC	Infection prevention and control
KPC	<i>Klebsiella pneumoniae</i> carbapenemase
LRI	Lower respiratory infection
MBL	Metallo-beta-lactamase
MDR	Multi-drug resistant
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
NI	Nosocomial infection
PDR	Pan-drug resistant
SWI	Surgical wound infections
TASH	Tikur Anbessa Specialized Hospital
UTI	Urinary tract infections
VRE	Vancomycin-resistant <i>enterococcus</i>
VRSA	Vancomycin-resistant <i>staphylococcus aureus</i>
WHO	World health organization
XDR	Extensively drug-resistant

OPERATIONAL DEFINITION

Drug resistance category (DRC); In this study paper, it represented multi-drug resistance (MDR), Extensive drug resistance (XDR), and Pan drug resistance (PDR) bacteria.

Drug-resistant Type (DRT): One of ESBL, AmpC-BLs, MBLs, and Carbapenemase

Below is the list of wards and their respective department

Ward	Department	Ward	Department
PEOPD	In Pediatrics Emergency OPD	C5	In General surgery
EOPD	In Adult Emergency OPD	D5	In Neuro and general Surgery
C/W	In Causality	A6	In Labor gynecology
RT	In Radio therapy/Oncology	B6	In NICU
R/C	In Rehabilitation /Orthopedics	C6	In Gynecology & Obstetrics
CTR	In Central triage/Regular OPD	D6	In Gynecology & Obstetrics
InM	In Intermediate	B7	In Pedi Medical & Surgical
C3	In Gynecology & Obstetrics	C7	In Pedi Medical
D3	In Uro-surgery	D7	In Pedi Hematology
B4	In SICU, MICU/PMICU	B8	In Medical, cardiac and endocrine
C4	In Cardiac	C8	In Hematology
D4	In Cardiothoracic surgery & ENT	D8	In Medical renal and hematology
B5	In Medical and Neurosurgery		

ABSTRACT

Bloodstream infections are the major causes of morbidity and mortality worldwide. Alarming, Gram-negative bacteria that produce beta-lactamase and carbapenemase are causes for the rapid global spread of multi-drug resistance (MDR), which significantly jeopardizes the efficacy of existing antimicrobial treatments. This research aimed to analyze the microbial profile, antimicrobial susceptibility, genomic diversity, and associated risk factors of Gram-negative bacteria in bloodstream infections. A cross-sectional study was conducted between September 2018 and March 2019 on 1486 bloodstream infection suspected patients. In addition to biochemical identification and antimicrobial susceptibility tests, PCR and WGS were conducted for ESBL, AmpC, MBL and carbapenemase producing resistance genes. The prevalence of bloodstream infection was 417 (28.06%), and the most prevalent bacterial species were *Klebsiella pneumoniae* (17.6%) and *Acinetobacter spp.* (11%). Culture positivity was associated with age below 6 years, ICU admission, length of admission > 5 days, temperature greater than 38 °C, instrument usage during medical care, chronic illness, and neonatal incubation. Multi-drug resistance was 95% where 56%, 32% and 7% of the isolates exhibited MDR, XDR, and PDR respectively. *Klebsiella pneumoniae* and *Acinetobacter sp.* showed the greatest rates of XDR (37%) and PDR (77%), respectively.

Among the 231 phenotypically characterized isolates, 195 (84%) drug-hydrolyzing enzyme producers, 54% and 26% were ESBL and carbapenemase-producer, respectively. Again, *Klebsiella pneumoniae* was the highest drug-hydrolyzing enzyme-producer bacteria. 176 out of the 195 (76%) were PCR-confirmed resistant bacteria, of which ESBLs, MBLs, carbapenemase, and AmpC-BLs accounted for 53%, 22%, 20%, and 10%, respectively and blaCTX-M, blaFOXm, blaOXA-23, and blaNDM were the highest hits in *Klebsiella pneumoniae*, (75%), and *Escherichia coli* (24%).

Out of the 142 whole genome sequenced isolates, 1604 genes categorized into 168 resistance classed were identified. A total of 1055 virulence genes were identified belonging to 133 types. The majority of AMR genes and virulent genes were observed in *Klebsiella pneumoniae* (40%) and *E. coli* (71%), respectively. *Sul2* was the most prevalent gene (%), followed by blaCTX-M-15 (5%), and aph (3")-Ib (5%). *fimH* (6%), *iutA* (6%), and *traT* (5%) were the most prevalent virulent

genes. The most common ESBL producer genes were blaCTX-M-15 (7%) and blaTEM-1B (6%) and the most common AmpC genes were blaCMY-2 (2%) and blaCMY-6 (1%). The highest carbapenemase genes were blaOXA-23 (3%), blaOXA-66 (3%), blaNDM-1 (2%), and blaOXA-1 (2%). In 82 (58%) of the sequenced strains, eight types of gene mutations were identified: parC (30%), gyrA (30%), ramR (1%), rpoB (1%), ompK37 (45%), acrR (43%), and ompK36 (43%) with a total of 220 antimicrobial gene mutations and 956-point mutations. However, 22 (2%) of the mutations were frameshift mutations and the rest 934 were point mutations. Among the 142 drug-resistant strains, 103 (73%) have plasmid mlst, and 108 (76%) have plasmid genes. Resistance to disinfectants like benzalkonium chloride 77(52%) and cetylpyridinium chloride 77(52%) had OqxA 41(29%), OqxB 41(29%), and qacE 49(35%) disinfectant-resistant genes. Like other AMR genes, NICU (B6) still has the leading prevalence of disinfectant resistance genes (22%) followed by C/W (9%) and pediatric hematology (D7) (8%).

The study showed AMR has become a significant health hazard of bloodstream infection that most affects neonatal and ICU patients. The alarming result of this study was 7% PDR bacteria. The NICU by ESBL and SICU, General surgery, and caesarian section units by Carbapenemase producer strains were affected. A high burden of ESBL and Carbapenemase was seen in this study. The predominant ESBL, MBLs, and Carbapenemase genes were blaCTX-M, blaNDM, and blaOXA-23 respectively. Nine novel drug-resistant bacteria strains were identified. Surgical and inserted medical instruments are the main drug-resistant bacteria transmission roots. Carbapenemase and MBL drug-resistant genes were associated with NICU, general surgery, SICU, and C/S unit which needs infection prevention standard implementation.

Keywords: Bloodstream infections, antimicrobial resistance, Drug resistance category, Drug resistance mechanism, Multidrug resistance

1. CHAPTER ONE: INTRODUCTION

1.1. Background

Globally, Hospital Acquired Infections are the most frequent adverse event to occur during hospitalization (Labib *et al.*, 2018). Bloodstream infections represent about 15% - 20% of all nosocomial infections and affect approximately 1% of all hospitalized patients globally (Bassetti *et al.*, 2013). In Sub-Saharan countries, including Ethiopia, bloodstream infections in children, have a mortality rate of nearly 53% (Aiken *et al.*, 2011; Dagnew *et al.*, 2013). Studies conducted in Ethiopia on septicemia suspected cases showed a prevalence of 13%-39.2% bloodstream infections (Negussie *et al.*, 2015; Seboxa *et al.*, 2015). Depending on the risk factors, the mortality rate varies between 4.0 and 41.5% (Ahmed *et al.*, 2017). Patients with positive blood cultures had a fivefold higher death rate than those without bacteremia i.e., 50.0% as compared to 9.8% which is the case of those without bacteremia (Seboxa *et al.*, 2015).

Bloodstream infections are infectious diseases defined by the existence of viable microorganisms in the bloodstream, which are later confirmed by the positivity of one or more blood cultures (Viscoli, 2016). It is an important cause of morbidity and mortality worldwide (Gaynes and Band, 2009; Timsit *et al.*, 2014) and has of great impact on healthcare operations (Martinez and Wolk, 2016). It is a preventable infection but it is the leading cause of complications especially in critically ill patients (Hugonnet *et al.*, 2004). Clinical signs and symptoms of bloodstream infections include fever, chills, and/or hypotension. Other common symptoms include rapid breathing, confusion, sweating, pain and discomfort, fast heartbeat, light-headedness, and dizziness (Martinez and Wolk, 2016).

Bloodstream infections might be either community-acquired bloodstream infections or healthcare-acquired bloodstream infections (Friedman *et al.*, 2002; Mun *et al.*, 2022). Recent studies have reported a rapid increase in the number of bloodstream infections from both community-acquired bloodstream infections and healthcare-acquired bloodstream infections (Ahmed *et al.*, 2017). Nosocomial infection (NI) or hospital-acquired infections (HAI) are defined as infections that are acquired during their stay in the hospital within 48–72 hours following admission (Ghadiri *et al.*, 2012). It includes infections acquired in the hospital or health care facility but appearing after discharge, and occupational infections among staff (WHO, 2014).

Bloodstream infections may be either a primary infection or a secondary infection (Gaynes and Band, 2009). Diffusion of bacteria into the bloodstream from a localized illness results in secondary infection. When no focus is found, it can be considered a primary infection (Russotto *et al.*, 2015). There are several known risk factors for primary bloodstream infections, these include immuno-suppressive conditions, environmental exposures, ICU stay, the prevalence of specific nosocomial pathogens, surgery, invasive devices, invasive procedures (such as mechanical ventilation, dialysis, insertion of central vein catheter (CVC)), increased age, length of hospital stay, impaired conscious levels, and steroid therapy (Bond *et al.*, 2017; Hugonnet *et al.*, 2004; Ozdemir and Dizbay, 2015; Russotto *et al.*, 2015; von Baum *et al.*, 2005). Secondary infections such as urinary tract infections (UTI), surgical wound infections (SWI), gastrointestinal tract infections (GII), lower respiratory infections (LRI) peritonitis, malignancies, and skin infection increases the risk of acquiring bloodstream infection (Gaynes and Band, 2009; Ghadiri *et al.*, 2012; Goto and Al-Hasan, 2013; Timsit *et al.*, 2014).

Bloodstream infections are also becoming problematic due to the escalation of antimicrobial resistance (AMR) (Ghadiri *et al.*, 2012). Therefore, AMR is a global health and development threat. Consequently, the World Health Organization (WHO) has declared AMR to be one of the top ten worldwide public health hazards for human beings (WHO, 2021). The rapid global spread of multi-resistant bacteria that cause common infections and resist treatment with existing antimicrobial medicines is alarming (CDC, 2013a; WHO, 2015 2016). These AMRs in a wide range of infectious agents are a growing public health threat and concern of health care management (WHO, 2015). During the past decades, a shift in the multidrug-resistant (MDR) dilemma has been noted from gram-positive to gram-negative bacteria, due to the scarceness of new antimicrobial agents (Brusselaers *et al.*, 2011). Therefore, in this study multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) were classified according to Magiorakos definition (Magiorakos *et al.*, 2012).

Gram-negative bacteria, whose incidence and spread are on the rise right now and that are resistant to antibiotics, are major causes of illness and death, and significant economic costs worldwide (Holmes *et al.*, 2021; Leski *et al.*, 2013; Yungyuen *et al.*, 2021). The major global health concerns of MDR are due to beta-lactamase and carbapenemase (CPs) production, which leads to antimicrobial resistance (AMR) (Elamreen, 2007; Vasoo *et al.*, 2015). These antibiotic resistance

determinants are passed from one bacterial community to another through the transduction by bacteriophages, conjugation with plasmids, transformation with free deoxyribonucleic acid (DNA) or mobile genetic elements, or other genetic exchange mechanisms (transposons and integrons) (Bush, 2010; Leski *et al.*, 2013; Peterson and Kaur, 2018). On the other hand, a faster rate of β -lactamase gene mobility from the chromosome to the plasmids was observed (Peterson and Kaur, 2018). β -Lactamase-mediated resistance is increasingly associated with plasmid-encoded extended-spectrum β -lactamases (ESBLs) and Carbapenemase sometimes in combination with other resistance mechanisms (e.g., porin loss, efflux) (Bush, 2010; Noster *et al.*, 2021).

Infections with ESBL-harboring bacteria have been successfully treated with carbapenems, but the massive use of this antibiotic accelerated the dissemination of carbapenemase (Noster *et al.*, 2021). The most common drug-resistant gram-negative bacteria in clinical practice include *K. pneumoniae*, *Acinetobacter baumannii*, *Escherichia coli* (*E. coli*), and *Pseudomonas aeruginosa* (Yungyuen *et al.*, 2021). These strains are frequently left by clinicians without therapeutic options (Leski *et al.*, 2013). Although beta-lactam antibiotics are heavily utilized in many developing countries, the diversity of beta-lactamase genes (*bla*) and carbapenemase genes are highly overlooked (Kiiru *et al.*, 2012).

Due to its great accuracy in describing the entire genomic structure of isolates, whole genome sequencing (WGS) is now in a good position to establish relatedness across strains and to become the gold standard in bacterial typing (Lee *et al.*, 2014; Simar *et al.*, 2021). It can be used to identify the particular genes that lead to antibiotic and other medicine resistance as well as to give light on how these genes are distributed throughout different bacterial populations (Köser *et al.*, 2014). WGS of bacterial isolates is recommended to improve our understanding of transmission dynamics and AMR gene diversity. (Moghnia and Al-Sweih, 2022). It has been clear that these tests are useful for genotyping, and the quick and thorough identification of bacterial clones involved in outbreak transmission. (Didelot *et al.*, 2012).

A study done in Ethiopia shows mortality was significantly associated with AMR. There was a report of multi-resistant *Klebsiella spp.* in the pediatrics department of TASH in 1988 with resistance rates for chloramphenicol, gentamicin, and co-trimoxazole with 96%, 89%, and 86% occurrences, respectively (Seboxa *et al.*, 2015). As Balew *et al.*, study in Addis Ababa from cancer

patients nine isolates tested for nine sets of antibiotics, 89% were resistant to amoxicillin-clavulanic acid, ampicillin, and trimethoprim-sulphamethoxazole (Arega *et al.*, 2017). A study done in Mekele shows that 59% of the bacterial isolates were MDR. AMR pattern for gram-positive and gram-negative bacteria was 0 - 83.3% and 0 - 100%, respectively. High resistance was seen to Trimethoprim-sulphamethoxazole 70.1%, Oxacillin 62.5%, Ceftriaxone 58.9% and Doxycycline 49.3% (Wasihun *et al.*, 2015). Overall, drug resistance for gram-positive bacteria was 7 to 61%, and for gram-negative 6.9 to 82.6%. The gram-negative bacterial isolates showed 66 to 82.6% resistance to ampicillin, ceftriaxone, and trimethoprim-sulfamethoxazole (Hailu *et al.*, 2016a). In Kumalo *et al.*, study MDR was observed in 80% of isolates. Of this, 87.5% and 71.4 % accounted for Gram-positive and Gram-negative bacteria respectively (Kumalo *et al.*, 2016). One of the bacterial drug-resistance transmission roots is medical tourism. The Ethiopian middle class now attends hospitals in Thailand and other destinations overseas for medical treatment including surgery. The identification of meropenem-resistant *K. pneumoniae* in Addis Ababa before carbapenems were used inside the country can be due to the healthcare tourism of recent years (Seboxa *et al.*, 2015).

Antibiotic-resistant bacterial infections are becoming increasingly hard to treat. (Su *et al.*, 2019). Antibiotic resistance is a global health emergency, with resistance detected to all antibiotics currently in clinical use and only a few novel drugs in the pipeline (Darby *et al.*, 2023). Rising rates of antimicrobial resistance amplify the morbidity, mortality, and economic burden associated with infections. Even successful treatment can come with increased complications, as more-toxic drugs of last resort (Su *et al.*, 2019).

During these last few years, clinicians have witnessed a growing incidence of bloodstream infections by bacteria with resistance against commonly used antimicrobials (Russotto *et al.*, 2015). The ongoing emergence of resistance in the community and hospital is considered a major threat to public health (Brusselaers *et al.*, 2011). The incidence of infections caused by beta-lactam-resistant (ESBL, MBL), Carbapenem-resistant Enterobacteriaceae, AmpC producing and colistin resistance organisms due to the production of various enzymes has increased in recent years (Shaikh *et al.*, 2015).

Updated information regarding etiological agents of bloodstream infection and their antimicrobial susceptibility patterns is central for empirical treatment success. In this regard, continuous assessment of the antibiogram profile (in which used for starting treatment with out arrival of laboratory result) is crucial to track changes in etiological agents and antimicrobial susceptibility patterns. Besides these studies, to the knowledge of the researchers, most of them did not address the magnitude of ESBL and carbapenem-producing gram-negative bacteria and associated factors from all age groups of bloodstream infection. Therefore, the objective of this study is to address the identified gaps of insufficient information regarding the diversity of these drug-resistance genes. Accordingly, this study aims to determine the prevalence of bloodstream infections, microbial profiles, anti-microbial susceptibility patterns, and associated risk factors as well as genomic diversity of the isolates at Tikur Anbessa Specialized Hospital (TASH). Mainly the study aims to show the magnitude of ESBL, AmpC-BL, MBL, and Carbapenemase-producing gram-negative bacteria and the diversity of beta-lactamase and carbapenemase genes with associated risk factors among bloodstream infection patients in Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

1.2. Statement of the Problem

Bloodstream infections represent a common complication among critically ill patients and a leading cause of death (Mantzaris *et al.*, 2022). It has a significant negative effect on patient treatment outcomes, it increases the death rate, lengthens admission to the hospital, and incurs many more significant additional expenses (Finkelstein *et al.*, 2004; Hugonnet *et al.*, 2004). Even if several strategies are successful the aforementioned factors make bloodstream infection monitoring and prevention top goals (Hugonnet *et al.*, 2004; Patel *et al.*, 2010). A prospective analysis shows an estimated 250,000 cases occurring each year in the United States (U.S.) (Gaynes and Band, 2009), and cases due to antibiotic-resistant organisms, in particular, have increased over the last decades (WHO, 2010). Unfortunately, HAIs affect 5% to 10% of hospitalized patients in the U.S. each year. Each year, around 1.7 million HAIs in U.S. hospitals result in \$20 billion in medical expenses (CDC, 2014). Bloodstream infection represents about 15% - 20% of all nosocomial infections and affects approximately 1% of all hospitalized patients globally (Bassetti *et al.*, 2013). As a result, the ICU population has one of the highest incidences of nosocomial infections, which account for 20–30% of all ICU admissions (Brusselsaers *et al.*, 2011). Higher

mortality rates of 40%–50% have been observed in surveys of patients with bloodstream infections or septic shock in the hospital ICU (Kontula *et al.*, 2021; Timsit *et al.*, 2014). A recent study shows bloodstream infections are thought to be the cause of 250,000 deaths annually in North America and Europe combined, despite improvements in antimicrobial medication therapy, intensive care, and preventative methods (Kontula *et al.*, 2021).

Developing countries generally have higher rates of morbidity and mortality, high healthcare-related expenses, and longer hospital stays than developed countries (Ahmed *et al.*, 2017). A study conducted in Africa showed that bloodstream infection has a high mortality rate of 43.5%, which is two times higher than malaria's mortality rate (20.2%) which is primarily caused by gram-negative bacteria (Endalafer *et al.*, 2011; Ndir *et al.*, 2016). It is one of the top seven causes of death in all of the nations of North America and Europe. However, due to a scarcity of studies, it is difficult to acquire reliable estimates of bloodstream infection in developing countries (Goto and Al-Hasan, 2013). In sub-Saharan Africa, few facilities can maintain long-term surveillance (Musicha *et al.*, 2017).

In Sub-Saharan countries including Ethiopia, septicemia is an important cause of illness and death in children. The mortality rate approaches 53% which makes it a significant health problem in developing countries (Aiken *et al.*, 2011). Studies conducted in Ethiopia from patients admitted to surgical wards and intensive care units found that an overall incidence of nosocomial infections was 35.8/100 patients but bloodstream infection accounts for 20.8% (Endalafer *et al.*, 2011). In another similar study conducted in Ethiopia from suspected cases the prevalence of bloodstream infection was 18.2% in Gonder (Dagneu *et al.*, 2013), 28% in Mekelle (Wasihun *et al.*, 2015), 39.2%, in Bahirdar (Hailu *et al.*, 2016a), 15.8 % in Jimma (Kumalo *et al.*, 2016), 27.9% and 15.1% in Addis Ababa (Negussie *et al.*, 2015; Seboxa *et al.*, 2015). Severe sepsis, septic shock, and multisystem organ dysfunction related to bloodstream infection frequently require admission to the ICU for appropriate management (Timsit *et al.*, 2014).

The problem will worsen when the infection is an MDR organism due to the escalation of drug-resistant bacteria (Ghadiri *et al.*, 2012). The impacts of MDR also include extended hospital stays, permanent disabilities, enormous financial burdens on health systems, substantial expenses for patients and their families, preventable deaths, and increased microbial resistance as a result of

gene transfer (WHO, 2010). According to a study conducted in Belgium, the cost of MDR infections in hospitalized patients ranged from \$6,000 to \$30,000 per patient due to inappropriate empirical treatment (Brusselaers et al., 2011) and increased risk of hospital mortality (Lueangarun and Leelarasamee, 2012). According to research made by Kraker et al. in Europe, fatality rates increased up to fivefold when the causing organisms were an MDR (de Kraker *et al.*, 2011).

Resistance is constantly emerging in the community and hospitals, which is viewed as a serious threat to public health (Russotto *et al.*, 2015, Brusselaers et al., 2011). These public health threats of infections are caused by the production of various enzymes of beta-lactam-resistant, ESBL, AmpC, MBL, and carbapenemase-producing bacteria (Shaikh et al., 2015). In gram-negative bacteria, the resistance is mainly due to the rapid increase of ESBLs and carbapenem in *K. pneumoniae*, *E. coli*, *P. mirabilis*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* *Enterobacter spp.* and *Citrobacter species* (Brusselaers et al., 2011; Timsit et al., 2014).

In 1988, the pediatrics department at TASH received a report of multi-resistant *Klebsiella spp.* with resistance rates for co-trimoxazole, gentamicin, and chloramphenicol of 96%, 89%, and 86%, respectively (Seboxa et al., 2015). In Balew's study in Addis Ababa from cancer patients nine isolates tested for nine sets of antibiotics, 89% were resistant to amoxicillin-clavulanic acid, ampicillin, and trimethoprim-sulphathiazole (Arega *et al.*, 2017). A similar study undertaken in Mekelle shows an AMR pattern for gram-positive bacteria and gram-negative bacteria of, 0–83.3% and 0–100%, respectively. Oxacillin (62.5%), Ceftriaxone (58.9%), Trimethoprim-Sulphathiazole (70.1%), and Doxycycline (49.5%) all showed high levels of resistance (Wasihun et al., 2015). The overall, drug resistance for gram-positive bacteria was 7 to 61%, and for gram-negative bacteria 6.9 to 82.6%. The gram-negative bacteria isolates showed 66 to 82.6% resistance to ampicillin, ceftriaxone, and trimethoprim-sulfamethoxazole (Hailu *et al.*, 2016a). In Kumalo et al. study, MDR was observed in 80% of isolates. Of this, 87.5% and 71.4 % accounted for gram-positive bacteria and gram-negative bacteria respectively (Kumalo *et al.*, 2016). Medical tourism is one of the causes of microbial transmission that is resistant to drugs. The recent increase in medical tourism may have contributed to the discovery of meropenem-resistant *Klebsiella pneumoniae* (*K. pneumoniae*) in Addis Ababa before the domestic usage of carbapenems (Seboxa et al., 2015).

Understanding the etiologic agents, the burden of drug resistance, the type of drug resistance organism, and their antibiogram profile of bloodstream infection is an important concern for patients, physicians, healthcare managers, and policymakers (Barlow and Nathwani, 2005). Therefore, constant evaluation of current practice based on trends in MDR and antibiotic resistance patterns is essential to reduce this burden.

1.3. Significance of the Study

As explained above the problem due to bloodstream infection needs urgent intervention. The severity of the case is higher in developing countries including Ethiopia having poor infection prevention practices in the health care service. So far, there is no well-established national bloodstream infection surveillance and antibiogram profile for bacterial treatment. What is true in the West might not hold elsewhere in the world. There is a paucity of objective data on the causative agents and their susceptibility in these hospital populations, particularly in our study area. Besides, most patients are treated empirically based on their clinical symptoms.

Up-to-date etiological data for major pathogens causing bloodstream infection and their drug susceptibility pattern may play a positive role in better healthcare management as antibiotic sensitivity patterns to common pathogens have been changing day to day. Over time, researchers have noticed notable shifts in the microbiology, epidemiology, clinical, and prognostic importance of positive blood cultures. For these reasons, surveillance of bloodstream infections from blood cultures and their antibiotic resistance patterns is vital to treatment and prevention. Many studies have been conducted in Ethiopia on bacterial isolation and drug susceptibility patterns from different clinical samples in tertiary hospitals but to the researcher's knowledge, few of these studies address the molecular characterization of drug resistance bloodstream infection and their responsible genes found in Ethiopia. In addition, virulence genes, plasmid multilocus, plasmid genes, point mutations, disinfectants resistant cluster, and disinfectants resistant genes were identified to understand the virulence factor, the transmission dynamics, how it becomes AMR and how we control this drug resistance strains were studied.

Therefore, assessing the prevalent gram-negative bacteria, testing their antibiotic susceptibility, and understanding responsible genes for drug resistance helps clinicians provide safe and effective empirical therapies, develop rational prescription programs to help policymakers decisions on the national drug list, and finally understand novel drug resistance genes that will help for drug

formulation. Hence, the purpose of this thesis paper is to show the overall burden and drug resistance pattern of bloodstream infection-causing bacteria focusing on gram-negative isolates in a healthcare setting. The epidemiology, microbiology of etiological agents, and drug susceptibility patterns were analyzed and antibiogram profiles were prepared. Characterization for ESBL, Carbapenem-resistant Enterobacteriaceae, AmpC β -lactamase, and MBL was conducted. In addition to this, whole genome sequencing was done for AMR isolates to identify chromosomal and plasmid drug resistance genes, point of mutation, virulent genes and their disinfectant resistant, and selection of gram-negative bacteria. Finally, risk factors and intervention mechanisms were discussed to minimize disease burden.

1.4. General Objective

1.4.1. General Objective

- To determine the prevalence and Molecular Characterization of Multi-Drug Resistant Bloodstream Infections of gram-negative Clinical Isolates among Patients Admitted at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

1.4.2. Specific Objectives

- To assess the prevalence of bloodstream infection, epidemiology of microbial profiles, antimicrobial susceptibility test (AST) of each bacterial isolate, and their associated factors, in bloodstream infection.
- To determine the phenotyping characteristics of multi-drug resistant (MDR), Extensively drug-resistant (XDR), and Pan-drug resistant (PDR) gram-negative bacteria among bloodstream infections.
- To determine phenotyping characteristics of ESBL, AmpC, MBL, and Carbapenemase-producing gram-negative bacteria.
- To investigate the molecular characterization of ESBLs, AmpC-BLs, Carbapenemase, and MBLs producing gram-negative bacteria in bloodstream infection.
- To identify AMR and virulent genes of ESBLs, AmpC-BLs, Carbapenemase, and MBLs isolates by using whole-genome sequencing.
- To identify AMR point mutation, plasmid gene, and disinfectant resistance genes.
- To identify AMR and virulent genes of PDR isolates by using whole-genome sequencing.

2. CHAPTER TWO: LITERATURE REVIEW

2.1. Epidemiology of Bloodstream Infection

2.1.1. World Epidemiology of Bloodstream Infection

Bloodstream infection due to bacteria is an important cause of morbidity and mortality in the world. It is a life-threatening condition that may be complicated by septic shock and death (Bauer *et al.*, 2022; Moyo *et al.*, 2010). According to a population-based study conducted by the Centers for Disease Control and Prevention (CDC), Globally, bloodstream infection affects about 30 million people leading to 5.3 million deaths annually (Fleischmann *et al.*, 2016). A systematic review of an estimated number of episodes and deaths of bloodstream infection from population-based studies in North America estimated to be 575,000 – 677,000 episodes and 79,000 – 94,000 deaths per year whereas in Europe it is estimated over 1,200,000 episodes and 157,000 deaths per year (Goto and Al-Hasan, 2013)

The incidence of bloodstream infection has increased over time and reported bloodstream infection rates range from 122 to 220 cases/100,000 population (Kontula *et al.*, 2021). Using estimates from one population-based study in each of the following countries: in North America USA (536,000 – 628,000 episodes and 72,000 – 85,000 deaths), Canada (40,000 – 49,000 episodes and 7,000 – 9,000 deaths), in Europe Denmark (9100 episodes and 1900 deaths), Finland (8700 episodes and 1100 deaths) and England (96,000 episodes and 12,000 – 19,000 deaths) (Goto and Al-Hasan, 2013). A study conducted in Finland during 2004–2018 shows a total of 173,715 bloodstream infections recorded and the annual incidence increased from 150 to 309 cases/100,000 population (Kontula *et al.*, 2021). A study done in Bangladesh by Ahmed *et al.* shows from an overall prevalence of bloodstream infection, 13.6% of the blood culture samples were positive, and gram-negative bacteria (72.1%) were predominant throughout the study period (Ahmed *et al.*, 2017).

From a retrospective cohort study which was conducted in US healthcare facilities, the average sepsis mortality rate for 30 days was 24.4%, and that for 90 days was 32.2%. The Average septic shock mortality for 30 days was 34.7%, and for 90 days was 38.5% (Bauer *et al.*, 2020). The mortality rate in Finland from 2004–2018 rose from 20 to 39 deaths per 100,000 population (Kontula *et al.*, 2021). A study in America showed mortality rate varied among regions, with 30-

day septic shock mortality being 33.7% (95% CI 31.5–35.9) in North America, 32.5% (95% CI 31.7–33.3) in Europe and 26.4% (95% CI 18.1–34.6) in Australia. The average mortality rate has increased by 1.8–3.3% from 2011 to 2019 (Bauer *et al.*, 2020). A similar study conducted in Ontario Canada shows a mortality rate of 17.0% for positive culture episodes, while the mortality rate associated with a negative culture episode was 9.5% (Verway *et al.*, 2022). Another study undertaken in South Asia, Bangladesh shows the bloodstream infection mortality rate varies between 4.0 and 41.5% (Ahmed *et al.*, 2017). Patients infected by ARMs are more likely to develop complications and up to 3 times the probability of losing their lives (Cecchini *et al.*, 2015). Internationally, there is a growing concern over antimicrobial resistance (AMR) which is currently estimated to account for more than 700,000 deaths per year worldwide (Tadesse *et al.*, 2017). A recent report estimated that 10 million deaths will be attributed to an AMR by 2050 (Founou *et al.*, 2017).

2.1.2. World Economy Impact

A study done in G7 and OECD countries shows an AMR is hastily becoming a top health problem that causes challenges to the running of healthcare systems and their budget (Cecchini *et al.*, 2015). Hospitals spend, on average, an additional USD 10,000 to 40,000 to treat a patient infected by an AMR (Cecchini *et al.*, 2015). If no effective strategy is put in place soon, it has been estimated that the health and economic burden produced by ARMs will be much larger (Cecchini *et al.*, 2015). Research conducted across the whole globe indicates that the world economic output will deteriorate by 100 trillion USD if substantive efforts are not made to contain this threat (Founou *et al.*, 2017). The associated impact of lost economic outputs due to increased mortality, prolonged sickness, and reduced labor efficiency is likely to double this figure (Cecchini *et al.*, 2015).

2.1.3. Bloodstream Infection in Africa

The study was conducted in Ghana from 2009 to 2017, the incidence of bloodstream infection was recorded in the wet seasons of May and October at 8.9 per 10,000 and 10.1 per 10,000 population respectively. But among bloodstream infection suspected patients 13.1% were culture-positive (Deku *et al.*, 2019). In Ghana, bloodstream infection rates as published in hospital and laboratory surveillance reports are estimated at 9.3% to 11.2% (Obeng-Nkrumah *et al.*, 2016; Opintan and Newman, 2017). In Eastern African countries, the proportion of patients with bloodstream

infections is reported to range from 11% to 28%. In Sub-Saharan countries, including Ethiopia, bloodstream infection in children, has a mortality rate approaching 53% (Aiken et al., 2011; Dagnew et al., 2013). A meta-analysis of different proportions of patients with bloodstream infection in Eastern African countries is reported to range from 11% to 28% (Reddy *et al.*, 2010).

2.1.4. Bloodstream Infection in Ethiopia

A meta-analysis of 26 studies with 8,958 blood specimens and 2,382 culture-positive bacterial isolates in Ethiopia shows the prevalence of bloodstream infection was 25.78% (Alemnew *et al.*, 2020). In a study conducted in Ethiopia of bacteremia-suspected patients, 71 (18.2%) of them were confirmed with culture (Dagnew et al., 2013) and another similar study in Ethiopia shows from bloodstream infection overall mortality rate of 15.1% was in adults and this figure reached 53% in pediatrics. Patients with positive blood cultures have a nearly fivefold death probability which is a higher death rate than those without bacteremia (50.0% as compared to 9.8%) (Seboxa et al., 2015). As indicated above, by Dagnew *et al.* study, out of 18.2% prevalence, bacterial isolates of gram-positive and gram-negative bacteria were 49(69%) and 22(31%) respectively with predominant isolates of Coagulase-negative staphylococci (CoNS) 30(42.3%) followed by *staphylococcus aureus* (*S. aureus*) 17(23.9%) and *klebsiella spp.* 9 (12.7%). Similarly to Seboxa *et al.* finding, in the Dagnew *et al.* study there was the highest proportion of bloodstream infection was found in neonates, which accounts for 35.3% (Dagnew et al., 2013). In a comparison of hospitalized and outpatient bloodstream infection bacterial isolates, a high prevalence was found in inpatients 69 (97%) and 2 (3%) from outpatients (Dagnew et al., 2013). This result shows nosocomial infection is a major cause of bloodstream infection. Studies conducted in Ethiopia from septicemia suspected cases of the prevalence of bloodstream infection showed 18.2% in Gonder (Dagnew et al., 2013), 28% in Mekelle (Wasihun et al., 2015), 39.2% in Bahirdar (Hailu *et al.*, 2016a), 15.8 % in Jimma (Kumalo *et al.*, 2016), 28.2%, 27.9%, and 13.% in Addis Ababa (Beshah et al., 2022; Negussie et al., 2015; Seboxa et al., 2015). Patients with positive blood cultures had a fivefold higher death rate than those without bacteremia i.e., 50.0% as compared to 9.8% which is the case of those without bacteremia (Seboxa et al., 2015).

2.2. Factor Associated with Bloodstream Infection

Nosocomial infections are a major source of bloodstream infections (Ribas *et al.*, 2007). According to the WHO report, the overall prevalence of NI in developed countries varies between 5.1% and 11.6%, and in developing countries, it is from 5% to 19%, markedly higher than the developed countries but most studies report these figures to be higher than 10%. Among hospital-born babies in developing countries, NI is the cause of 4% to 56% of all causes of death in the neonatal age, with 75% occurring in South East Asia and Sub-Saharan Africa (WHO, 2010). From the total NI, bloodstream infections are estimated to be from 7% to 16%, and the mortality rate which is directly attributable to bloodstream infection in critically ill patients has been estimated to be 16–40%, and the increase in length of stay to be between 7.5–25 days (WHO, 2010). Positive blood culture has increased the risk of 5-year mortality (44.7% vs 19.6%, adjusted hazard ratio 2.10, 95% CI 1.18–3.71) (Tauriainen *et al.*, 2017).

A study done at the University of Geneva Hospitals analyzed prospective onsite surveillance data from suspected 113 episodes of primary nosocomial bloodstream infection in MICU, 33 (29%) were microbiologically documented (Hugonnet *et al.*, 2004). The BSI leading sites from 554 ICU-acquired infections have LRI pneumonia (28.7%), bloodstream infection (20.4%), skin and soft tissue (15.3%), CVC (13.5%), and UTI (11.2%). It shows nine episodes of secondary bloodstream infection, six secondary infections from UTI, two from an LRI, and one from the skin and soft tissue infection (Emori and Gaynes, 1993). A similar study conducted in Italy shows the source of bloodstream infections which accounts for 21.1% was attributed to the LRI, and 21.4% of bloodstream infections were catheter-related. Among 23.7% of total patients, no clear source of infection was identified (Russotto *et al.*, 2015). The French national network study showed that the main causes of ICU-acquired bacteremia were CVC (29.2%), LRI (18.0%), and GII (13.6%). The sources of ICU-acquired bacteremia remained unknown in 24.9% of cases. The same network study result shows, the main causes of catheter-related bloodstream infections were *coagulase-negative staphylococci* (28%), *S. aureus* (23%), *P. aeruginosa* (12%), *E. coli* (8%), and multiple causes (11.5%) (Timsit *et al.*, 2014). It is more difficult for empiric treatment, especially for patients admitted to pediatric and adult ICUs with a major incidence of multidrug-resistant microorganisms (Timsit *et al.*, 2014). A cohort study in the UK revealed from a total of 559 home parenteral nutrition (HPN) patients, 134 catheter-related bloodstream infections (CR-BSI) were

recorded in 92(16.45%) patients (62 patients with a single CR-BSI and 30 patients with more than 1 CR-BSI). *CoNS* were the most common isolates 41/134 (30.5%), followed by polymicrobial infections 14/134 (10.4%), *Klebsiella spp.* 16/134 (11.9%) and methicillin-sensitive *S. aureus* (MSSA) 5/134 (3.7%) (Bond *et al.*, 2017).

2.3. Microbial Profile of Bloodstream Infection

According to research done in Tehran and Australia gram-positive bacteria, *S. aureus*, *CoNS*, and *Enterococcus species*. gram-negative bacteria *Pseudomonas aeruginosa* (*P. aeruginosa*), *Klebsiella pneumoniae*, *E. coli*, *Enterobacter spp.*, and *Acinetobacter spp.* are the most prevalent bacterial pathogens found in bloodstream infections (Ghadiri *et al.*, 2012; Prowle *et al.*, 2011). Similar studies conducted in Ethiopia show the major bloodstream infection-causing gram-negative bacteria are *E. coli*, *P. aeruginosa*, *Klebsiella species*, *Neisseria meningitidis*, *Haemophilus influenzae*, and gram-positive bacteria are *CoNS*, *S. aureus*, *S. pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, and *Enterococcus faecium* (Dagnew *et al.*, 2013; Hugonnet *et al.*, 2004; Seboxa *et al.*, 2015).

2.4. MDR-Causing Bacteria in Bloodstream Infection

Recently, it has been observed that the MDR problem has shifted from gram-positive bacteria to gram-negative bacteria, which in turn worsens the problem by the dearth of new antimicrobial drugs having an effect against them (Brusselsaers *et al.*, 2011). The ESBL enzyme can hydrolyze penicillin, monobactam, and third-generation cephalosporins. AmpC beta-lactamase (AmpC-BL) can also break down penicillins, third-generation cephalosporins, and cephameycin, while carbapenemase and metallo beta-lactamase (MBL) can break down carbapenems drugs (CDC, 2013a; CLSI, 2018; Jacoby, 2009; Shaikh *et al.*, 2015). Common examples of MDR of clinical concern include ESBL-producing gram-negative bacteria, carbapenem-resistant *Enterobacteriaceae*, and multidrug-resistant *Acinetobacter spp* (CDC, 2013b; Rossolini *et al.*, 2014; SNH, 2015). In gram-negative bacteria, the resistance is mainly due to the rapid increase of ESBLs and carbapenem in *K. pneumoniae*, *E. coli*, *P. mirabilis*, *Acinetobacter baumannii*, *P. aeruginosa*; (Timsit *et al.*, 2014) *Enterobacter spp.* and *Citrobacter spp.* infections (Brusselsaers *et al.*, 2011). Considering gram-negative bacteria, *Enterobacteriaceae*, *Acinetobacter spp.* and *pseudomonas spp.* are Multidrug-resistant due to the rapid increase of extended-spectrum Beta-

lactamases (ESBLs), carbapenemase, AmpC b-lactamases and Metallo beta-lactamases (MBLs). Some strains of *A. baumannii* have been detected as drug-resistant to all antibiotics (Cao *et al.*, 2014; Gamal *et al.*, 2016; Kao *et al.*, 2015; Timsit *et al.*, 2014).

Among gram-positive organisms, methicillin-resistant *Staphylococcus aureus* (MRSA), *S. aureus* with resistance to vancomycin (VISA/VRSA), vancomycin-resistant *Enterococci* (VRE), and multidrug-resistant *S. pneumoniae* are the major (CDC, 2013b; Rossolini *et al.*, 2014; SNH, 2015). Due to resistance to several other (beta-lactam) antimicrobials, treatment of MRSA often relies on vancomycin. However, the transmission of the Vancomycin-resistant *Enterococcus* (VRE) *vanA* gene from *enterococci* to *staphylococci* has increased in high-level vancomycin-resistance *S. aureus* which leads to the development of PDR bacteria. *Enterococci* represent the third-most prevalent nosocomial pathogens worldwide. Inherently and due to genetic exchange, *Enterococci* are resistant to most antibiotics (Brusselaers *et al.*, 2011; Gharsa *et al.*, 2012; Miller *et al.*, 2014; Russotto *et al.*, 2015; Timsit *et al.*, 2014). Multidrug-resistant *Enterobacteriaceae* can accumulate many antibiotic-resistance genes through plasmid-mediated horizontal transfer of genetic elements (Hudson *et al.*, 2014; Kocsis and Szabó, 2013; Kumarasamy *et al.*, 2010).

The study conducted in Ethiopia shows that Gram-negative bacteria exhibited a greater level of AMR ranging between 20%– 100% (Dagneu *et al.*, 2013). The most important change in the epidemiology of ICU-related bloodstream infection in recent years is the emergence of highly resistant organisms, particularly XDR Gram-negative bacteria including *P. aeruginosa*, *Acinetobacter baumannii*, and *K. pneumoniae* (Timsit *et al.*, 2014). ESBLs are plasmid-mediated, and their potential for transfer makes effective control and treatment difficult, which has resulted in endemic and epidemic outbreaks (Brusselaers *et al.*, 2011). Besides this, the number of new antibiotics developed and approved for use has drastically decreased over the past three decades (Ventola, 2015). In developing countries, the issue is even more serious. This arose from the vast disease load, large population density, the fast spreading of the disease due to overcrowding, poor hygiene culture, and inappropriate use of the available treatments, on top of the economic effects which in turn has the share to contribute to this drug resistance bacteria development (Sosa, 2010).

2.5. MDR Burden Due to Extra Cost for Patients

In the United States, at least 2 million people become infected with bacteria that are resistant to antibiotics and at least 23,000 people die each year as a direct result of these infections at an estimated cost of \$20 billion and additional productivity losses of \$35 billion (CDC, 2013a). In Europe, an estimated 25,000 deaths are attributable to antibiotic-resistant infections, costing € 1.5 billion annually in direct and indirect costs (EMA-ECDC, 2009). Little is known about the excess mortality caused by multidrug-resistant (MDR) bacterial infections in low and middle-income countries (LMICs). Lim *et al.* estimated 19,122 of 45,209 (43%) deaths in patients with hospital-acquired infection due to MDR bacteria in Thailand in 2010 (Lim *et al.*, 2016). So, per capita, this estimation was about 3 to 5 times larger than those for the United States and the European Union (Lim *et al.*, 2016). Although there are no reliable estimates of economic losses in developing countries, it is estimated that 58,000 neonatal sepsis deaths are attributable to drug-resistant infections in India alone (Laxminarayan *et al.*, 2013). The global data shows that MRSA-reported deaths in America were around 90%, in Africa 80%, in the Mediterranean Region >50%, in the European region around 60%, in South East Asia Region >25%, and in some parts of West Pacific Region, it is as many as 80%. On average, people with MRSA are estimated to be 64% more likely to die than people with a non-resistant form of the infection (WHO, 2016). Consequently, this shows that treatment with standard antibiotics is not effective (WHO, 2014).

2.6. MDR, XDR, and, PDR Prevalence

In 2050, multidrug-resistant bacteria are expected to be the cause of 10 million annual deaths (Dadgostar, 2019). Research conducted in an Ethiopian context showed that there is a significant association between death and AMR in bloodstream infection (Seboxa *et al.*, 2015). Both gram-positive bacteria and gram-negative bacteria are affected by the emergence and rise of AMR (CDC, 2013a; Magiorakos *et al.*, 2012; WHO, 2016). However, gram-negative bacteria have a higher mortality rate than gram-positive bacteria. Despite the anti-microbial treatment, mortality due to septic shock can be as high as 60% (Moyo *et al.*, 2010). The report attributed 45% of deaths in both Africa and Southeast Asia to multi-drug resistant (MDR) bacteria (Founou *et al.*, 2017). Similar research conducted in Kenya with an analysis of 624 isolates revealed that the AMR rates are higher than most recent local and international reports. 88% of the isolates tested were multi-

drug resistant (MDR) whereas 26% were extensively drug-resistant (XDR) (Wangai *et al.*, 2019). A study on MDRs by Hassen *et al.* has reported that 52.6% of the isolates were MDR. Likewise, as per a study conducted in Italy shows an MDR bacteria were detected in 50.7% of patients with bloodstream infection (Russotto *et al.*, 2015). In a study undertaken in the USA on *Acinetobacter baumannii*, over 80% of the isolates exhibited an MDR. It increased the risk of receiving inappropriate empiric therapy fivefold in turn, increased the risk of in-hospital mortality (Zilberberg *et al.*, 2016). A study on gram-negative bacteria in Ethiopia revealed 94.5% MDR of which 8.8% and 0.8% were extensively and pan-drug resistant, respectively (Beyene *et al.*, 2019). Similarly, a study conducted in Ethiopia shows that multidrug resistance organisms were found in 89.1% (n=49/55) of all bacterial isolates. Accordingly, Gram-positive and gram-negative isolates showed 71.42% and 95.11% MDR respectively. In this study, Amoxicillin (89.1%) and cefotaxime (85.5%) showed the highest level of resistance (Hailu *et al.*, 2016b). In most studies of bloodstream infection, the prevalence of Gram-positive is higher than Gram-negative but in terms of drug susceptibility Gram-negative organisms have higher multi-drug resistance.

2.7. ESBL, AmpC, MBL, and Carbapenemase Prevalence

Production of ESBL and carbapenemase are the causes of drug resistance, particularly in gram-negative bacteria, and this is a serious challenge to the effectiveness of contemporary antibiotic treatment (CDC, 2013a; Shaikh *et al.*, 2015). Over 19% of nosocomial infections are caused by extended-spectrum beta-lactamase (ESBL) enzyme-producing bacteria. They are also expected to have a 57% higher mortality rate among bloodstream infections than a strain that doesn't produce ESBL (CDC, 2013a). A study conducted in Ethiopia reported a prevalence of ESBL and AmpC-BL in gram-negative bacteria was reported to be 38.8% and 2.4% (Tekele *et al.*, 2020), 67% and 2% (Beyene *et al.*, 2019), 51% and 25% (Gashaw *et al.*, 2018a; Gashaw *et al.*, 2018b), and in *Enterobacteriaceae* was 78.6% and 12.1% (Legese *et al.*, 2017), respectively. Another research conducted on gram-negative bacteria in an Ethiopian context has revealed 94.5% MDR, of which 8.8% and 0.8% were extensively drug-resistant and pan-drug resistant, respectively (Beyene *et al.*, 2019).

In Developing countries, likewise, rapid increases in the rates of infections due to carbapenemase-producing *K. pneumonia*, metallo-beta-lactamase-producing *A. baumannii* (MBL-AB), metallo-

beta-lactamase-producing *P. aeruginosa* (MBL-PA), and Extended-spectrum beta-lactamase (ESBL) producing *Enterobacter spp.* have been reported across the world (Founou *et al.*, 2017). For Gram-negative isolates, 20% were ESBL producers, 16% were AmpC producers, and 48% were carbapenemase producers (Hassan *et al.*, 2017). A study conducted in Italy shows carbapenem resistance was detected in 69% of *Acinetobacter spp.*, 38% of *K. pneumoniae*, and 37% of *Pseudomonas species*, having exposure to prior hospitalization and antibiotic exposure (Russotto *et al.*, 2015). In Ethiopia, the reported prevalence of ESBL and AmpC-BL in gram-negative bacteria was 38.8% and 2.4%, respectively (Tekele *et al.*, 2020). Another study also showed the overall prevalence of ESBL and Carbapenemase from *Enterobacteriaceae* was 78.6% and 12.1%, respectively (Legese *et al.*, 2017) while additional studies from gram-negative bacteria were reported at 67% and 2% respectively by (Beyene *et al.*, 2019), and 51% and 25%, respectively by (Gashaw *et al.*, 2018a). However, most of these studies did not address the magnitude of ESBL, carbapenem-producing gram-negative bacteria and associated factors from all age groups of bloodstream infection in tertiary hospitals, this study aims to fill this identified gap.

2.8. ESBL, AmpC, MBL, and Carbapenemase Genes Prevalence

Enterobacter spp. are intrinsically resistant to aminopenicillins, cefazolin, and ceftiofur, due to the production of constitutive chromosomal AmpC-BLs (Russotto *et al.*, 2015). A growing number of *K. pneumoniae*, *E. coli*, and *Enterobacter spp.* have evolved resistance to third-generation cephalosporins over the past few years due to ESBL. Resistance to ampicillin, amoxicillin, and the first generation of cephalosporins is caused by the ESBL genes TEM-1, TEM-2, and SHV-1, which are often expressed in Gram-negative bacteria (Russotto *et al.*, 2015). In Enterobacteriaceae, the blaCTX-M-15 ESBL encoded genes are the means for acquired resistance to third-generation cephalosporins (Kumarasamy *et al.*, 2010). Some b-lactamases are active on a broad spectrum of b-lactams including the last-resort carbapenems. The gene for the broad-spectrum and carbapenem-active Metallo-beta-lactamase NDM-1 (carbapenem resistance gene bla_{NDM-1}) is rapidly spreading (Hudson *et al.*, 2014). NDM-1 was mostly found among *E. coli* and *K. pneumoniae*, which were highly resistant to all antibiotics except tigecycline and colistin. The potential of NDM-1 is going to be a worldwide public health problem (Kumarasamy *et al.*, 2010). The most important beta-lactamases are the cephalosporinases, for instance, extended-spectrum beta-lactamases (ESBLs), and the most important carbapenemases are KPC, Metallo-beta-

lactamases (MBLs), and oxacillinase OXA-48 enzymes. Other resistance mechanisms against beta-lactams are the outer membrane permeability change and efflux pumps (Kocsis and Szabó, 2013).

Different nomenclatures have been proposed and debated for β -lactamases and Carbapenemases (Jacoby and Bush, 2014). The definition of Giske *et al* divides ESBL enzymes into three main groups namely ESBL_A, ESBL_M, and ESBL_{CARBA}. This is a functional classification depending on the biochemical function and susceptibility towards β -lactamase inhibitors. ESBL_A includes the most frequently identified CTX-M enzymes as well as SHV and TEM enzymes that are horizontally transferrable and degraded by clavulanic acid. ESBL_M are miscellaneous ESBL among those ESBL_M AmpC represents the most common type. ESBL_{CARBA} are enzymes conferring carbapenemase activity, such as Metallo β -lactamases and *Klebsiella pneumoniae* carbapenemase (KPC) (Giske *et al.*, 2008).

The Ambler categorization was the most adopted category in the classification of drug resistance mechanisms. This classification system, based on amino acid homology, is the most widely used approach to categorize beta-lactamases as the source of bacterial resistance to beta-lactam antibiotics (Ambler, 1980). He classified beta-lactamases into four classes in 1980 based on the similarity of these sequences. Classes A, C, and D operate by the serine ester hydrolysis mechanism, but class B Metallo-lactamases require a bivalent metal ion, usually Zn^{2+} , for its activity (Hall and Barlow, 2005; Nordmann and Poirel, 2019).

The Class A ESBLs are capable of hydrolyzing penicillin, monobactams, and third-generation cephalosporins (Biutifasari, 2018; Munoz-Price *et al.*, 2019). The class A major ESBL encoding genes *blaSHV*, *blaCTX-M*, and *blaTEM* (Trung *et al.*, 2015) and the class A minor ESBLA, *blaPER*, *blaGES*, *blaBEL*, and *blaVEB* (Bogaerts *et al.*, 2013) were included in this study (Table 1). Class C AmpC β -lactamases hydrolyze penicillin, monobactams, broad and extended-spectrum cephalosporins, and cephamycin but sensitive to Cefepime and that are not inhibited by β -lactamase inhibitors such as clavulanic acid (Aryal *et al.*, 2020; Jacoby, 2009; Nordmann and Poirel, 2019). The AmpC genes for AmpC-BL families *blaMOXM*, *blaCITM*, *blaDHAM*, *blaACCM*, *blaEBCM*, and *blaFOX*M are the focus areas in this research (Pérez-Pérez and Hanson, 2002) (Table 1). Class B carbapenems are metalloproteins and are capable of hydrolyzing all

antibiotics except monobactams (Hall and Barlow, 2005; Nordmann and Poirel, 2019). Metallo beta-lactamase genes were grouped into major groups; *bla*NDM, *bla*VIM, and *bla*IMP (Bogaerts *et al.*, 2013); and minor groups, *bla*GIM-1, *bla*SIM-1, *bla*AIM-1, *bla*SPM-1 (Poirel *et al.*, 2011) (Table 1). Class D Carbapenemases are oxacillinase derivatives (OXA-23-group, OXA-24-group, OXA-48b, OXA-58-group). Like some Class A (KPC, GES, and IMI) carbapenemase, their hydrolytic activity against carbapenems and some 3rd generation cephalosporins are not inhibited by clavulanic acid and tazobactam (Nordmann and Poirel, 2019; Noster *et al.*, 2021) (Table 1).

Table 1: ESBL and carbapenem drug resistance genes detected in bacterial isolates from bloodstream infection patients (Lutgring and Limbago, 2016; Schmitt *et al.*, 2007)

Category	Drug resistance category	Drug sensitive category	Drug resistance	Indicator drugs	Genes
ESBLs	Cephalosporins	Cephameycins and Carbapenems	Cefotaxime Ceftriaxone Ceftazidime Cefepime	Clavulanate, Sulbactam, and Tazobactam	<i>bla</i> CTX-M, <i>bla</i> SHV, <i>bla</i> TEM, <i>bla</i> PER, <i>bla</i> GES, <i>bla</i> BEL, and <i>bla</i> VEB
AmpC BLs	Cephameycin and Cephalosporins	Carbapenems	Cefotaxime Ceftriaxone Ceftazidime Cefepime Cefoxitin Cefotetan	Cefoxitin Cefotetan Benzo-thiophene-2-boronic acid	<i>bla</i> MOXM, <i>bla</i> CITM, <i>bla</i> DHAM, <i>bla</i> ACCM, <i>bla</i> EBCM, <i>bla</i> FOXM
Carbapenem resistance gram-negative bacteria (CR gram-negative bacteria)	Carbapenems	Colistin	Imipenem Ertapenem, Meropenem Doripenem	Clavulanic acid, boronic acid; EDTA, Dipicolinic acid	<i>bla</i> KPC, <i>bla</i> SMe, <i>bla</i> OXA, OXA-23-group, OXA-24-group, OXA-48b, OXA-58-group

Metallo-beta-lactamase (MBL)	Carbapenems	Colistin	Imipenem Ertapenem, Meropenem Doripenem	Sodium mercaptoacetic acid	<i>Bla</i> VIM, <i>bla</i> IMP, <i>bla</i> NDM-1 <i>bla</i> GIM-1, <i>bla</i> SIM-1, <i>bla</i> AIM-1, <i>bla</i> SPM-1

3. CHAPTER THREE: MATERIALS AND METHODS

3.1. Study Area

This study was conducted at TASH, located at Lideta sub-city (Figure 1). TASH was established in 1973 to serve the needs of the whole country as a specialized referral hospital and as a medical university. It is the largest specialized hospital in Ethiopia, with over 700 beds of patients admitted at a time. It also acts as a teaching hospital for medical students pursuing undergraduate and graduate degrees, dentists, nurses, midwives, pharmacists, medical laboratory technologists, radiology technologists, and other health professionals who assume responsibility for the community's and the nation's health issues. The laboratory is an advanced laboratory with four sample collection areas, a sample processing unit, hematology, immunohematology, blood bank, chemistry, serology, immunochemistry, urinalysis, parasitology, microbiology, and molecular biology sections.

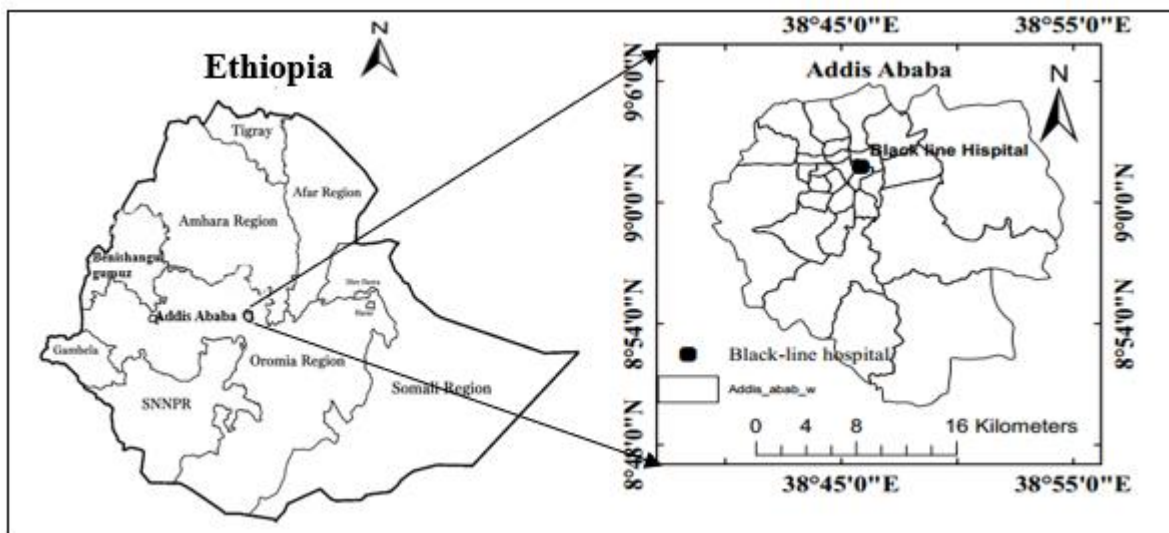


Figure 1: Map the study area of Tikur Anbessa specialized hospital in Addis Abeba, Ethiopia, located at 9.0203°N latitude and 38.74905° E longitude.

The Hospital has 201 physicians, 627 nurses, and over 115 other health professionals working at the hospital who are committed to giving healthcare service. Patient care services are provided by the School of Medicine's faculty, residents, and various departments under their specialty training within the hospital. In order to support hospital operations, the hospital employs over 1,300 contract and permanent administrative staff members. Furthermore, the majority of Addis Ababa's

regional and federal hospitals are connected to the School of Medicine as a training and clinical service center (AAU, 2021). Patients are referred to the hospital from all over the nation because it is a specialized referral facility with a wide range of specialties, which helped the researcher to get different types of drug-resistant organisms.

3.2. Study Design and Period

An Institutional-based cross-sectional study was conducted for about seven months between September 2018 and March 2019 G.C.

3.3. Source Population

- Patients who sought medical services at TASH during the study period were included.

3.4. Study Population

- All patients from all age groups suspected of bloodstream infection and who visited the study site during the study period were included. The conditions of sepsis definitions were established by the 1991 Consensus Conference, which was updated in 2001 (Bone *et al.*, 1992; Levy *et al.*, 2003).

3.5. Inclusion and Exclusion Criteria

3.5.1. Inclusion Criteria

- Patients suspected of septicemia by physicians during the study period.
- Patients who are willing to give blood samples and volunteer to sign a Consent paper participated in the study.

3.5.2. Exclusion Criteria

- Individuals who took antibiotics within ten days of taking the sample were excluded.
- Individuals who were not willing to participate in the study were excluded.

3.6. Sample Size

The sample size calculation is based on a single sample size estimation with slight modification (Daniel and Cross, 1995; Naing *et al.*, 2006). A prevalence of 32.8% was taken from the study which was conducted in Addis Ababa Ethiopia (Kitila *et al.*, 2018).

$n = Z^2 p(1-p)/d^2$. $n = (1.96)^2 0.328(1-0.328)/(0.025)^2 = 1354.80$ with 10% contingency = 1490. But 4 of them were excluded and 1486 patients were involved in this study.

Where n = sample size, Z = Z-score at 95% confidence interval; which is 1.96. P = expected prevalence or proportion (in proportion to one; if 32.8%, $P = 0.328$), and d = precision (in proportion to one; is 5%, but for this research case $d = 0.025$).

3.7. Sampling Method and Procedure

Consecutive convenient sampling techniques were used and a total of 1486 study participants from all age groups were recruited.

3.8. Study Variable

3.8.1. The Dependent Variables

- Prevalence of bacterial isolates in septicemia suspected patient.
- Antibiotic Susceptibility pattern of the bacterial isolated.
- Enzyme-producing drug resistance mechanisms, and
- Genes responsible for Enzyme producing drug resistance mechanisms

3.8.2. Independent Variable

- Assumed risk factors for all age groups.
 - Demographic characteristics: Age, Sex...
 - Admission ward and admission department, length of admission, and ICU admission
 - Antibiotic treatment ten days before sample collection

- Insertion instrumentation usage during medical care: mechanical ventilator, central venous catheter, urinary catheter, suction machine, tracheostomy, intravenous lines, drainage tube, shunt, NG tube
- Clinical symptoms: body temperature, (fever, chills, shivers, vomiting, and fast heartbeat),
- Clinical diagnosis
- Assumed risk factors for the age group of “above neonatal ages”
 - Chronic illness, (RVI patients, diabetic patients, hypertension, surgical wound infections, wound infections on the skin, gastrointestinal infection, ureteral tract infection, respiratory tract infection, heart disease, coronary artery diseases, stroke, gangrene, tuberculosis, cirrhosis, renal failure, hematologic malignancy, and tissue sarcomas)
- Assumed risk factors for neonates:
 - The educational level of the family, Place of delivery, history of incubation, Mode of delivery, Gestational age, premature rupture of membrane, Body weight during delivery, and History of maternal infection during pregnancy.

3.9. Data Collection

Data collection was conducted using standardized pretested questionnaires (Appendix 1-6) which later was appended by the information of the respondent from medical records. The demographic data, clinical data, and blood samples were collected by qualified professional nurses and laboratory technologists with experience in collecting research data and blood samples. All data and sample collectors were trained by the principal investigator on the detailed instructions of using prepared standardized questionnaires, blood sample collection procedures, and sample transportation to the laboratory (Appendix 7-8). Follow-up of microbial growth in the blood culture bottle and further processing was accomplished by the recruited professional microbiologists and the principal investigator in the laboratory.

3.10. Sample Collection

Samples were collected aseptically from patients for routine blood cultures before taking antibiotic treatment in the hospital. The vein puncture site was disinfected with 70% alcohol and 2% tincture of iodine before collecting the sample. The sample was approximately 1-4 ml of blood for pediatrics (less than 12 years) and 5- 10 ml of blood for adults (greater than 12 years) collected in two bottles. It is inoculated into a ready-made BacT/ALERT blood culture bottle according to Dreyer (Dreyer, 2012). Two aerobic BacT/Alert FA Plus bottles for adults and BacT/ALERT PF Plus bottles for pediatric blood culture bottles were used for each patient and similar growth in both bottles was considered positive. Blood culture bottles were incubated in an automatic BacT/ALERT® 3D machine at 37°C of 5% CO₂ gas concentration and were inspected daily for signs of bacterial growth for up to 5 days (Flayhart *et al.*, 2007; Lee *et al.*, 2013).

3.11. Microbial Culture

Bacterial pathogens were identified in the TASH microbiology department using standard bacteriological procedures (CLSI, 2017). Two aerobic BacT/Alert FA Plus bottles for adults and BacT/ALERT PF Plus bottles for pediatric blood culture bottles were used for each patient and similar growth in both bottles was considered as positive. Blood culture bottles were incubated in an automatic BacT/ALERT® 3D machine at 37°C of 5% CO₂ gas concentration and were inspected daily for signs of bacterial growth for up to 5 days.

Upon BacT/ALERT® 3D blood culture, if microorganisms are present in the test sample, carbon dioxide will be produced as the microorganisms metabolize the substrates in the culture medium. When the growth of the microorganisms produces CO₂, the color of the sensors at the bottom of each culture bottle changes from dark color to light color. A light-emitting diode (LED) projects light onto the sensor. The light reflected is measured by a photodetector. As more CO₂ is generated, more light is reflected. This information is compared to the initial sensor reading (Peter-Getzlaff *et al.*). If there is a high initial CO₂ content, it means that there is an unusually high rate of CO₂ production, and/or a sustained production of CO₂, therefore, the sample is considered to be positive for microbial growth (Flayhart *et al.*, 2007; Lee *et al.*, 2013). Turbid blood samples from the blood culture bottle were sub-cultured on blood agar (Oxoid, England) (5% sheep blood plates), MacConkey agar (BD, USA), and chocolate agar (Oxoid, England) at 37°C for 24 hours. MC

plates were incubated at 35°C in aerobic conditions. Chocolate and blood agar plates were incubated at 35°C in microaerophilic conditions (containing 5% CO₂) (Cheesbrough, 2006). The positive culture from blood samples was characterized by a gram stain and Gram-negative bacteria which were further classified at the species level by using conventional biochemical tests (Rods, 2014).

3.12. Antimicrobial Susceptibility Test

Antimicrobial susceptibility tests: Antimicrobial susceptibility tests were performed in the TASH microbiology department by using disk diffusion and susceptibility patterns which were determined following CLSI guidelines. Using a sterile wire loop, 3-5 pure colonies were picked from pure blood agar culture and emulsified in nutrient broth. Standard inoculums adjusted to 0.5 McFarland were swabbed onto Muller-Hinton agar (dispensed on a 150mm plate). The disk diffusion method was performed after 16-18 hours of incubation on Mueller-Hinton agar at a 37°C zone of inhibition and was measured and interpreted as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2017). The antimicrobials for disc diffusion testing was obtained from BD and Oxoid for gram-negative bacteria with the following concentrations: for gram-negative bacteria Amikacin (AN) (30 µg), Amoxicillin + clavulanic acid (AMC) (20/10µg), Ampicillin – Sulbactam (SAM) (10/10 µg), Ampicillin (AM) (10 µg), Azithromycin (ATM) (30 µg), Cefepime (FEP) (30 µg), Cefotaxime–clavulanate (CTL) (30/20µg), Cefotaxime (CTX) (30 µg), Cefotetan (CTT) (30 µg), Cefoxitin (FOX) (30 µg), Ceftaroline (CPT) (30 µg), Ceftazidime–clavulanate (CAL) (30/10 µg), Ceftazidime (CAZ) (30 µg), Ceftriaxone (CRO) (30 µg), Cefuroxime (CXM) (30 µg), Chloramphenicol (C) (30 µg), Ciprofloxacin (5 µg), Doripenem (DOR) (10 µg), Doxycycline (DOX) (30 µg), Ertapenem (ETP) (10 µg), Gentamicin (GM) (10 µg), Imipenem (IMP) (10 µg), Levofloxacin (LEV) (5 µg), Meropenem (MEM) (10 µg), Piperacillin–Tazobactam (TZP) (100/10µg), Tetracycline (TET) (30 µg), Tobramycin (NN) (10 µg), and Trimethoprim-Sulfamethoxazole (SXT) 1(.25/23.75µg) (CLSI, 2015, 2017).

Double Disk Synergy Test (DDST): The antibiotic disks that are used for this research are Ceftriaxone (30 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Aztreonam (30µg), and Amoxicillin/ Clavulanic acid (20/10 µg). The four antibiotics were placed at a distance of 20 mm (edge to edge) from the Amoxicillin/Clavulanic acid disk which was placed in the middle of the

plate. After 24-h incubation, if an enhanced zone of inhibition between either of the cephalosporin antibiotics and the Amoxicillin/Clavulanic acid disk has occurred, the test was considered ESBL positive.

Combined Disk Test (CDT): A Ceftazidime (30 µg) disk and Cefotaxime disks were used for this research's sake. They were arranged in a way that the ceftazidime drug and its combination with Clavulanic acid were placed side by side. Likewise, Cefotaxime (30µg) was arranged in such a way that the Cefotaxime drug and its combination with Clavulanic acid were placed side by side so that their combination with Clavulanic acid (30 µg/10 µg) was considered for confirmation of the presence of ESBL. An increase in zone diameter greater than 5mm for either of the Cephalosporin disks and their respective Cephalosporin/ Clavulanate disk were interpreted as ESBL Positive (CLSI, 2017). E-tests were done for ESBL detection by Ceftazidime 0.5-32 µg/ml, and Cefotaxime 0.25-16 µg/ml strips. This test was used alone and their combination with Clavulanic acid i.e., Ceftazidime -Clavulanic acid 0.064-4 µg/ml and Cefotaxime- Clavulanic acid 0.016-1.0 µg/ml respectively for phenotypic confirmation. AmpC detection was carried out using cefotetan 0.60-32µg/ml with cefotetan and cloxacillin 0.5-32µg/ml combined E-test. The resistance and susceptibility were interpreted according to the CLSI Laboratory Standards (CLSI, 2012). *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), and *Pseudomonas aeruginosa* (ATCC 27853) were used as reference strains for the culture and susceptibility testing.

3.12.1. ESBL Primary Screening

Primary identification of drug-resistant bacteria was done by antibiotic susceptibility testing. Resistant or intermediate to Ceftazidime (30µg), Ceftriaxone (30µg), and Cefotaxime (30µg) were suspected to ESBLs producers and additionally resistant to cephamycin (cefotetan) were indicator for AmpC-BLs producer within the CLSI screening criteria. However, we did a confirmatory test for all cefoxitin-resistant gram-negative bacteria. These breakpoints indicative of thought for ESBL production were CAZ≤22mm, CRO, ≤ 25 mm, and CTX≤ 27mm. Initial screening for AmpC was done by the diameters of zones of inhibition produced by Ceftazidime (30µg), Ceftriaxone (30µg), Cefotaxime (30µg), Cefepime (30 µg), and Cefoxitin (30 µg) which is found to be within the CLSI screening criteria. These breakpoints are indicative of AmpC production when CAZ≤22mm, CRO, ≤ 25 mm, CTX≤ 27mm, FEP ≥ 25, and FOX ≤ 18mm. Phenotypic

detection of ESBL and AmpC production was confirmed by a double-disk synergy test and combined disk test by using an E-test according to EUCAST and CLSI guidelines respectively (CLSI, 2017; EUCAST, 2017).

3.12.1.1. Double disk synergy test (DDST) for ESBL detection

The bacterial isolates to be tested were inoculated onto a Mueller–Hinton agar plate. The antibiotic disks that deployed for this purpose were Ceftriaxone (30 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Aztreonam (30µg), and Amoxicillin/ Clavulanic acid (20/10 µg). The four antibiotics were then placed at distances of 20 mm (edge to edge) from the Amoxicillin/Clavulanic acid disk, which was placed in the middle of the plate. After 24 hours of incubation, the occurrence of an enhanced zone of inhibition between either of the cephalosporin antibiotics and the Amoxicillin/Clavulanic acid disk was ESBL positive (Abdeta *et al.*, 2022; CLSI, 2017). The primary AST screening integrated the DDST and later on, the confirmatory E-test for ESBL and AmpC-BL identification was conducted.

3.12.1.2. E-Test for both ESBL and AmpC beta-lactamase detection

The E-test was conducted for ESBL detection using Ceftazidime (CAZ) 0.5-32 µg/ml alone with Ceftazidime+ Clavulanic acid (CAL) 0.064-4 µg/ml combination, and Cefotaxime (CTX) 0.25-16 µg/ml alone with Cefotaxime+ Clavulanic acid (CTL) 0.016-1.0 µg/ml combination strip were used for phenotypic confirmation. For detection of ESBL, $CTX \geq 0.5$ and CTX/CTL ratio ≥ 8 or $CAZ \geq 1$ and CAZ/CAL ratio ≥ 8 were considered as positive, whereas $CTX < 0.5$ or CTX/CTL ratio < 8 and $CAZ < 1$ or CAZ/CAL ratio < 8 was considered as negative. The AmpC-BL detection was done by using cefotetan 0.60-32µg/ml alone with cefotetan-cloxacillin 0.5-32µg/ml combination. The ratio of $CTT/CXT \geq 8$ indicates the production of an AmpC BL (CLSI, 2012; Liofilchem, 2014a, b).

3.12.2. Carbapenem-Resistant Detection

The presence of Carbapenemase detection was implemented by using the modified Carbapenemase inactivation method (mCIM) and EDTA-modified Carbapenemase inactivation method (eCIM), except for the case of *Acinetobacter spices*. This was conducted for all the

Carbapenem-resistant or intermediate with either Imipenem, Meropenem, Doripenem, or Ertapenem except for non-fer gram-negative bacteria where Ertapenem was not used. To perform the mCIM, a suspension was made by taking 1 µl loopful of bacteria from an overnight blood agar plate and then adding it to a 2 ml trypticase soy broth. Subsequently, the Meropenem (10 µg) disc was immersed in the suspension and incubated for 4 hours ± 15 minutes at 37°C. After the incubation, the disc was removed from the suspension using 10 µl inoculation loops and placed on a Mueller-Hinton agar plate inoculated with a susceptible *E. coli* indicator strain (ATCC 29522). Then, the results were recorded after 18-24 hours of incubation at 37°C. Besides, for the eCIM case taking 1 µl loopful of bacteria from an overnight blood agar plate and then adding it to 2 ml having 5 molar EDTA concentration trypticase soy broth. Then MBL production was detected using the mCIT test procedure. For mCIM zone of inhibition of 6 to 15 mm around a disk and a zone of inhibition of 16 to 18 mm around a disk including the presence of colonies in the zone of inhibition was considered positive. A zone of inhibition with a distance of 19 mm or greater around a disk was considered negative. A negative result suggests that the isolate is resistant to carbapenems by a mechanism other than the production of carbapenemase. If mCIT shows carbapenem production and an increased diameter of eCIT greater or equal to 5mm then mCIT is considered MBL positive (CDC, 2013c; CLSI, 2017; EUCAST, 2017; Kadri, 2020).

3.12.3. Determination of MDR, XDR, and PDR Isolates

Multidrug resistance (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) isolates were categorized by using standard definitions of Magiorakos *et al.* MDR microorganism has been defined as an isolate with non-susceptibility to at least one agent in three or more antimicrobial categories. Extensively drug-resistant (XDR) was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories) and pan-drug-resistant (PDR) was defined as non-susceptibility to all agents in all antimicrobial categories (Magiorakos *et al.*, 2012).

3.13. Molecular Detection of ESBL, AmpC-BL, MBL, and Carbapenemase

ESBL, AmpC, Carbapenemase, and MBLs producer genes were detected using PCR. Gene selection is based on Drug resistance type.

3.13.1. DNA Extraction

The DNA was extracted from fresh colonies of gram-negative bacteria from the blood agar plate (Oxoid). An aliquot of 1000µL of cell suspension containing 10^7 cells/mL from each gram-negative bacteria was transferred to microtubes and incubated overnight at 37°C. Cell suspensions were centrifuged at 4,500 rpm for 5 min at 4°C, and the pellets obtained were used for DNA extraction by boiling method with a modification. The gram-negative bacteria obtained from the pellets were suspended in a tube containing 50 µL nuclease-free water and then subjected to boiling at 100°C for five minutes. The mixture was centrifuged at 3000g (5175rpm) for 10 minutes. The DNA-containing upper aqueous phase was transferred into a separate 2 ml Eppendorf tube and 0.7 volumes of cold absolute ethanol were added. The aqueous phase was recovered by centrifugation for 20 min, and genomic DNA was precipitated by ethanol. The pellet was washed in cold 70% ethanol then after further centrifugation steps the ethanol was removed, and the nucleic acid pellet was allowed to dry before being re-suspended in aqueous TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) (Ahmed and Dablood, 2017; Dashti *et al.*, 2009). The final concentration of 70-100ng/µl of the template was prepared by using a NanoDrop Spectrophotometer.

3.13.2. Conventional PCR

PCR was performed using hot start Multiplex PCR (5x HOT FIREPol Multiplex Master Mix) containing HOT FIREPol DNA polymerase, 5x multiplex buffer, 10 mM MgCl₂, dNTPs, BSA, and loading dye that increased sample density were imported from (Solis Biodyne Estonia), according to manufacturer's instruction. The primers used are indicated in Tables 2 and 3. Oligonucleotide yield or lyophilized primer was reconstituted by nuclease-free TE buffer (10mMTris pH 8.0; 0.1 m MEDTA; pH 8.0) using integrated DNA technology (IDT) protocol. The PCR amplification was done by T3000 Biometra thermocycler using a Bio-Rad gel electrophoresis machine and DNA bands were viewed under Bio-Rad gel doc Imaging System and results were taken through the internet. All primers were obtained from IDT except AmpC-BLs and *bla*VIM and *bla*IMP primers which were imported from LIGO Macrogen Europ.

The choice of PCRs to perform was subject to the sensitive data. We analyze at least 20 resistance gene determinants. Multiple PCR assays were used for the detection of Carbapenemases, including both laboratory-developed tests and commercial assays. Commercial assays include the Xpert

Carba-R test (detecting KPC, NDM, IMP, VIM, and OXA-48), the hyper plex SuperBug ID (detecting KPC, NDM, IMP, VIM, GIM, OXA-48, OXA-162, OXA-181, and OXA-204), and the Check Direct carbapenem resistance gram-negative bacteria assay (detecting KPC, NDM, VIM, and OXA-48). The sensitivities for all these assays are between 97 and 100% (Lutgring and Limbago, 2016).

3.13.3. ESBL Gene Detection

The isolates that were screened positive for ESBLs were subjected to ESBL multiplex 1 and 2 PCR tests using specific primers for different families. In multiplex 1, ESBL, *bla*SHV, *bla*CTX-M, and *bla*TEM genes were performed using the Trung et al with slight modifications during optimization (Trung *et al.*, 2015) (Table 2). Multiplex PCRs were performed in a final volume of 20 µl of the amplification mixture containing 4 µl Multiplex master mix, 0.6 µl reverse and 0.6 µl forward primers each containing (10 ng/µl and 0.2 µl), 13.8 µl nuclease-free water, and 1 µl of 70-100 ng/µl DNA template. The thermal cycling conditions were: initial denaturation at 95°C for 240 seconds, followed by 35 cycles of denaturation at 94°C for 25 s, annealing at 58°C for 45 s and extension at 72°C for 1 min, and final extension at 72°C for 5 min. Multiplex PCR 2 was performed for ESBL, *bla*PER, *bla*GES, *bla*BEL, and *bla*VEB, using the protocol of Bogaerts et al. (Bogaerts *et al.*, 2013) (Table 2). Multiplex PCRs were performed in a final volume of 20 µl of the amplification mixture containing 4 µl Multiplex master mix, 0.8 µl reverse and 0.8 µl forward primers each containing (10 ng/µl), 13.4 nucleases free water, and 1 µl of 70-100 ng/µl DNA template. The thermal cycling conditions were: initial denaturation at 95°C for 300 seconds, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 90 seconds and extension at 72°C for 90 seconds, and final extension at 72°C for 5 min. The multiplex PCR products were separated using 1.5% agarose gel and stained with 3µl Ethidium bromide; 2µl loading dye and 6 µl PCR reaction of sample and control were loaded and gel electrophoresis was conducted using 120V, 400MA, for 45 minutes. DNA bands were viewed under the Biorad gel doc Imaging System.

Table 2: Primers used for detection of ESBL and AmpC-BLs genes detection

		Primer sequence (5' to 3')^a	Amp licon	

PCR name	Targeted gene			size in bp	References	
ESBL Multiplex 1	blaCTX-M	Forward	ATGTGCAGYACCAGTAARGTKATGGC	590	(Trung, 2015 #576)	
		Reverse	GGTRAARTARGTSACCAGAAAYCAGCGG			
	blaTEM	Forward	TCGCCGCATACACTATTCTCAGAATGAC	422		
		Reverse	CAGCAATAAACCAGCCAGCCGGAAG			
	blaSHV	Forward	TGTATTATCTC(C/T)CTGTTAGCC(A/G)CCCTG	739		
		Reverse	GCTCTGCTTTGTTATTCGGGCCAAGC			
MIN ESBL Multiplex 2	blaPER	Forward	AGTGTGGGGGCCTGACGAT	725	(Bogaerts <i>et al.</i> , 2013)	
		Reverse	GCAACCTGCGCAATRATAGCTT			
	blaGES	Forward	CTGGCAGGGATCGCTCACTC	600		
		Reverse	TTCCGATCAGCCACCTCTCA			
	blaBEL	Forward	CGACAATGCCGCAGCTAACC	448		
		Reverse	CAGAAGCAATTAATAACGCCC			
	blaVEB	Forward	CGACTTCCATTTCCCGATGC	376		
		Reverse	TGTTGGGGTTGCCCAATTTT			
ESBL Multiplex 3	blaMOX M	Forward	GCT GCT CAA GGA GCA CAG GAT	520	(Pérez-Pérez, 2002 #578)	
		Reverse	CAC ATT GAC ATA GGT GTG GTG C			
	blaCITM	Forward	TGG CCA GAA CTG ACA GGC AAA	462		
		Reverse	TTT CTC CTG AAC GTG GCT GGC			
	blaDHA M	Forward	AAC TTT CAC AGG TGT GCT GGG T	405		
		Reverse	CCG TAC GCA TAC TGG CTT TGC			
	blaACC M	Forward	AAC AGC CTC AGC AGC CGG TTA	346		
		Reverse	TTC GCC GCA ATC ATC CCT AGC			
	Vitoria-Gasteiz, Spain	blaEBC M	Forward	TCG GTA AAG CCG ATG TTG CGG		302
			Reverse	CTT CCA CTG CGG CTG CCA GTT		
		blaFOX M	Forward	AAC ATG GGG TAT CAG GGA GAT G		190
			Reverse	CAA AGC GCG TAA CCG GAT TGG		
A For degenerate primers: D = A, G or T; R = A or G; Y = C or T; K = G or T; W = A or T						

(Avison *et al.*, 2004; Bogaerts *et al.*, 2013; Trung *et al.*, 2015)

3.13.4. AmpC-Beta Lactamase Genes Detection

The isolates that were positive for AmpC-BLs were subjected to ESBL three multiplex PCR tests using specific primers for different families of ESBLs and AmpC-BLs (Tables 1 and 2):

PCR for ESBL Multiplex 3:- AmpC-BLs family *bla*MOXM, *bla*CITM, *bla*DHAM, *bla*ACCM, *bla*EBCM, and *bla*FOXm were detected (Pérez-Pérez and Hanson, 2002) (Table 2). Multiplex PCR reactions were performed in a final volume of 20 µl of the amplification mixture containing 4 µl Multiplex master mix, 1.0 µl reverse, and 1.0 µl forward primers (each containing 0.6µM for *bla*MOXM, *bla*CITM, *bla*DHAM; 0.5 µM for *bla*ACCM, *bla*EBCM and 0.4 µM for *bla*FOXm), 13.0 µl nuclease-free water, and 1 µl of 70-100 ng/µl DNA template. The thermal cycling conditions were: initial denaturation at 95°C for 240 seconds, followed by 25 cycles of denaturation at 94°C for 30 s, annealing at 64°C for 60 s and extension at 72°C for 1 min, and final extension at 72°C for 7 min. The multiplex PCR products were separated using 2% agarose gel prepared and stained using 3µl Ethidium bromide; 5 µl 100bp ladder, 2µl loading dye, and 6 µl PCR reaction of sample and control were loaded into the gel, and electrophoresis was carried out using 120V, 400MA, for 60 minutes. DNA bands were viewed under the Biorad gel doc Imaging System after staining with ethidium bromide.

3.13.5. Carbapenemase, Oxacillinase, and MBLs Gene Detection

For carbapenem resistant isolates Carbapenemase and MBLs were subjected to ESBL1, 2, and 3 (Tables 2) and CARBA multiplex 1, 2, and 3 PCR tests using specific primers for different families of beta-lactamase and carbapenemase production (Table 3). Multiplex CARBA PCR 1, using Bogaert's method with slight modifications, was employed to examine the positive isolates in the initial screening test for carbapenemase *bla*_{KPC}, *bla*_{OXA-48}, and Metallobeta-lactamase genes of *bla*_{NDM}, *bla*_{VIM}, and *bla*_{IMP} (Bogaerts *et al.*, 2013) (Table 3). It was performed in a final volume of 20 µl of the amplification mixture containing 4 µl Multiplex master mix, 0.6 µl reverse and 0.6 µl forward primers each containing (10 ng/µl), 13.8 µl nuclease-free water, and 1 µl of 70-100 ng/µl DNA template. The thermal cycling conditions were: initial denaturation at 95°C for 300 seconds, followed by 35 cycles of denaturation at 94°C for the 30s, annealing at 57°C for 90s and extension at 72°C for 90s, and final extension at 72°C for 5 minutes. Multiplex PCR 2 for OXA family Carbapenemase-producing genes of *bla*OXA-23group, *bla*OXA-24/143 group, and *bla*OXA-58

group were examined on the positive isolates in the initial screening test for carbapenem-resistant. The same method was employed with the Multiplex CARBA PCR 1 (Bogaerts *et al.*, 2013).

Table 3: Primers used for Carbapenemase and MBLs gene detection

PCR name	Targeted gene	Primer sequence (5' to 3') ^a		Amplicon size in bp	References
CARBA Multiplex 1	blaNDM	Forward	ACTTGGCCTTGCTGTCCTT	603	(Bogaerts <i>et al.</i> , 2013)
		Reverse	CATTAGCCGCTGCATTGAT		
	blaVIM	Forward	TGTCCGTGATGGTGATGAGT	437	
		Reverse	ATTCAGCCAGATCGGCATC		
	blaIMP	Forward	ACAYGGYTTRGTDGKCTTG	387	
		Reverse	GGTTTAAYAAARCAACCACC		
	blaKPC	Forward	TCGCCGTCTAGTTCTGCTGTCTTG	353	
		Reverse	ACAGCTCCGCCACCGTCAT		
blaOXA-48	Forward	ATGCGTGTATTAGCCTTATCG	265		
	Reverse	CATCCTTAACCACGCCCAAATC			
CARBA Multiplex 2 OXA	blaOXA-23 group	Forward	CCCCGAGTCAGATTGTTCAAGG	330	
		Reverse	TACGTCGCGCAAGTTCCTGA		
	blaOXA-24/143 group	Forward	GCAGAAAGAAGTAAARCGGGT	271	
		Reverse	CCAACCWGTCAACCAACCTA		
	blaOXA-58 group	Forward	GGGGCTTGTGCTGAGCATAGT	688	
		Reverse	CCACTTGCCCATCTGCCTTT		
A. For degenerate primers: D = A, G or T; R = A or G; Y = C or T; K =G or T; W = A or T.					
CARBA Multiplex 3	blaAIM	Forward	CTGAAGGTGTACGGAAACAC	322	(Poirel, 2011 #579)
		Reverse	GTTTCGGCCACCTCGAATTG		
	blaGIM	Forward	TCGACACACCTTGGTCTGAA	477	
		Reverse	AACTTCCAACCTTGCCATGC		
	blaSIM	Forward	TACAAGGGATTCGGCATCG	570	
		Reverse	TAATGGCCTGTTCCCATGTG		
	blaDIM	Forward	GCTTGTCTTCGCTTGCTAACG	699	
		Reverse	CGTTCGGCTGGATTGATTG		

a Nucleotide numbering begins at the initiation codon of genes. b D = A or G or T; Y = C or T.					
CARBA -1 additional	blaVIM	Forward	TTTGGTCGCATATCGCAACG	500	(Amudhan, 2012#580)
		Reverse	CCATTCAGCCAGATCGGCAT		
	blaIMP	Forward	GTTTATGTTTCATACWTCG	432	
		Reverse	GGTTTAAYAAAACAACCAC		

(Amudhan *et al.*, 2012; Bogaerts *et al.*, 2013; Poirel *et al.*, 2011)

3.13.6. Minor Metallo-Beta-Lactamase Genes

The multiplex 3 was used for the detection of Metallo-beta-lactamase (MBL) producing genes, *bla*AIM, *bla*GIM, *bla*SIM, and *bla*DIM (Poirel *et al.*, 2011) for carbapenems resistance strains (Table 3). It was performed in a final volume of 20 µl of the amplification mixture containing 4 µl Multiplex master mix, 0.8 µl reverse and 0.8 µl forward primers each containing (10 ng/µl), 13.4 µl nuclease-free water, and 1 µl of 70-100 ng/µl DNA template. The thermal cycling conditions were: initial denaturation at 94°C for 300 seconds, followed by 36 cycles of denaturation at 94°C for the 30s, annealing at 52°C for 40s and extension at 72°C for 50s, and final extension at 72°C for 350 sec. For all Ca7rbapenemase 1-3 multiplex 1.5 agarose gel prepared by using 3µl Ethidium bromide with 2µl loading dye and 6 µl PCR reaction of sample and control for gel electrophoresis by using 120V, 400MA, for 45 minutes. DNA bands were viewed under the Biorad gel doc Imaging System.

3.14. Whole Genome Sequencing

Phenotypically identified and PCR-confirmed drug-resistant gram-negative bacteria were subjected to whole-genome sequencing using the Illumina NextSeq 500 platform with the support of the Fleming Fund SeqAfrica project in Kilimanjaro Clinical Research Institute (KCRI) Moshi, Kilimanjaro Tanzania. Genome sequencing was done for (n=191) drug resistance isolates by using Illumina paired-end sequencing to enable their complete genomic characterization. The three fundamental steps of the next-generation sequencing procedure are library preparation, sequencing, and data analysis.

3.14.1. DNA Extraction and Preparation

Genomic DNA was extracted using a QIAamp DNA min kit (Qiagen GmbH, Hilden, Germany). The quality and quantity of genomic DNA were confirmed using a Qubit 2.0 fluorometer, (Thermal Fisher Scientific, Waltham, MA, USA). Genome sequencing was done using Illumina DNA prep kit ref (ref: 10000000025416 version 9).

3.14.2. DNA Sequencing

Library preparation (dual indexing) was performed using an Illumina DNA prep kit, (Illumina Inc., San Diego, CA, USA). Whole genome sequencing of the library was completed on an Illumina NextSeq 500 platform using a paired-end 2 × 150 bp protocol. The readings are removed after reading the forward DNA strand, and the procedure is then repeated for the reverse strand.

3.14.3. Analysis of Data

After sequencing, base calling a procedure that identifies nucleotides was performed by the instrument software, along with a prediction of how accurate each base call will be. The sequencing data was transferred into a common analytic tool during data analysis. Sequence alignment, variant calling, data visualization, and interpretation were conducted at Fleming Fund SeqAfrica project in Kilimanjaro Clinical Research Institute (KCRI) Moshi, Kilimanjaro Tanzania. The NextSeq sequencer output was base called with BCL Convert 3.9.3 (Illumina, 2023) Fastq files were examined for sequencing issues with FastQC 0.11.9 (Andrews, 2017), and screened for human and vector contamination using FastQScreen 0.15.1 (Gautam *et al.*, 2019) against the KneadData (Kneaddata, 2023) human reference and NCBI UniVec Core.

Untrimmed reads were then processed by the open-source KCRI-CGE Bacterial Analysis Pipeline (BAP) 3.6.5 (Thomsen *et al.*, 2016; Zwetselaar, 2022b), using default settings. The BAP workflow encompassed: the computation of reads metrics with fastq-utils 1.3.0 (Zwetselaar, 2022a); and genome assembly was done with SKESA 2.4.0 (Souvorov *et al.*, 2018). The computation of assembly metrics was done with uf-stats 1.3.1 (Zwetselaar, 2022c). The species identification was done with KmerFinder 3.0.2 (Clausen *et al.*, 2018; Hasman *et al.*, 2014; Larsen *et al.*, 2014), then MLST typing was processed by CGE MLST 2.0.9 (Jolley and Maiden, 2010; Larsen *et al.*, 2012)

and KCST 1.2.6 for bacteria species Multilocus sequence typing (MLST) (Zwetselaar, 2021). Then AMR detection was done with ResFinder/PointFinder 4.2.3 for antimicrobial resistance gene (amr_gen), and antimicrobial resistance gene mutation (amr_mut) (Bortolaia *et al.*, 2020; Clausen *et al.*, 2018). The plasmid identification was done with PlasmidFinder 2.1.6 and pMLST 2.0.3 for plasmid identification and plasmid multilocus sequence typing (pMLSTs) (Carattoli *et al.*, 2014; Clausen *et al.*, 2018). The virulence gene (vir_gen) detection was done by VirulenceFinder 2.0.4 (Clausen *et al.*, 2018; Joensen *et al.*, 2014); and core genome MLST assignment done with cgMLSTFinder 1.1.5 (Clausen *et al.*, 2018; Jolley and Maiden, 2010). The antimicrobial resistance cluster (amr_cls), antimicrobial resistance (amr_res), disinfectant resistant gene (dis_res), and disinfectant resistance gene (dis_gen) identification were done (Appendix 9).

3.15. Quality Control

Standard operating procedures were strictly followed to verify that quality control parameters were fulfilled. Visual inspections of cracks in media, unequal fill, hemolysis, evidence of freezing, bubbles, and contamination were performed. According to standard operating procedures, blood samples were drawn and then transferred to blood culture broth and immediately transported to the TASH microbiology laboratory (Dreyer, 2012; Lee *et al.*, 2013). Quality control was performed to check the quality of the media. The resistance and susceptibility were interpreted according to the CLSI laboratory standards. *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), and *P. aeruginosa* (ATCC 27853) were used as reference strains for culture and susceptibility tests (CLSI, 2017). *K. pneumoniae* ATCC® 700603 and *E. coli* ATCC® 35218 were used for positive control and negative control for E-test. *K. pneumoniae* ATCC® 700603 (ESBL positive) was used as a negative control and *K. pneumoniae* ATCC® BAA-1144 was used as a positive control for E-test AmpC detection. *K. pneumoniae* ATCC® BAA-1705™ and *K. pneumoniae* ATCC® BAA-1706 were used as positive and negative controls for the carbapenemase test respectively (Liofilchem, 2014a, b). These strains were obtained from the TASH.

3.15.1. Culture and Sensitivity

Standard Operating Procedures were strictly followed as per CLSI 2017 standards. Visual inspections of cracks in media or plastic Petri dishes, unequal fill, hemolysis, evidence of freezing, bubbles, and contamination were performed. QC was performed to check the quality of the

medium. The resistance and susceptibility were interpreted according to the CLSI 2017 Laboratory Standards (CLSI, 2012). *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), and *Pseudomonas aeruginosa* (ATCC 27853), for non-fastidious *K. pneumoniae* ATCC 700603 *E. coli* ATCC 35218 were used as reference strains for culture and susceptibility testing.

3.15.2. ESBL and Carbapenemase Identification

For the ESBL confirmatory test, *K. pneumoniae* ATCC 700603 (ESBLs positive) and *E. coli* ATCC 25922 (ESBLs negative) control strains were used. For carbapenemase, confirmatory tests control strains *K. pneumoniae* ATCC BAA-1705 and *E. coli* ATCC 25922 (negative), and ATCC BAA-1706 (negative) were used. For optimization of the multiplex PCR known control strains were used as the positive control. DNA samples from reference *bla*TEM, *bla*SHV, and *bla*CTX-M positive strains were used as positive controls during ESBL detection. AmpC was extracted from the ATCC 27853 *P. aeruginosa* strain and known *K. pneumoniae* isolates carrying *bla*KPC and *bla*NDM were used as a positive control along with the carbapenemase detection. Positive DNA samples for ESBL AmpC, Carbapenemase, and Metallo-beta-lactamase served as controls for tests. Before multiplexing the primers for ESBL and carbapenemase genes, each primer was tested by a mono plex PCR reaction.

3.15.3. Whole Genome Sequencing

The quality and quantity of genomic DNA were confirmed using a Qubit 2.0 fluorometer. Assemblies were assessed for genome completeness and bacterial contamination with CheckM 1.2.2 (Parks *et al.*, 2015) and GUNC 1.0.5 (Orakov *et al.*, 2021). Assemblies were then assigned a 1-5 quality score, as follows. Assemblies were marked “mixed species” (Q1) at CheckM contamination >10%, or at CheckM contamination >5%, and GUNC CSS >0.45. Assemblies were marked “poor quality” (Q2) when any of the following applied: average coverage <15x, completeness <90%, human reads >4%, vector reads >4%, number of contigs >1000, or N50 <5000. Assemblies passing the Q2 threshold were rated “acceptable” (Q3) by default, “good” (Q4) at coverage >30x, contig count <480, completeness >98%, and contamination <2%, or “excellent” (Q5) at coverage >40x, contig count <160, completeness >99.5%, and contamination <0.5%.

3.16. Statistical Analysis and Interpretation

Data captured into Epi-data were cleaned and analyzed by using SPSS version 24 software for further analysis. The data were stored in a password-protected computer and backups were saved by the external hard disk. The quantitative data collected using different techniques were analyzed using simple descriptive statistics i.e. percentages, frequency, mean, and standard deviation, and the data collected were also analyzed using cross-tabulation, Chi-square, correlation, and multiple linear regression model with the help of statistical package. The bi-variant logistic regression analysis was used to see the relationship between a dependent variable and independent variables. The association was assessed by using the x²-test. Variables that showed a significant association were selected for further analysis using multiple logistic regression models with a p-value < 0.05 as statistically significant. The strength of the association was interpreted using an odds ratio in a 95% confidence interval. Finally, the results were presented on charts, graphs, and tables.

3.17. Ethical Considerations

The study was approved by “The College of Natural and Computational Sciences Institutional Research Ethics Review Board (CNS-IRB) on 30/03/2018 with minutes of meeting reference no IRB/032/2018 at Addis Ababa University”. Permission was also obtained from the College of Health Sciences Institutional Review Board (IRB). This study procedure has fulfilled the requirements set by the declaration of Helenski (Association, 2013). For participants whose age was below 18 years, data were collected with the assent (12–<18) and Consent of the patient's legal guardians. However, in the cases of ≥ 18 years of age, we have collected Consent from the patients themselves. The purpose and procedures of the study were explained to the participants' parents, and/or guardians during the study period. Those patients who were given informed consent were selected and enrolled as the study participants and patient results were communicated to the attending physicians.

4. CHAPTER FOUR: RESULTS

4.1. Epidemiology of Bloodstream Infection

4.1.1. Socio-Demographic Characteristics

A total of 1486 patients suspected of bacteremia were included in this study. Among the recruited patients, 844 (56.8%) were males and 642 (43.2%) were females with a ratio of 1.3:1. The age range of the patients was from 1 day to 91 years with a mean age of 18.24 and a standard deviation of 20.5; 433(29.14%) were below 2 years. The majority of them 1473 (99.1%), were in-patient, while the rest 13 (0.9%), were outpatients. The frequent blood culture requests were from Casualty Ward (C/W) 353(23.8%), the Emergency Outpatient Department (EOPD) 222 (14.9%), and the neonatal ICU 195(13.1%). The majority of participants in their departments were from Medical 728/1486 (48.99%), Hematology 306/1486 (20.59%), and NICU 178/1486 (11.98%) (Table 4).

Table 4: Sociodemographic characteristics of gram-negative bacteria identified in bloodstream infection patients at TASH n=1486.

Variables		Culture Results				Total	
		Negative N(%)		Positive N(%)		N(%)	
Age of participant	Birth to 1 month	122	53.30%	107	46.70%	229	15.40%
	>1-<24 months	137	67.20%	67	32.80%	204	13.70%
	02-<06 years	137	71.70%	54	28.30%	191	12.90%
	06-<13 years	147	76.60%	45	23.40%	192	12.90%
	13-<19 years	81	77.10%	24	22.90%	105	7.10%
	19-<45 years	285	80.30%	70	19.70%	355	23.90%
	45-<65 years	113	76.90%	34	23.10%	147	9.90%
	>65 years	47	74.60%	16	25.40%	63	4.20%
Total		1069	71.90%	417	28.10%	1486	100.00%
Sex of participant	Male	607	71.90%	237	28.10%	844	56.80%
	Female	462	72.00%	180	28.00%	642	43.20%
	Total	1069	71.90%	417	28.10%	1486	100.00%
Type of patient	In-patient	1058	71.80%	415	28.20%	1473	99.10%
	out-patient	11	84.60%	2	15.40%	13	0.90%
	Total	1069	71.90%	417	28.10%	1486	100.00%

Ward or unit of follow-up	C/W	271	76.80%	82	23.20%	353	23.80%
	EOPD	181	81.50%	41	18.50%	222	14.90%
	B6	94	48.20%	101	51.80%	195	13.10%
	D7	103	71.50%	41	28.50%	144	9.70%
	B4	69	58.00%	50	42.00%	119	8.00%
	C8	68	84.00%	13	16.00%	81	5.50%
	D8	56	75.70%	18	24.30%	74	5.00%
	C7	42	67.70%	20	32.30%	62	4.20%
	B8	53	89.80%	6	10.20%	59	4.00%
	B5	45	84.90%	8	15.10%	53	3.60%
	B7	26	63.40%	15	36.60%	41	2.80%
	InM	20	83.30%	4	16.70%	24	1.60%
	PEOPD	6	42.90%	8	57.10%	14	0.90%
	other	35	77.80%	10	22.20%	45	3.03%
	Total	1069	71.90%	417	28.10%	1486	100.00%
Department of Follow-up	Medical	573	78.70%	155	21.30%	728	49.00%
	Hematology	229	74.80%	77	25.20%	306	20.60%
	NICU	87	48.90%	91	51.10%	178	12.00%
	MICU	48	65.80%	25	34.20%	73	4.90%
	Emergency	52	73.20%	19	26.80%	71	4.80%
	General Surgery	28	60.90%	18	39.10%	46	3.10%
	SICU	14	41.20%	20	58.80%	34	2.30%
	Oncology	25	80.60%	6	19.40%	31	2.10%
	Gynecology	6	75.00%	2	25.00%	8	0.50%
	other	7	63.60%	4	36.40%	11	0.70%
	Total	1069	71.90%	417	28.10%	1486	100.00%

4.1.2. Blood Culture Result

The overall culture-positive result among the patients was 417/1486 (28.06%), and ten of them were found with double bacterial infections of which the total bacterial isolates were 427. Among the total of 427 bacterial strains, 233(54.57%) were Gram-negative, and 194(45.43%) were Gram-positive. Among the Gram-negative isolates, *K. pneumoniae* accounted for 75(17.56%),

Acinetobacter spp 47(11.01%), and *E. coli* 38(8.9%) were the dominant ones. Among 191 Gram-positive isolates, the predominant strain was *CoNS*, 65(15.2%), followed by *S. aureus* 44(10.3%), and *Enterococcus spp* 24(5.6%) (Table 5).

Table 5: Bacterial isolates from bloodstream infection patients at TASH n=1486.

Gram -ve	f	%	%Gram -ve	Gram +ve	f	%	%Gram +ve
<i>K. pneumoniae</i>	75	17.56%	32.19%	<i>CoNS</i>	65	15.22%	33.5%
<i>Acinetobacter spp</i>	47	11.01%	20.17%	<i>S. aureus</i>	44	10.30%	22.7%
<i>E. coli</i>	38	8.90%	16.31%	<i>Enterococcus spp</i>	24	5.62%	12.4%
<i>K. oxytoca</i>	27	6.32%	11.59%	<i>S. viridian</i>	17	3.98%	8.76%
<i>E. coli (A-D)</i>	11	2.58%	4.72%	<i>S. lugdunensis</i>	13	3.04%	6.70%
<i>C. diversus</i>	5	1.17%	2.15%	<i>Bacillus spp</i>	13	3.04%	6.70%
<i>Pseudomonas spp</i>	5	1.17%	2.15%	<i>Corynebacterium spp</i>	7	1.64%	3.61%
<i>K. rhinoscleromatis</i>	4	0.94%	1.72%	<i>E. faecium</i>	5	1.17%	2.58%
<i>S. marcescens</i>	4	0.94%	1.72%	<i>S. pneumoniae</i>	2	0.47%	1.03%
<i>K. ozaenae</i>	3	0.70%	1.29%	<i>Micrococcus</i>	2	0.47%	1.03%
<i>E. cloacae</i>	2	0.47%	0.86%	<i>S. pyogenes</i>	1	0.23%	0.52%
<i>E. agglomerans</i>	2	0.47%	0.86%	<i>S. milarie</i>	1	0.23%	0.52%
<i>M. morgani</i>	2	0.47%	0.86%	Total Gram +ve	194	45.4%	100%
<i>P. mirabilis</i>	2	0.47%	0.86%	Total All bacteria	427	100%	
<i>P. rettgeri</i>	2	0.47%	0.86%	Total sample	1486		
<i>C. gleum</i>	1	0.23%	0.43%				
<i>E. aerogenes</i>	1	0.23%	0.43%				
<i>S. typhi</i>	1	0.23%	0.43%				
<i>N. meningitidis</i>	1	0.23%	0.43%				
Total Gram -ve	233	54.6%	100%				

f= frequency; *CoNS*= *Coagulase negative staphylococcus*

4.1.3. Factors Associated with Positive Blood Culture

Prevalence of positive blood culture results against the age of participants showed an increased trend at very young ages and the difference in prevalence is associated with age (Figure1) as shown by the adjusted odds ratios for different age groups; birth to one month $p \leq 0.001$; greater than 1

month up to less than 24 months, $p \leq 0.001$; and two years up to less than six years, $p = 0.002$, (Table 6). A slightly increasing trend of positivity was also shown at old age (>45 years); (Figure 2) the difference was not statistically significant.

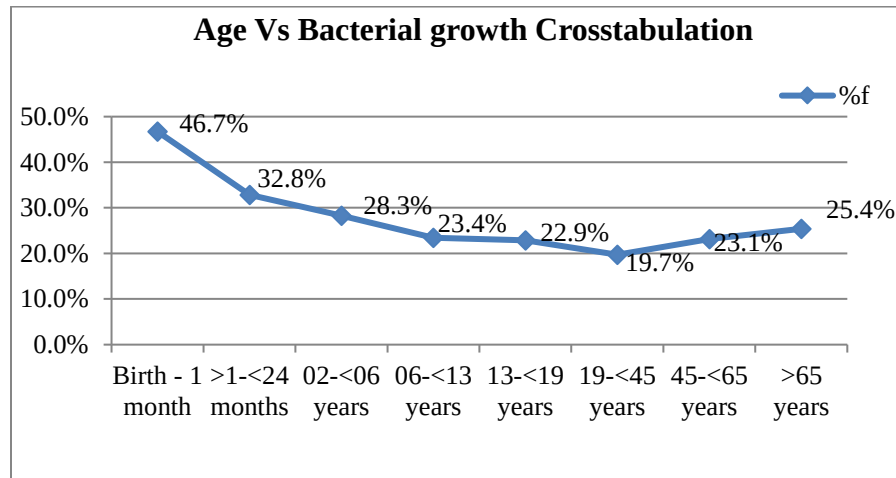


Figure 2: Proportion of bacterial identification among the age of the participants in bloodstream infection suspected patients at Tikur Anbessa Specialized Hospital.

We analyzed if there was a difference in the type of pathogens isolated with age; as shown in Figure 3 *K. pneumoniae* at 33% was the predominant pathogen isolated from neonates followed by *Klebsiella oxytoca* (*K. oxytoca*) at 15.17% and *Acinetobacter species* at 11.6%. Identified bacteria in neonate compared with all age levels *K. pneumoniae* 49.3%, *K. oxytoca* 62.96%, and *Acinetobacter spp* 27.7% were predominantly found. The highly prevalent bacteria in neonates were *K. pneumoniae* and the high percentage of prevalent organisms compared in all age levels was *K. oxytoca*.

In the age group between 1-24 months, the most prevalent bacteria were again *K. pneumoniae*, 17.39%, Coagulase negative staphylococcus (CoNS), 15.9%, and *Acinetobacter spp*, 13%. In the age group of 2- <6 *K. pneumoniae*, *Acinetobacter spp*, and *S. aureus* were the most predominant. Another trend we observed in terms of pathogens isolated is the rate of CoNS, 30.7%, in adults. The larger proportion of the CoNS was isolated from the age group 19-45 years. In the case of those above 65 years, the most prevalent bacteria were *E. coli*, 24%, *K. pneumoniae*, 18%, and CoNS, 12%, (Figure 3).

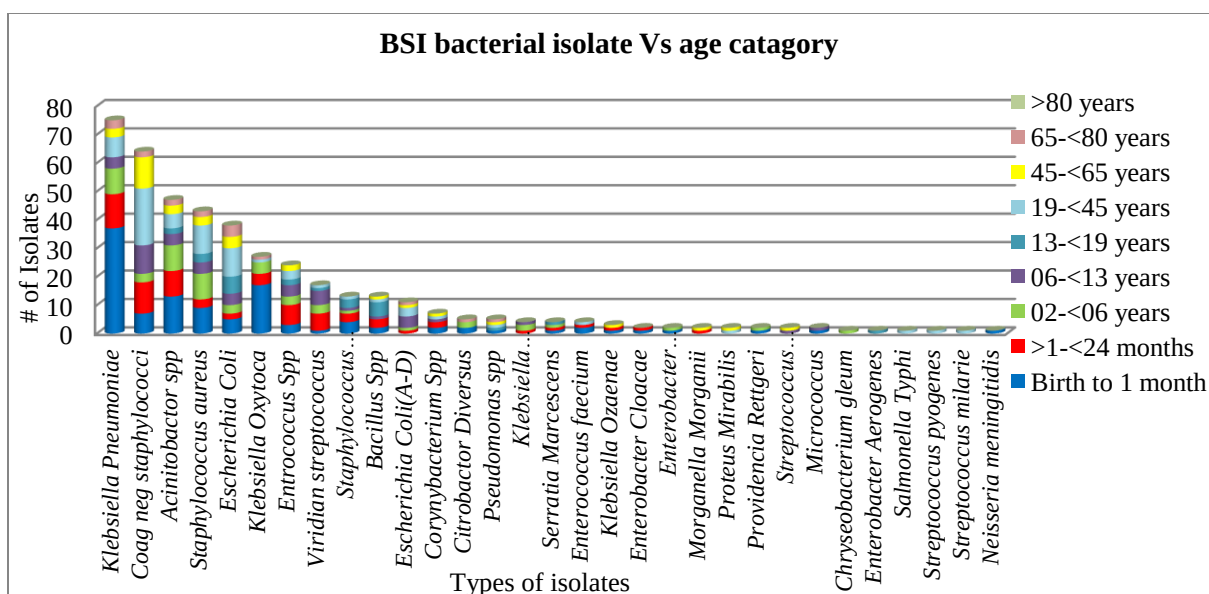
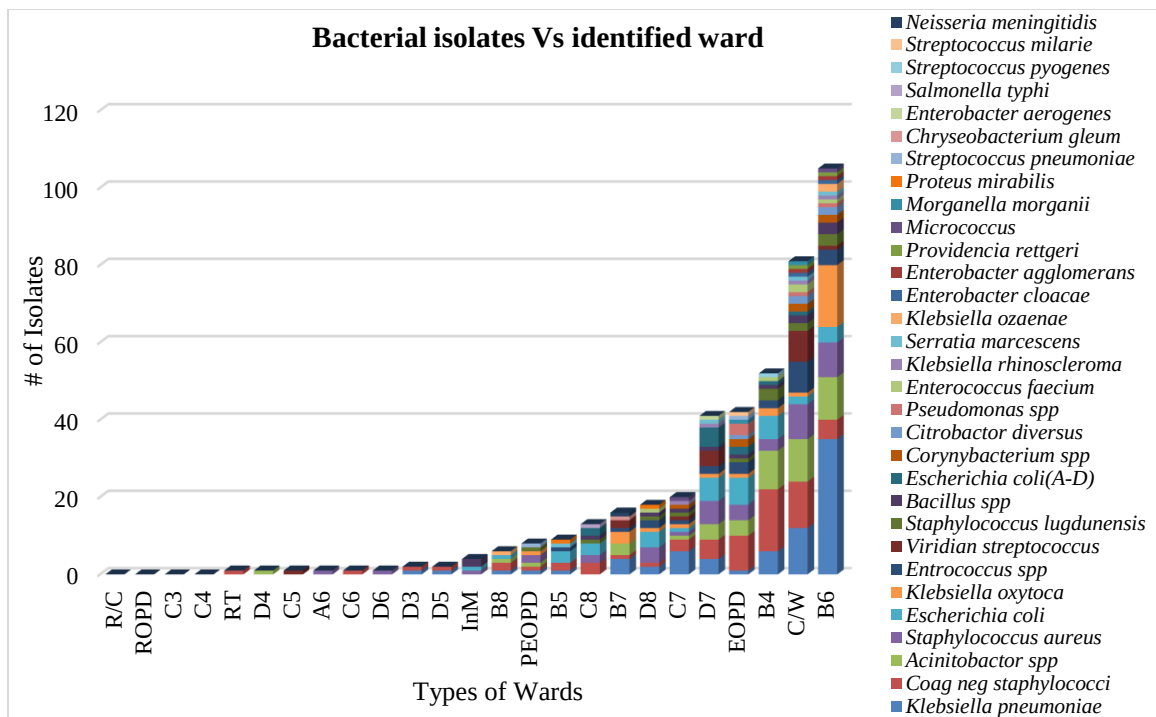


Figure 3: Distribution of identified bacterial isolates in the age category from bloodstream infection suspected patients in Tikur Anbessa Specialized Hospital Addis Ababa Ethiopia.

In this study, the top five departments of the blood culture request were internal medicine followed by hematology, NICU, MICU, and Emergency OPD. However, the highest rate of isolation was observed in SICU, with about 20(59 %) AOR $p < 0.001$, NICU, 91(51%) AOR $p = 0.002$, general surgery, 18(39.1%) AOR $P = 0.044$, ICU, 25(34.2%) COR $p = 0.013$, and emergency, 19(26.8%) AOR $P = 0.024$ (Table 6). Among wards, the highest number of patients involved in this study was from causality, 23.8%, EOPD, 14.9%, and B6, 13.1%, and the highest isolation was recorded in B6 (24.5%), C/W (19%), and B4 (12.2) bacteria were identified. Bacterial identification among the wards shows that *K. pneumoniae* has the highest prevalence in this study; 35(46.7%), 12(16 %), and 6(8%) have been identified from B6 (NICU), C/W, and B4(MICU), respectively. The second prevalent were *CoNS* at rates of 16(25.0%), 12(18.8), and 9(14.1%) identified in B4(MICU) C/W, and EOPD, respectively. The third one is *Acinetobacter spp* which was detected at the rates of 47 11.6(23.4%), 11.6(23.4%), 10(21.2%) from B6, C/W, and B4, respectively (Figure 4).



R/C, Rehabilitation center (Orthopedics); CTR, Central triage (Regular OPD); C3, Gynecology & Obstetrics; C4, Cardiac; RT, Radio therapy (Oncology department); D4, Cardiothoracic surgery & ENT; C5, General surgery; A6, Labor gynecology; C6, Gynecology & Obstetrics; D6, Gynecology & Obstetrics; D3, Uro-surgery; D5, Neuro and general Surgery; InM, Intermediate; B8, Medical, cardiac and endocrinology; PEOPD, Pediatrics Emergency OPD; B5, Medical and Neurosurgery; C8, Hematology; B7, Pedi Medical & Surgical; D8, Medical renal and hematology; C7, Pedi Medical; D7, Pedi Hematology; EOPD, Adult Emergency OPD; B4, SICU, MICU/PMICU; C/W, Causality; B6, NICU;

Figure 4: Bacterial isolates and their frequency from blood culture of bloodstream infection suspected patients in the different wards of Tikur Anbessa Specialized Hospital Addis Ababa Ethiopia.

Cross tabulation shows that when the admission date increases the bacterial prevalence also increases (Figure 5). The length of stay in the hospital with positive blood culture results is shown in Table 6. In our study, bivariate analysis has shown an increased trend of positivity following a longer stay > 5 days COR p=0.016, and > 9 days COR p=0.001. The multivariate analysis was at the borderline and did not show a significant association of > 9 days on AOR p=0.062 (Table 6).

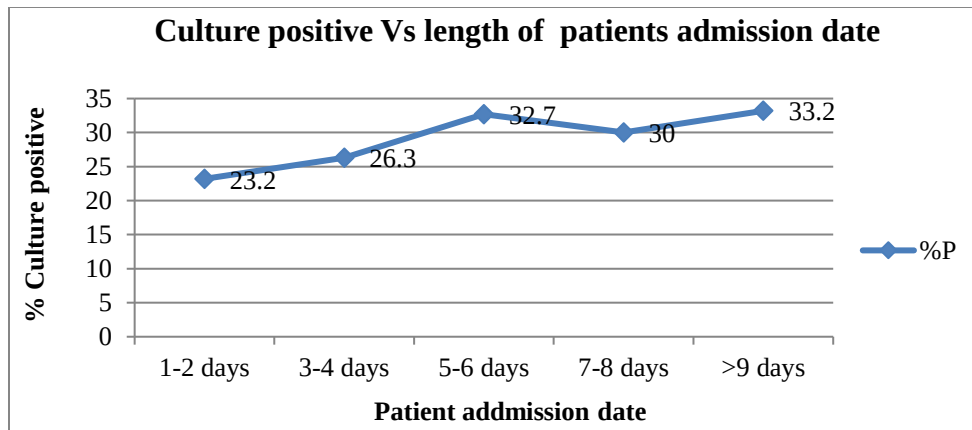


Figure 5: Hospital stay of patients from admission to sample collection in Tikur Anbessa Specialized Hospital.

We observed 867(58.35%) of patients utilized indwelling medical devices but 619 (41.65%) are not. Culture positivity was higher in indwelling medical device users at 35.4% than in non-users at 17.4% with a p-value of AOR <0.001. The top three usages of indwelling medical devices were the intravenous line, 782 (52.6%), mechanical ventilator, 137 (9.2%), and suction machine, 121 (8.1%). Intravenous line usage is high in patients but it didn't have an association with microbial growth. Among patients 962(64.7%) have a chronic illness, and 524(35.3%) were not. In this study having chronic illness has an association with being culture positive with an AOR of p=0.027. History of incubation shows out of 230 neonates 168 were incubated and 62 were not incubated. Among the total 230 neonates, 122 were culture-negative, and 108 were culture-positive. Of 108 culture-positive 89(53%) of them were incubated and culture positive and 62 (30.6%) were non-incubated and culture positive. There is an association between Incubation and microbial growth AOR of p= 0.016 (Table 6).

Mode of delivery, premature rupture of membrane, history of maternal fever, and neonatal body weight (in kg) during delivery do not have an association with positive blood culture. The normal range of body weight is 2500g - 4500g. The average body weight in this study was 2539.05g with a standard deviation of 771.15 a minimum weight was 800 and a maximum weight was 6502g. Of the total 86 low birth weights, 51% (44/86) were culture-positive while 45% (64/142) of normal body weight were culture-positive, and none of (0/2) high birth weights were culture-positive (Table 6).

Table 6: Socio-demographic characteristics and clinical history of BSI suspected patients with blood culture-positive results crude and adjusted odds ratio at TASH n=1486.

Variables	Culture results in N(%)		Bivariate regression analysis		Multivariate regression analysis	
	Negative	Positive	P-value	COR (95%CI)	P-value	AOR (95%CI)
Age of participants						
Birth to 1 mo	122(53.3%)	107(46.7%)	<0.001	3.571(2.470-5.162)	<0.001	3.96(2.026-7.738)
>1-<24 mont	137(67.2%)	67(32.8%)	0.001	1.991(1.345-2.947)	<0.001	3.237(2.043-5.128)
02-<06 years	137(71.7%)	54(28.3%)	0.024	1.605(1.066-2.416)	0.002	2.035(1.292-3.206)
06-<13 years	147(76.6%)	45(23.4%)	0.309	1.246(0.816-1.905)	0.104	1.479(0.923-2.368)
13-<19 years	81(77.1%)	24(22.9%)	0.484	1.206(0.713-2.040)	0.128	1.555(0.881-2.745)
19-<45 years	285(80.3%)	70(19.7%)	0			
45-<65 years	113(76.9%)	34(23.1%)	0.391	1.225(0.770-1.948)	0.318	1.288(0.784-2.114)
>65 years	47(74.6%)	16(25.4%)	0.306	1.386(0.742-2.589)	0.594	1.205(0.607-2.391)
Department of Follow-up						
Medical	573(78.7%)	155(21.3%)	0			
Emergency	52(73.2%)	19(26.8%)	0.288	1.351(0.776-2.352)	0.024	2.011(1.098-3.686)
MICU	48(65.8%)	25(34.2%)	0.013	1.925(1.150-3.222)	0.076	1.651(0.948-2.874)
NICU	87(48.9%)	91(51.1%)	<0.001	3.867(2.743-5.451)	0.002	2.638(1.423-4.890)
G. Surgery	28(60.9%)	18(39.1%)	0.006	2.376(1.281-4.410)	0.044	2.015(1.018-3.989)
SICU	14(41.2%)	20(58.8%)	<0.001	5.281(2.608-10.695)	<0.001	6.823(3.112-14.96)
Gyn & Obs	2(66.7%)	1(33.3%)	0.617	1.848(0.167-20.518)	0.502	2.456(0.178-33.951)
Gynecology	6(75.0%)	2(25.0%)	0.799	1.232(0.246-6.165)	0.177	3.201(0.592-17.313)
Oncology	25(80.6%)	6(19.4%)	0.796	0.887(0.358-2.201)	0.735	0.847(0.325-2.212)
Hematology	229(74.8%)	77(25.2%)	0.174	1.243(0.909-1.700)	0.874	1.03(0.713-1.488)
Orthopedics	3(60.0%)	2(40.0%)	0.325	2.465(0.408-14.879)	0.346	2.594(0.358-18.811)
Others	3(75.0%)	1(25.0%)	1			
Inpatient, Length of admission						
1-2 days	443(76.6%)	135(23.4%)	0.008		0.341	
3-4 days	157(73.7%)	56(26.3%)	0.393	1.17(0.816-1.679)	0.969	1.008(0.677-1.501)
5-6 days	109(67.3%)	53(32.7%)	0.016	1.596(1.091-2.335)	0.24	1.29(0.843-1.975)
7-8 days	104(69.3%)	46(30.7%)	0.066	1.451(0.976-2.159)	0.296	1.27(0.811-1.987)
>9 days	256(66.8%)	127(33.2%)	0.001	1.628(1.222-2.169)	0.062	1.402(0.983-2.001)
ICU Admission						
No	903(76.2%)	282(23.8%)	0			
Yes	166(55.1%)	135(44.9%)	<0.001	2.604(2.001-3.389)		
Body Temperature in °c						
<36.0	78(64.5%)	43(35.5%)	0.186	1.523(0.817-2.838)	0.238	1.495(0.766-2.919)
36.0-36.5	35(70.0%)	15(30.0%)	0.673	1.184(0.540-2.593)	0.78	1.13(0.480-2.661)
36.6-37.5	58(73.4%)	21(26.6%)	0		0	
37.6-38.0	327(83.8%)	63(16.2%)	0.029	0.532(0.302-.938)	0.354	0.748(0.406-1.380)
>38	571(67.5%)	275(32.5%)	0.282	1.33(0.791-2.236)	0.013	2.064(1.165-3.659)
History of using the instrument during medical care						
No	510(82.4%)	109(17.6%)	0		0	
Yes	559(64.5%)	308(35.5%)	<0.001	2.578(2.009-.308)	<0.001	1.988(1.493-.646)
History of using NG tube						
No	537(65.2%)	286(34.8%)	0			
Yes	22(50.0%)	22(50.0%)	0.042	1.878(1.022-.449)		
History of having a chronic illness						

No	346(66.0%)	178(34.0%)	0		0	
Yes	723(75.2%)	239(24.8%)	<0.001	0.643(0.509-.811)	0.027	1.503(1.048-2.156)
History of having wound infection on the skin						
No	712(75.7%)	228(24.3%)	0			
Yes	11(50.0%)	11(50.0%)	0.009	3.123(1.336-.298)		
History of having ureteral tract infection						
No	703(76.2%)	220(23.8%)	0			
Yes	20(51.3%)	19(48.7%)	0.001	3.036(1.591-.791)		
History of having respiratory tract infection						
No	663(76.0%)	209(24.0%)	0.002			
Yes	60(66.7%)	30(33.3%)	0.052	0.63(0.396-1.004)		
The educational level of the family						
Illiterate	19(61.3%)	12(38.7%)	0		0.204	0.417(0.108-1.610)
Read & write	48(71.6%)	19(28.4%)	0.307	0.627(0.256-1.537)	0.021	0.23(0.066-.799)
Prim. School	30(53.6%)	26(46.4%)	0.488	1.372(0.562-3.353)	0.242	0.476(0.137-1.651)
Seco school	20(32.8%)	41(67.2%)	0.01	3.246(1.321-.975)	0.916	1.07(0.308-3.720)
High Education	5(33.3%)	10(66.7%)	0.081	3.167(0.868-1.551)	0.003	
History of incubation						
No	43(69.4%)	19(30.6%)	0		0.003	
Yes	79(47.0%)	89(53.0%)	0.003	2.55(1.373-.735)	0.013	2.318(1.194-4.500)
Premature rupture of membrane						
>18 hours	31(68.9%)	14(31.1%)	0.33	0	0.137	
<18 hours	81(50.9%)	78(49.1%)	0.035	2.132(1.055-4.309)	0.118	1.809(0.861-3.804)
No rupture	10(38.5%)	16(61.5%)	0.014	3.543(1.289-9.739)	0.059	2.904(0.958-8.802)

COR: Crude odds ratio, AOR: Adjusted odds ratio, CI: Confidence interval

Values in bold are statistically significant.

4.1.4. Antimicrobial Resistance of Blood Culture

Among the total 26 commonly used antibiotics against Gram-ve bacteria 25 for *Enterobacteriaceae*, 16 for *Acinetobacter*, and 12 for *Pseudomonas* were used for the antimicrobial susceptibility test according to CLSI 2018 standard. Among all gram-negative bacteria, 9 of them were resistant to more than 50% of commonly used antibiotics. The percentage of drug resistance of gram-negative bacteria from commonly used antibiotics was as follows *S. marcescens* accounts 52/76(68.4%), *Acinetobacter spp* 489/750 (65.2%), *K. pneumoniae* 1152/1797(64.1%), *E. coli (A-D)* 165/274 (60.2%), *E. cloacae* 21/38 (55.3%), *K. oxytoca* 370/675 (54.8%), *E. coli* 519/950 (54.6%), *Klebsiella rhinoscleromatis (K. rhinoscleromatis)* 53/100 (53.0%), *Morganella morganii (M. morganii)* 22/44 (50.0%), *Proetus mirabilis (P. mirabilis)* 23/48 (47.9%), *Providencia rettgeri (P. Rettgeri)* 16/44 (36.4%), *Citrobacter diversus (C. diversus)* 45/125 (36.0%), *Enterobacter aerogenes (E. aerogenes)* 6/19 (31.6%), *Klebsiella ozaenae (K. ozaenae)* 23/75 (30.7%), *Pseudomonas spp* 15/60(25.0%), *Enterobacter agglomerans (E. agglomerans)* 12/50 (24.0%), and *Salmonella typhi (S. typhi)* 0/25 (0.0%) (Table 7).

Table 7: Frequencies of gram-negative bacterial species and their AST pattern from blood cultures of bloodstream infection patients attended at TASH n=231.

Bacteria identified		<i>S. marcescens</i>	<i>Acinetobacter spp</i>	<i>K. pneumoniae</i>	<i>E. coli (A-D)</i>	<i>E. cloacae</i>	<i>K. oxytoca</i>	<i>E. coli</i>	<i>K. Rhinosclomatis</i>	<i>M. morganii</i>	<i>P. mirabilis</i>	<i>P. rettgeri</i>	<i>C. diversus</i>	<i>E. aerogenes</i>	<i>K. ozaenae</i>	<i>Pseudomonas spp</i>	<i>E. agglomerans</i>	<i>S. typhi</i>	Total DR
	Total	n=4	n=47	n=75	n=11	n=2	n=27	n=38	n=4	n=2	n=2	n=2	n=5	n=1	n=3	n=5	n=2	n=1	n=231
Ampicillin	R(%R)	NA	NA	NA	11(100)	NA	26(96.3)	38(100)	3(75)	NA	2(100)	NA	4(80)	NA	3(100)	NA	1(50)	0(0)	88(94.6)
Tetracycline	R(%R)	4(100)	NA	72(96)	11(100)	2(100)	25(92.6)	37(97.4)	2(50)	2(100)	NA	NA	4(80)	1(100)	3(100)	NA	2(100)	0(0)	165(94.3)
Amp-Sulbactam	R(%R)	NA	40(85.1)	75(100)	10(90.9)	NA	26(96.3)	37(97.4)	3(75)	1(50)	2(100)	2(100)	5(100)	NA	3(100)	NA	2(100)	0(0)	206(94.1)
Cefuroxime	R(%R)	NA	NA	75(100)	11(100)	NA	23(85.2)	33(86.8)	3(75)	NA	1(50)	2(100)	2(40)	NA	3(100)	NA	2(100)	0(0)	155(91.2)
Augmentin	R(%R)	NA	NA	70(93.3)	10(90.9)	NA	23(85.2)	33(86.8)	3(75)	NA	2(100)	NA	5(100)	NA	1(33.3)	NA	0(0)	0(0)	147(87.5)
Doxycycline	R(%R)	NA	41(87.2)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	41(87.2)
Tri-sulfamethoxazole	R(%R)	4(100)	36(76.6)	69(93.2)	10(90.9)	2(100)	25(92.6)	32(84.2)	2(50)	1(50)	2(100)	2(100)	3(60)	0(0)	3(100)	NA	1(50)	0(0)	192(85.3)
Cefotaxime	R(%R)	4(100)	43(91.5)	70(93.3)	9(81.1)	1(50)	23(85.5)	27(71.1)	2(50)	1(50)	1(50)	2(100)	2(40)	1(100)	1(33.3)	NA	1(50)	0(0)	188(83.2)
Ceftriaxone	R(%R)	4(100)	42(89.4)	71(94.7)	9(81.8)	1(50)	21(77.8)	28(73.7)	2(50)	1(50)	1(50)	1(50)	2(40)	1(100)	1(33.3)	NA	1(50)	0(0)	186(82.3)
Ceftaroline	R(%R)	2(50)	NA	71(95.9)	8(80.0)	1(50)	23(85.2)	28(73.7)	2(50)	1(50)	1(50)	2(100)	2(40)	1(100)	1(33.3)	NA	1(50)	0(0)	144(81.4)
Cefepime	R(%R)	3(75)	36(76.6)	69(92)	9(81.8)	1(50)	23(85.2)	25(65.8)	2(50)	1(50)	1(50)	1(50)	2(40)	0(0)	0(0)	2(40)	0(0)	0(0)	175(75.8)
Aztreonam	R(%R)	4(100)	NA	68(90.7)	8(72.7)	1(50)	18(66.7)	26(68.4)	2(50)	1(50)	1(50)	1(50)	2(40)	0(0)	1(33.3)	3(60)	0(0)	0(0)	136(73.9)
Ceftazidime	R(%R)	4(100)	32(68.1)	69(92)	9(81.8)	1(50)	18(66.7)	26(68.4)	2(50)	1(50)	1(50)	0(0)	2(40)	0(0)	1(33.3)	1(20)	1(50)	0(0)	168(72.7)
Gentamycin	R(%R)	3(75)	26(56.5)	60(80)	6(54.5)	1(50)	20(74.1)	16(42.1)	2(50)	1(50)	1(50)	0(0)	0(0.0)	0(0)	1(33.3)	1(20)	0(0)	0(0)	138(60.0)
Ciprofloxacin	R(%R)	2(50)	25(53.2)	50(66.7)	10(90.9)	1(50)	10(37.0)	24(63.2)	2(50)	2(100)	0(0)	0(0)	2(40)	0(0)	0(0)	0(0)	0(0)	0(0)	128(55.4)
Chloramphenicol	R(%R)	3(75)	NA	42(56)	2(18)	1(50)	19(70)	10(26)	2(50)	1(50)	1(50)	2(100)	1(20)	1(100)	0(0)	NA	0(0)	0(0)	85(47)
Pip-Tazobactam	R(%R)	2(50)	27(57.4)	36(48)	7(63.6)	1(50)	7(25.9)	21(55.3)	2(50)	1(50)	0(0)	0(0)	1(20)	0(0)	0(0)	2(40)	0(0)	0(0)	107(46.3)
Tobramycin	R(%R)	2(50)	24(52.2)	41(54.7)	3(27.3)	1(50)	10(37)	8(21.1)	1(25)	0(0)	0(0)	0(0)	1(20)	0(0)	0(0)	0(0)	0(0)	0(0)	91(39.6)
Cefoxitin	R(%R)	NA	NA	31(41.3)	3(27.3)	NA	7(25.9)	15(39.5)	3(75)	1(50)	1(50)	0(0)	3(60)	NA	1(33.3)	0(0)	0(0)	0(0)	65(37.8)
Levofloxacin	R(%R)	2(50)	19(40.4)	21(28.4)	9(81.9)	1(50)	4(14.8)	23(60.5)	2(50)	1(50)	0(0)	0(0)	1(20)	0(0)	0(0)	0(0)	0(0)	0(0)	83(36.1)
Doripenem	R(%R)	2(50)	30(63.8)	20(26.7)	2(18.2)	2(100)	5(18.5)	7(18.4)	2(50)	1(50)	1(50)	0(0)	0(0)	0(0)	0(0)	2(40)	0(0)	0(0)	74(32.0)
Meropenem	R(%R)	2(50)	28(59.6)	15(20)	2(18.2)	1(50)	3(11.1)	4(10.5)	2(50)	1(50)	1(50)	0(0)	0(0)	0(0)	0(0)	2(40)	0(0)	0(0)	61(26.4)
Imipenem	R(%R)	1(25)	22(46.8)	17(22.7)	2(18.2)	1(50)	3(11.1)	3(7.9)	2(50)	1(50)	1(50)	1(50)	0(0)	0(0)	0(0)	2(40)	0(0)	0(0)	56(24.2)
Cefotetan	R(%R)	NA	NA	19(25.3)	2(18.2)	NA	4(14.8)	12(31.6)	2(50)	1(50)	1(50)	0(0)	0(0)	NA	0(0)	NA	0(0)	0(0)	41(23.8)
Ertapenem	R(%R)	3(75)	NA	18(24)	2(18.2)	1(50)	4(14.8)	5(13.2)	2(50)	1(50)	1(50)	0(0)	0(0)	1(100)	0(0)	NA	0(0)	0(0)	38(21.2)
Amikacin	R(%R)	1(25)	18(38.3)	3(4)	0(0)	0(0)	0(0)	1(2.6)	1(25)	0(0)	0(0)	0(0)	1(20)	0(0)	0(0)	0(0)	0(0)	0(0)	25(10.8)
Total DR	R(%R)	52(68.4)	489(65.2)	1152(64.1)	165(60.2)	21(55.3)	370(54.8)	519(54.6)	53(53)	224(50)	238(48)	164(36)	45(36)	6(31.6)	23(30.7)	15(25)	12(24)	0(0)	2983(57.9)

Intrinsic-resistant drugs were not included according to the CLSI 2018 Standard.

Ampicillin was the most resisted drug, 88/93(94.6%); whereas, Amikacin, 25/231(10.8%), was the least resisted (Table 7). *S. marcescens* was 100% resistant for CTX, CRO, CAZ, ATM, and 75% for FEP. *Acinetobacter* spp were resistant to CTX, FEP, and CAZ at the levels of 91.5%, 89.4%, 76.6%, and 68.1%, respectively. *K. pneumoniae* was resistant to CTX, CRO, FEP, and CAZ at the levels of 93.3%, 94.7%, 92%, and 92%. *E. coli* (A-D) were 81.1% resistant to CTX, and 81.8% for CRO, FEP, and CAZ. *K. oxytoca* resistant to CTX 85.5%, CRO 77.8%, FEP 85.2%, CAZ 66.7%, and *E. coli* resistant to CTX 71.1%, CRO 73.7%, FEP 65.8%, CAZ 68.4% (Table 7).

Among the WHO global priority list, Carbapenem-resistant in *Acinetobacter* spp were about 30(63.8%), Carbapenem-resistant *Pseudomonas* spp were 40% (2/5), Carbapenem-resistant *K. pneumoniae* were about 20(26.7%), 3rd generation cephalosporin-resistant *K. pneumoniae* were 71(94.7%), Carbapenem-resistant *E. coli* were 7(18.4%); and 3rd generation cephalosporin-resistant *E. coli* 28(73.7%) (Table 7).

4.2. Multidrug Resistance in Gram-Negative Bacteria

4.2.1. Prevalence of Multidrug Resistance

In this study, MDR organisms were highly prevalent, especially in gram-negative organisms. As shown in Figure 6 among 233 gram-negative organisms, 222(95.3%) were multi-drug resistant and 11(4.7) were not. Of the total gram-negative organisms, 130(55.8%) were MDR, 75 (32.2%) were XDR, and 17(7.3%) were PDR according to the Megiorakos classification (Magiorakos *et al.*, 2012) (Figure 6).

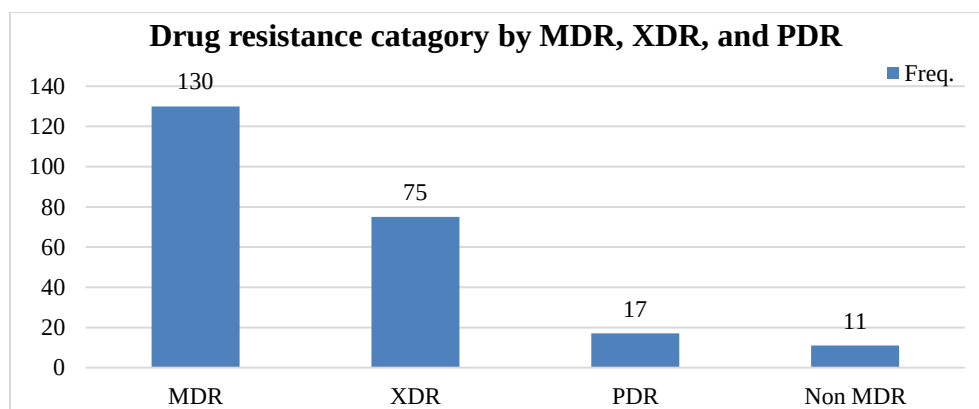


Figure 6: MDR category in bloodstream infection

When we looked at the type of pathogens that were either MDR or XDR the large majority of them were *K. pneumoniae*, *Acinetobacter spp*, *E. coli*, and *K. oxytoca* (Figure 7). The large majority of PDR pathogens were *Acinetobacter spp*.

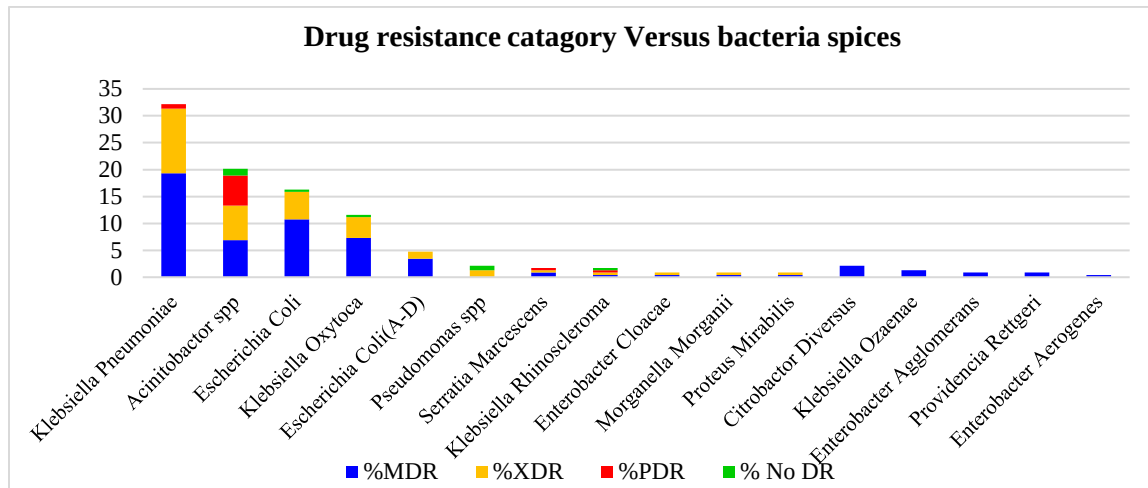


Figure 7: Drug resistance category in gram-negative bacteria

The highest percentage of drug resistance was found in neonates and children. Among the total drug resistance bacteria, 148(66.67%) of them were identified in patients below six years, and from the total of 17 PDR bacteria 35.29% of them were among the age of < 28 days (Figure 8). This finding shows children especially neonates were highly affected by drug-resistant bacteria.

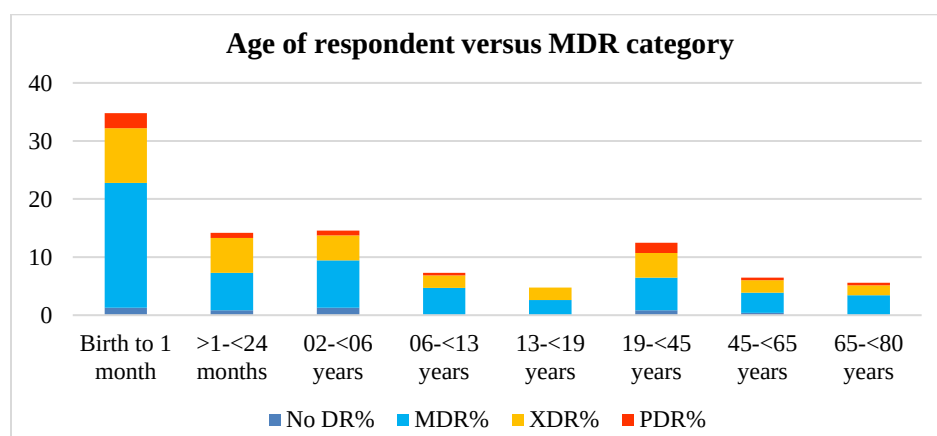


Figure 8: MDR Category in the different age groups of participants from bloodstream infection suspected patients.

4.2.2. Multi-Drug Resistance in Gram-Negative Bacteria

Multi-drug resistance gram-negative bacteria and factors associated with MDR were analyzed with a total of 231(54.1%) gram-negative bacteria. In this study, the majority of gram-negative bacteria s were MDR. Among 231 gram-negative bacteria, 222(96.1%) of them were MDR, and 9(3.89%) of them were not MDR. In the drug resistance category, 130 (56.28%) were MDR, 75(32.47%) were XDR, and 17(7.36%) were possible PDR according to Megiorakos classification (Magiorakos *et al.*, 2012).

The most prevalent among MDR in gram-negative bacteria were *K. pneumoniae*, 45(34.62%) $p<0.001$, *E. coli*, 25(19.23%) $p<0.001$, and *K. oxytoca*, 17(13.08%) $p=0.001$; and among 17 PDR isolates the dominant bacterial spp were *Acinetobacter spp*, 13(76.47%) $p=0.006$, *K. pneumoniae*, 2(11.76%), *S. marcescens*, 1(5.88%), and *K. rhinoscleromatis*, 1(5.88%) (Table 8).

Table 8: Gram-negative bacteria associated with multidrug resistance category from bloodstream infection patients n=231.

Drug resistance categories of		Prevalence		Bivariate regression analysis	
MDR, XDR, PDR		N	%	P-value	COR(95%CI)
MDR	<i>K. pneumoniae</i>	45	34.62%	<0.001	63.578 (6.262-645.542)
	<i>E. coli</i>	25	19.23%	<0.001	76.825 (6.758-873.342)
	<i>K. oxytoca</i>	17	13.08%	0.001	70.044 (5.631-871.247)
	<i>Acinetobacter spp</i>	16	12.31%		
	<i>E. coli (A-D)</i>	8	6.15%	0.003	96.384 (4.924-1886.795)
	<i>C. diversus</i>	5	3.85%	0.018	235.086 (2.59-21377.56)
XDR	<i>K. pneumoniae</i>	28	37.33%	0.004	74.164 (3.885-1415.636)
	<i>E. coli</i>	12	16.0%	0.011	53.527 (2.483-1153.824)
	<i>K. oxytoca</i>	9	12.0%	0.011	59.122 (2.546-1373.146)
	<i>Acinetobacter spp</i>	15	20.0%	0.073	54.556 (0.691-4308.650)
	<i>E. coli (A-D)</i>	3	4.0%	0.047	41.937 (1.051-1673.475)
PDR	<i>Acinetobacter spp</i>	13	76.47%	0.006	9005.7 (13.5-6023435.7)
	<i>K. pneumoniae</i>	2	2.7%		
	<i>K. rhinoscleromatis</i>	1	25.0%		
	<i>S. marcescens</i>	1	25.0%		

Among 130 MDR bacteria, the majority, 103(79.23%) belonged to *K. pneumoniae*, *Acinetobacter spp*, *E. coli*, and *K. oxytoca* at the rates of 45(34.62%), 16 (12.31%), 25 (19.23%), and 17(13.08%), respectively. Among 75 XDR bacteria, the majority, 64(85.33%), were covered by *K. pneumoniae*, *Acinetobacter spp*, *E. coli*, and *K. oxytoca* at the rates of 28(37.33%), 15(20.0%), 12(16.0%), and

9(12.00%), respectively. This result shows from the total 222 drug resistance bacteria 199(89.64%) of MDR, XDR, and PDR were covered by *Klebsiella spp*, *E. coli spp*, and *Acinetobacter spp* accounting for 107(48.20%), 48 (21.62%), and 44 (19.82%) respectively. Among 17 Possible PDR bacteria, 15(88.24%), were *Acinetobacter spp* and *K. pneumoniae* accounted for 13(76.47%) and 2(11.76%), respectively (Table 9).

Table 9: Gram-negative bacteria and their drug resistance category of bloodstream infection suspected patients n=231.

Bacteria identified	MDR		XDR		PDR		Total		Species N&%
	N	%	N	%	N	%	N	%	
<i>K. pneumoniae</i>	45	34.62%	28	37.33%	2	11.76%	75	33.78%	107(48.2%)
<i>K. oxytoca</i>	17	13.08%	9	12.00%	0	0.00%	26	11.71%	
<i>K. ozaenae</i>	3	2.31%	0	0.00%	0	0.00%	3	1.35%	
<i>K. rhinoscleroma</i>	1	0.77%	1	1.33%	1	5.88%	3	1.35%	
<i>E. coli</i>	25	19.23%	12	16.00%	0	0.00%	37	16.67%	48(21.62%)
<i>E. coli (A-D)</i>	8	6.15%	3	4.00%	0	0.00%	11	4.95%	
<i>Acinetobacter spp</i>	16	12.31%	15	20.00%	13	76.47%	44	19.82%	44(19.82%)
Total									199(89.64%)
<i>E. cloacae</i>	1	0.77%	1	1.33%	0	0.00%	2	0.90%	5(2.25%)
<i>E. agglomerans</i>	2	1.54%	0	0.00%	0	0.00%	2	0.90%	
<i>E. aerogenes</i>	1	0.77%	0	0.00%	0	0.00%	1	0.45%	
<i>C. diversus</i>	5	3.85%	0	0.00%	0	0.00%	5	2.25%	
<i>S. marcescens</i>	2	1.54%	1	1.33%	1	5.88%	4	1.80%	4(1.80%)
<i>Pseudomonas spp</i>	0	0.00%	3	4.00%	0	0.00%	3	1.35%	3(1.35%)
<i>M. morgani</i>	1	0.77%	1	1.33%	0	0.00%	2	0.90%	2(0.90%)
<i>P. mirabilis</i>	1	0.77%	1	1.33%	0	0.00%	2	0.90%	2(0.90%)
<i>P. rettgeri</i>	2	1.54%	0	0.00%	0	0.00%	2	0.90%	2(0.90%)
Total									23(10.36%)
Total	130	100%	75	100%	17	100%	222	100%	222(100%)

MDR: Multidrug resistance; XDR: Extensively drug-resistant; PDR: Pan drug-resistant.

4.2.3. Factors Associated with MDR Gram-Negative Bacteria

Among 417 culture-positive individuals, 226(54.2%) and 191(45.8%) were gram-negative bacteria and gram-positive bacteria, respectively. Excluding one *Corynebacterium gleum* and one *N. meningitis* isolated patient 224(53.72%) individuals were culture-positive for gram-negative bacteria and of them 9(3.89%) were not MDR isolates. Therefore, 215(51.56%) patients who acquired MDR gram-negative bacteria were analyzed to determine the associated factors. The bacterial species with maximum drug resistance were selected for associated risk factors.

Among the total 215 drug-resistant gram-negative bacteria drug resistance gram-negative bacteria, 152(70.70%) were identified as below thirteen years. The highest amount of MDR recorded in the age group of birth to <28 days accounted for 74(32.3%), and the second in the age group of >1-<13 years accounted for 78(13.3%) (Figure 9). Among the total 17 PDR, 6 (35.3%) of them were found at the age of <28 days. Multivariate analysis showed that the highest percentage of drug resistance was found in neonates <28 days with AOR p= 0.007 (Table 10). This finding showed that neonates were highly affected by drug-resistant gram-negative bacteria bloodstream infections.

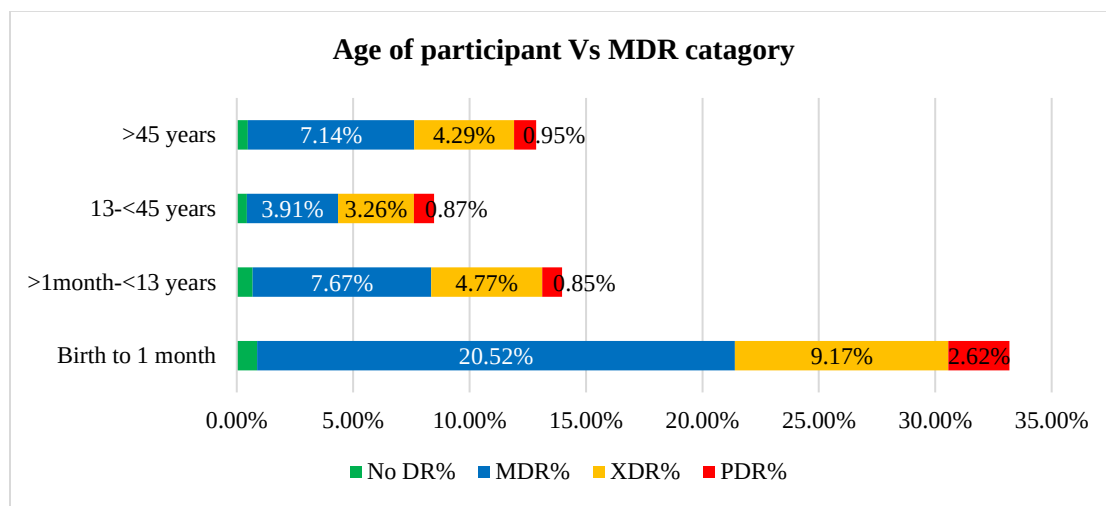
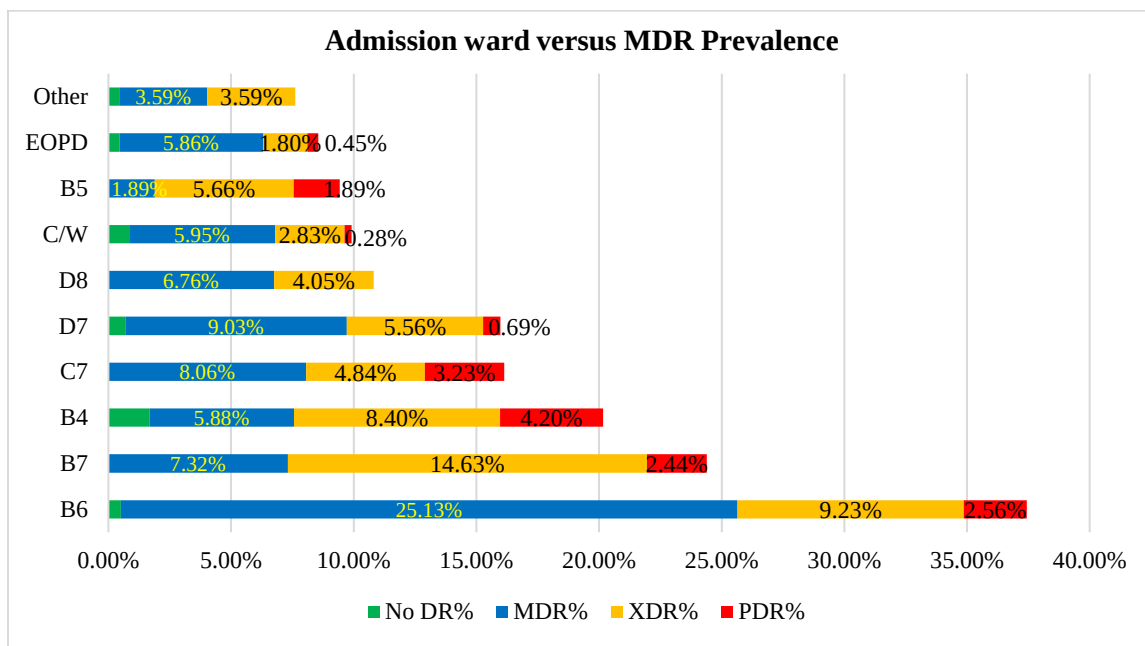


Figure 9: Drug resistance category relating to age in gram-negative bacteria from bloodstream infection -suspected patients

When we look at the prevalence of MDR bacteria in the 25 wards B6 (NICU), B7 (pediatrics medical & surgical), B4 (pediatrics & adult SICU, MICU), C7 (Pediatrics Medical), and D7 (Pedi Hematology) were the top five most affected wards (Figure 10). Among the total of 215, MDR and Above MDR gram-negative bacteria B6 (NICU) accounted for 72 (36.92%), COR p=<0.001. Among the total 72 drug-resistant bacteria MDR was 49 (25.13%), XDR was 18(9.23%), and PDR was 5(2.56%) were the most vulnerable areas for drug-resistant bacteria. The second vulnerable ward was B7 (pediatrics medical & surgical) accounting for 10(24.39%) COR p=0.001; and of this MDR gram-negative bacteria, MDR was 3(7.32%), XDR was 6(14.63%), and PDR was 1(2.44%). The third vulnerable ward was B4(pediatrics & adult SICU, MICU) accounting for 22(18.49%) COR p=0.002, MDR gram-negative bacteria. Among these MDR gram-negative bacteria, each

accounted for MDR 7(5.88%), XDR 10(8.40%), and PDR 5(4.2%). There is a high burden of MDR in C/W and EOPD. The MDR in C/W were 32(9.08%) each accounted for MDR 21(5.95%), XDR 10(2.83%), and PDR 1(0.28%), and EOPD were 18(8.1%) each accounted for MDR 13(5.86%), XDR 4(1.80%), PDR 1(0.45%), no statistical association was observed with MDR prevalence COR p=0.425 (Table 10, Figure 10). Among the total 17 PDR, the top two wards, B6 and B4, accounted for 5(29.4%) and 5(29.4%), respectively; thus, in ICU both pediatrics and adult were affected by pan-drug-resistant strains.



B6, NICU; B7, pediatrics medical & surgical; B4, pediatrics & adult SICU, MICU; C7, Pediatrics Medical; D7, Pedi Hematology; D8, Medical; C/W, Causality; B5, Medical & Neurosurgery; EOPD, Emergency outpatient department

Figure 10: Drug resistance category related to admission ward in gram-negative bacteria from bloodstream infection suspected.

Percentage prevalence and multivariate logistic regression analysis showed that NICU, 63(35.39%) AOR p=0.027, SICU, 10(29.41%) AOR p=0.001, and general surgery, 11 (23.91%) AOR p=0.023, departments were the most vulnerable areas for MDR infection. This finding aligned with ward results and it implies that ICU patients have a high chance of acquiring MDR bacteria 84(39.07%). ICU with general surgery accounted for 95(44.19%). Univariate analysis

showed a strong association with MDR bacterial infection, $p < 0.001$ (Table 10). Next to ICU patients, general surgery patients were highly exposed to XDR and PDR infection (Figure 11).

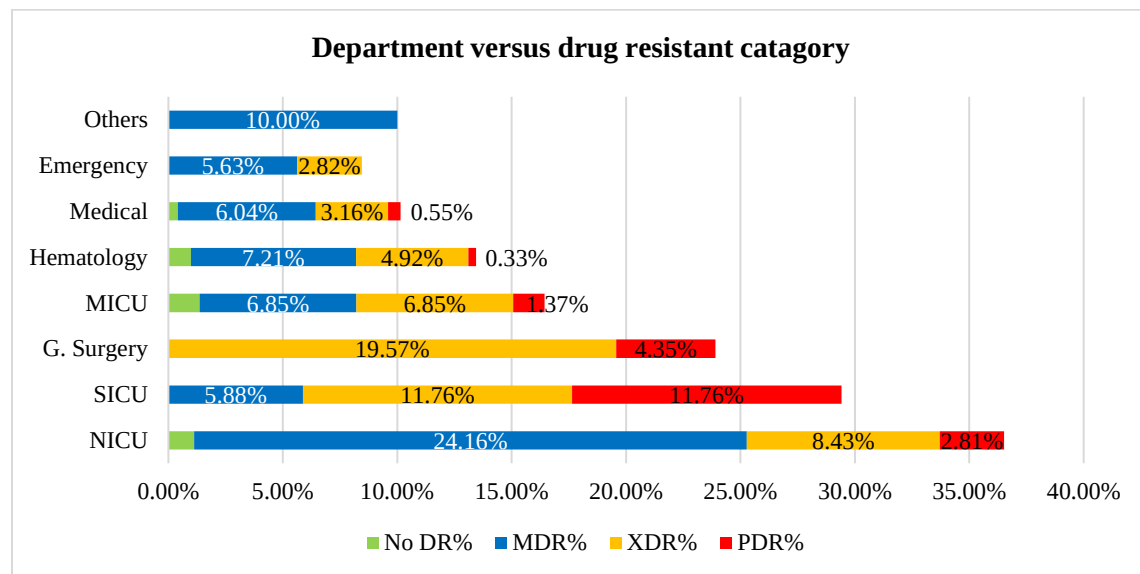


Figure 11: Prevalence drug resistance category of gram-negative bacteria with the patient admission departments from bloodstream infection suspected patients

Regarding the association of length of admission with 215 MDR gram-negative bacteria-identified bloodstream infection patients, the majority, 118(54.9%), were admitted for more than five days (Figure 12). Univariate logistic regression analysis showed patients admitted for greater than five days have one-half folds of acquiring bloodstream infection of MDR, XDR, and possible PDR gram-negative bacteria bloodstream infection COR $p = 0.015$ (Table 10).

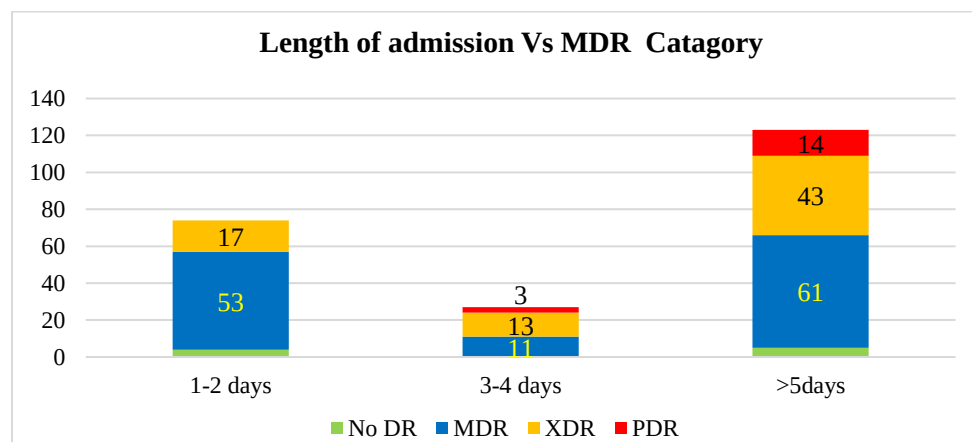


Figure 12: Drug resistance category with the length of admission in gram-negative bacteria from bloodstream infection suspected patients

As observed in Figure 12 the prevalence of XDR and PDR increases when the length of admission increases. Patients who have an infection within less than 48-72 hours of admission were considered to be exposed to community-acquired infections; as you see in the graph also, there are no possible PDR patients in less than 48 hours of admission.

Antibiotic treatment before ten days of sample collection, COR $p=0.019$, has an association with the prevalence of MDR bacteria (Table 10). Among 215 MDR bacterial infections, 173(80.46%) of them used instrumentation during medical care, and 42(19.53%) of them were not used, AOR $p<0.001$ (Figure 13 and Table 10). This implies that the usage of indwelling medical devices has more than double the chance of exposure to MDR bloodstream infection.

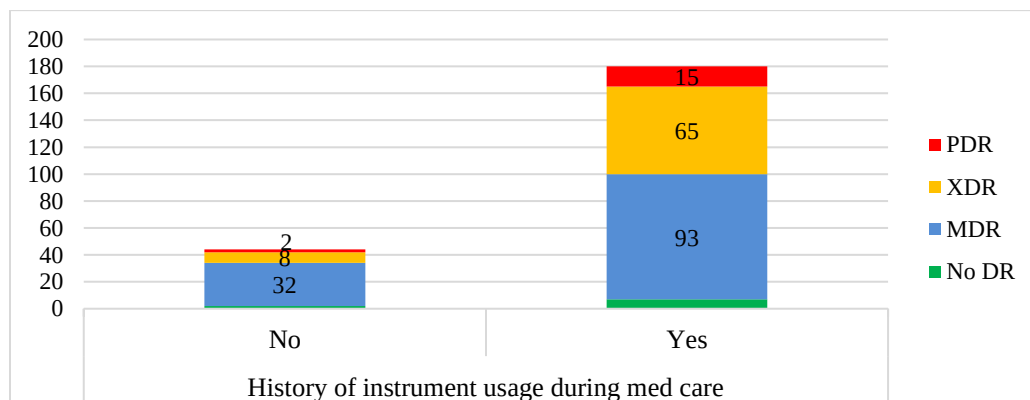


Figure 13: Drug resistance category with indwelling medical device usage in gram-negative bacteria from bloodstream infection -suspected patients.

Among nosocomial infections causing suspected medical devices IV lines, drainage tubes, and NG tubes have been associated with MDR prevalence (Figure 14). IV line and drainage tube users have more than a double chance of acquiring MDR and NG tube users have nearly double the chance of acquiring MDR bacteria. Multivariate analysis showed that IV-line users accounted for 21.2% and not users were 12.9% AOR $P=0.029$; drainage tube users accounted for 35.5% and not users were 19.8% AOR $P=0.033$, likewise, NG tube users accounted for 34.8% and not users were

19.6% COR P=0.047. Urinary catheter users accounted for 25.6% and not users were 19.4% but this does not have an association with MDR prevalence (Table 10).

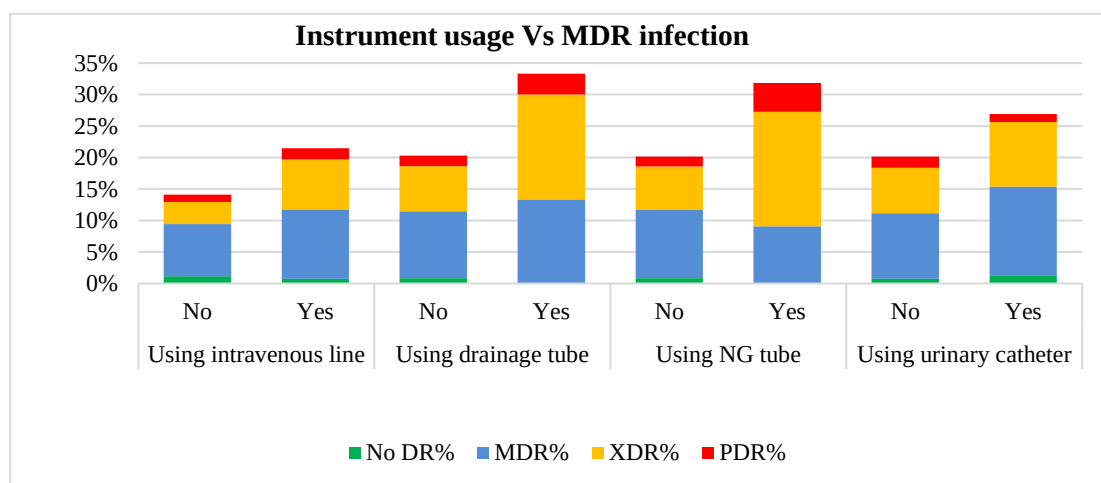


Figure 14: Drug resistance category concerning the type of indwelling medical device usage in gram-negative bacteria from bloodstream infection suspected patients.

Among chronic illnesses, people with wound infection (WI), ureteral tract infection (UTI), and respiratory tract infection (RTI) showed more than three, five, and two-fold acquiring MDR bloodstream infection (Figure 15). Of this, wound infection (WI) accounted for 31.82%, not having WI was 11.48% COR P=0.006, having ureteral tract infection (UTI) accounted for 41.03%, not having UTI was 10.7% AOR P=<0 .001, and having respiratory tract infection (RTI) accounted for 18.89% and not having RTI was 11.23% AOR P=0.027 (Table 10).

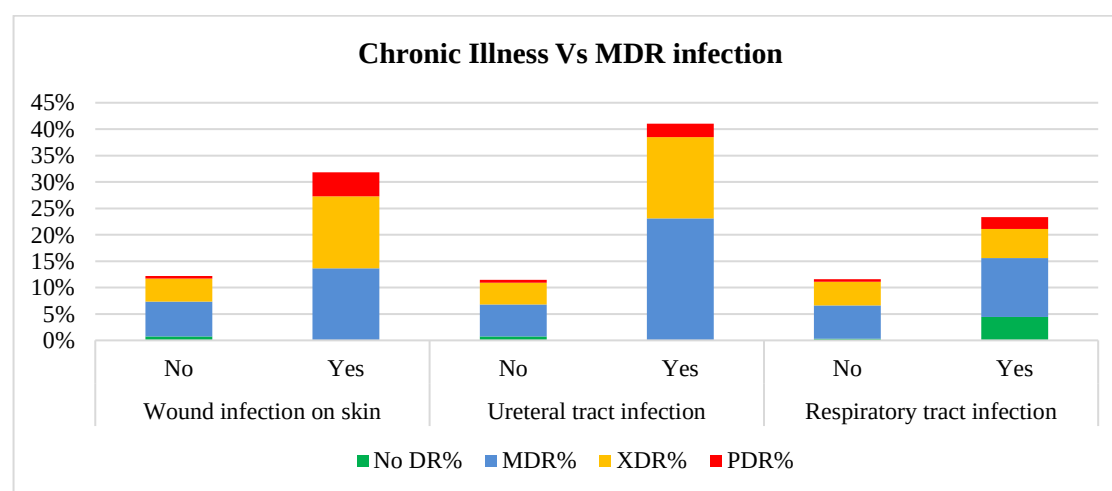


Figure 15: Type of chronic illness associated with MDR category in gram-negative bacteria from bloodstream infection suspected patients.

Concerning neonatal bloodstream infection neonatal incubation, premature rupture of the membrane had three-fold exposure to MDR infection and the mode of delivery had two-fold exposure to multidrug resistance bloodstream infection (Figure 16). History of incubation, 38.1%, had a double prevalence of MDR gram-negative bacteria than not having a history of neonatal incubation, 16.1% AOR $p=0.005$. However, the prevalence of possible PDR bacteria in not having a neonatal history of incubation was more than 5 times that of having neonatal incubation. Delivery by cesarian section (C/S) at 43.8% had double the prevalence of normal vaginal delivery (NVD) at 27.7% COR $p=0.021$. Among factors associated with MDR bacterial infections in premature rupture of membrane >18 hours were 17.8%, <18 hours were 32.7%, and no rupture of the membrane due to cesarian section was 53.8% during delivery, showing that both factors did not show an association with MDR infection but no rupture of the membrane due to cesarian section has an association with MDR prevalence AOR $P=0.04$ (Table 10). MDR association with patient admission ward in general surgery has a strong association with MDR prevalence. Surgical procedures done in gynecology and the surgery departments showed a strong association with MDR infection (Figures 10 and 15).

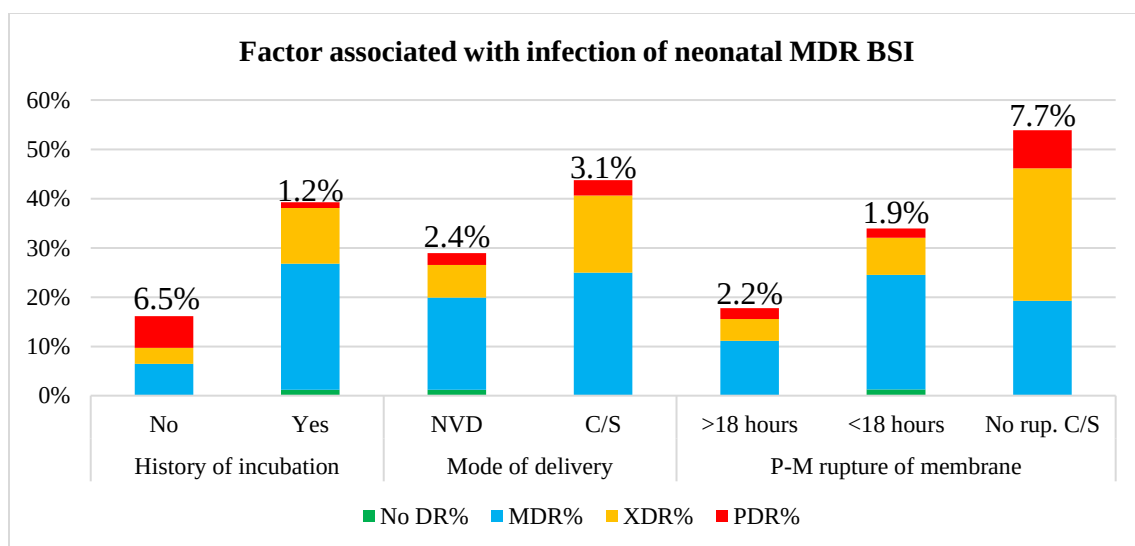


Figure 16: Factors associated with MDR category in gram-negative bacterial bloodstream infection suspected neonatal patients

Table 10: Factors associated with MDR prevalence crude and adjusted odds ratio of bloodstream infection suspected patients n=1486.

Variables	Bacterial MDR prevalence N(%)		Bivariate logistic regression analysis		Multivariate logistic regression analysis	
	Positive	Negative	P-value	COR (95% C.I.)	P-value	AOR (95% C.I.)
Age of respondent						
Birth to 1 month	74(32.3%)	155(67.7%)	<0.001	3.379(2.059-5.544)	0.007	3.149(1.376-7.209)
>1 month-<13 yrs	78(13.3%)	509(86.7%)	0.738	1.084(0.675-1.743)	0.172	1.426(0.857-2.371)
13-<45 years	37(8%)	423(92%)	0.076	0.619(0.364-1.052)	0.125	0.649(0.373-1.128)
>45 years	26(12.4%)	184(87.6%)	0	0.141	0	
Sex of respondent						
Male	120(14.2%)	724(85.8%)	0.753	0.954(0.713-1.277)		
Female	95(14.8%)	547(85.2%)	0	0.174		
Type of patient						
In-patient	214(14.5%)	1259(85.5%)	0.495	2.04(0.264-15.767)		
Out-patient	1(7.7%)	12(92.3%)	0.017	0.083		
Admission Ward						
B6(NICU)	72(36.9%)	123(63.1%)	<0.001	7.573(4.215-13.61)	Duplicate data with ward	
C/W	32(9.1%)	321(90.9%)	0.425	1.290(0.69-2.410)		
B4(SICU, MICU)	22(18.5%)	97(81.5%)	0.002	2.934(1.475-5.836)		
D7(Pedi Hemat))	22(15.3%)	122(84.7%)	0.015	2.333(1.18-4.613)		
EOPD	18(8.1%)	204(91.9%)	0.711	1.142(0.567-2.30)		
B7(Ped med&surg)	10(24.4%)	31(75.6%)	0.001	4.173(1.739-10.02)		
C7(Pedi Medical)	10(16.1%)	52(83.9%)	0.035	2.488(1.067-5.801)		
D8(Medical)	8(10.8%)	66(89.2%)	0.323	1.568(0.642-3.829)		
B5(Med. & Neusur)	5(9.4%)	48(90.6%)	0.578	1.348(0.471-3.859)		
Other	16(7.2%)	207(92.8%)	0	0.077		
Patient Department						
Medical	71(9.7%)	658(90.3%)	0	0.108	0.017	
Emergency	6(8.5%)	65(91.5%)	0.725	0.855(0.358-2.045)	0.713	1.188(0.474-2.975)
MICU	11(15.1%)	62(84.9%)	0.156	1.644(0.828-3.266)	0.637	1.188(0.581-2.429)
NICU	63(35.4%)	115(64.6%)	<0.001	5.077(3.428-7.52)	0.027	2.205(1.093-4.451)
G. Surgery	11(23.9%)	35(76.1%)	0.004	2.913(1.417-5.986)	0.023	2.479(1.133-5.422)
SICU	10(29.4%)	24(70.6%)	0.001	3.862(1.775-8.401)	0.001	4.013(1.722-9.35)
Hematology	38(12.5%)	267(87.5%)	0.195	1.319(0.868-2.005)	0.677	1.104(0.694-1.755)
Others	5(10%)	45(90%)	0.952	1.03(0.396-2.678)	0.809	0.885(0.328-2.385)
Length of admission						
1-2 days	70(12.1%)	508(87.9%)	0.036		0.498	
3-4 days	27(12.7%)	186(87.3%)	0.83	1.053(0.655-1.694)	0.311	0.763(0.452-1.288)
>5days	118(17%)	577(83%)	0.015	1.484(1.079-2.041)	0.941	1.016(0.676-1.526)
ICU admission						
No	126(10.6%)	1059(89.4%)	0	0.119		
yes	89(29.6%)	212(70.4%)	<0.001	3.528(2.591-4.805)	Duplicate data with ward	
Body Temperature						
<36.5	36(21.2%)	134(78.8%)	0	0.269	0.655	
36.6-37.5	14(17.7%)	65(82.3%)	0.527	0.802(0.404-1.59)	0.381	0.701(0.316-1.554)
>37.6	165(13.3%)	1072(86.7%)	0.007	0.573(0.383-0.857)	0.477	0.731(0.308-1.734)
Antibiotic treatment 10 days before sample collection						
No	83(12.1%)	601(87.9%)	0	0.138		
Yes	132(16.5%)	670(83.5%)	0.019	1.427(1.061-1.918)	0.15	1.315(0.906-1.908)

Instrument usage during medical care?						
No	42(6.8%)	577(93.2%)	0	0.073		
Yes	173(20%)	694(80%)	<0.001	3.425(2.402-4.883)	<0.001	2.466(1.643-3.702)
History of using a mechanical ventilator						
No	150(20.5%)	580(79.5%)	0	0.259		
Yes	23(16.8%)	114(83.2%)	0.313	0.78(0.481-1.264)		
Central vain catheter						
No	169(20.3%)	663(79.7%)	0	0.255		
Yes	4(11.4%)	31(88.6%)	0.206	0.506(0.176-1.454)	0.32	0.58(0.198-1.697)
Urinary catheter						
No	153(19.4%)	636(80.6%)	0	0.241		
Yes	20(25.6%)	58(74.4%)	0.19	1.433(0.837-2.455)	0.151	1.526(0.857-2.72)
Suction machine						
No	150(20.1%)	596(79.9%)	0	0.252		
Yes	23(19%)	98(81%)	0.779	0.933(0.572-1.519)		
Tracheostomy						
No	169(20%)	678(80%)	0	0.249		
Yes	4(20%)	16(80%)	0.996	1.003(0.331-3.039)		
intravenous line						
No	11(12.9%)	74(87.1%)	0	0.149		
Yes	162(20.7%)	620(79.3%)	0.092	1.758(0.912-3.389)	0.029	2.194(1.083-4.444)
drainage tube						
No	163(19.5%)	674(80.5%)	0	0.242		
Yes	10(33.3%)	20(66.7%)	0.067	2.067(0.95-4.502)	0.033	2.449(1.074-5.587)
shunt						
No	171(19.9%)	690(80.1%)	0	0.248		
Yes	2(33.3%)	4(66.7%)	0.42	2.018(0.367-11.11)		
NG tube						
No	159(19.3%)	664(80.7%)	0	0.239	0	0.11
Yes	14(31.8%)	30(68.2%)	0.047	1.949(1.01-3.761)	0.099	1.77(0.899-3.488)
Having chronic illness						
No	100(19.1%)	423(80.9%)	0	0.236		
Yes	115(11.9%)	848(88.1%)	<0.001	0.574(0.428-0.768)	0.041	1.646(1.021-2.655)
RVI patient						
No	114(12.4%)	809(87.6%)	0	0.141		
Yes	1(2.5%)	39(97.5%)	0.094	0.182(0.025-1.337)	0.139	0.218(0.029-1.642)
Diabetic patients						
No	112(11.9%)	830(88.1%)	0	0.135		
Yes	3(14.3%)	18(85.7%)	0.738	1.235(0.358-4.26)	0.722	0.779(0.196-3.098)
Hypertension						
No	109(11.8%)	811(88.2%)	0	0.134		
Yes	6(14%)	37(86%)	0.678	1.207(0.498-2.925)	0.332	1.662(0.595-4.643)
Surgical wound infections						
No	111(11.9%)	824(88.1%)	0	0.135		
Yes	4(14.3%)	24(85.7%)	0.698	1.237(0.421-3.632)	0.912	0.935(0.284-3.082)
Wound infection on skin						
No	108(11.5%)	833(88.5%)	0	0.13		
Yes	7(31.8%)	15(68.2%)	0.006	3.599(1.435-9.025)	0.188	2.051(0.703-5.98)
Gastrointestinal infection						
No	114(11.9%)	840(88.1%)	0	0.136		
Yes	1(11.1%)	8(88.9%)	0.938	0.921(0.114-7.432)	0.764	1.389(0.162-11.92)
Ureteral tract infection						

No	99(10.7%)	825(89.3%)	0	0.12		
Yes	16(41%)	23(59%)	<0.001	5.797(2.963-11.34)	<0.001	5.59(2.633-11.872)
Respiratory tract infection						
No	98(11.2%)	775(88.8%)	0	0.126		
Yes	17(18.9%)	73(81.1%)	0.035	1.842(1.043-3.25)	0.027	2.146(1.092-4.217)
Ischemic heart disease						
No	102(12.6%)	709(87.4%)	0	0.144		
Yes	13(8.6%)	139(91.4%)	0.163	0.65(0.355-1.191)	0.532	0.778(0.354-1.71)
Coronary artery disease						
No	115(12.2%)	827(87.8%)	0	0.139		
Yes	0(0%)	21(100%)	0.998	0	0.999	0
Stroke						
No	115(12.1%)	834(87.9%)	0	0.138		
Yes	0(0%)	14(100%)	0.999	0	0.999	0
Diabetes mellitus						
No	114(11.9%)	847(88.1%)	0	0.135		
Yes	1(50%)	1(50%)	0.157	7.43(0.462-119.6)	0.998	185*109
Tuberculosis						
No	112(12%)	822(88%)	0	0.136		
Yes	3(10.3%)	26(89.7%)	0.788	0.847(0.252-2.844)	0.879	1.104(0.307-3.965)
Cirrhosis						
No	114(12%)	834(88%)	0	0.137		
Yes	1(6.7%)	14(93.3%)	0.533	0.523(0.068-4.011)	0.74	0.703(0.088-5.642)
renal failure						
No	109(11.8%)	818(88.2%)	0	0.133		
Yes	6(16.7%)	30(83.3%)	0.376	1.501(0.611-3.688)	0.478	1.445(0.523-3.989)
Hematologic Malignancy						
No	63(11.9%)	465(88.1%)	0	0.135		
Yes	52(12%)	383(88%)	0.992	1.002(0.678-1.482)	0.434	1.3(0.673-2.511)
Tissue Sarcomas						
No	99(11.8%)	741(88.2%)	0	0.134	0	0.098
Yes	16(13%)	107(87%)	0.696	1.119(0.636-1.971)	0.442	1.35(0.628-2.903)
Neonates						
The educational level of the family						
Illiterate	9(29%)	22(71%)	0.243	0.468(0.13-1.676)	0.652	0.724(0.178-2.942)
Read and write	8(11.9%)	59(88.1%)	0.004	0.155(0.044-0.543)	0.014	0.188(0.049-0.714)
Primary School	16(28.1%)	41(71.9%)	0.175	0.446(0.139-1.433)	0.404	0.58(0.161-2.084)
Secondary school	34(56.7%)	26(43.3%)	0.488	1.495(0.48-4.653)	0.424	1.647(0.484-5.604)
Higher Education	7(46.7%)	8(53.3%)	0.796	0.875	0	
Place of delivery						
In hospital	74(33.5%)	147(66.5%)	0.999	8.13*108		
At home	0(0%)	9(100%)	0.999	0		
history of incubation						
No	10(16.1%)	52(83.9%)	0	0.192		
Yes	64(38.1%)	104(61.9%)	0.002	3.2(1.519-6.74)	0.005	3.186(1.419-7.151)
Mode of delivery						
NVD	46(27.7%)	120(72.3%)	0	0.383		
C/S	28(43.8%)	36(56.3%)	0.021	2.029(1.114-3.695)	0.46	1.322(0.63-2.776)
Gestational age						
>37 weeks	41(29.5%)	98(70.5%)	0.283	1.36(0.775-2.385)		
<37 weeks	33(36.3%)	58(63.7%)	0	0.418		
Premature rupture of membrane						

>18 hours	8(17.8%)	37(82.2%)	0.01		0.12	
<18 hours	52(32.7%)	107(67.3%)	0.057	2.248(0.977-5.17)	0.249	1.691(0.692-4.135)
No rupture	14(53.8%)	12(46.2%)	0.002	5.396(1.822-15.98)	0.04	3.725(1.064-13.04)
Body weight range						
Normal	36(29.8%)	85(70.2%)	0	0.424		
Low	38(35.5%)	69(64.5%)	0.354	1.3(0.746-2.266)		
High	0(0%)	2(100%)	0.999	0		
Maternal fever						
No	65(31.1%)	144(68.9%)	0	0.451		
Yes	9(42.9%)	12(57.1%)	0.275	1.662(0.667-4.138)		

COR: Crude odds ratio; AOR: Adjusted odds ratio; CI: Confidence interval; MDR: Multidrug resistance. Values in bold are statistically significant.

4.3. Phenotyping Characterization

4.3.1. Antimicrobial Susceptibility Profile

4.3.1.1. AST for Screening of ESBL and Cephamycin Detection

After this chapter phenotyping and molecular characterization were analyzed for a total of 231(54.1%) gram-negative bacteria. Among 231 gram-negative bacteria in primary screening and in confirmatory tests ESBL and AmpC-BLs phenotypically identified were 188 (81.4%) and 81 (35.1%), respectively (Table 11). Among all, gram-negative bacteria ESBL suspected resistance strains for Cefotaxime, Ceftazidime, Ceftriaxone, and Cefepime accounted for 81.4%, 72.7%, 80.5%, and 75.8%, respectively and AmpC suspected resistance strains for Cefotetan and Cefoxitin accounted for 35.1% and 22.1%. Among all *Klebsiella pneumoniae*, 71(94.7%) and 31 (41.3%) were the highest resistant for extended-spectrum cephalosporin (Ceftriaxone) and Cephamycin (Cefotetan) drugs n >5 (Table 11).

Table 11: Phenotypical identification of ESBLs and AmpC-BLs producer bloodstream infection bacteria n=231.

Bacteria identified	#	Cefotaxime		Ceftazidime		Ceftriaxone		Cefepime		Cefoxitin		Cefotetan	
		R#	R%	R#	R%	R#	R%	R#	R%	R#	R%	R#	R%
<i>K. pneumoniae</i>	75	70	93.3%	69	92.0%	71	94.7%	69	92.0%	31	41.3%	19	25.3%
<i>Acinetobacter spp</i>	47	43	91.5%	32	68.1%	42	89.4%	36	76.6%	14	29.8%	14	29.8%
<i>E. coli</i>	38	27	71.1%	26	68.4%	28	73.7%	25	65.8%	15	39.5%	12	31.6%
<i>K. oxytoca</i>	27	23	85.2%	18	66.7%	21	77.8%	23	85.2%	7	25.9%	4	14.8%
<i>E. coli (A-D)</i>	11	9	81.8%	9	81.8%	9	81.8%	9	81.8%	3	27.3%	2	18.2%

<i>C. diversus</i>	5	2	40%	2	40%	2	40%	2	40%	3	60.0%	0	0.0%
<i>Pseudomonas spp</i>	5	NA	NA	1	20%	NA	NA	2	40%	0	0.0%	0	0.0%
<i>K. rhinoscleroma</i>	4	2	50%	2	50%	2	50%	2	50%	2	50.0%	2	50.0%
<i>S. marcescens</i>	4	4	100%	4	100%	4	100%	3	75.0%	3	75.0%	2	50.0%
<i>K. ozaenae</i>	3	1	33.3%	1	33.3%	1	33.3%	0	0.0%	1	33.3%	0	0.0%
<i>E. cloacae</i>	2	1	50%	1	50%	1	50%	1	50%	NA	NA	NA	NA
<i>E. agglomerans</i>	2	1	50%	1	50%	1	50%	0	0.0%	1	50.0%	1	50.0%
<i>M. morgani</i>	2	1	50%	1	50%	1	50%	1	50%	1	50.0%	1	50.0%
<i>P. mirabilis</i>	2	1	50%	1	50%	1	50%	1	50%	0	0.0%	0	0.0%
<i>P. rettgeri</i>	2	2	100%	0	0.0%	1	50%	1	50%	0	0.0%	0	0.0%
<i>S. typhi</i>	1	0	0.0%	0	0.0%	0	0.0%	0	0.0%	NA	NA	NA	NA
<i>E. aerogenes</i>	1	1	100%	0	0.0%	1	100%	0	0.0%	0	0.0%	0	0.0%
Total	231	188	81.4%	168	72.7%	186	80.5%	175	75.8%	81	35.1%	51	22.1%

NA- Not applicable because of intrinsically resistant species. R- resistant

4.3.1.2. AST for Screening Carbapenem-Resistant Detection

The number of drug-resistant strains to carbapenem and MBL drug-resistance strains was 74(32.0%); each accounted for Meropenem 61(26.4%), Imipenem 56 (24.2%), Doripenem 74 (32.0%) and Ertapenem 38 (16.5%). However, during the confirmatory testing, 69(29.9%) carbapenem and Metallo beta-lactam drug resistance strains were selected for the PCR test. The highest carbapenem drug resistance was detected in *Acinetobacter spp* with 28(59.6%) Meropenem and 30(63.8%) Doripenem (Table 12).

Table 12: Phenotypical identification of Carbapenemases and metallo-beta-lactamase producer bloodstream infection bacteria n=231.

Bacteria identified	#	Meropenem		Imipenem		Doripenem		Ertapenem		All Average	
		R#	R%	R#	R%	R#	R%	R#	R%	R#	R%
<i>K. pneumoniae</i>	75	15	20.0%	17	22.7%	20	26.7%	18	24.0%	17.5	23.3%
<i>Acinetobacter spp</i>	47	28	59.6%	22	46.8%	30	63.8%	NA	NA	26.7	56.7%
<i>E. coli</i>	38	4	10.5%	3	7.9%	7	18.4%	5	13.2%	4.8	12.5%
<i>K. oxytoca</i>	27	3	11.1%	3	11.1%	5	18.5%	4	14.8%	3.8	13.9%
<i>E. coli (A-D)</i>	11	2	18.2%	2	18.2%	2	18.2%	2	18.2%	2.0	18.2%
<i>C. diversus</i>	5	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0.0	0.0%
<i>Pseudomonas spp</i>	5	2	40%	2	40%	2	40%	NA	NA	2.0	40.0%
<i>K. rhinoscleromatis</i>	4	2	50%	2	50%	2	50%	2	50%	2.0	50.0%
<i>S. marcescens</i>	4	2	50%	1	25.0%	2	50%	3	75.0%	2.0	50.0%
<i>K. ozaenae</i>	3	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0.0	0.0%
<i>E. cloacae</i>	2	1	50%	1	50%	2	100%	1	50%	1.3	62.5%

<i>E. agglomerans</i>	2	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0.0	0.0%
<i>M. morgani</i>	2	1	50%	1	50%	1	50%	1	50%	1.0	50.0%
<i>P. mirabilis</i>	2	1	50%	1	50%	1	50%	1	50%	1.0	50.0%
<i>P. rettgeri</i>	2	0	0.0%	1	50%	0	0.0%	0	0.0%	0.3	12.5%
<i>S. typhi</i>	1	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0.0	0.0%
<i>E. aerogenes</i>	1	0	0.0%	0	0.0%	0	0.0%	1	100%	0.3	25.0%
Total	231	61	26.4%	56	24.2%	74	32.0%	38	16.5%	57.3	24.8%

Out of the total 231 gram-negative bacteria ESBL, Carbapenemase, AmpC-BL, and MBL producers accounted for 52.8%, 25.1%, 14.3%, and 5.6%, respectively. Most *K. pneumoniae* (77.3%) and *K. oxytoca* (77.8%) were ESBL producers; likewise, most *Acinetobacter spp* (53.2%) were carbapenemase producers

4.3.1.3. Confirmatory Result for ESBL And AmpC

Phenotyping confirmatory tests done by DDST, CDT, and E-test reduced screening results from 188 (81.4%) and 81(35.1%) to 122(52.8%) and 33(14.3%), for ESBL and AmpC, respectively. Confirmatory test results showed that the highest bacterial ESBL prevalence result had a little bit of difference from primary screening it showed *K. oxytoca*, 21(77.8%), was dominated by *K. pneumoniae*, with 58 (77.3%) strains exhibiting the resistance. Similarly, in AmpC *E. coli*, 8(21.1%), comes ahead of *Acinetobacter spp*, 9 (19.1%). After confirmatory testing, *K. oxytoca*, 21(77.8%), *K. pneumoniae*, 58(77.3%), and *E. coli* (A-D), 7(63.6%), were the leading ESBL producers; among AmpC-BLs *E. coli*, 8(21.1%), *Acinetobacter spp*, 9 (19.1%), and *K. oxytoca*, 4 (14.8%), were the top three. On the contrary, *C. diversus*, *K. rhinoscleromatis*, *E. cloacae*, *M. morgani*, and *S. typhi* were not ESBL and AmpC producers (Table 13).

Table 13: Phenotypical confirmatory identification of bloodstream infection enzyme-producing drug-resistant bacteria n=231.

Gram-negative bacteria	Total strains	Not DRM	Enzyme-producing drug resistance mechanism identified				
			ESBL	AmpC-BL	CPs	MBL	CP&MBL
			N (%)	N (%)	N (%)	N (%)	N (%)
<i>K. pneumoniae</i>	75	3	58(77.3%)	6(8%)	15(20%)	4(5.3%)	19(25.3%)
<i>Acinetobacter spp</i>	47	6	10(21.3%)	9(19.1%)	25(53.2%)	5(10.6%)	30(63.8%)
<i>E. coli</i>	38	10	17(44.7%)	8(21.1%)	4(10.5%)	2(5.3%)	6(15.8%)
<i>K. oxytoca</i>	27	2	21(77.8%)	4(14.8%)	5(18.5%)	0(0%)	5(18.5%)
<i>E. coli</i> (A-D)	11	2	7(63.6%)	1(9.1%)	1(9.1%)	1(9.1%)	2(18.2%)

<i>C. diversus</i>	5	0	2(40%)	0(0%)	0(0%)	0(0%)	0(0.0%)
<i>S. marcescens</i>	4	1	2(50%)	1(25.0%)	2(50%)	0(0%)	2(50.0%)
<i>Pseudomonas spp</i>	5	3	0(0%)	2(40%)	2(40%)	0(0%)	2(40.0%)
<i>K. ozaenae</i>	3	2	1(33.3%)	1(33.3%)	0(0%)	0(0%)	0(0.0%)
<i>K. rhinoscleromatis</i>	4	2	0(0%)	0(0%)	1(25.0%)	1(25%)	2(50.0%)
<i>P. mirabilis</i>	2	1	1(50%)	0(0%)	1(50%)	0(0%)	1(50.0%)
<i>E. cloacae</i>	2	1	0(0%)	0(0%)	1(50%)	0(0%)	1(50.0%)
<i>E. agglomerans</i>	2	1	1(50%)	0(0%)	0(0%)	0(0%)	0(0.0%)
<i>M. morganii</i>	2	0	0(0%)	0(0%)	1(50%)	0(0%)	1(50.0%)
<i>P. rettgeri</i>	2	0	2(100%)	0(0%)	0(0%)	0(0%)	0(0.0%)
<i>E. aerogenes</i>	1	1	0(0%)	1(100%)	0(0%)	0(0%)	0(0.0%)
<i>S. typhi</i>	1	1	0(0%)	0(0%)	0(0%)	0(0%)	0(0.0%)
Total	231	36	122(52.8%)	33(14.3%)	58(25.1%)	13(5.6%)	71(30.7%)

EP-DRB- enzyme producer drug resistance bacteria; ESBL- Extended-spectrum beta-lactamase;
AmpC BLs- AmpC beta-lactamase

4.3.1.4. Confirmatory Result for CPs and MBLs

During confirmatory testing, carbapenem and MBL-resistant strains were 71(30.7%), which was reduced from 74(32.0%) to 71(30.7%) (Table 12 & 13). The highest result was observed in *Acinetobacter spp*, 30(63.8%), and *K. pneumoniae*, 19(25.3%) in n>5; whereas, *C. diversus*, *K. ozaenae*, *E. agglomerans* *P. rettgeri*, *E. aerogenes*, and *S. typhi* were not Carbapenemase and Metallo beta-lactamase producers (Table 13).

4.3.1.5. Prevalence of Double Drug Resistance

Among the 231 gram-negative bacteria s, 195 (84.4%) of the isolates were positive for one or two enzyme-producing drug resistance mechanisms. Among this, 13.4% of gram-negative bacteria have a double drug resistance mechanism, with ESBL + AmpC-BL producers accounting for 6.9%, ESBL + Carbapenemase producers for 5.2%, and ESBL + MBL producers for 1.3%. *K. pneumoniae* (35.5%) and *Acinetobacter spp* (25.8%) comprise the dominant species possessing double drug resistance mechanisms (Table 14).

Table 14: Prevalence of gram-negative bacteria that produce ESBL or AmpC-BL or MBL or Carbapenemase among bloodstream infection suspected patients n=231.

Gram-negative bacteria	ESBL or AmpC-BL or MBL or Carbapenemase			
	Producer	Non-producer	Total	Double producer N
	N (%)	N (%)	N	
<i>K. pneumoniae</i>	72(96%)	3(4%)	75	11
<i>Acinetobacter spp</i>	41(87.2%)	6(12.8%)	47	8
<i>E. coli</i>	28(73.7%)	10(26.3%)	38	3
<i>K. oxytoca</i>	25(92.6%)	2(7.4%)	27	5
<i>E. coli (A-D)</i>	9(81.8%)	2(18.2%)	11	1
<i>C. diversus</i>	2(40%)	3(60%)	5	0
<i>S. marcescens</i>	4(100%)	0(0%)	4	1
<i>Pseudomonas spp</i>	4(80%)	1(20%)	5	0
<i>K. ozaenae</i>	1(33.3%)	2(66.7%)	3	1
<i>K. rhinoscleromatis</i>	2(50%)	2(50%)	4	0
<i>P. mirabilis</i>	1(50%)	1(50%)	2	1
<i>E. cloacae</i>	1(50%)	1(50%)	2	0
<i>E. agglomerans</i>	1(50%)	1(50%)	2	0
<i>M. morgani</i>	1(50%)	1(50%)	2	0
<i>P. Rettgeri</i>	2(100%)	0(0%)	2	0
<i>E. aerogenes</i>	1(100%)	0(0%)	1	0
<i>S. typhi</i>	0(0%)	1(100%)	1	0
Total	195(84.4%)	36(15.6%)	231	31(13.4%)

ESBL: Extended-spectrum beta-lactamase; AmpC-BL: AmpC beta-lactamase; MBL: Metallo beta-lactamase

4.3.2. Factors Associated with ESBLs, AmpC-BLs, MBLs, and Carbapenemase

Among 417(28.06%) culture-positive patients 224(53.72%) individuals were infected with gram-negative bacteria of which 189(84.4%) patient isolates showed one or two enzyme producer drug resistance bacteria (Table 15). However, polymicrobial growth and double enzyme producer bacteria, the higher-grade ones were selected for associated factor analysis.

Table 15: Gram-negative bacteria and their drug resistance mechanism from bloodstream infection suspected patients n=224.

Bacteria identified	Non-DRM		ESBL		AmpC-BL		CARBA		MBL		All EP-DRM		Indiv iduals Total
	N	%	N	%	N	%	N	%	N	%	T.	T %	
<i>K. pneumoniae</i>	3	4.2%	43	60.6%	6	8.5%	15	21.1%	4	5.6%	68	95.8%	71
<i>Acinetobacter spp</i>	6	13.0%	2	4.3%	9	19.6%	24	52.2%	5	10.9%	40	87.0%	46
<i>E. coli</i>	9	25.0%	13	36.1%	8	22.2%	4	11.1%	2	5.6%	27	75.0%	36

<i>K. oxytoca</i>	2	7.4%	16	59.3%	4	14.8%	5	18.5%	0	0.0%	25	92.6%	27
<i>E. coli (A-D)</i>	2	18.2%	6	54.5%	1	9.1%	1	9.1%	1	9.1%	9	81.8%	11
<i>C. diversus</i>	3	60.0%	2	40.0%	0	0.0%	0	0.0%	0	0.0%	2	40.0%	5
<i>Pseudomonas spp</i>	1	20.0%	0	0.0%	2	40.0%	2	40.0%	0	0.0%	4	80.0%	5
<i>K. rhinoscleroma</i>	2	50.0%	0	0.0%	0	0.0%	1	25.0%	1	25.0%	2	50.0%	4
<i>S. marcescens</i>	0	0.0%	1	25.0%	1	25.0%	2	50.0%	0	0.0%	4	100.0%	4
<i>K. ozaenae</i>	2	66.7%	0	0.0%	1	33.3%	0	0.0%	0	0.0%	1	33.3%	3
<i>E. cloacae</i>	1	50.0%	0	0.0%	0	0.0%	1	50.0%	0	0.0%	1	50.0%	2
<i>E. agglomerans</i>	1	50.0%	1	50.0%	0	0.0%	0	0.0%	0	0.0%	1	50.0%	2
<i>M. morgani</i>	1	50.0%	0	0.0%	0	0.0%	1	50.0%	0	0.0%	1	50.0%	2
<i>P. mirabilis</i>	1	50.0%	0	0.0%	0	0.0%	1	50.0%	0	0.0%	1	50.0%	2
<i>P. rettgeri</i>	0	0.0%	2	100.0%	0	0.0%	0	0.0%	0	0.0%	2	100.0%	2
<i>E. aerogenes</i>	0	0.0%	0	0.0%	1	100.0%	0	0.0%	0	0.0%	1	100.0%	1
<i>S. typhi</i>	1	100.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	1
Total	35	15.6%	86	38.4%	33	14.7%	57	25.4%	13	5.8%	189	84.4%	224

EP-DRB: enzyme producer drug resistance mechanisms; ESBL: Extended-spectrum beta-lactamase; AmpC-BL: AmpC beta-lactamase; CARBA: Carbapenemase; MBL: Metallo beta-lactamases; N: number

4.3.2.1. Factors Associated with ESBL Producers of Gram-negative Bacteria in Bloodstream Infection

Furthermore, factors associated with ESBLs and AmpC-BLs gram-negative bacteria were analyzed. Among participants of different age groups neonates less than one month, $p < 0.001$, were strongly associated with ESBLs and AmpC-BLs bacterial bloodstream infection. Similar results were found in admission ward B6 (NICU), AOR $p < 0.001$, and admission of the department in NICU, COR $p < 0.001$. Among pediatric wards, D7 (Pediatrics Hematology), AOR $p = 0.044$, and B7 (Pediatrics Medical & Surgery), AOR $p = 0.014$, were also affected by ESBLs and AmpC-BLs producer gram-negative bacteria (Table 16).

Among other factors; ICU admission, COR $p < 0.001$, antibiotic treatment before 10 days of sample collection, COR $p < 0.001$, indwelling medical instrument usage during med care, AOR $p < 0.001$, chronic illness, AOR $p = 0.027$, UTI, AOR $p = 0.001$, RTI, AOR $p = 0.018$, tissue sarcomas, AOR $p = 0.022$, and in neonates' history of incubation, COR $p = 0.006$, had strong associations with ESBLs and AmpC-BLs producer bacterial infections (Table 16).

Table 16: Sociodemographic characteristics of ESBL and AmpC gram-negative bacteria from bloodstream infection suspected patients at TASH n=1486.

Variables	ESBL &/or AmpC-BL		Bivariate and multivariate analysis			
	Negative	Positive	P-value	COR (95%CI)	P-value	AOR (95%CI)
Age of participant						
Birth -1 mth	181(79.04%)	48(20.96%)	<0.001	3.448(1.866-6.371)		
>1mth-<13 yrs	546(93.02%)	41(6.98%)	0.939	0.976(0.528-1.803)		
13-<45 years	445(96.74%)	15(3.26%)	0.028	0.438(0.21-0.914)		
>45 years	195(92.86%)	15(7.14%)	0.000	0.077		
Admission ward						
B6(NICU)	149(76.41%)	46(23.59%)	<0.001	8.297(3.81-18.087)	<0.001	15.15(5.61-40.922)
C/W	336(95.18%)	17(4.82%)	0.482	1.36(0.577-3.206)	0.182	1.81(0.757-4.328)
B4(SICU, MICU)	110(92.44%)	9(7.56%)	0.115	2.199(0.826-5.857)	0.17	2.005(0.743-5.413)
D7(Pedi Hemat)	130(90.28%)	14(9.72%)	0.020	2.894(1.182-7.087)	0.044	2.524(1.025-6.211)
EOPD	210(94.59%)	12(5.41%)	0.358	1.536(0.615-3.833)	0.193	1.848(0.733-4.655)
B7(ped med& surg)	36(87.8%)	5(12.2%)	0.028	3.733(1.156-12.05)	0.014	4.565(1.36-15.286)
C7 (Pedi Medical)	57(91.94%)	5(8.06%)	0.146	2.357(0.743-7.482)	0.121	2.515(0.784-8.068)
D8(Medical)	71(95.95%)	3(4.05%)	0.854	1.136(0.293-4.397)	0.934	1.059(0.272-4.117)
B5(Med & Neurosurg)	53(100%)	0(0%)	0.997		0.997	
Other	215(96.41%)	8(3.59%)	0.000			
Admission Department						
Medical	686(94.1%)	43(5.9%)	0.000	0.063		
Emergency	68(95.77%)	3(4.23%)	0.565	0.704(0.213-2.329)		
MICU	68(93.15%)	5(6.85%)	0.744	1.173(0.45-3.061)		
NICU	138(77.53%)	40(22.47%)	<0.001	4.624(2.897-7.382)		
G. Surgery	43(93.48%)	3(6.52%)	0.862	1.113(0.332-3.734)		
SICU	31(91.18%)	3(8.82%)	0.487	1.544(0.454-5.253)		
Hematology	287(94.1%)	18(5.9%)	0.998	1.001(0.567-1.764)		
Others	46(92%)	4(8%)	0.548	1.387(0.477-4.033)		

ICU admission						
No	1114(94.0%)	71(5.99%)	0.000	0.064		
yes	253(84.1%)	48(15.95%)	<0.001	2.977(2.014-4.4)		
Antibiotic treatment 10 days before sample collection						
No	1268(93.17%)	93(6.83%)	0.000	0.073		
Yes	99(79.2%)	26(20.8%)	<0.001	3.581(2.215-5.79)		
Inserted instrument Usage during medical care						
No	593(95.8%)	26(4.2%)	0.000	0.044		
Yes	774(89.27%)	93(10.73%)	<0.001	2.74(1.751-4.288)	<0.001	2.369(1.476-3.802)
Chronic illness						
No	466(89.1%)	57(10.9%)	0.000	0.122		
Yes	901(93.56%)	62(6.44%)	0.003	0.563(0.386-0.82)	0.027	2.128(1.088-4.162)
Ureteral tract infection						
No	870(94.2%)	54(5.84%)				
Yes	31(79.49%)	8(20.51%)	<0.001	6.324(2.46-16.252)	0.001	4.102(1.778-9.466)
Respiratory tract infection						
No	822(94.2%)	51(5.84%)				
Yes	79(87.78%)	11(12.22%)	0.004	3.322(1.465-7.533)	0.018	2.35(1.156-4.777)
Tissue Sarcomas						
No	791(94.2%)	49(5.83%)	0.000	0.039	.000	0.049
Yes	110(89.4%)	13(10.57%)	0.037	2.778(1.062-7.268)	0.022	2.153(1.116-4.154)
Neonatal incubation						
No	57(91.94%)	5(8.06%)	0.000	0.088		
Yes	125(74.4%)	43(25.6%)	0.006	3.92(1.475-10.424)		

Wards = B6, NICU; C/W, causality; B4, SICU, MICU/PMICU; D7, Pediatrics Hematology; EOPD Emergency outpatient department; B7, pediatrics medical & surgery; C7, Pediatrics Medical; D8, Medical; B5, Medical & Neurosurgery; COR: Crude odds ratio, AOR: Adjusted odds ratio, CI: Confidence interval; Values in bold are statistically significant.

4.3.2.2. Factors associated with carbapenem-resistant gram-negative bacteria

Among all age groups, neonates with less than one month showed an association with Carbapenemase-producing gram-negative bacteria infection on univariate logistic regression, COR p=0.016, but not on multivariate logistic regression analysis. These results were confirmed

by association with the admission ward, B6(NICU) COR $p=0.004$, and the admission department, NICU COR $p<0.001$, findings (Table 17).

Multivariate logistic regression analysis in the department showed that carbapenemase producer carbapenem drug-resistant infections were highly associated with General Surgery AOR $p=0.001$ and SICU, AOR $p=0.007$. Similar results were found in admission wards of General Surgery, B7(Pedi surgical & medical ward), COR $p=0.011$, and SICU B4(SICU/MICU ward), COR $p=0.006$. ICU admission in which MICU, Ped MICU, NICU, and SICU, AOR $p=0.008$, showed strong associations with carbapenem drug-resistant bacterial infection (Table 17).

Third-generation cephalosporin resistance ESBLs and AmpC-BLs producer bacterial infections were more prevalent in neonates, and pediatrics (NICU, Pediatrics Medical and Surgical, and Pediatrics Hematology) but carbapenem Carbapenemase producer gram-negative bacteria was more prevalent in general surgery and SICU for both pediatrics and adult. In addition, Surgical ICU was strongly associated with carbapenem-resistant bacterial infection (Table 17).

Unlike ESBLs and AmpC-BLs producers, Carbapenemase producer gram-negative bacteria bloodstream infection was highly associated with usage of antibiotic treatment ten days before sample collection and length of admission, greater than 3 days, AOR $p=0.023$, and COR, $p=0.003$, respectively. The major factor associated with Carbapenem-resistant bloodstream infection was instrument usage during medical care, AOR $p=0.001$. The instrumentations used were a suction machine, AOR $p=0.040$, an intravenous line, AOR $p=0.045$, and a drainage tube, AOR $p=0.032$ (Table 18).

In the case of chronic illness of WI on the skin, AOR $p=0.030$, and UTI, AOR $p=0.007$, were associated with Carbapenemase producer gram-negative bacteria but in the case of ESBLs and AmpC-BLs producer Chronic illness, UTI, AOR $p=0.001$, RTI, AOR $p=0.018$, and tissue Sarcomas, AOR $p=0.022$, were associated (Table 17).

Regarding neonatal bloodstream infection, mode of delivery, COR $p=0.012$, and premature rupture of membrane, AOR $p=0.036$, were associated with Carbapenemase producer gram-negative bacterial bloodstream infection in case of ESBLs and AmpC-BLs history of incubation, COR $p=0.006$, had a strong association with bloodstream infection (Table 17).

Table 17: Sociodemographic characteristics of Carbapenemase and MBLs producer gram-negative bacteria from bloodstream infection suspected patients at TASH n=1486.

Variables	CPs &/or MBL		Bivariate and multivariate analysis			
	Negative	Positive	P-value	COR (95%CI)	P-value	AOR (95%CI)
Age of participant						
Birth -1 month	208(90.8%)	21(9.17%)	0.016	2.928(1.218-7.04)	0.093	3.277(0.819-13.18)
>1mth-<13 yrs	562(95.7%)	25(4.26%)	0.559	1.29(0.55-3.028)	0.057	2.47(0.972-6.279)
13-<45 years	443(96.3%)	17(3.7%)	0.815	1.113(0.454-2.73)	0.709	1.197(0.466-3.07)
>45 years	203(96.7%)	7(3.33%)	0.009		0.930	
Admission ward						
B6(NICU)	176(90.3%)	19(9.74%)	0.004	3.904(1.526-9.986)		
C/W	344(97.5%)	9(2.55%)	0.918	0.946(0.332-2.696)		
B4(SICU, MICU)	107(89.9%)	12(10.1%)	0.006	4.056(1.482-11.11)		
D7(Pedi Hemat)	139(96.5%)	5(3.47%)	0.669	1.301(0.39-4.344)		
EOPD	219(98.6%)	3(1.35%)	0.325	0.495(0.122-2.006)		
B7(pedi med&sui	36(87.8%)	5(12.2%)	0.011	5.023(1.456-17.31)		
C7(Pedi Medi.)	58(93.5%)	4(6.45%)	0.168	2.494(0.681-9.133)		
D8(Medical)	71(95.9%)	3(4.05%)	0.556	1.528(0.373-6.269)		
B5(Med& Neurosu	49(92.5%)	4(7.55%)	0.103	2.952(0.803-10.86)		
Other	217(97.3%)	6(2.69%)	0.000			
Admission department						
Medical	710(97.4%)	19(2.61%)	0.000		0.019	
Emergency	70(98.6%)	1(1.41%)	0.544	0.534(0.07-4.048)	0.952	1.066(0.134-8.51)
MICU	68(93.2%)	5(6.85%)	0.051	2.748(0.995-7.59)	0.963	0.973(0.308-3.073)
NICU	161(90.4%)	17(9.55%)	<0.001	3.946(2.006-7.76)	0.462	1.549(0.483-4.974)
G. Surgery	38(82.6%)	8(17.39%)	<0.001	7.867(3.237-19.12)	0.001	5.102(1.938-13.44)
SICU	27(79.4%)	7(20.59%)	<0.001	9.688(3.754-25)	0.007	4.787(1.533-14.95)
Hematology	293(96.1%)	12(3.93%)	0.257	1.53(0.734-3.193)	0.576	1.25(0.571-2.738)
Others	49(98%)	1(2%)	0.794	0.763(0.1-5.816)	0.627	0.599(0.076-4.72)
Length of admission						
1-2 days	569(98.4%)	9(1.56%)	0.000			
3-4 days	201(94.4%)	12(5.63%)	0.003	3.774(1.567-9.092)		

>5days	646(92.9%)	49(7.05%)	<0.001	4.795(2.335-9.849)		
ICU admission						
No	1150(97%)	35(2.95%)	0.000	0.03		
yes	266(88.4%)	35(11.63%)	0.000	4.323(2.656-7.037)	0.008	2.611(1.288-5.292)
Antibiotic treatment 10 days before sample collection						
No	667(97.5%)	17(2.49%)				
Yes	749(93.4%)	53(6.61%)	0.000	0.338(0.193-0.592)	0.023	2.045(1.104-3.787)
Instrument usage during medical care						
No	613(99%)	6(0.97%)	0.000	0.01		
Yes	803(92.6%)	64(7.38%)	<0.001	8.143(3.503-18.93)	0.001	4.297(1.773-10.41)
Using suction machine						
No	696(93.3%)	50(6.7%)				
Yes	107(88.4%)	14(11.57%)	0.071	2.285(0.932-5.602)	0.040	1.974(1.032-3.772)
Using intravenous line						
No	82(96.5%)	3(3.53%)				
Yes	721(92.2%)	61(7.8%)	0.036	3.812(1.091-13.32)	0.045	3.502(1.03-11.94)
Using drainage tube						
No	778(93%)	59(7.05%)				
Yes	25(83.3%)	5(16.67%)	0.030	3.296(1.122-9.678)	0.032	3.22(1.108-9.36)
Wound infection on skin						
No	907(96.4%)	34(3.61%)				
Yes	18(81.8%)	4(18.18%)	0.102	2.949(0.81-10.775)	0.030	3.826(1.14-12.883)
Ureteral tract infection						
No	892(96.5%)	32(3.46%)				
Yes	33(84.6%)	6(15.38%)	0.017	3.678(1.261-10.73)	0.007	3.887(1.437-10.52)
Mode of delivery in neonates						
NVD	156(94%)	10(6.02%)	0.000	0.064		
C/S	53(82.8%)	11(17.19%)	0.012	3.238(1.302-8.054)		
Premature rupture of membrane						
>18 hours	44(97.8%)	1(2.22%)	0.006		0.027	
<18 hours	146(91.8%)	13(8.18%)	0.194	3.918(0.498-30.79)	0.327	2.872(0.348-23.68)
No rupture	19(73.1%)	7(26.92%)	0.012	16.211(1.86-141.0)	0.036	10.94(1.166-102.6)

Wards = B6, NICU; C/W, casualty; B4, SICU, MICU/PMICU; D7, Pediatrics Hematology; EOPD Emergency outpatient department; B7, pediatrics medical & surgery; C7, Pediatrics Medical; D8, Medical; B5, Medical & Neurosurgery. NCPR: not carbapenem resistance; CPs: carbapenemase; MBLs: Metallo beta-lactamase;

4.3.2.3. Factors associated with all drug resistance types

Regarding age groups, neonates were 5 times $p < 0.001$, acquired gram-negative bacteria having all types of enzyme producer drug resistance bacteria than all other age groups. This result was confirmed by the association ESBLs, AmpC-BLs, MBLs, and Carbapenemase producer gram-negative bacteria bloodstream infection in the admission ward, COR B6(NICU) $p < 0.001$, and admission department, AOR NICU $p = 0.03$ (Table 18; Figure 17).

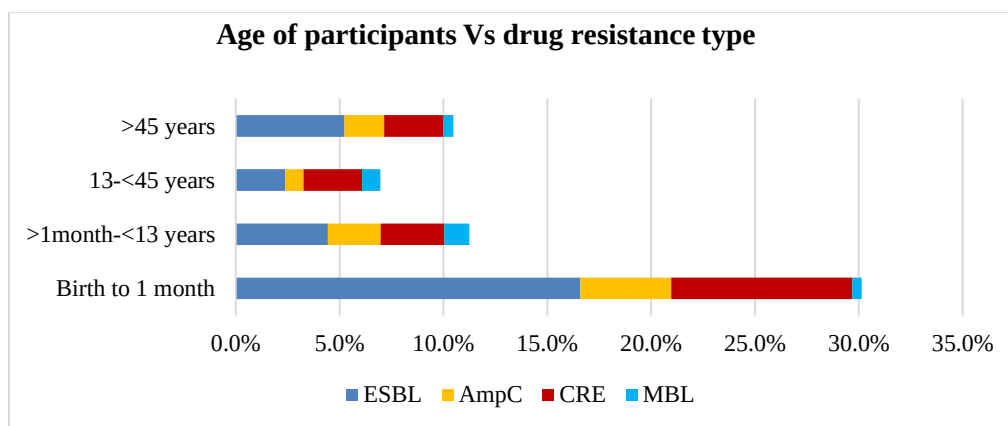
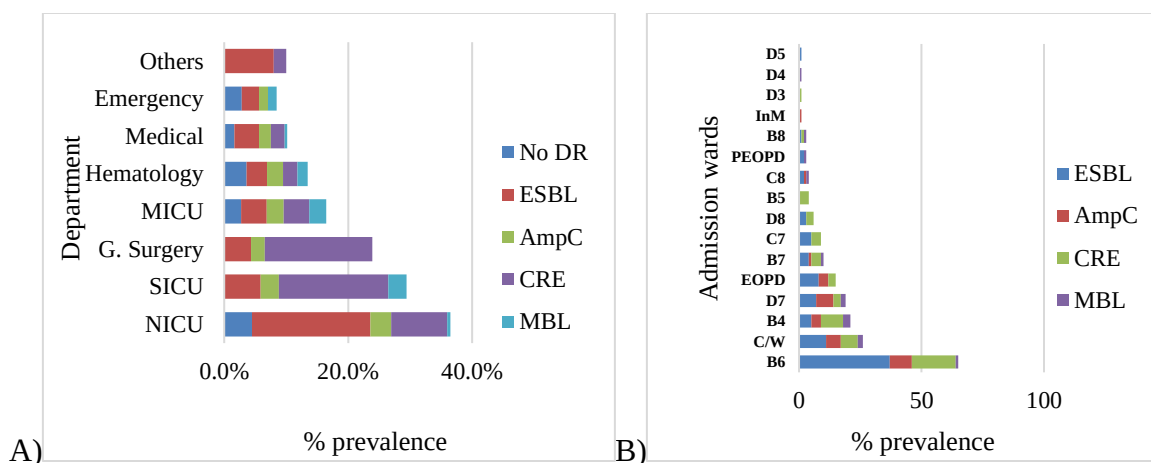


Figure 17: Age of participants concerning all drug resistance mechanisms

Multivariate logistic regression analysis showed patients of NICU, SICU, and General surgery (Figure 18a) and wards B6(NICU) $p < 0.001$, B4 (SICU and MICU) $p = 0.001$, D7(Pedi hematology) $p = 0.027$, B7 (Pedi med & Surg) $p = 0.001$, C7(Pedi. medical) $p = 0.041$ were infected by all enzyme producer drug resistance bacteria of bloodstream infection (Figure 18b). Again, admission in ICU $p < 0.001$ had a four-fold chance to acquire all ESBLs, AmpC-BLs, Carbapenemase, and MBLs drug-resistant types (Table 18).



B6: NICU; C/W: Causality; B4: pediatrics & adult SICU, MICU; D7: Pedi Hematology; EOPD: Adult emergency; B7: pediatrics medical & surgical; C7: Pediatrics Medical; D8: Medical; B5: Medical & Neurosurgery; C8: Hematology; PEOPD: Pediatrics emergency; B8: Medical, cardiac and endocrine; InM: Intermediate; D3: Uro-surgery; D4: Cardiothoracic surgery & ENT; D5: Neuro and general Surgery.

Figure 18: a) Department with all drug resistance mechanisms and b) Ward with all drug resistance mechanisms

Admission for greater than 5 days was one of the factors in acquiring all types of enzyme-activated drug-resistant bacteria in bloodstream infection. Patients who were taking antibiotics ten days before sample collection, $p=0.033$, showed an association with acquiring enzyme-produced drug resistance bacteria in bloodstream infection. Supportive instrumentation usage during medical care led to three-fold exposure than those who did not use it. Using a urinary catheter, IV line, and drainage tube showed twice the chance of acquiring enzyme-produced drug resistance bacterial bloodstream infection compared to non-users. Having chronic illness showed greater than two-fold exposure to enzyme-produced drug-resistant bacteria. Among chronic illnesses, UTI and RTI had five-fold and two-fold chances of acquiring enzyme-produced drug-resistant bacteria (Table 18).

Again, bivariate logistic regression analysis in neonates delivered by cesarean section (C/S) showed a high prevalence of all enzyme producer drug resistance bacteria. The bivariate and multivariate logistic regression analysis showed that neonates who were admitted to the NICU

incubation room, $p=0.002$, had five-fold exposure than other patients by all enzyme producer drug resistance bacteria (Table 18).

Table 18: Factors associated with all drug resistance types (ESBL, AmpC, MBL, and Carbapenemase) prevalence infection crude and adjusted odds ratio of bloodstream infection suspected patients at TASH $n=1486$.

Variables	Drug resistance types		Bivariate and multivariate analysis			
	Positive	Negative	P-value	COR (95%CI)	P-value	AOR (95%CI)
Age of participant						
Birth - 1mth	69(30.1%)	160(69.9%)	<0.001	3.685(2.182-6.224)	<0.001	4.824(2.019-11.53)
>1mth-<13ys	66(11.2%)	521(88.8%)	0.761	1.083(0.65-1.804)	0.14	1.508(0.873-2.606)
13-<45 yrs	32(7.0%)	428(93.0%)	0.123	0.639(0.362-1.129)	0.195	0.675(0.373-1.223)
>45 years	22(10.5%)	188(89.5%)	0		0	
Admission Ward						
B6(NICU)	65(33.3%)	130(66.7%)	<0.001	7.464(4.025-13.84)		
C/W	26(7.4%)	327(92.6%)	0.617	1.187(0.606-2.325)		
B4(SICU, MICU)	21(17.6%)	98(82.4%)	0.001	3.199(1.561-6.556)		
D7(Pedi Hemat)	19(13.2%)	125(86.8%)	0.027	2.269(1.099-4.685)		
EOPD	15(6.8%)	207(93.2%)	0.838	1.082(0.509-2.298)		
B7(Ped med & Sur)	10(24.4%)	31(75.6%)	0.001	4.816(1.968-11.78)		
C7(Pedi Medical)	9(14.5%)	53(85.5%)	0.041	2.535(1.041-6.173)		
D8(Medical)	6(8.1%)	68(91.9%)	0.587	1.317(0.487-3.562)		
B5(Med & Neurosur)	4(7.5%)	49(92.5%)	0.737	1.219(0.384-3.864)		
Other	14(6.3%)	209(93.7%)	0			
Admission Department						
Medical	62(8.5%)	667(91.5%)	0	0.093	0.003	
Emergency	4(5.6%)	67(94.4%)	0.405	0.642(0.227-1.82)	0.999	1.001(0.34-2.947)
MICU	10(13.7%)	63(86.3%)	0.143	1.708(0.834-3.495)	0.508	1.286(0.612-2.702)
NICU	57(32%)	121(68%)	<0.001	5.068(3.368-7.625)	0.03	2.182(1.08-4.407)
G. Surgery	11(23.9%)	35(76.1%)	0.001	3.381(1.636-6.986)	0.009	2.879(1.305-6.355)
SICU	10(29.4%)	24(70.6%)	<0.001	4.483(2.05-9.801)	<0.001	4.884(2.08-11.468)
Hematology	30(9.8%)	275(90.2%)	0.493	1.174(0.742-1.855)	0.807	0.94(0.575-1.537)
Others	5(10%)	45(90%)	0.716	1.195(0.458-3.122)	0.908	1.06(0.393-2.863)
Length of admission						
1-2 days	55(9.5%)	523(90.5%)	0	0.105		
3-4 days	26(12.2%)	187(87.8%)	0.269	1.322(0.806-2.17)		
>5days	108(15.5%)	587(84.5%)	0.002	1.75(1.239-2.471)		
ICU admission						
No	106(8.9%)	1079(91.1%)	0	0.098		
yes	83(27.6%)	218(72.4%)	<0.001	3.876(2.809-5.348)		
Antibiotic treatment 10 days before sample collection						
No	67(9.8%)	617(90.2%)	0	0.109		

Yes	122(15.2%)	680(84.8%)	0.002	1.652(1.203-2.269)	0.033	1.501(1.034-2.178)
Instrumentation usage during medical care						
No	32(5.2%)	587(94.8%)	0	0.055		
Yes	157(18.1%)	710(81.9%)	<0.001	4.056(2.731-6.024)	<0.001	2.917(1.898-4.483)
History of using a urinary catheter						
No	138(17.5%)	651(82.5%)	0	0.212		
Yes	19(24.4%)	59(75.6%)	0.135	1.519(0.878-2.63)	0.047	1.8(1.008-3.212)
History of using an IV line						
No	9(10.6%)	76(89.4%)	0	0.118		
Yes	148(18.9%)	634(81.1%)	0.062	1.971(0.966-4.024)	0.011	2.674(1.25-5.719)
History of using a drainage tube						
No	148(17.7%)	689(82.3%)	0	0.215		
Yes	9(30%)	21(70%)	0.091	1.995(0.896-4.444)	0.042	2.406(1.034-5.598)
Chronic illness						
No	89(17%)	434(83%)	0	0.205		
Yes	100(10.4%)	863(89.6%)	<0.001	0.565(0.415-0.769)	0.009	2.003(1.187-3.381)
Ureteral tract infection						
No	86(9.3%)	838(90.7%)	0	0.103		
Yes	14(35.9%)	25(64.1%)	<0.001	5.457(2.74-10.889)	<0.001	5.646(2.595-12.28)
Respiratory tract infection						
No	85(9.7%)	788(90.3%)	0	0.108		
Yes	15(16.7%)	75(83.3%)	0.043	1.854(1.02-3.371)	0.023	2.269(1.119-4.602)
Neonates' history of incubation						
No	10(16.1%)	52(83.9%)	0	0.192		
Yes	59(35.1%)	109(64.9%)	0.007	2.815(1.333-5.942)	0.002	4.855(1.766-13.35)
Mode of delivery						
NVD	42(25.3%)	124(74.7%)	0	0.339		
C/S	27(42.2%)	37(57.8%)	0.013	2.154(1.174-3.954)	0.673	1.187(0.536-2.629)

Wards = B6, NICU; C/W, causality; B4, SICU, MICU/PMICU; D7, Pediatrics Hematology; EOPD Emergency outpatient department; B7, pediatrics medical & surgery; C7, Pediatrics Medical; D8, Medical; B5, Medical & Neurosurgery; Values in bold are statistically significant.

4.4. PCR Based Molecular Characterization

4.4.1. PCR-Based Detection of Antimicrobial Resistance

Among 231 gram-negative bacteria tested for combination drugs, 195 (84.4%) had one or more drug resistance mechanisms, while 36 (15.6%) did not. However, in PCR confirmation 176(76.2%) showed drug resistance mechanisms and 55(23.8%) did not. The 19(9.7%) phenotypically confirmed drug-resistant mechanisms were negative for PCR confirmation (Table 14). Among gram-negative bacteria the majority were *K. pneumoniae*, 75(32.5%), *Acinetobacter* spp, 47(20.3%), *E. coli*, 38(16.5%), *K. oxytoca*, 27(16.7%), *E. coli* (A-D), 11(4.8%), and

Pseudomonas spp, 5(2.2%) (Table 19). Among 195 having drug-resistant mechanisms *K. pneumoniae*, 70(93.3%), *K. oxytoca*, 23(85.2%), and *E. coli* (A-D), 9 (81.8%) were the dominant species (Table 19).

Table 19: Phenotyping versus molecular characterization of gram-negative bacteria from bloodstream infection n=231.

Bacteria identified	Phenotyping confirmed drug resistance Mechanisms			PCR confirmed drug resistance mechanisms		
	No DRT	DRT All	Total	No PDRT	PDRT All	Total
	N(%)	N(%)	N	N(%)	N(%)	Count
<i>K. pneumoniae</i>	3(4%)	72(96%)	75	5(6.7%)	70(93.3%)	75
<i>Acinetobacter spp</i>	6(12.8%)	41(87.2%)	47	15(31.9%)	32(68.1%)	47
<i>E. coli</i>	10(26.3%)	28(73.7%)	38	14(36.8%)	24(63.2%)	38
<i>K. oxytoca</i>	2(7.4%)	25(92.6%)	27	4(14.8%)	23(85.2%)	27
<i>E. coli</i> (A-D)	2(18.2%)	9(81.8%)	11	2(18.2%)	9(81.8%)	11
<i>C. diversus</i>	3(60%)	2(40%)	5	3(60%)	2(40%)	5
<i>Pseudomonas spp</i>	1(20%)	4(80%)	5	2(40%)	3(60%)	5
<i>K. rhinoscleromatis</i>	2(50%)	2(50%)	4	2(50%)	2(50%)	4
<i>S. marcescens</i>	0(0%)	4(100%)	4	1(25%)	3(75%)	4
<i>K. ozaenae</i>	2(66.7%)	1(33.3%)	3	2(66.7%)	1(33.3%)	3
<i>E. cloacae</i>	1(50%)	1(50%)	2	1(50%)	1(50%)	2
<i>E. agglomerans</i>	1(50%)	1(50%)	2	1(50%)	1(50%)	2
<i>M. morgani</i>	1(50%)	1(50%)	2	1(50%)	1(50%)	2
<i>P. mirabilis</i>	1(50%)	1(50%)	2	1(50%)	1(50%)	2
<i>P. rettgeri</i>	0(0%)	2(100%)	2	0(0%)	2(100%)	2
<i>E. aerogenes</i>	0(0%)	1(100%)	1	0(0%)	1(100%)	1
<i>S. typhi</i>	1(100%)	0(0%)	1	1(100%)	0(0%)	1
Total	36(15.6%)	195(84.4%)	231	55(23.8%)	176(76.2%)	231

4.4.2. Molecular Characterization of AMR Genes in Gram-Negative Bacteria

Among 195 (84.4%) enzyme-producing drug resistance mechanisms 176(76.2%) were PCR-confirmed drug resistance enzyme-producing bacterial strains, and 415 drug-resistance encoding genes were identified. Among PCR-confirmed drug resistance mechanisms encoding genes, ESBL were the highest followed by MBLs, Carbapenemase, and AmpC producers which accounted for 122 (52.8%), 50 (21.6%), 46 (19.9%), and 22(9.5%), respectively (Table 20). The number of AST-confirmed drug resistance mechanisms was reduced by PCR-confirmed drug resistance

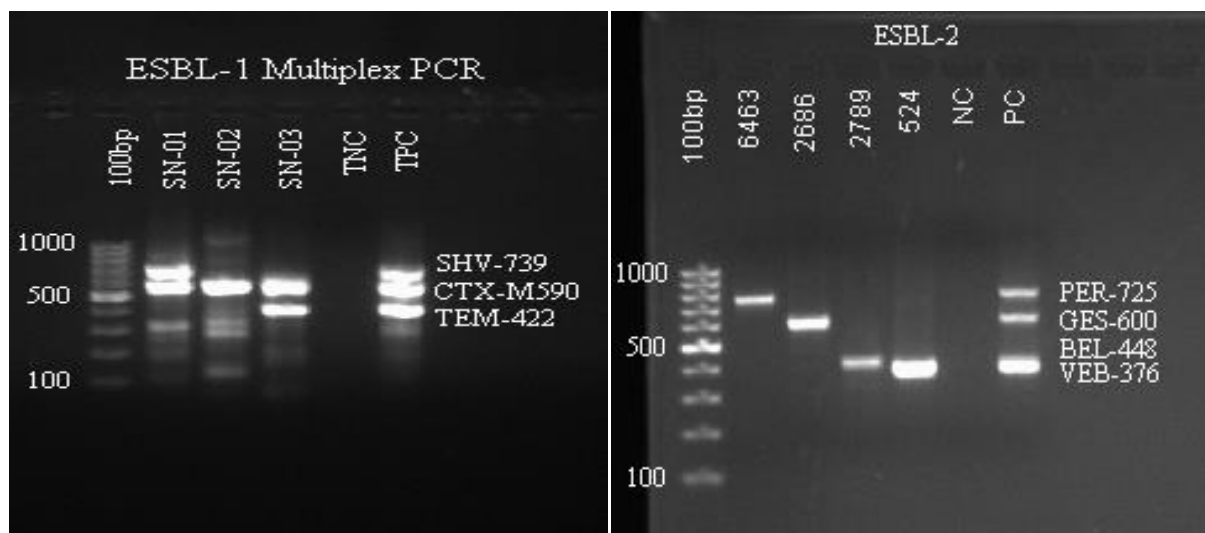
mechanisms encoding gene from ESBL 122(52.8%) to 122 (52.8%), AmpC 33(14.3%) to 22(9.5%), and Carbapenemase with MBLs producer 71(30.7%) reduced to 69(29.9%) (Table 20).

Table 20: PCR-confirmed drug resistance types in Gram-negative isolates n=231.

Bacteria identified	Total	PCR Positive	PCR Negative	ESBL	AmpC	Carbapenemase	MBLs
	f	N&%	N&%	N&%	N&%	N&%	N&%
<i>K. pneumoniae</i>	75	70(93.3%)	5(6.7%)	56(74.7%)	7(9.3%)	12(16%)	13(17.3%)
<i>Acinetobacter spp</i>	47	32(68.1%)	15(31.9%)	8(17%)	2(4.3%)	20(42.6%)	22(46.8%)
<i>E. coli</i>	38	24(63.2%)	14(36.8%)	19(50%)	9(23.7%)	3(7.9%)	5(13.2%)
<i>K. oxytoca</i>	27	23(85.2%)	4(14.8%)	21(77.7%)	2(7.4%)	3(11.1%)	1(3.7%)
<i>E. coli (A-D)</i>	11	9(81.8%)	2(18.2%)	7(63.6%)	0(0%)	1(9.1%)	2(18.2%)
<i>C. diversus</i>	5	2(40%)	3(60%)	2(40%)	0(0%)	0(0%)	0(0%)
<i>Pseudomonas spp</i>	5	3(60%)	2(40%)	2(40%)	0(0%)	0(0%)	2(40%)
<i>K. rhinoscleroma</i>	4	2(50%)	2(50%)	0(0%)	0(0%)	2(50%)	1(25%)
<i>S. marcescens</i>	4	3(75%)	1(25%)	1(25%)	1(25%)	1(25%)	2(50%)
<i>K. ozaenae</i>	3	1(33.3%)	2(66.7%)	1(33.3%)	1(33.3%)	0(0%)	0(0%)
<i>E. cloacae</i>	2	1(50%)	1(50%)	0(0%)	0(0%)	1(50%)	1(50%)
<i>E. agglomerans</i>	2	1(50%)	1(50%)	1(50%)	0(0%)	0(0%)	0(0%)
<i>M. morgani</i>	2	1(50%)	1(50%)	0(0%)	0(0%)	1(50%)	1(50%)
<i>P. mirabilis</i>	2	1(50%)	1(50%)	1(50%)	0(0%)	1(50%)	0(0%)
<i>P. rettgeri</i>	2	2(100%)	0(0%)	2(100%)	0(0%)	0(0%)	0(0%)
<i>E. aerogenes</i>	1	1(100%)	0(0%)	1(100%)	0(0%)	1(100%)	0(0%)
<i>S. typhi</i>	1	0(0%)	1(100%)	0(0%)	0(0%)	0(0%)	0(0%)
Total	231	176(76.2%)	55(23.8%)	122(52.8%)	22(9.5%)	46(19.9%)	50(21.6%)

4.4.2.1. ESBL Encoding Gene Identification

ESBL encoding gene identification was done by using multiplex one primer of SHV 739bp, CTX-M 590bp, and TEM 422 bp and multiplex two primers of PER 725 bp, GES 600bp, BEL 448bp, and VEB 376 bp (Figure 19).



a. ESBL multiplex PCR 1, Major ESBL for the detection of *blaSHV*, *blaCTXM*, and *blaTEM* genes from 3 patient samples with TNC-negative and TPC-positive control

b. ESBL multiplex PCR 2, Minor ESBL for the detection of *blaPER*, *blaGES*, *blaBEL*, and *blaVEB* genes from 4 patient samples with NC-negative and PC-positive control

Figure 19: Multiplex PCR for the detection of genes encoding ESBL 1 (left) and ESBL 2 (right)

Among 122 (52.8%) ESBL encoding genes, multiplex one *blaCTX-M*, *blaTEM*, and multiplex two *blaBEL* were the top three drug-resistant genes accounted for 104(78.8%), 97(73.5%), and 21(15.9%), respectively (Figure 20).

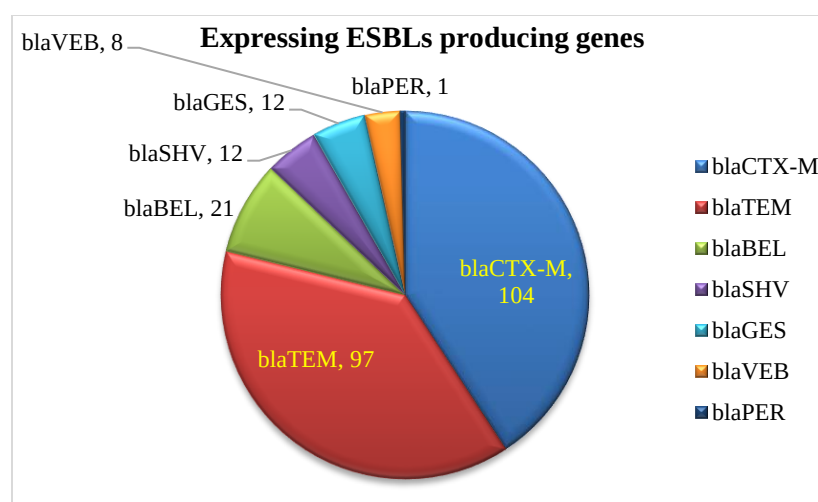


Figure 20: ESBL gene prevalence among 132 phenotypic confirmed gram-negative bacteria

Eshecrchia cloacae were positive for ESBL confirmatory E-test and do not express the ESBL gene. *bla*CTX-M was found in 10 bacteria and *bla*PER was found in *E. coli* only. The rest were found in the range of 5-9 bacteria. Among 17 strains four gram-negative bacteria s, *K. rhinoscleromatis*, *M. morganii*, *P. rettgeri*, and *S. typhi* were negative for AST confirmatory and did not express ESBL-producing genes in the PCR test. ESBL-producing bacteria having the highest drug-resistance genes were *K. pneumoniae*, *K. oxytoca*, *E. coli* (A-D), and *E. coli* accounting for 56 (74.7%), 22(81.5%), 7(63.6%), and 19(50%) n>5 respectively (Table 21).

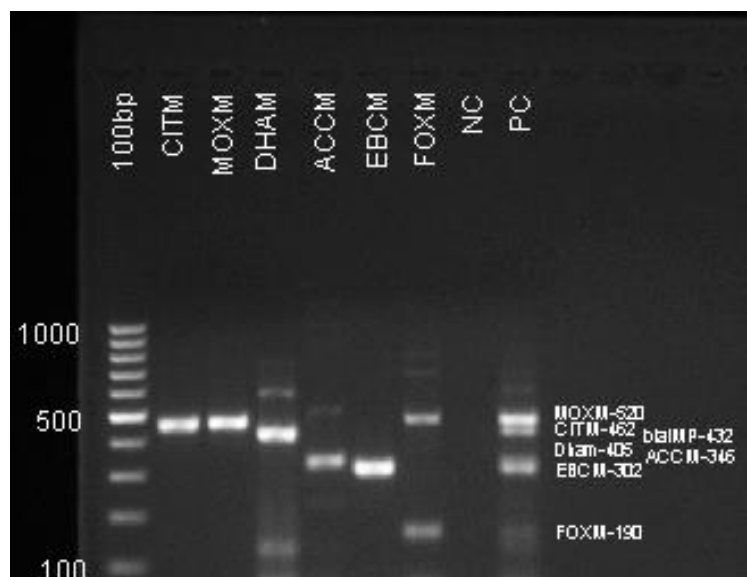
Table 21: PCR identification of ESBLs producer genes from suspected bloodstream infection bacteria n=231.

Bacteria identified	ESBLs gene positive among 231 gram-negative bacteria								PCR conf. ESBL
	<i>bla</i> TEM		<i>bla</i> CTX-M	<i>bla</i> SHV	<i>bla</i> VEB	<i>bla</i> BEL	<i>bla</i> GES	<i>bla</i> PER	
	N	N&%	N&%	N&%	N&%	N&%	N&%	N&%	N&%
<i>K. pneumoniae</i>	75	51(91.1%)	51(91.1%)	8(14.3%)	2(3.6%)	6(10.7%)	4(7.1%)	0(0%)	56(74.7%)
<i>K. oxytoca</i>	27	20(87%)	20(87%)	1(4.3%)	0(0%)	2(8.7%)	1(4.3%)	0(0%)	22(81.5%)
<i>E. coli</i>	38	11(50%)	16(72.7%)	0(0%)	1(4.5%)	5(22.7%)	5(22.7%)	1(4.5%)	19(50%)
<i>Acinetobacter spp</i>	47	0(0%)	2(16.7%)	0(0%)	2(16.7%)	5(41.7%)	1(8.3%)	0(0%)	8(17%)
<i>E. coli</i> (A-D)	11	7(100%)	7(100%)	0(0%)	1(14.3%)	2(28.6%)	0(0%)	0(0%)	7(63.6%)
<i>Pseudomonas spp</i>	5	1(50%)	1(50%)	1(50%)	0(0%)	1(50%)	1(50%)	0(0%)	2(40%)
<i>S. marcescens</i>	4	1(50%)	1(50%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(25%)
<i>E. aerogenes</i>	1	0(0%)	0(0%)	0(0%)	1(100%)	0(0%)	0(0%)	0(0%)	1(100%)
<i>E. agglomerans</i>	2	1(100%)	1(100%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(50%)
<i>P. mirabilis</i>	2	0(0%)	1(100%)	1(100%)	0(0%)	0(0%)	0(0%)	0(0%)	1(50%)
<i>K. ozaenae</i>	3	1(100%)	0(0%)	1(100%)	0(0%)	0(0%)	0(0%)	0(0%)	1(33.3%)
<i>E. cloacae</i>	2	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
<i>C. diversus</i>	5	2(100%)	2(100%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	2(40%)
<i>Others</i>	9	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Total	231	97(73.5%)	104(78.8%)	12(9.1%)	8(6.1%)	21(15.9%)	12(9.1%)	1(0.8%)	123(53.2%)

Other = *K. rhinoscleromatis*, *M. morganii*, *P. rettgeri*, and *S. typhi*; ESBL extended-spectrum beta-lactamase; PCR = polymerase chain reaction.

4.4.2.2. AmpC-BLs Encoding Gene Identification

AmpC-BL encoding gene identification was done by multiplex three primers of MOXM 520bp, CITM 462bp, and DHAM 405 bp, ACCM 346 bp, EBCM 302bp, and FOXM 190bp by using negative and positive control (Figure 21).



ESBL Multiplex PCR 3, AmpC-BLs gene detection of *bla*MOXM, *bla*CITM, *bla*DHAM, *bla*ACCM, *bla*EBCM, and *bla*FOXM genes of 6 patient samples with NC-negative and PC-positive control

Figure 21: Multiplex PCR for the detection of genes encoding AmpC

Six drug resistance genes of AmpC beta-lactamases were analyzed, *bla*FOXM, *bla*CITM, *bla*MOXM, *bla*EBCM, *bla*ACCM, and *bla*DHAM accounted for 13(36.10%), 12(33.30%), 5(13.90%), 5 (13.90%), 3(8.30%), and 1(2.80%) respectively (Figure 22).

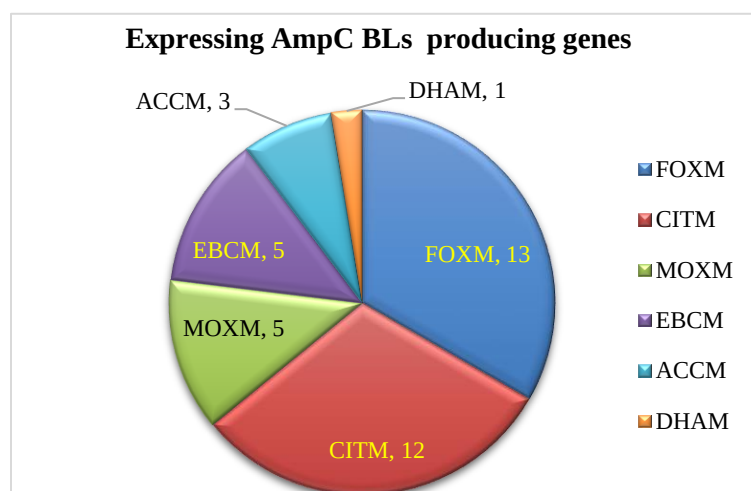


Figure 22: AmpC-BLs gene prevalence among 36 phenotypic confirmed gram-negative bacteria

Six strains of gram-negative bacteria were identified to have AmpC BLs-producing genes, and the highest prevalence of drug resistance genes was identified in *E. coli* and *K. pneumoniae* accounting for 9(23.7%) and 7(9.3%), n>5 respectively (Table 22). *Pseudomonas spp* were positive for AmpC confirmatory E-test (Table 13) and do not express AmpC-BLs genes (Table 23). The rest 10 species of *E. coli* (A-D), *C. diversus*, *P. rettgeri*, *E. aerogenes*, *P. mirabilis*, *E. agglomerans*, *K. rhinoscleromatis*, *E. cloacae*, *M. morgani*, *S. typhi* were negative for AmpC E-test and PCR AmpC encoding genes.

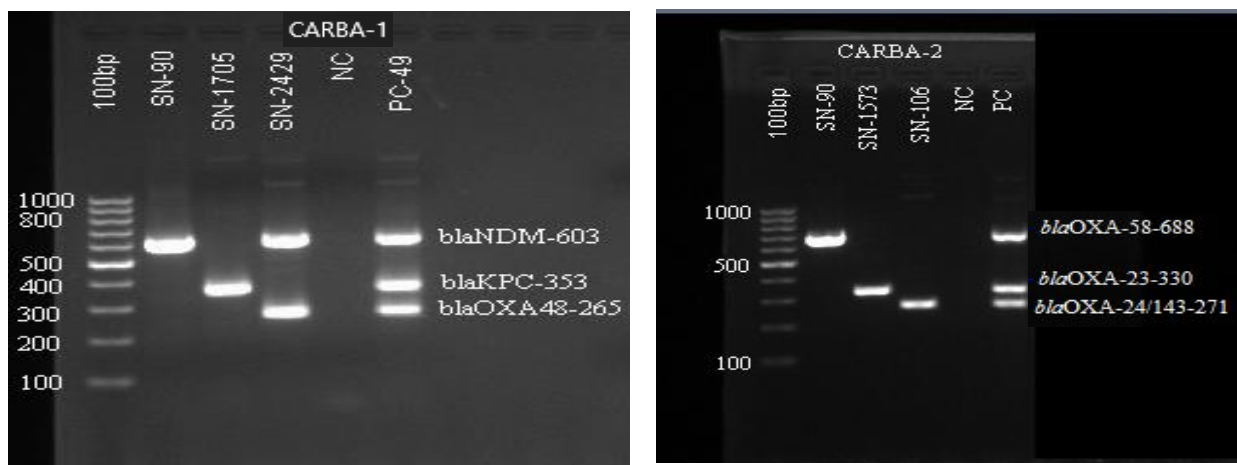
Table 22: PCR identification of AmpC-BLs producer genes from suspected bloodstream infection bacteria n=231.

Bacteria identified	AmpC BLsgene positive							PCR AmpC
		<i>bla</i> MOXM	<i>bla</i> CITM	<i>bla</i> DHAM	<i>bla</i> CCM	<i>bla</i> EBCM	<i>bla</i> FOXM	
	N	N&%	N&%	N&%	N&%	N&%	N&%	N&%
<i>K. pneumoniae</i>	75	1(10%)	4(40%)	0(0%)	0(0%)	2(20%)	5(50%)	7(9.3%)
<i>E. coli</i>	38	1(10%)	7(70%)	1(10%)	1(10%)	1(10%)	6(60%)	9(23.7%)
<i>Acinetobacter spp</i>	47	1(12.5%)	1(12.5%)	0(0%)	0(0%)	0(0%)	1(12.5%)	2(4.3%)
<i>K. oxytoca</i>	27	1(25%)	0(0%)	0(0%)	2(50%)	0(0%)	1(25%)	2(7.4%)
<i>Pseudomonas spp</i>	5	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
<i>S. marcescens</i>	4	0(0%)	0(0%)	0(0%)	0(0%)	1(100%)	0(0%)	1(25%)
<i>K. ozaenae</i>	3	1(100%)	0(0%)	0(0%)	0(0%)	1(100%)	0(0%)	1(33.3%)
<i>Other</i>	32	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Total	231	5(13.9%)	12(33.3%)	1(2.8%)	3(8.3%)	5(13.9%)	13(36.1%)	22(9.5%)

AST conf. = AST confirmatory test AmpC-BLs AmpC beta-lactamase; PCR = polymerase chain reaction.

4.4.2.3. Carbapenemase encoding gene identification

Carbapenem encoding gene identification was done on multiplex CARBA one *bla*KPC 353 bp, *bla*OXA-48 265 bp, *bla*OXA-23 330bp, *bla*OXA -24/143 271 bp, and *bla*OXA -58 688bp by using negative and positive control (Figure 23).



- a. CARBA Multiplex PCR 1, Carbapenemase gene detection of *bla*NDM, *bla*KPC, and *bla*OXA-48, genes of 3 patient samples with NC-negative and PC-positive control
- b. CARBA Multiplex PCR 2, Oxacillinase gene detection of *bla*OXA-58, *bla*OXA-23, and *bla*OXA24/143, genes of 3 patient samples with NC-negative and PC-positive control

Figure 23: Multiplex PCR CARBA 1 (left) and Multiplex PCR CARBA 2 (right)

Three multiplexes were run to amplify Carbapenemase and MBLs-producing genes. However, *bla*VIM and *bla*IMP were analyzed separately with different primers. For carbapenemase detection, *bla*KPC and *bla*OXA48 results were taken from the 1st Carbapenem multiplex 1 (Figure22a), and the OXA family was taken from multiplex 2 (Figure22b). Among carbapenemase genes *bla*OXA-23 were the highest 28(40.6%) followed by *bla*KPC accounted 10(14.5%) (Figure 24).

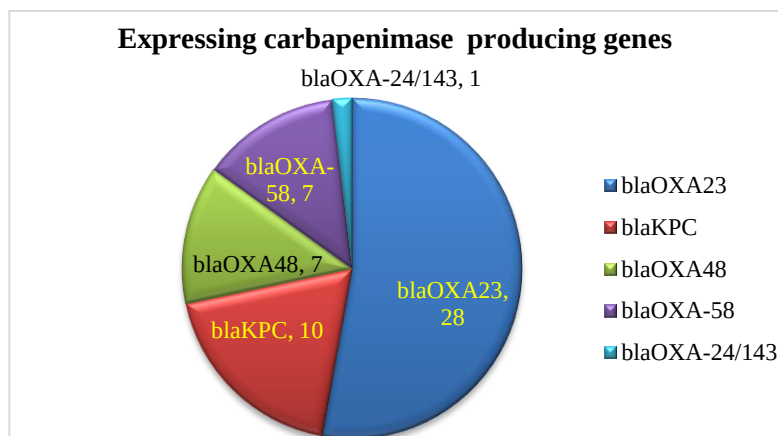


Figure 24: Carbapenemase encoding gene prevalence among 69 phenotypic confirmed gram-negative bacteria

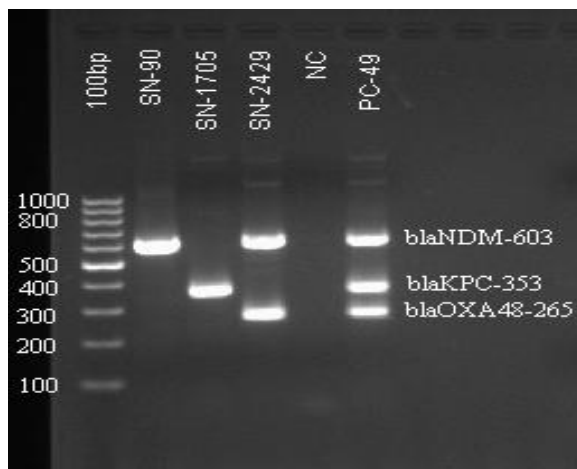
Among all gram-negative bacteria, Carbapenemase was 46(19.9%), and the highest prevalence was recorded in *Acinetobacter spp* 20(42.6%) and *K. pneumoniae* 12(16.0%). Among carbapenemase genes, *blaOXA-23* 28(40.6%) was the highest prevalent followed by *blaKPC* 10(14.5%) (Table 23).

Table 23: PCR identification of carbapenemase producer genes from suspected bloodstream infection bacteria n=231.

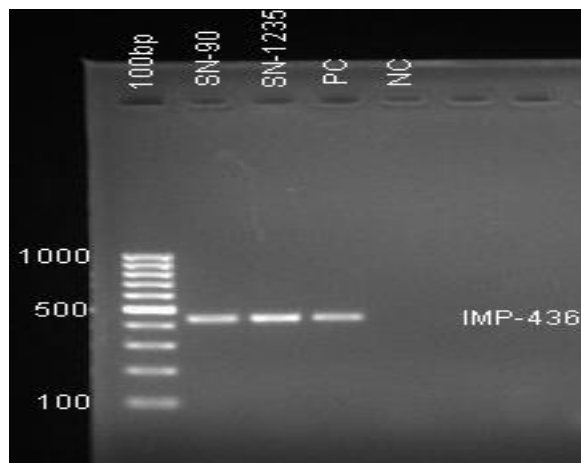
Bacteria identified	Carbapenemase gene positive						PCR Carbapenemase
	<i>blaKPC</i>		<i>blaOXA-48</i>	<i>blaOXA-23</i>	<i>blaOXA-24/143</i>	<i>blaOXA-58</i>	
	N	N&%	N&%	N&%	N&%	N&%	N&%
<i>Acinetobacter spp</i>	47	1(3.4%)	2(6.9%)	16(55.2%)	1(3.4%)	4(13.8%)	20(42.6%)
<i>K. pneumoniae</i>	75	6(33.3%)	1(5.6%)	5(27.8%)	0(0%)	0(0%)	12(16%)
<i>E. coli</i>	38	1(16.7%)	0(0%)	1(16.7%)	0(0%)	1(16.7%)	3(7.9%)
<i>K. oxytoca</i>	27	2(66.7%)	1(33.3%)	1(33.3%)	0(0%)	0(0%)	3(11.1%)
<i>Pseudomonas spp</i>	5	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
<i>E. coli (A-D)</i>	11	0(0%)	1(50%)	1(50%)	0(0%)	0(0%)	1(9.1%)
<i>S. marcescens</i>	4	0(0%)	0(0%)	1(50%)	0(0%)	0(0%)	1(25%)
<i>K. rhinoscleromatis</i>	4	0(0%)	0(0%)	1(50%)	0(0%)	1(50%)	2(50%)
<i>E. aerogenes</i>	1	0(0%)	1(100%)	0(0%)	0(0%)	0(0%)	1(100%)
<i>P. mirabilis</i>	2	0(0%)	1(100%)	1(100%)	0(0%)	0(0%)	1(50%)
<i>E. cloacae</i>	2	0(0%)	0(0%)	1(100%)	0(0%)	0(0%)	1(50%)
<i>M. morganii</i>	2	0(0%)	0(0%)	0(0%)	0(0%)	1(100%)	1(50%)
<i>Others</i>	13	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Total	231	10(14.5%)	7(10.1%)	28(40.6%)	1(1.4%)	7(10.1%)	46(19.9%)

4.4.2.4. MBLs encoding gene identification

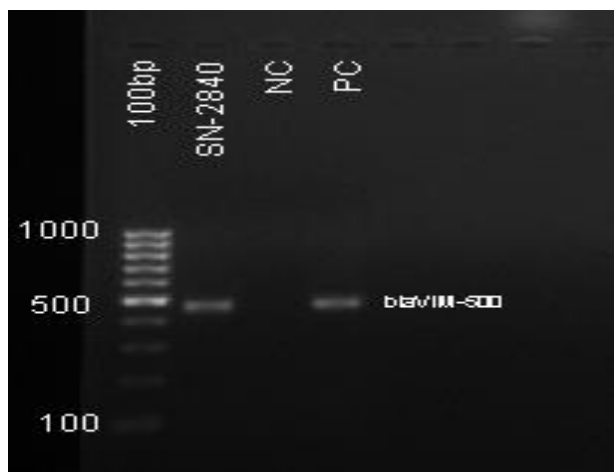
Metallo-beta lactamase encoding genes were detected by Multiplex CARBA-1 *blaNDM* 603bp, *blaIMP* 436bp, *blaVIM* 500bp primers, and from minor groups multiplex CARBA-3 were *blaDIM* 699 bp, *blaSIM* 570 bp, *blaGIM* 477 bp, and *blaAIM* 322 bp primers were used (Figure 25).



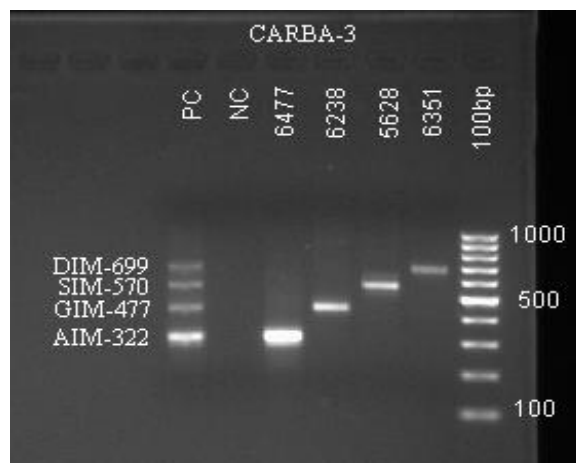
a. CARBA Multiplex PCR 1, Carbapenemase gene detection of *bla*NDM, *bla*KPC, and *bla*OXA-48, genes of patient samples with NC-negative and PC-positive control



b. CARBA Multiplex PCR 1, MBLs gene detection of *bla*IMP genes of a patient samples with NC-negative and PC-positive control



c. CARBA Multiplex PCR 1, MBLs gene detection of *bla*VIM genes of a patient samples with NC-negative and PC-positive control



d. CARBA Multiplex PCR 3, Minor MBLs gene detection of *bla*DIM, *bla*SIM, *bla*GIM, and *bla*AIM genes of 4 patient samples with NC-negative and PC-positive control

Figure 25: Metallo-beta lactamase encoding gene (a) CARBA Multiplex PCR 1 *bla*NDM, (b) CARBA Multiplex PCR 1, *bla*IMP, (c) CARBA Multiplex PCR 1 *bla*VIM, (d) CARBA Multiplex PCR 3, *bla*DIM, *bla*SIM, *bla*GIM, and *bla*AIM.

Among carbapenemase genes *bla*NDM were the highest prevalence accounted for 27(39.1%) followed by *bla*VIM accounted for 24(34.8%) (Figure 26).

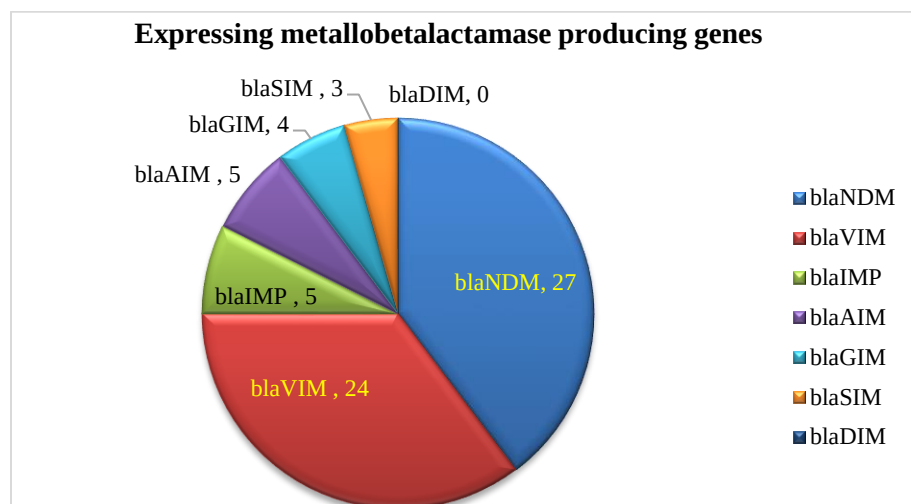


Figure 26: MBLS gene prevalence among 132 phenotypic confirmed gram-negative bacteria

Among all gram-negative bacteria, 50(21.6%) Metallo beta-lactamase encoding genes were detected. *Acinetobacter spp.* 22 (46.8%), *K. pneumoniae* 13 (17.3%), and *E. coli* 5 (13.2%) were the most common expressions of Metallo beta-lactamase-producing genes. The two most common metallo beta-lactamase genes were *bla*NDM 27 (39.10%) and *bla*VIM 24 (34.80%) (Table 24).

Table 24: PCR identification of Metallo-beta-lactamase producer genes from suspected bloodstream infection bacteria n=231.

Bacteria identified	MBLs gene positive								PCR
		<i>bla</i> NDM	<i>bla</i> VIM	<i>bla</i> IMP	<i>bla</i> AIM	<i>bla</i> GIM	<i>bla</i> SIM	<i>bla</i> DIM	MBLs
	N	N&%	N&%	N&%	N&%	N&%	N&%	N&%	N&%
<i>Acinetobacter spp</i>	47	8(27.6%)	18(62.1%)	0(0%)	1(3.4%)	2(6.9%)	1(3.4%)	0(0%)	22(46.8%)
<i>K pneumoniae</i>	75	13(72.2%)	0(0%)	3(16.7%)	0(0%)	1(5.6%)	1(5.6%)	0(0%)	13(17.3%)
<i>E. coli</i>	38	3(50%)	2(33.3%)	1(16.7%)	2(33.3%)	0(0%)	0(0%)	0(0%)	5(13.2%)
<i>K. oxytoca</i>	27	0(0%)	1(33.3%)	0(0%)	0(0%)	0(0%)	1(33.3%)	0(0%)	1(3.7%)
<i>Pseudomonas spp</i>	5	0(0%)	0(0%)	1(33.3%)	1(33.3%)	0(0%)	0(0%)	0(0%)	2(40%)
<i>E. coli (A-D)</i>	11	1(50%)	0(0%)	0(0%)	1(50%)	1(50%)	0(0%)	0(0%)	2(18.2%)
<i>S. marcescens</i>	4	0(0%)	2(100%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	2(50%)
<i>K. rhinoscleromat</i>	4	1(50%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(25%)
<i>E. aerogenes</i>	1	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
<i>P. mirabilis</i>	2	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
<i>E. cloacae</i>	2	0(0%)	1(100%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(50%)
<i>M. morganii</i>	2	1(100%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(50%)

Others	13	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Total	231	27(39.1%)	24(34.8%)	5(7.2%)	5(7.2%)	4(5.8%)	3(4.3%)	0(0%)	50(21.6%)

GNB, Gram-negative bacteria.

4.4.3. Load of Beta-Lactamase and Carbapenemase Gene in Strains

Among 75 *K. pneumoniae* 70(93.3%) of them have phenotyping drug resistance mechanisms and 5(6.7%) were not. A total of 88 drug resistance mechanisms with 1.3 mean values were identified in the PCR confirmatory test. Compared with AST 7 additional drug resistance mechanisms were identified in the PCR test so it increased from 82 AST confirmatory tests to 88 PCR tests. Among 70 drug-resistant bacteria, 164 Drug resistance genes were identified with the range of 1-7 and a mean value of 2.34 for each bacterium (Table 25). Among 27 *K. oxytoca*, 21(77.8%) were positive for both methods and 6(22.2%) of them were negative for PCR identification. Among the 6 not having drug-resistant genes one of them was positive for the phenotyping drug resistance mechanisms test and negative for the PCR test. One of the *K. oxytoca* was found to be positive for all drug resistance mechanisms having 7 drug resistance genes. The range of DR genes was 1-7 and on average 2.19 for each. An average of 1.4 drug resistance mechanisms was observed. Among the minor *Klebsiella species*, All *K. ozaenae* have a double drug resistance mechanism and on average each has 4 drug resistance genes. But in *K. rhinoscleromatis* half of them were drug-resistant and each of them has a mean of 1.5 drug-resistance mechanisms and drug-resistant genes (Appendix 10).

Among 47 *Acinetobacter species*, 68.09% have PCR-confirmed drug resistance mechanisms 19.14% of them don't have drug resistance mechanisms and 12.8% of them do have drug resistance mechanisms in the phenotyping test but during molecular characterization, it doesn't have drug-resistant genes. From 32 drug-resistant bacteria having drug resistance mechanisms, 69 DR genes were identified with an average of 2.16 and in the range of 1-5. Among the confirmed drug-resistant bacteria with both methods, drug resistance mechanisms increased from 49 by phenotyping to 53 with PCR-confirmatory test, on average from 1.2 to 1.66 for each. Among 38 *E. coli*, 24 of them were positive and 14 were negative by PCR method. however, 11 of them were not drug-resistant by both PCR and phenotyping detection methods; 3 of ESBL-positive by phenotyping detection were negative by PCR test. Of 24 *E. coli*, 66 drug resistance genes were

identified with an average of 2.75 drug-resistant genes in the range of 1-6 for each, and 35 drug resistance mechanisms in an average of 1.5 for each in the range of 1-3 were identified. Among 11 *E. coli* (A-D) 9(81.8%) of them were positive for drug resistance mechanisms and 2(18.2%) were not. Of 9 drug resistance, we found 22 drug resistance genes with a 1-4 range and an average of 2.4 for each. All positive for phenotyping were positive for the PCR test (Appendix 10).

Among 5 *Pseudomonas spp* 2(40%) of them don't have drug resistance mechanisms and 3(60%) of them have drug resistance mechanisms in the range of 1-2. Among 3 bacteria 7 DR genes have identified an average of 2.3 for each. A total of 7 drug resistance genes was identified with a mean value of 2.3. Among 4 *S. marcescens* 3(75%) of them have drug resistance mechanisms with a mean of 2 drug-resistant genes in each. Of these 4 species, 5 drug-resistance mechanisms were identified. Both the two *P. rettgeri* have one drug-resistance mechanism with 5 drug-resistant genes. Among 2 gram-negative bacteria s of *M. morganii*, *Proteus Mirabilis*, *E. agglomerans*, and *E. cloacae* have 50% drug resistance mechanisms and from 5 *C. diversus* 40% have drug resistance mechanisms. One *E. aerogenes* 100%, and one *S. typhi* 0% have drug resistance mechanisms (Appendix 10).

4.4.4. Association of Factors for Resistance Gene Types

One patient was infected with two *Acinetobacter spp* and *E. coli* both are carbapenemase producers of gram-negative bacteria so we took one and it became a total number of 62 carbapenemase producers. Compared to other age groups, neonates showed the most drug resistance mechanisms (drug resistance mechanisms), and men had 85.8% more drug resistance mechanisms than females 76.9%. Among factors associated with ESBL and AmpC-BLs producing genes age > 30 days p=0.003, NICU p=0.004, general surgery p=0.002, SICU p=0.019, instrument usage during medical care p=0.011, and chronic illness p=0.013 were associated. Among chronic illnesses, UTI p=0.049 and RTI p=0.028 were associated with ESBL and AmpC BLs-producing genes (Table 25).

Table 25: Socio-demography and clinical data for associated factors for ESBL and AmpC BLs n=1486.

Variables	ESBL and AmpC		Bivariate regression analysis		Multivariate regression analysis	
	f(R%)	f(R%)	P-value	COR (95%CI)	P-value	AOR (95%CI)
Age of participants						
Birth to 1 m	179(78.2%)	50(21.8%)	< 0.001	3.387(1.862-6.162)	0.003	4.683(1.68-13.053)
>1m-<13 yrs	546(93%)	41(7%)	0.76	0.91(0.499-1.66)	0.581	1.196(0.634-2.255)
13-<45 years	441(95.9%)	19(4.1%)	0.064	0.522(0.263-1.037)	0.089	0.542(0.267-1.099)
>45 years	194(92.4%)	16(7.6%)	0	(-)	0	0
Admission Wards						
C/W	337(95.5%)	16(4.5%)	0	0	0.277	0
Eopd	214(96.4%)	8(3.6%)	0.588	0.787(0.331-1.872)	0.998	0.998(0.257-3.885)
B6	147(75.4%)	48(24.6%)	< 0.001	6.878(3.782-12.507)	0.004	8.786(1.98-38.978)
B7	35(85.4%)	6(14.6%)	0.012	3.611(1.327-9.822)	0.105	2.494(0.827-7.525)
C7	56(90.3%)	6(9.7%)	0.104	2.257(0.847-6.013)	0.352	1.633(0.581-4.589)
D7	131(91%)	13(9%)	0.057	2.09(0.978-4.466)	0.905	1.061(0.4-2.812)
D8	69(93.2%)	5(6.8%)	0.424	1.526(0.541-4.305)	0.831	1.182(0.256-5.459)
C8	78(96.3%)	3(3.7%)	0.743	0.81(0.23-2.849)	0.54	0.588(0.108-3.214)
B8	57(96.6%)	2(3.4%)	0.692	0.739(0.165-3.301)	0.648	0.654(0.106-4.041)
PEOPD	13(92.9%)	1(7.1%)	0.652	1.62(0.199-13.163)	0.52	2.05(0.229-18.357)
Other	223(92.5%)	18(7.5%)	0.134	1.7(0.849-3.404)	0.593	0.683(0.169-2.763)
Admission Departments						
Medical	691(94.8%)	38(5.2%)	0	0	0.034	0
Emergency	69(97.2%)	2(2.8%)	0.384	0.527(0.124-2.232)	0.665	0.721(0.165-3.159)
MICU	67(91.8%)	6(8.2%)	0.287	1.628(0.664-3.992)	0.548	1.329(0.525-3.36)
NICU	137(77%)	41(23%)	< 0.001	5.442(3.375-8.776)	0.08	2.076(0.916-4.705)
G. Surgery	37(80.4%)	9(19.6%)	< 0.001	4.423(1.991-9.827)	0.002	4.092(1.704-9.823)
SICU	29(85.3%)	5(14.7%)	0.026	3.135(1.149-8.554)	0.019	3.646(1.238-10.73)
Hematology	283(92.8%)	22(7.2%)	0.211	1.414(0.821-2.433)	0.529	1.204(0.675-2.148)
Others	47(94%)	3(6%)	0.81	1.161(0.345-3.9)	0.95	1.041(0.301-3.6)
ICU admission						
No	1114(94%)	71(6%)	0	0.0640		0
yes	246(81.7%)	55(18.3%)	< 0.001	3.508(2.403-5.12)		0
Antibiotic treat. 10 days before						
No	1262(92.7%)	99(7.3%)	0	0.0780		0
Yes	98(78.4%)	27(21.6%)	< 0.001	3.512(2.189-5.634)	0.15	1.507(0.862-2.637)
Total	1360(91.5%)	126(8.5%)		0		0
Instrument usage during med care						
No	593(95.8%)	26(4.2%)	0	0.0440		0
Yes	767(88.5%)	100(11.5%)	< 0.001	2.974(1.906-4.638)	0.011	1.909(1.161-3.141)
Chronic illness						
No	463(88.5%)	60(11.5%)	0	0.0740	0	0.0150
Yes	897(93.1%)	66(6.9%)	0.003	1.761(1.22-2.543)	0.013	2.367(1.199-4.671)
UTI						
No	864(93.5%)	60(6.5%)	0	0.0690		0
Yes	33(84.6%)	6(15.4%)	0.038	2.618(1.056-6.494)	0.049	2.728(1.005-7.404)
RTI						
No	818(93.7%)	55(6.3%)	0	0.0670		0
Yes	79(87.8%)	11(12.2%)	0.038	2.071(1.042-4.118)	0.028	2.49(1.105-5.611)
History of incubation						

No	57(91.9%)	5(8.1%)	0	0.0880		0
Yes	123(73.2%)	45(26.8%)	0.004	4.171(1.572-11.067)	0.007	4.214(1.49-11.917)

Among Carbapenemase and MBLs producers NICU p=0.019, G. Surgery P=0.002, and SICU P=0.009, length of admission greater than 3 days, Instrument usage during medical care P=0.04, were associated with carbapenemase producer gram-negative bacteria. Among Instrument usage during medical care, using a suction machine P=0.039, using a drainage tube P=0.017 and from chronic illness patients, surgical wound infections P=0.039 and ureteral tract infection P=0.035 were associated with carbapenemase producer (Table 26).

Table 26: Socio-demography and clinical data for associated factors for Carbapenemase and MBLs n=1486.

Variables	PCs and MBLs		Bivariate analysis		Multivariate regression analysis	
	f(R%)	f(R%)	P-value	COR(95%CI)	P-value	AOR(95%CI)
Admission Department						
Medical	713(97.8%)	16(2.2%)	0		0.011	
Emergency	70(98.6%)	1(1.4%)	0.664	0.637(0.083-4.872)	0.877	1.178(0.149-9.308)
MICU	70(95.9%)	3(4.1%)	0.313	1.91(0.543-6.715)	0.701	0.765(0.195-3.002)
NICU	164(92.1%)	14(7.9%)	<0.001	3.804(1.82-7.95)	0.019	2.944(1.192-7.274)
G. Surgery	38(82.6%)	8(17.4%)	<0.001	9.382(3.779-23.29)	0.002	4.584(1.78-11.789)
SICU	27(79.4%)	7(20.6%)	<0.001	11.553(4.39-30.408)	0.009	4.277(1.43-12.788)
Hematology	293(96.1%)	12(3.9%)	0.121	1.825(0.853-3.905)	0.468	1.337(0.61-2.932)
Others	49(98%)	1(2%)	0.927	0.909(0.118-7.001)	0.729	0.696(0.089-5.429)
Length of admission						
1-2 days	571(98.8%)	7(1.2%)	0	0	0.038	0
3-4 days	203(95.3%)	10(4.7%)	0.005	4.018(1.51-10.697)	0.051	2.755(0.995-7.633)
>5days	650(93.5%)	45(6.5%)	<0.001	5.647(2.527-12.621)	0.011	3.161(1.303-7.672)
Antibiotic treat. 10 days before						
No	671(98.1%)	13(1.9%)	0	0.0190		0
Yes	753(93.9%)	49(6.1%)	<0.001	3.359(1.806-6.245)	0.055	1.976(0.986-3.961)
Instrument usage during medical care						
No	612(98.9%)	7(1.1%)	0	0.0110		0
Yes	812(93.7%)	55(6.3%)	<0.001	5.922(2.678-13.094)	0.04	2.442(1.041-5.73)
Using suction machine						
No	704(94.4%)	42(5.6%)	0	0.060		0
Yes	108(89.3%)	13(10.7%)	0.035	2.018(1.049-3.881)	0.039	2.698(1.051-6.926)
Using drainage tube						
No	787(94%)	50(6%)	0	0.0640		0
Yes	25(83.3%)	5(16.7%)	0.025	3.148(1.156-8.573)	0.017	3.728(1.26-11.016)
Chronic illness						
No	496(94.8%)	27(5.2%)	0	0.0540		0
Yes	928(96.4%)	35(3.6%)	0.162	0.693(0.415-1.158)	0.323	1.491(0.675-3.296)

Surgical wound infections						
No	902(96.5%)	33(3.5%)	0	0.0340		0
Yes	26(92.9%)	2(7.1%)	0.001	6.523(2.084-20.418)	0.039	4.04(1.072-15.224)
Ureteral tract infection						
No	894(96.8%)	30(3.2%)	0	0.0340		0
Yes	34(87.2%)	5(12.8%)	0.004	4.382(1.601-11.994)	0.035	3.489(1.089-11.18)
Place of delivery						
In hospital	204(92.3%)	17(7.7%)	0	0.0510		
At home	9(100%)	0(0%)	0.022	3.232(1.188-8.79)		
Have a history of incubation						
No	58(93.5%)	4(6.5%)	0	0.0690		
Yes	155(92.3%)	13(7.7%)	0.741	1.216(0.381-3.882)		
Mode of delivery						
NVD	158(95.2%)	8(4.8%)	0	0.0510		
C/S	55(85.9%)	9(14.1%)	0.022	3.232(1.188-8.79)		
Premature rupture of membrane						
>18 hours	44(97.8%)	1(2.2%)	0.01	0		
<18 hours	149(93.7%)	10(6.3%)	0.308	2.953(0.368-23.707)		
No rupture	20(76.9%)	6(23.1%)	0.02	13.2(1.489-117.004)		

4.5. Whole Genome Sequencing

4.5.1. Drug Resistance Gram-Negative Bacteria Selected for Whole Genome Sequencing

Among 195/233(83.7%) ESBL, AmpC, CPs, and MBLs enzyme producers' gram-negative bacteria, 142 strains qualified quality scores from 137 patient samples were selected for whole genome sequencing. Phenotypically and PCR-confirmed ESBL and carbapenemase-producer drug resistance bacteria were analyzed by whole genome sequencing. The following strains were most common: *K. pneumoniae* (32.4%), *E. coli* (25.4%), and *A. baumannii* (24.6%). The majority of the species belong to the genus *Klebsiella* (40.1%). B6 ward showed the higher prevalence, 46(32.4%), followed by C/W, 18(12.7%) (Table 27).

Table 27: Gram-negative bacteria selected for whole genome sequencing with the admission wards used for isolation n=142.

Species MLST confirmed	Patient admission Wards																					
	C/W		PEOPD		B6		B7		C7		D7		D8		C8		B8		Other		Total	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
<i>K. pneumoniae</i>	7	15.2	1	2.2	24	52.2	4	8.7	3	5.6	2	4.3	2	4.3	0	0.0	0	0.0	3	5.6	46	32.4
<i>K. oxytoca</i>	0	0.0	1	9.1	8	72.7	2	18.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	11	7.7
<i>E. coli</i>	3	8.3	6	16.7	1	2.8	0	0.0	1	2.8	8	22.2	3	8.3	4	11.1	1	2.8	9	25.0	36	25.4
<i>A. baumannii</i>	4	11.4	3	8.6	9	25.7	3	8.6	1	2.9	4	11.4	0	0.0	0	0.0	1	2.9	10	28.6	35	24.6

<i>E. cloacae</i>	2	22.2	0	0.0	3	33.3	0	0.0	1	11.1	1	11.1	0	0.0	0	0.0	1	11.1	1	11.1	9	6.3
<i>S. maltophilia</i>	1	50.0	0	0.0	1	50.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	1.4
<i>P. monteilii</i>	0	0.0	1	100	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.7
<i>P. Rettgeri</i>	1	100	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.7
<i>C. shandongense</i>	0	0.0	0	0.0	0	0.0	1	100	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.7
Total	18	12.7	12	8.5	46	32.4	10	7.04	6	4.2	15	10.6	5	3.5	4	2.8	3	2.1	23	16.2	142	100

4.5.2. Whole Genome Sequencing and In Silico Analysis

The WGS data included in this study were ≥ 3 q-scores quality assessment indicators. Some strains failed to be sequenced due to mixed species in $>10\%$ of WGS, and fragmented, and incomplete sequences $<90\%$ were excluded from this study (Appendix 11).

Over all the bacterial complete genome assembly (Checkm_complete) was 99.97% Median, 99.74% Mean, 0.54 Standard Deviation, and 96.35% Minimum and 100.0% Maximum value. The mean and median contamination check was 0.62% and 0.7%, whereas, in detail contamination with the human DNA was 0.10%, and contamination with vector was 0.70%. The overall bacteria mean values of percentage G-C content, N50, and nucleotide read were 50.7%, 113751.8, and 4028944.1. The number of contigs in the assembly was 39 to 965 (Appendix 12).

The *K. pneumoniae* completeness of sequence mean and median were 99.63% and 99.85%, respectively. The mean total contamination check was 0.74%. The isolates' final assembly range was from 86 to 667 contigs per assembly with a mean of 5549217 and a range of 4094201 to 5963878 total number of contigs per sample in *K. pneumoniae* isolates. The range of N50 values was 10709 to 216350 with a mean of 88976 and a median of 77897. The mean nucleotides read and mean total nucleotides read were 4000450 and 593358946, respectively. The average G-C content mean was 57% and the median was 57.1%.

The *E. coli* completeness of sequence mean and median were 99.86% and 99.93%, respectively. The mean total contamination check was 0.39%. The isolates' final assembly range was from 82 to 347 contigs per assembly with a mean of 5049692 and a range of 4619307 to 5500912 total number of contigs per sample in *E. coli* isolates. The range of N50 values was 41529 to 224194 with a mean of 137131 and a median of 133320. The mean nucleotides read and mean total nucleotides read were 3945645 and 58917423, respectively. The average G-C content mean was 50.6% and the median was 50.7% (Appendix 13).

The *A. baumannii* completeness of sequence mean, and median were 99.93%, and 99.98%, respectively. The mean total contamination check was 0.51%. The isolates' final assembly range was from 39 to 189 contigs per assembly with a mean of 3932646 and a range of 3440462 to 4128817 total number of contigs per sample in *A. baumannii* isolates. The range of N50 values was 52525 to 367993 with a mean of 130755 and median of 104028. The mean nucleotides read and mean total nucleotides read were 4358872 and 645968164, respectively.

The *K. oxytoca* completeness of sequence mean and median were 99.73 and 99.99%, respectively. The mean total contamination check was 1.9%. The isolates' final assembly range was from 86 to 667 contigs per assembly with a mean of 6143467 and a range of 5810144 to 6438312 total number of contigs per sample in *K. oxytoca* isolates. The range of N50 values was 24855 to 161880 with a mean of 95494 and median of 98586. The mean nucleotides read and mean total nucleotides read were 4122133 and 607875148, respectively. The average G-C content mean was 55.2% and the median was 55.1% (Appendix 13).

4.5.3. Whole Genome Sequencing Based Multilocus Sequence Typing for Species Identification

Among the 142 gram-negative bacterial strains of MLST, based on kcst_MLST species identification 9 gram-negative bacteria mark an allele with less than 100% identity with having unique gene clusters and hence is a novel allelic profile.

Among the six *Klebsiella* species, there were three novel drug-resistant *K. pneumoniae* isolates ST1799*, ST45*, and ST973* that represent three distinct allelic types of drug-resistant bacteria. The third isolate, *K. pneumoniae* ST973*, shared three other isolates' allelic profiles while being novel. Among the *Acinetobacter* species, *A. baumannii* ST85* and *A. baumannii* ST63* had novel allelic profiles. In addition, one of the *E. coli* ST131*, had a novel allelic profile (Table 28).

Table 28: Novel bacterial isolates identified using Kcst_MLST species identification n=142.

No	Bio_id	bio_id	Bap-species	kcst_MLST and novel allele	MLST
1	824	FF02_824	<i>A. baumannii</i>	ST85*Pas_rplB:3*(STnov)	ST1089
4	1686	FF02_1686	<i>K. pneumoniae</i>	ST45*infB:1*(STnov)	
5	3799	FF02_3799	<i>K. pneumoniae</i>	ST1799*tonB:34*(STnov)	

3	3862	FF02_3862	<i>E. coli</i>	ST131*adk:53*(STnov)	ST506
2	5055.2	FF02_5055	<i>A. baumannii</i>	ST63*Pas_pyrG:10*(STnov)	ST1030
6	5876	FF02_5876	<i>K. pneumoniae</i>	ST973*gapA:16*(STnov)	
7	5971	FF02_5971	<i>K. pneumoniae</i>	ST973*gapA:16*(STnov)	
8	6480	FF02_6480	<i>K. pneumoniae</i>	ST973*gapA:16*(STnov)	
9	6568	FF02_6568	<i>K. pneumoniae</i>	ST973* gapA:16*(STnov)	

4.5.4. Molecular Epidemiology of Antimicrobial Resistance in Gram-Negative Bacteria from Bloodstream Infection.

Antimicrobial resistance gene identification was done using the ResFinder tool for antibiotics and their cluster resistance. Among antibiotic clusters, the highest number of drug resistance genes were identified in Beta-Lactam, Aminoglycoside, and Folate Pathway Antagonist, accounting for 136 (95.8%), 128 (90.1%), and 128 (90.1%), respectively (Table 29). The dominant resistance was recorded for the antibiotics Amoxicillin, 124 (87.32%), Ampicillin, 124 (87.32%), and Piperacillin, 121 (85.21%) (Table 29). In GNB the highest rates of resistance were recorded in *K. oxytoca*, 64.20%, *K. pneumoniae* 60.90%, and *E. coli*, 56.70%. The total average drug-resistant genes identified among 40 antibiotics was 51.1%. Among 142 GNB the average carbapenem resistance identified was 21.8% and third-generation cephalosporin resistance was 75.4%. The highest number of bacteria having resistance genes were *Acinetobacter baumannii*, 48.6%, for Imipenem and Meropenem (Table 29).

Table 29: Antimicrobial resistance cluster (amr_cls), and antimicrobial resistance (amr_res) among gram-negative bacteria from Blood stream infection n=142.

Antimicrobial-resistant character		<i>K. pneumoniae</i>	<i>E. coli</i>	<i>A. baumannii</i>	<i>K. oxytoca</i>	<i>E. cloacae</i>	<i>S. maltophilia</i>	<i>P. Rettgeri</i>	<i>P. monteilii</i>	<i>C. shandonense</i>	Total	Total %
		N=46	N=36	N=35	N=11	N=9	N=2	N=1	N=1	N=1	142	%
WGS	Aminocyclitol	13	29	6	1	1	0	1	0	0	51	35.9%
Antimicrobial resistance cluster	Aminoglycoside	43	35	26	12	9	1	1	1	0	128	90.1%
	Amphenicol	43	3	6	10	4	0	1	1	0	68	47.9%
	All Beta-Lactam	45	35	30	12	9	3	1	1	0	136	95.8%
	Folate Pathway Antagonist	44	35	25	12	9	1	1	1	0	128	90.1%

detection	Fosfomycin	32	0	2	3	3	0	0	0	0	40	28.2%
	Glycopeptide	0	1	0	0	0	0	0	0	0	1	0.7%
	Lincosamide	0	4	1	0	1	0	0	0	0	6	4.2%
	Macrolide	5	26	19	3	4	0	0	0	0	57	40.1%
	Quinolone	44	31	3	11	8	0	1	0	0	98	69.0%
	Rifamycin	15	2	2	1	1	0	0	0	0	21	14.8%
	Streptogramin B	1	4	18	1	2	0	0	0	0	26	18.3%
	Tetracycline	23	27	18	9	3	1	0	0	0	81	57.0%
	Total	308	232	156	75	54	6	6	4	0	841	
WGS Antimicrobial resistance	Amikacin	35	25	15	11	4	1	1	0	0	92	64.8%
	Amoxicillin	45	35	20	12	9	2	1	0	0	124	87.3%
	Amoxici+clavu. acid	28	28	8	12	9	1	1	0	0	87	61.3%
	Ampicillin	45	35	20	12	9	2	1	0	0	124	87.3%
	Ampicil+clavu. acid	28	28	8	12	9	2	1	0	0	88	62.0%
	Azithromycin	4	26	18	3	0	0	0	0	0	51	35.9%
	Aztreonam	43	31	2	11	9	0	1	0	0	97	68.3%
	Cefepime	43	34	6	12	9	2	1	0	0	107	75.4%
	Cefixime	6	6	4	1	1	1	0	0	0	19	13.4%
	Cefotaxime	43	34	6	12	9	0	1	0	0	105	73.9%
	Cefoxitin	6	11	8	10	8	0	0	0	0	43	30.3%
	Ceftazidime	43	34	8	12	9	0	1	0	0	107	75.4%
	Ceftriaxone	43	31	0	11	9	0	1	0	0	95	66.9%
	Cephalothin	40	22	10	10	7	0	1	0	0	90	63.4%
	Chloramphenicol	44	3	5	10	3	0	1	0	0	66	46.5%
	Ciprofloxacin	45	31	0	11	8	0	1	0	0	96	67.6%
	Doxycycline	24	27	16	9	3	0	0	0	0	79	55.6%
	Ertapenem	6	6	4	1	1	0	0	0	0	18	12.7%
	Fosfomycin	33	0	0	3	2	0	0	0	0	38	26.8%
	Gentamicin	34	19	20	11	7	0	1	0	0	92	64.8%
	Imipenem	6	6	17	1	1	0	0	0	0	31	21.8%
	Kanamycin	1	2	14	2	0	0	0	0	0	19	13.4%
	Meropenem	6	6	17	1	1	0	0	0	0	31	21.8%
	Minocycline	0	16	15	0	1	0	0	0	0	32	22.5%
	Nalidixic acid	40	30	0	0	0	0	0	0	0	70	49.3%
	Netilmicin	37	23	12	11	4	0	1	0	0	88	62.0%
	Piperacillin	45	35	19	12	9	1	1	0	0	121	85.2%
	Piper+ clav. acid	4	0	0	0	0	0	0	0	0	3	2.1%
	Piper+ tazobactam	24	28	6	12	9	1	1	0	0	81	57.0%
	Streptomycin	38	35	19	12	7	0	1	0	0	112	78.9%
	Sulfamethoxazole	40	33	23	12	9	0	1	0	0	118	83.1%
	Tetracycline	23	27	16	9	3	0	0	0	0	78	54.9%

Ticarcillin	45	35	14	11	9	0	1	0	0	114	80.3%
Ticarc + clavul. acid	6	9	2	9	8	0	0	0	0	34	23.9%
Tigecycline	1	0	0	0	0	0	0	0	0	1	0.7%
Tobramycin	39	28	19	11	7	0	1	0	0	104	73.2%
Trimethoprim	42	33	5	11	7	0	1	0	0	99	69.7%
Unknown aminoglycoside	11	0	27	0	0	1	0	0	0	39	27.5%
Unknown beta-lactam	0	4	18	0	1	0	0	0	0	23	16.2%

4.5.5. Comparison of Phenotyping Combination Drug Test, Genotyping PCR Test, and Whole Genome Sequencing Results

The baseline result of phenotyping drug resistance detection by combination disk test is used to compare the performance of PCR and WGS methods in gram-negative bacteria. ESBL detection by 94.9% PCR method and 96.9% WGS detection have similarities with phenotyping detection. The PCR method for 65.4% AmpC detection had similarities with the WGS result of 61.5% and had a discrepancy with the initial phenotyping result. The MBL Phenotyping detection was 100% similar to the PCR test and 70% similar to WGS. The PCR Carbapenemase detection was 70% similar to phenotyping detection while WGS had 82.5% similarity with the phenotyping detection method. In general PCR method was 85.1% similar while the WGS method was 86.8% similar to phenotyping detection (Figure 27).

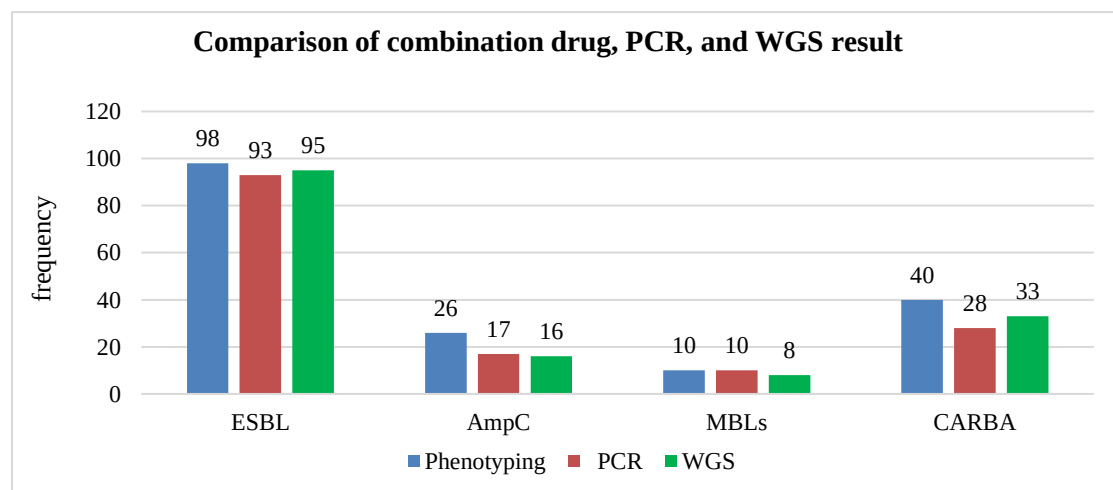


Figure 27: Shows the prevalence of drug resistance types by phenotyping combination drug test, genotyping PCR, and WGS analysis.

The identification of method comparison of phenotyping, genotyping by PCR, and WGS by categorizing based on the higher rank of drug resistance types was done. Phenotypically CDT-categorized gram-negative bacteria were exposed for that category of specific PCR primer, However, in the case of WGS all 142-gram-negative bacteria run for all types of drug resistance category. Therefore, for higher rank of antimicrobial resistance may have a higher number in WGS because of unexpressed genes found in the bacteria. Among 142 isolates of drug-resistant type categorization by using the higher rank of antimicrobial resistance enzyme production, during phenotyping combination drug test shows ESBL, AmpC, MBL, and Carbapenemase accounted for 74 (52.1%), 17 (12.0%), 10(7.0%), and 40(28.2%) respectively. The Genotyping PCR test result shows ESBL, AmpC, MBL, and Carbapenemase accounted for 78(54.9%), 9(6.3%), 13(9.2%), and 33(23.2%) respectively. The WGS result shows ESBL, AmpC, MBL, and Carbapenemase accounted for 32(22.5%), 10(7.0%), 5(3.5%), and 88(62.0%) respectively (Figure 28).

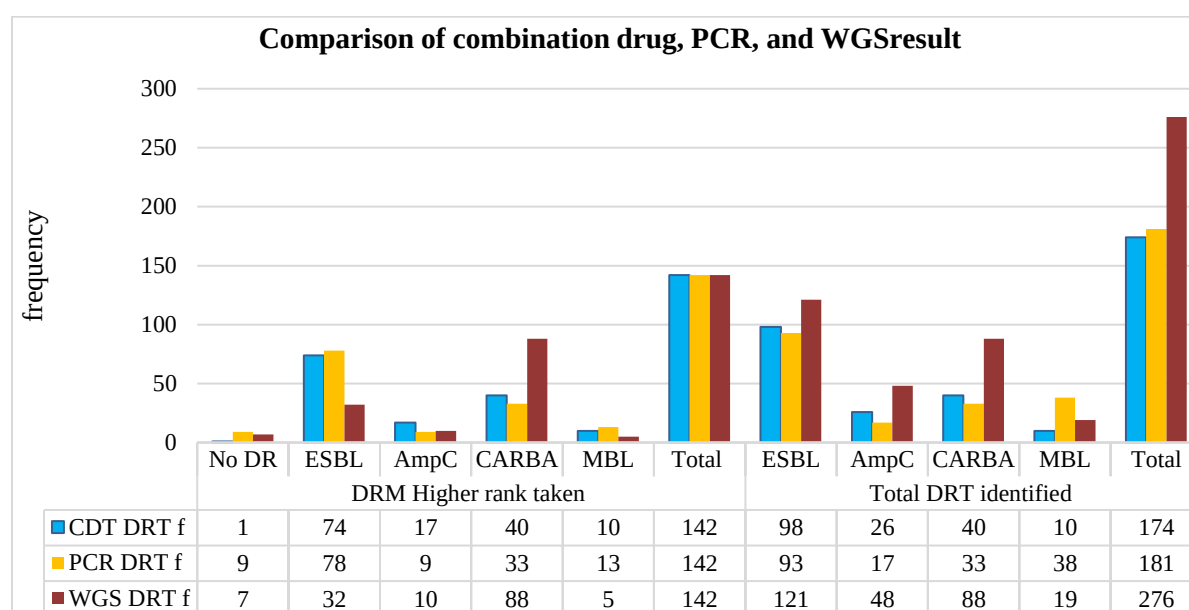


Figure 28: Comparison of phenotyping of combination drug test, Genotyping PCR, and WGS result of drug resistance types.

A comparison of all drug resistance types identified in all phenotyping (CDT), genotyping of PCR, and WGS analysis methods was done. Isolates may have more than one type of enzyme-producer drug-resistant mechanisms. Among 142 strains the phenotyping combination drug test shows a total of 174 drug-resistant types identified in which ESBL, AmpC, MBLs, and Carbapenemase

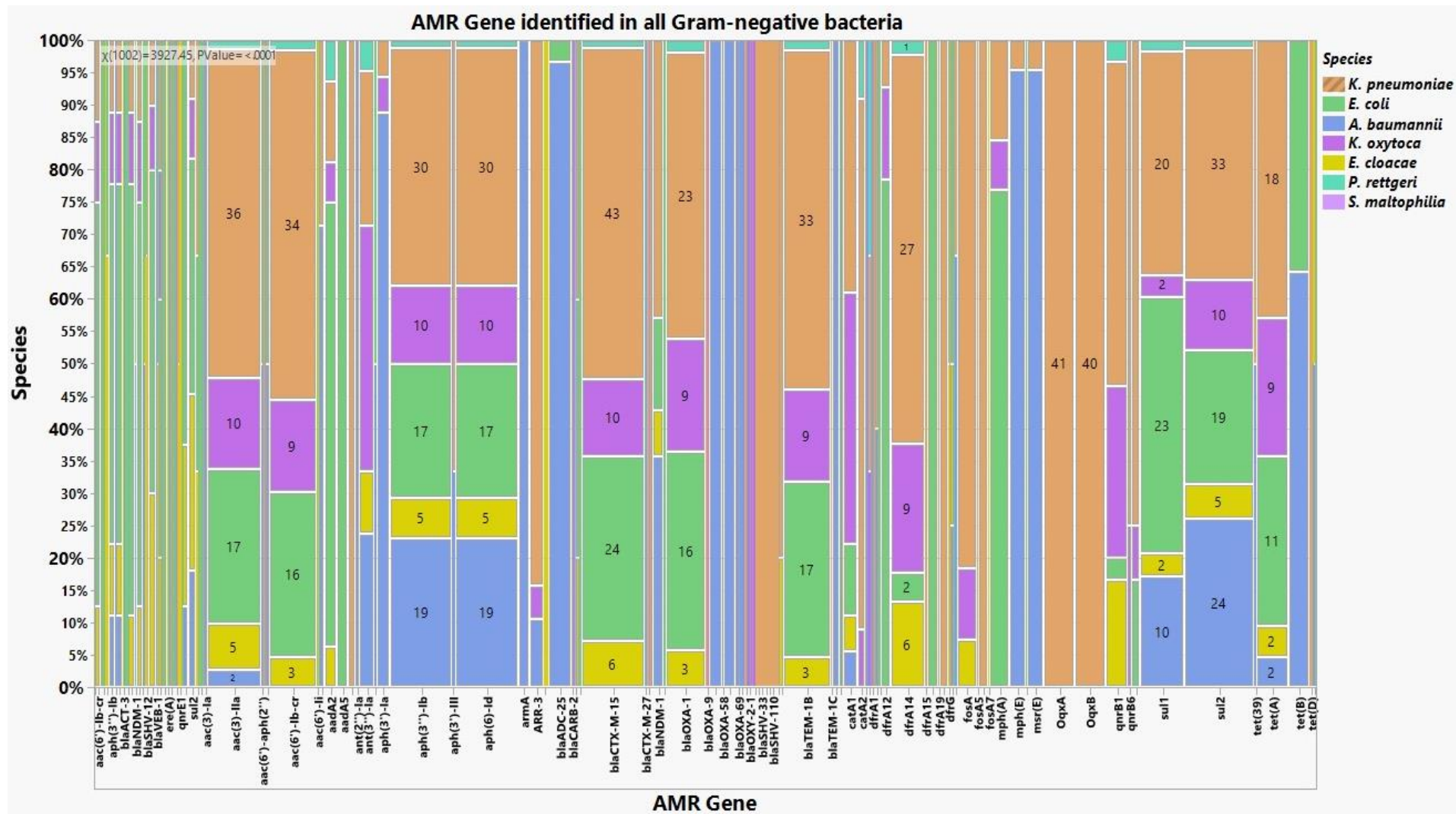
accounted for 98(69.0%), 26(18.3%), 10(7.0%), and 40(28.2%) respectively. The total Genotyping PCR test shows 181 drug-resistant types identified with ESBL, AmpC, MBLs, and Carbapenemase accounted for 93(65.5%), 17(12.0%), 38(26.8%) and 33(23.2%) respectively. While the total drug-resistant mechanisms of WGS results show 276 drug-resistant types recorded with ESBL, AmpC, MBLs, and Carbapenemase accounted for 121(85.2%), 48(33.8%), 19(13.4%), and 88(62.0%) respectively (Figure 28).

4.5.6. Genetic Diversity of Antimicrobial Resistance in Gram-Negative Bacteria Isolated from Bloodstream Infection

4.5.6.1. Antimicrobial Resistance in Gram-Negative Bacteria

Among the total 142 gram-negative bacteria, 97.1% of the strains were identified to have antimicrobial resistance genes. There were 168 different types of AMR genes discovered, for a total of 1604 genes. The most dominant gram-negative bacteria possessing AMR genes were *K. pneumoniae*, 639(39.8%), *A. baumannii*, 324(20.2%), and *E. coli*, 369(23.0%). The highest prevalence gene was recorded for *sul2*, 92(5.7%), *blaCTX-M-15*, 84(5.2%), *aph(3'')-Ib*, 82 (5.1%), *aph(6)-Id*, 82(5.1%), and *aac(3)-IIa*, 71(4.4%), genes.

Among beta-lactamase genes, 58 types of 391 genes were identified. The most dominant beta-lactamase genes were recorded in *K. pneumoniae*, 156(39.9%), *A. baumannii*, 100(25.6%), and *E. coli*, 69(17.6%). The most abundant beta-lactamase genes detected were *blaCTX-M-15*, 84(21.5%), *blaTEM-1B*, 63(16.1%), and *blaOXA-1*, 52(13.3%) (Figure 29).



4.5.6.2. Antimicrobial Resistance Gene in ESBL, AmpC, MBL, and Carbapenemase-producing Gram-Negative Bacteria

ESBL: Among 894 third-generation cephalosporin-resistant genes, which are grouped into 92 types, blaCTX-M-15, 66(7.38%), blaTEM-1B, 54(6.04%), and sul254, (6.04%), were predominant. (Figure 30).

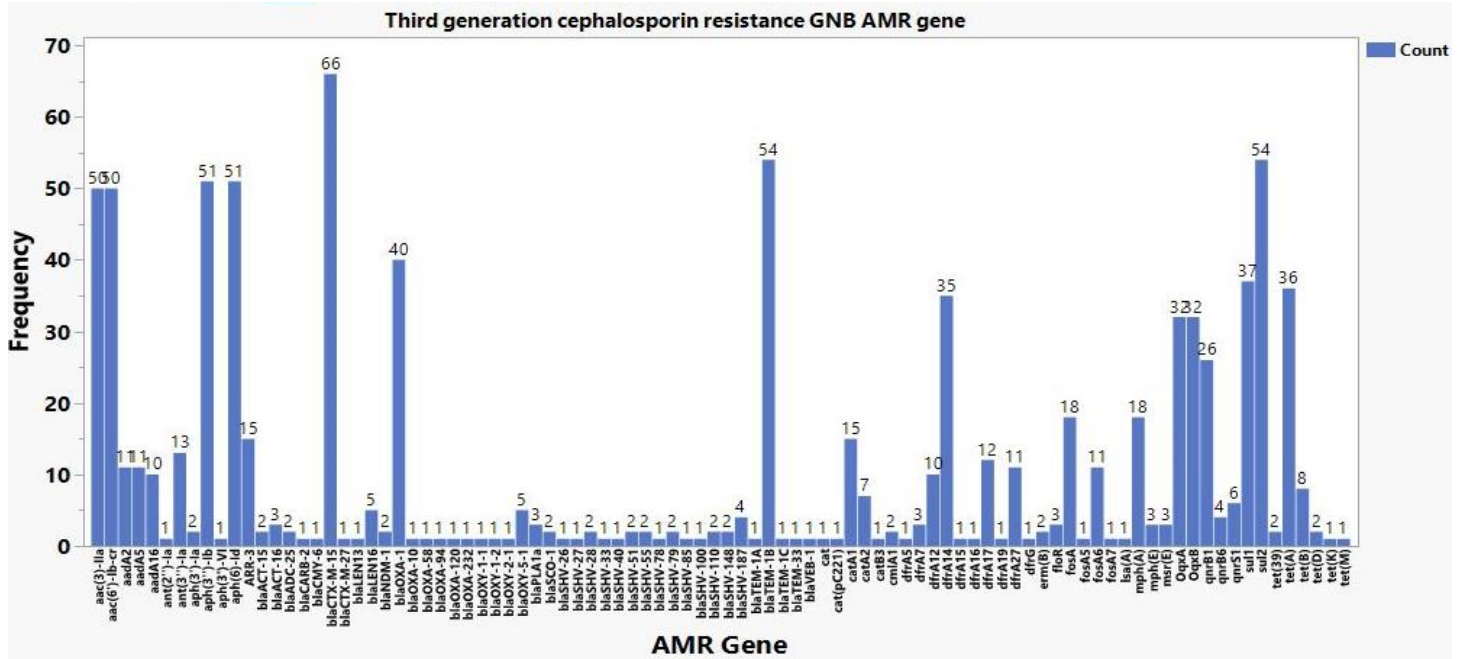


Figure 30: The AMR gene of third-generation cephalosporin-resistant gram-negative bacteria.

Two hundred twenty-three beta-lactam genes were found in 74 ESBL producer gram-negative bacteria. blaCTX-M-15 was the most common beta-lactamase (bla) gene, followed by blaTEM-1B, blaOXA-1, blaLEN16, blaOXY-5-1, and blaSHV-187, accounting for 66(7.38%), 54(6.04%), 40(4.47%), 5(0.56%), 5(0.56%), and 4(0.45%), respectively (Figure 31).

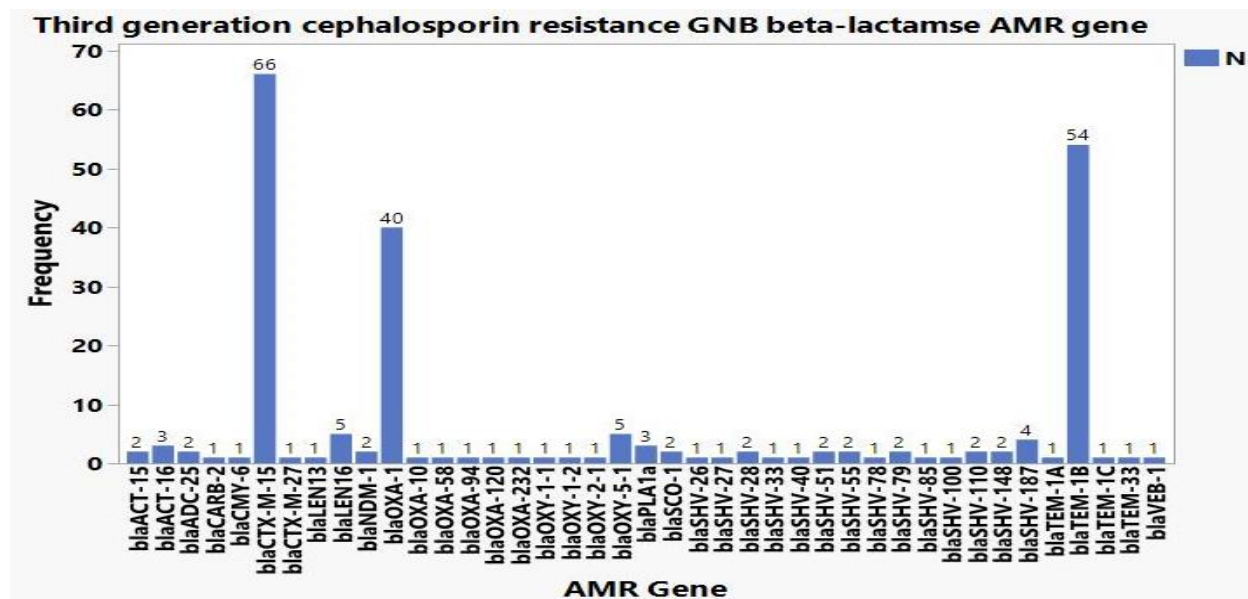


Figure 31: The beta-lactamase AMR genes of third-generation cephalosporin-resistant in gram-negative bacteria.

Among departments high load of beta-lactam drug-resistant bacteria and AMR genes were identified in NICU, followed by Medical, Hematology, General Surgery, MICU, Emergency, ENT Unit, Orthopedics, and SICU accounting for 369(41.3%), 280(31.3%), 145(16.2%), 42(4.7%), 15(1.7%), 12(1.3%), 11(1.2%), 11(1.2%), and 9(1.0%), respectively. In NICU AMR genes were found in *K. pneumoniae*, *K. oxytoca*, *E. cloacae*, and *E. coli* accounting for 241(27.0%), 104(11.6%), 14(1.6%), and 10(1.1%) respectively. The second higher beta-lactam drug-resistant bacteria and AMR gene prevalence was found in the medical department where *K. pneumoniae* and *E. coli* accounted for 108 (12.1%), and 64 (7.2%), respectively. At the third place Hematology department showed *E. coli* and *K. pneumoniae* that accounted for 72 (8.1%), and 62 (6.9%), respectively. The rest of the AMR genes were found in general surgery; *K. pneumoniae* 42 (4.7%), MICU *E. coli* 15 (1.7%), Emergency *E. coli* 12 (1.3%), ENT Unit *E. coli* 11 (1.2%), Orthopedics *E. coli* 11 (1.2%), and SICU *A. baumannii* 9 (1.0%) (Figure 32). Third-generation cephalosporin-resistant bacteria AMR genes in B6 (NICU) were the highest, with *K. pneumoniae*, 241 (27.0%), followed by *K. oxytoca*, 104 (11.6%). The second AMR drug-resistance gene dominated ward was causality, 110 (12.3%), and the predominant bacteria were *K. pneumoniae*, 59 (6.6%). The third one was D7 which accounted for 71(7.9%) and the major bacteria were *E. coli* 42(4.7%) (Figure 33).

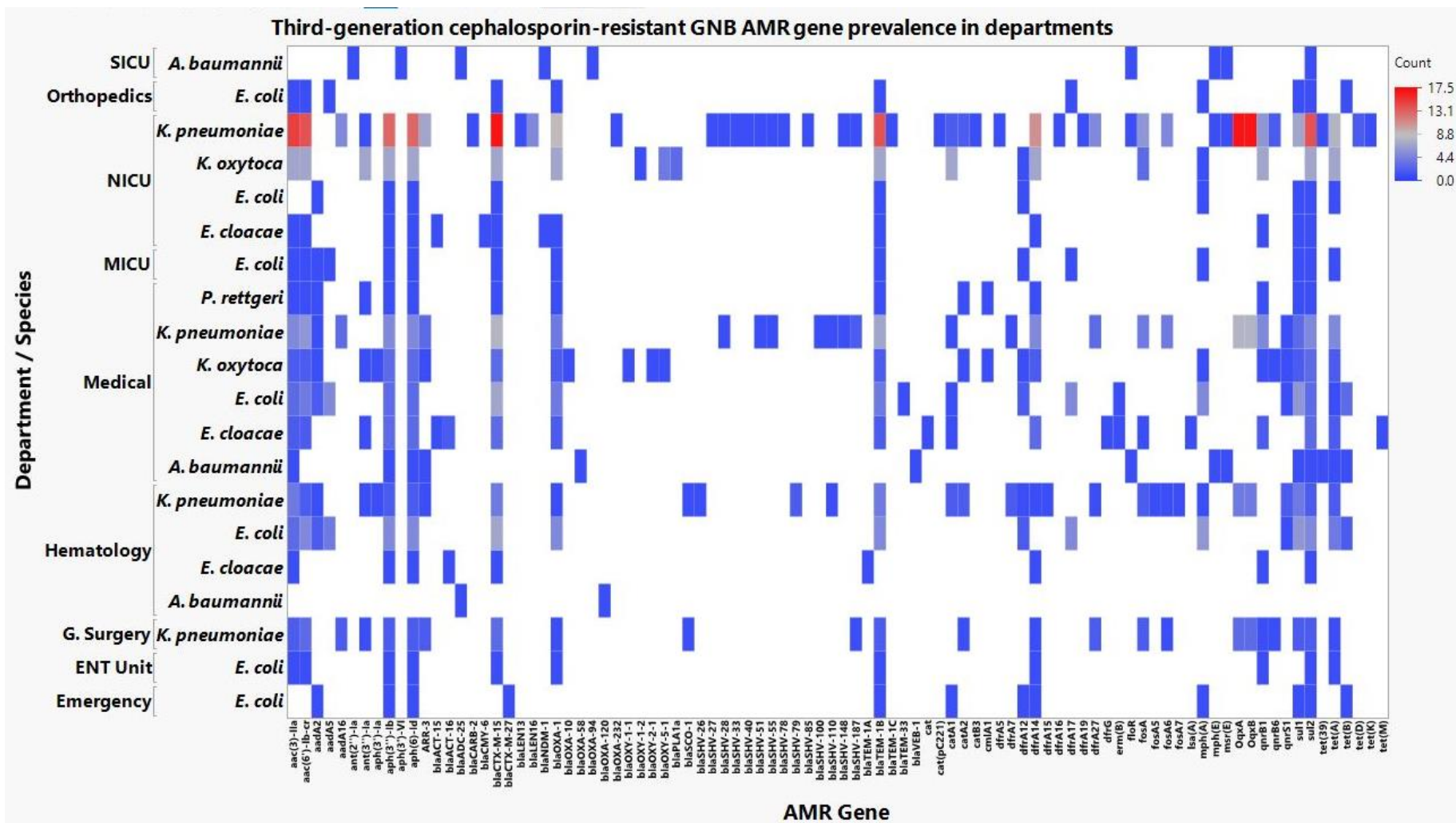


Figure 32: The third-generation cephalosporin-resistant gram-negative bacteria AMR gene distribution in departments

AmpC: Among 17 third-generation cephalosporin and cephamycin-resistant bacteria 160 antimicrobial resistance genes were identified. The most prevalent genes were sul2, 11(6.9%), blaTEM-1B, 10(6.3%), and aph(3'')-Ib 9(5.6%). However, the predominant genes from AmpC beta-lactamases producer were blaCMY-2 and blaCMY-6 which accounted for 3(1.9%) and 1(0.6%), respectively (Figure 34).

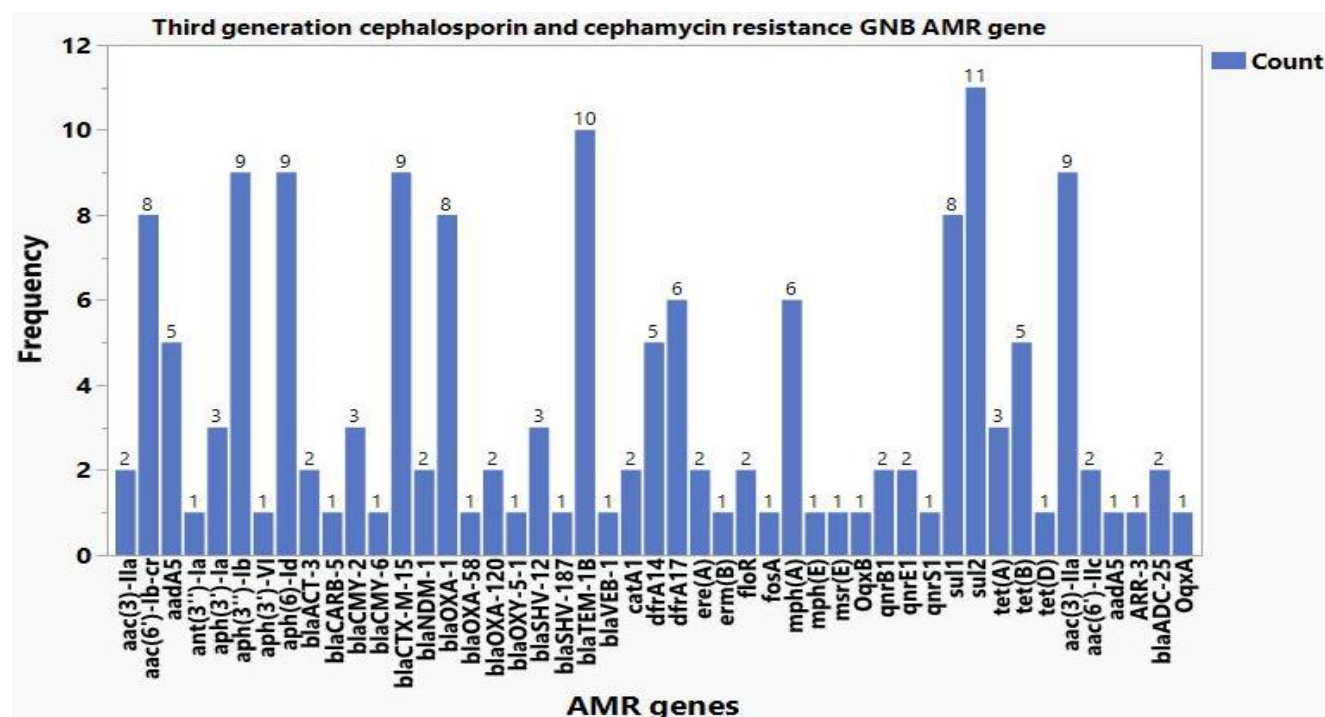


Figure 34: AmpC beta-lactam drug resistance bacteria antimicrobial resistance gene.

ESBL gene in AmpC BL resistant bacteria were CTX-M-15, blaSHV-12, blaSHV-187, blaTEM-1B, blaVEB-1, and blaOXA-1 at the rate of 9(5.6%), 3(1.9%), 1(0.6%), 1(6.3%), 1(0.6%) and 8(5.0%), respectively. Among carbapenemase genes blaNDM-1 and blaOXA-58 accounted for 2(1.3%) and 1(0.6%), respectively. The rest beta-lactamase families of blaOXA-120, blaOXY-5-1, blaCARB-5, blaOXA-1, blaADC-25, and blaACT-3 accounted for 2(1.3%), 1(0.6%), 1(0.6%), 8(5.0%), 2(1.3%), and 2(1.3%), respectively (Figure 34). The AmpC beta-lactam resistance was dominant in *E. coli*, 85(53.1%), *E. cloacae*, 31(19.4%), and *A. baumannii*, 19(11.9%). Almost all AmpC beta-lactam-resistant *K. pneumoniae* were identified in the General Surgery department (Figure 35).

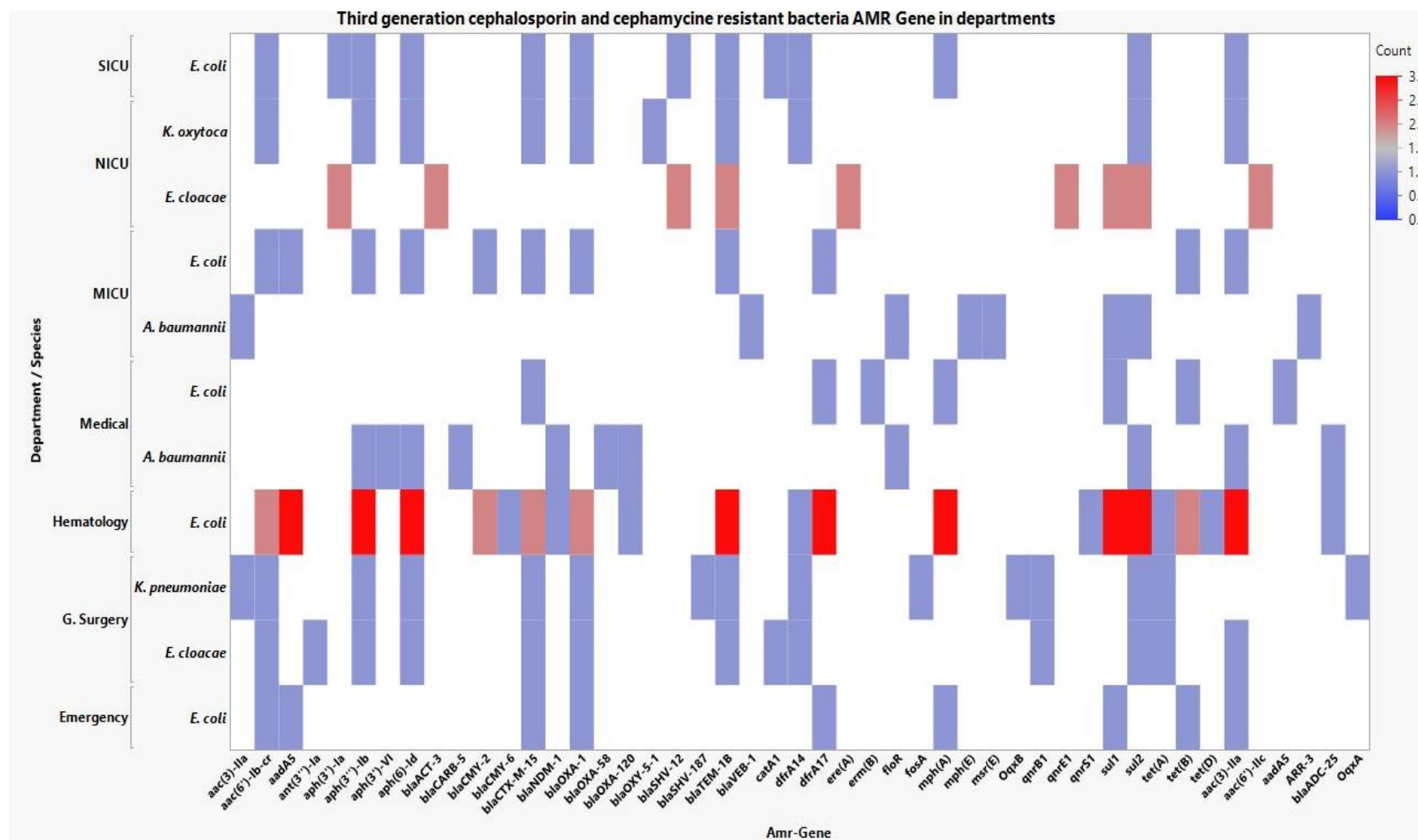


Figure 35: AmpC beta-lactam resistant gram-negative bacteria AMR genes in departments of bloodstream infection

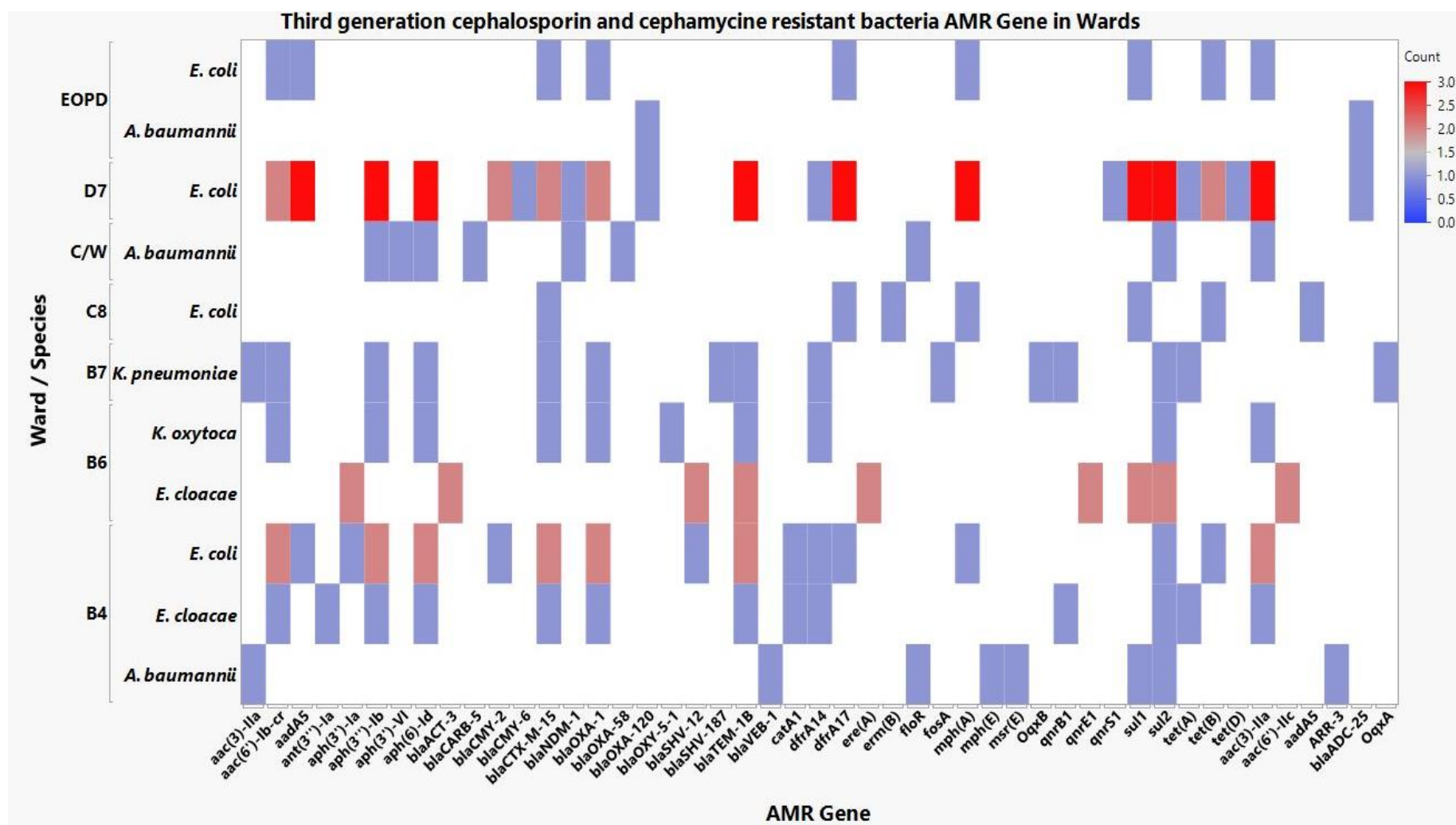


Figure 36: AmpC beta-lactam resistant gram-negative bacteria AMR genes in wards of bloodstream infection

In the same way, all AmpC beta lactam-resistant *A. baumannii* were found in the Medical and MICU departments. Among AmpC beta-lactam resistant bacteria *E. cloacae* and *K. oxytoca* AMR genes were found in NICU (Figure 36). The most abundant AmpC beta lactam-resistant AMR gene in-wards was found in B4 45(28.1%), D7 45(28.1%), B6 28(17.5%), B7 15(9.4%), EOPD 11(6.9%), C/W 9(5.6%), and C8 7(4.4%) (Figure 36).

Carbapenemase: Among 50(35.2%) carbapenem-resistant gram-negative bacteria *Acinetobacter baumannii*, *K. pneumoniae*, *E. coli*, *E. cloacae*, *Pseudomonas putida*, and *Stenotrophomonas maltophilia* accounted for 28(56.0%), 11(22.0%), 8(16.0%), 1(2.0%), 1(2.0%), and 1(2.0%), respectively. Among these carbapenem-resistant gram-negative bacteria, 550 AMR genes were identified. The most predominant AMR genes were *sul2* followed by *aph(3'')-Ib*, *aph(6)-Id*, *blaADC-25*, *sul1*, *tet(B)* at the rates of 38(6.9%), 31(5.6%), 31(5.6%), 27(4.9%), 21(3.8%), and 20(3.6%), respectively (Figure 37).

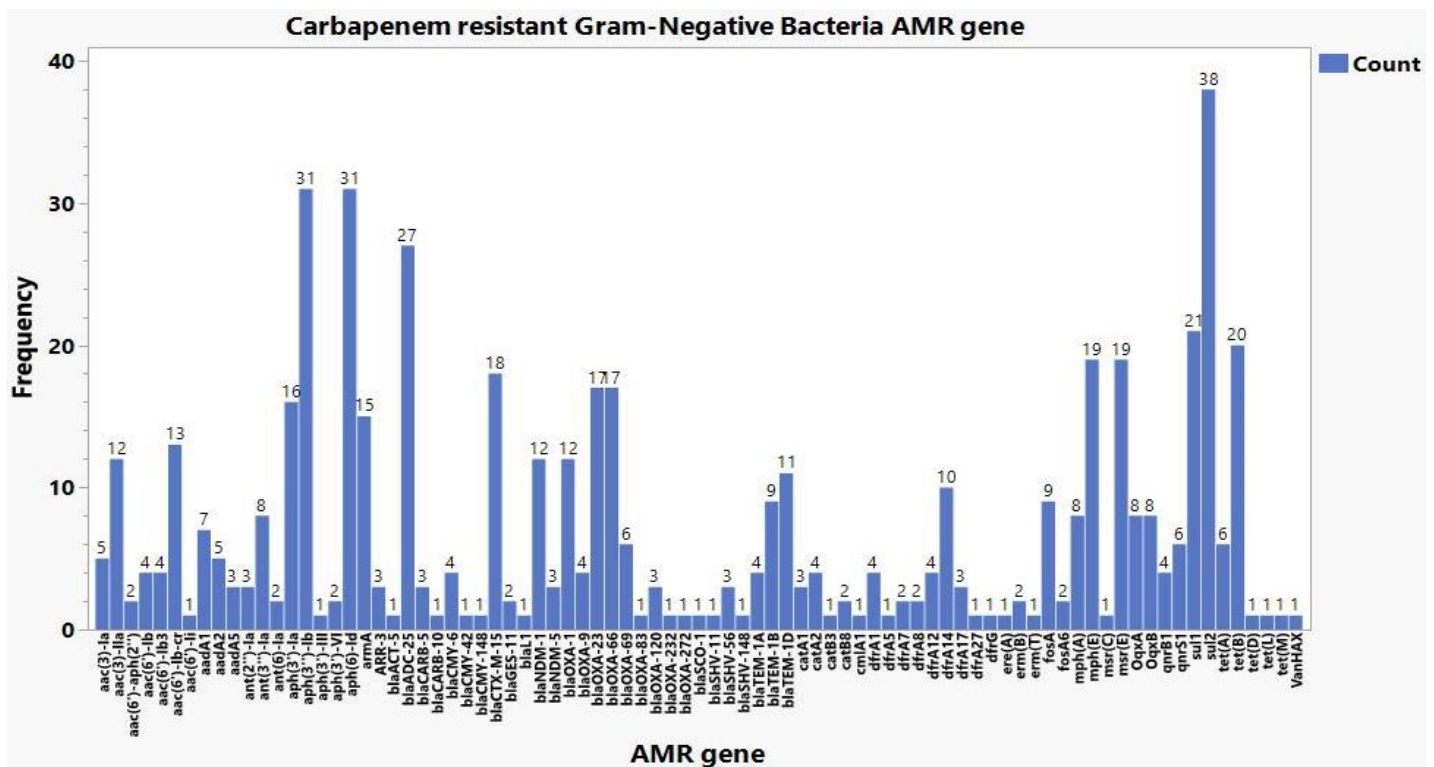


Figure 37: Antimicrobial-resistant genes among carbapenem drug-resistant gram-negative bacteria

Among 50 carbapenem resistance gram-negative bacteria 166(30.2%) beta-lactamase genes, grouped into 28 types, were identified. However, a total of 111(20.2%) specific carbapenem resistance genes, of 17 types, were identified. Among this blaOXA-23, blaOXA-66, blaNDM-1, blaOXA-1, blaOXA-69, blaOXA-9, blaCARB-5, blaNDM-5, blaOXA-120, blaOXA-232, blaOXA-272, and blaOXA-83, accounting for 17(3.1%), 17(3.1%), 12(2.2%), 12(2.2%), 6(1.1%), 4(0.7%), 3(0.5%), 3(0.5%), 3(0.5%), 1(0.2%), 1(0.2%), and 1(0.2%), respectively, have direct association with carbapenem resistance. However, genes including blaACT-5, blaL1, blaCARB-10, blaADC-25, and blaSCO-1 that accounted for 27(4.9%) were not frequently linked to resistance to carbapenems (Figure 38).

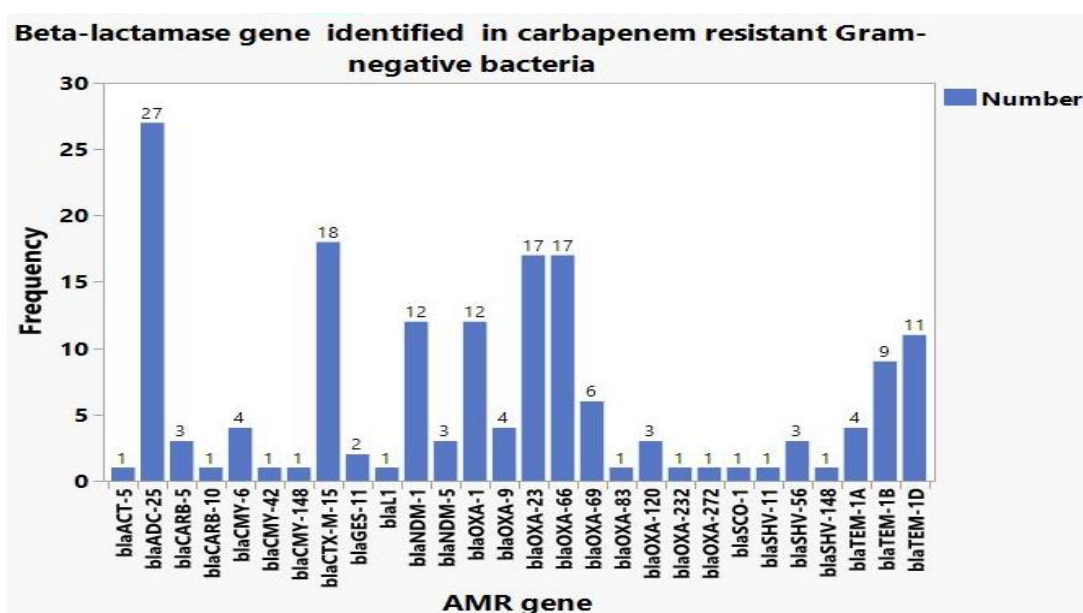


Figure 38: Beta-lactamase genes identified from carbapenem-resistant gram-negative bacteria.

Among 28(56%) carbapenem resistance *A. baumannii* 27 possessed blaADC-25 beta-lactam gene. Except for one novel strain of *A. baumannii* ST1030/ST63*, which carried the blaOXA-272 carbapenemase gene, the rest had the blaADC-25 gene. Next to the blaADC-25 gene, blaOXA-23, and blaOXA-66 genes, accounted for 17(34%) and all were identified from *Acinetobacter baumannii*. The carbapenem-resistant genes which are not identified in other gram-negative bacteria but found in *A. baumannii* were blaADC-25, blaOXA-23, blaOXA-66, blaOXA-69, blaOXA-83, and blaOXA-120, and accounted for 27(4.9%), 17(3.1%), 17(3.1%), 6(1.1%), 1(0.2%), and 3(0.5%), respectively. Additionally, blaCARB-5 and blaOXA-272 genes identified

in *A. baumannii* accounted for 3 (0.5%), and 1 (0.2%), respectively (Figure 39). Among *K. pneumoniae*, 11(22.0%), the most predominant carbapenemase producer genes were blaNDM-1, 6(1.1%) and blaOXA-9, 4(0.7%). The blaOXA-9, blaSCO-1, and blaOXA-232 genes were found only in carbapenem-resistant *Klebsiella pneumoniae*. Among *E. coli* 8(16.0%) the most prevalent carbapenem-resistant genes were blaNDM-5, 3(0.5%), and blaNDM-1, 2(0.4%). blaNDM-5 gene was found only in *E. coli*. Among *S. maltophilia* blaL1 and blaCARB-10 accounted for 1(0.2%) and 1(0.2%), respectively (Figure 39).

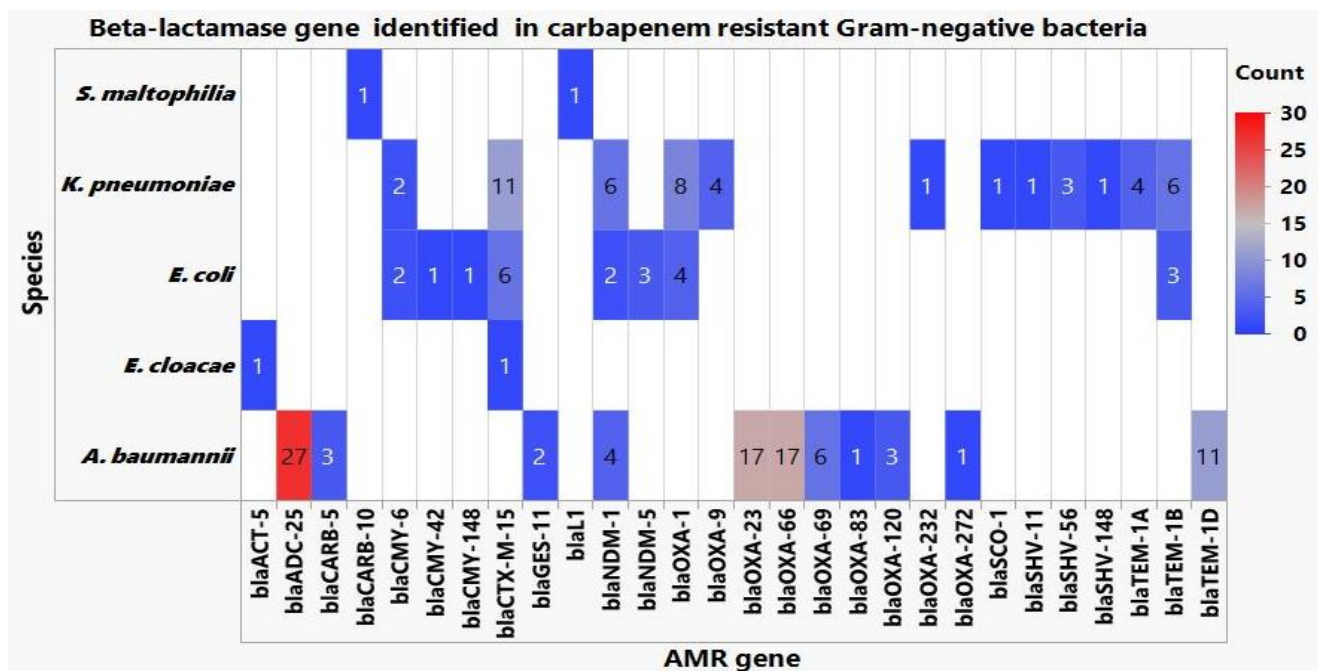


Figure 39: Beta-lactam genes associated with carbapenem resistance gram-negative bacteria

Among departments, a higher number of carbapenem-resistant gram-negative bacteria were found in NICU 12(24.0%), Hematology 12(24.0%), Medical 11(22.0%), and SICU 7(14.0%). Similarly, higher AMR genes were found in these departments NICU 142(25.8%), Hematology 110 (20.0%), and Medical 115 (20.9%). Among 12 carbapenem resistant gram-negative bacteria in NICU, 8(16.0%) *A. baumannii* and 4(8.0%) *K. pneumoniae* have 87(15.8%), and 55(10.0%) AMR genes. In Hematology department from 12 gram-negative bacteria 5(10.0%) *A. baumannii* 2(4.0%) *K. pneumoniae*, 4(8.0%) *E. coli*, 1(2.0%) *Stenotrophomonas maltophilia* accounted for 28(5.1%),50(9.1%), 30(5.5%), and 2(0.4%), AMR gene, respectively. Similarly in Medical departments among 11 carbapenem resistant gram-negative bacteria 6(12.0%) *Acinetobacter*

baumannii, 2(4.0%) *K. pneumoniae*, 2(4.0%) *E. coli*, have accounted for 60(10.9%), 21(3.8%), and 34(6.2%) AMR genes, respectively. Among 7 SICU carbapenem resistance gram-negative bacteria 6(12.0%) *Acinetobacter baumannii* and 1(2.0%) *K. pneumoniae* have 71(12.9%) and 15(2.7%) AMR genes, respectively (Appendix 14) (Figure 40).

Among wards, a higher number of carbapenem resistance gram-negative bacteria were observed in B6, B4, C/W, and B7 accounting for 12(24.0%), 10(20%), 8(16%), and 5(10%), respectively. In B6 carbapenem resistance gram-negative bacteria were *A. baumannii* 8(16%) and *K. pneumoniae* 4(8%) having 87(15.8%) and 55(10.0%), with a total of 142(25.8%) AMR genes, respectively. In B4 carbapenem resistance gram-negative bacteria were *A. baumannii*, 7(14%), *E. coli*, 2(4%), and *K. pneumoniae*, 1(2%), having 83(15.1%), 18(3.3%), and 15(2.7%), AMR genes, respectively with a total of 116(21.1%). In B7 ward carbapenem resistant gram-negative bacteria were *A. baumannii*, 3(6%), and *K. pneumoniae*, 2(4%), having 37(6.7%) and 38(6.9%) with a total of 75(13.6%) AMR genes, respectively. The fourth ward was C/W with *A. baumannii*, 2(4%), *E. coli*, 2(4%), *K. pneumoniae*, 2(4%), *E. cloacae*, 1(2%), and *Stenotrophomonas maltophilia*, 1(2%) having 14(2.5%), 21(3.8%), 28(5.1%), 8(1.5%), and 2(0.4%), with a total of 73(13.3%) AMR genes, respectively (Appendix 14) (Figure 41).

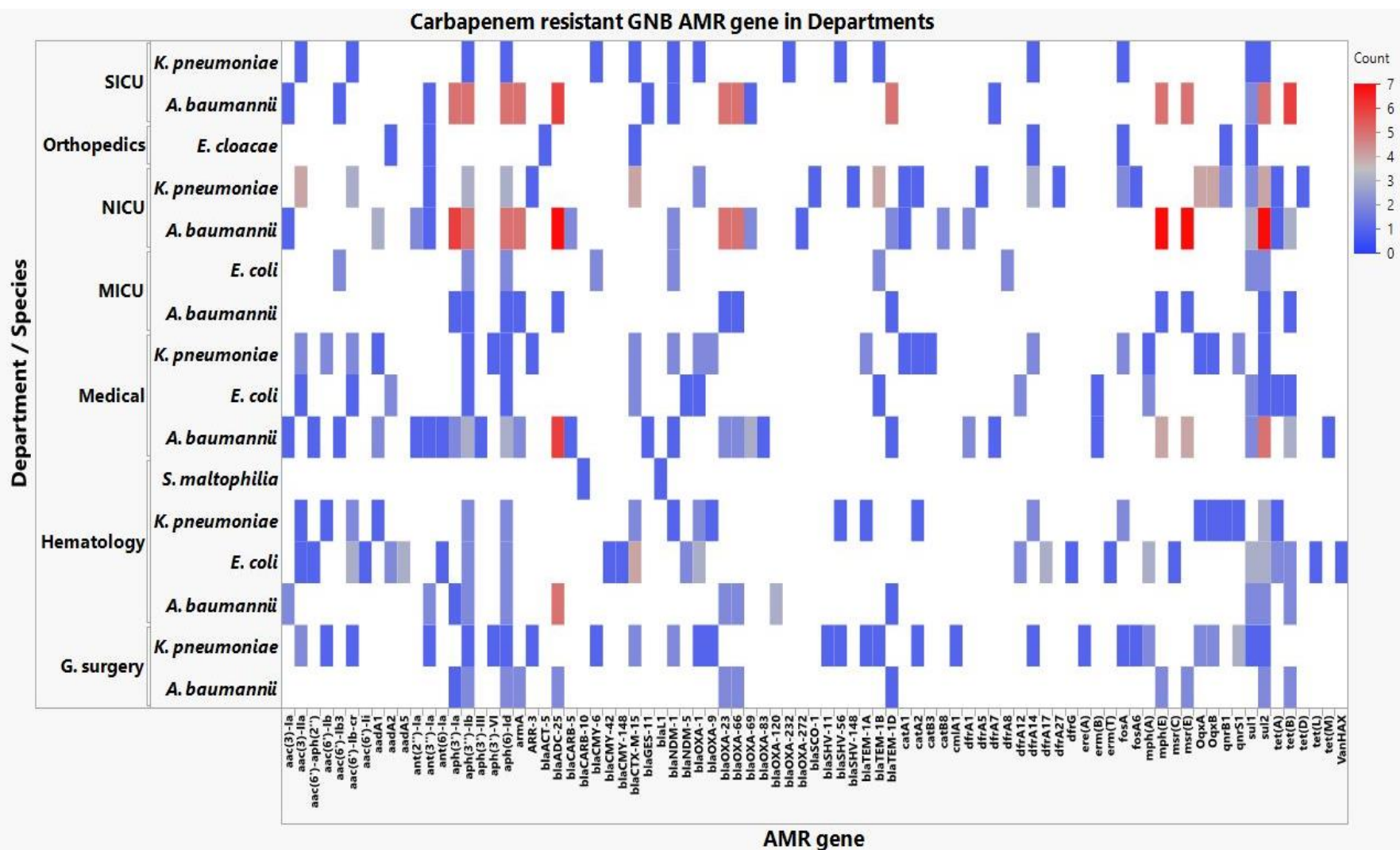


Figure 40: Beta-lactamase genes identified from carbapenem resistance gram-negative bacteria in departments

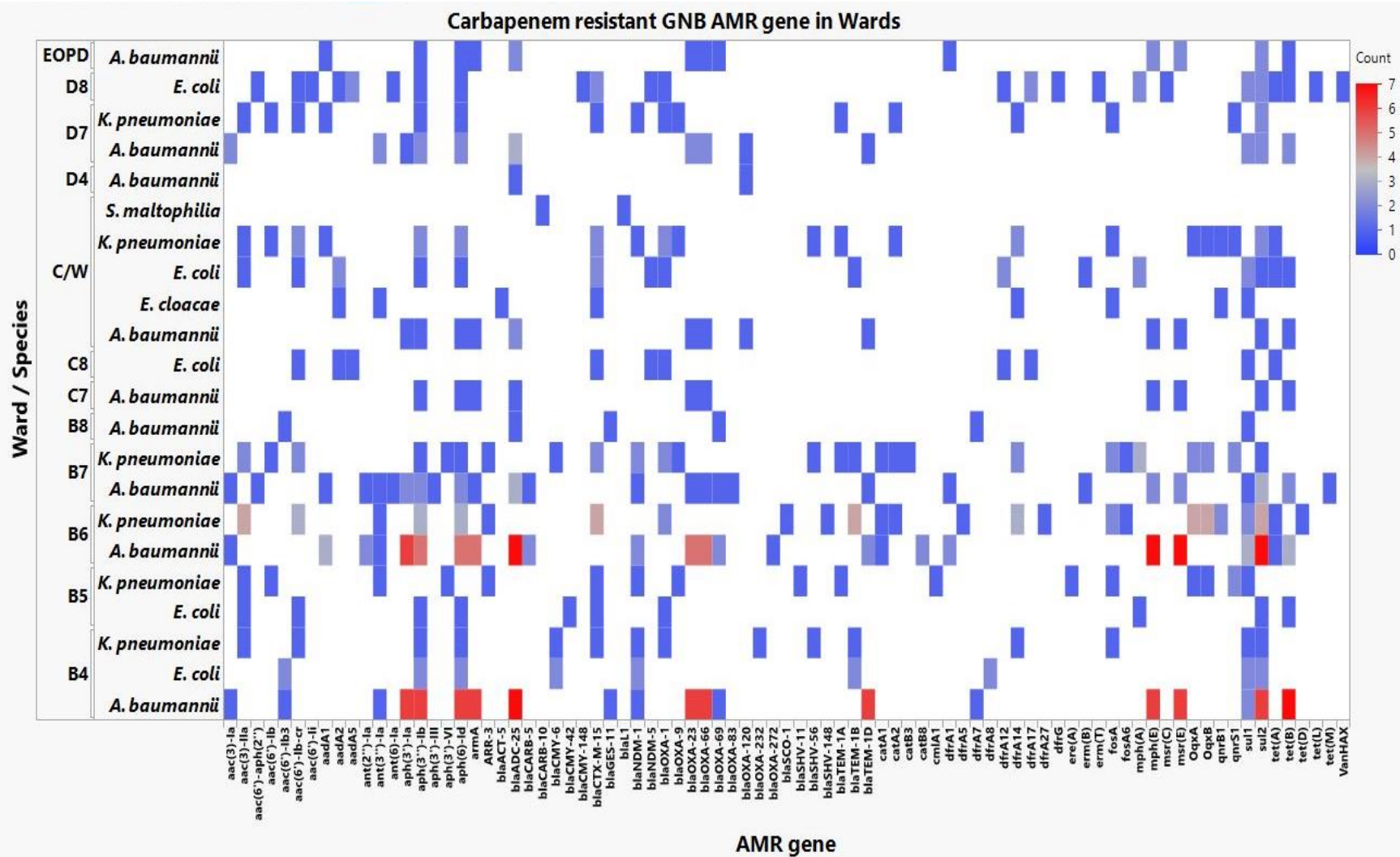


Figure 41: Beta-lactamase genes identified from carbapenem resistance gram-negative bacteria in admission wards

4.5.7. Genetic Diversity of Virulence Genes in Gram-Negative Bacteria Isolated from Bloodstream Infection

4.5.7.1. Virulence Genes in gram-negative bacteria

Among the total GNB isolates, VirulenceFinder analysis shows 101 (71.13%) possessed virulence genes; a total of 1055 genes, grouped into 133 types were identified. The most abundant virulence genes were detected in *E. coli*, 768(70.8%), *K. pneumoniae*, 233 (22.1%), and *E. cloacae*, 31 (2.9%). The highest prevalence virulence genes were recorded for *fimH*, *iutA*, *traT*, *terC*, *mrkA:ABW83989*, accounting for 63(6.0%), 58(5.5%), 54(5.1%), 48(4.5%), and 40(3.8%), respectively (Figure 42).

In *E. coli* a higher number of virulence genes, 768 out of 68 types, were found in 36 different species (Appendix 15). The virulence factors of *E. coli* are numerous and have a wide range of activities, including adhesins, toxins, iron acquisition factors, lipopolysaccharides, polysaccharide capsules, heat resistance, protectins, and invasins were explored. The ten most prevalent virulent genes in *E. coli* were; *gad* 36 (4.7%), Adhesins, *nlpI* 36 (4.7%) Adhesins, *terC* 36 (4.7%) Iron acquisition factors, *yehA* 30 (3.9%) Adhesins, *hlyE* 29 (3.8%) Toxin pore-forming, *traT* 27 (3.5%) Increased serum survival, *AslA* 25 (3.3%) Invasins of the brain, *yehB* 36 (4.7%), *yehD* 36 (4.7%), and *yehC* 34(4.4%) which helps bacterial cells to migrate into and multiply within the host (Appendix 15). Adhesins 24 types of 329(24.8%), Iron acquisition factors 7 types of 119(15.5%), Lipopolysaccharides 2 types 63(8.02%), Toxins 10 types of 54(7.0%), Increased serum survival 2 types of 33(4.3%), Polysaccharide capsule synthesis 6 types of 40(5.2%), Invasins 4 types of 38(4.9%), and Not well known 13 types of 92(12.0%) virulence gene were recorded. Among 36 drug-resistant *E. coli*, the highest 16.7% were found in PEOPD followed by 22.2% in D7 wards. On medical 27.8% and hematology 41.7% patients. Compared with other GNB *E. coli* in PEOPD and D7 prevalence were at 50% and 53.3% respectively. This GNB was found on patients of hematology medical and MICU patients 41.7%, 27.8%, and 13.9% patients respectively (Appendix 15).

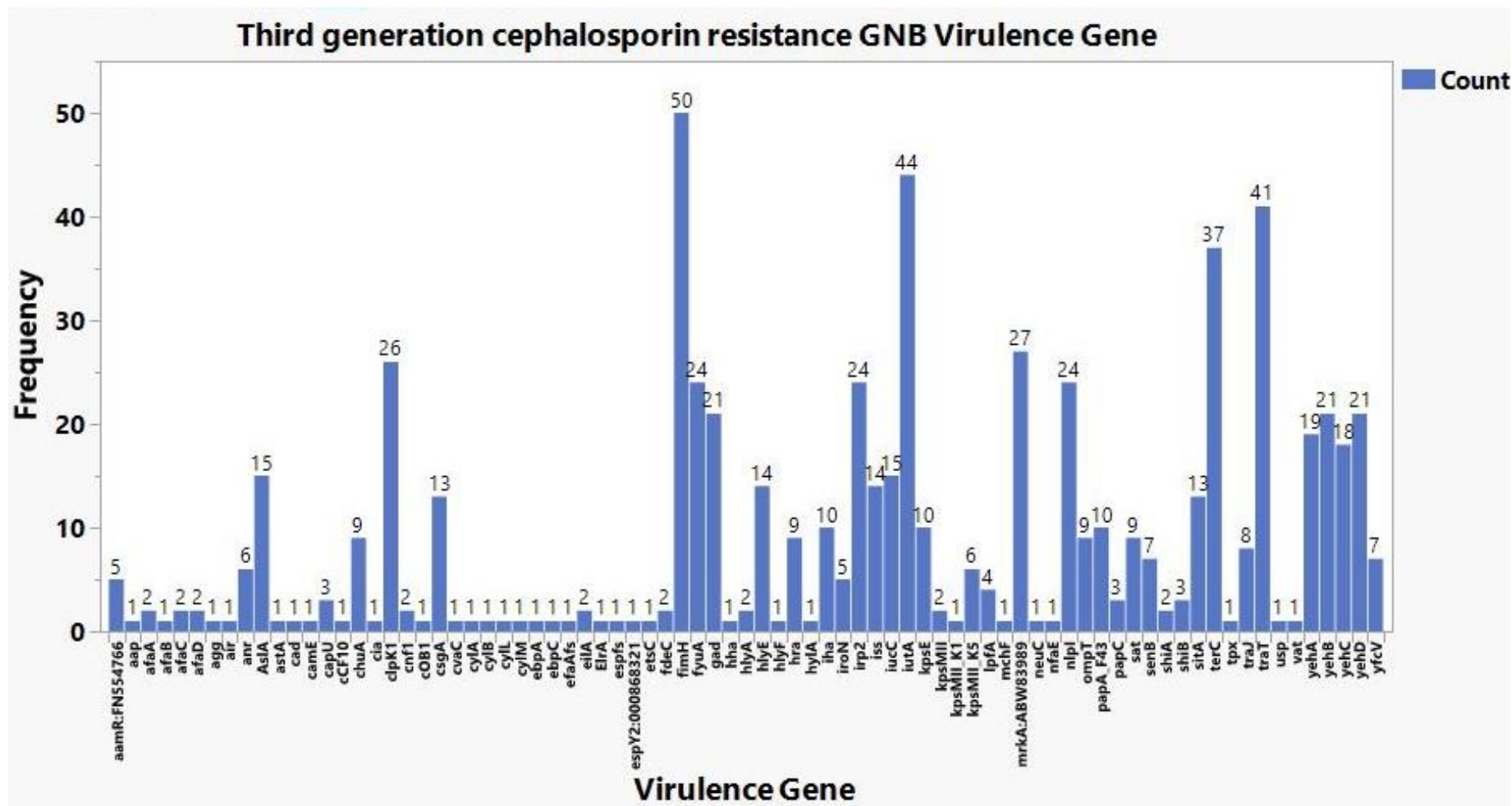
4.5.7.2. Virulence genes in ESBL, AmpC, MBL, and Carbapenemase, producing Gram-Negative Bacteria

ESBL: In the third-generation cephalosporin-resistant gram-negative bacteria, 654 genes, grouped into 80 types, were detected; fimH, 50(7.65%), iutA, 44(6.73%), traT, 41(6.27%), terC, 37(5.66%), and mrkA:ABW83989, 27(4.13%), were the predominant virulent genes (Figure 43).

The highest number of virulence gene of fimH 33(5.0%), iutA 29(4.4%), mrkA:ABW83989 27(4.1%), traT 20(3.1%) and clpK1 18(2.8%) identified in *K. pneumoniae*, whereas terC 8(1.2%), clpK1 6(0.9%), traT 5(0.8%), nlpI 1(0.2%) and gad 1(0.2%) identified in *K. oxytoca*. The highest number virulence gene of nlpI 20(3%), terC 20(3%), gad 20(3%), yehB 20(3%), and yehD 20(3%) identified in *E. coli* and nlpI 2(0.3%), iroN 2(0.3%) and terC 1(0.2%) identified in *E. cloacae* (Figure 44).

Among virulent gene in third-generation cephalosporin-resistant gram-negative bacteria Medical, Hematology, NICU, MICU, ENT unit, orthopedics, General surgery, and Emergency accounted for 214(32.7%), 171(26.1%), 140(21.4%), 51(7.8%), 33(5.0%), 18(2.8%), 15(2.3%), and 12(1.8%), respectively. The highest virulent gene was recorded in the Hematology, Medical, and NICU departments, accounting for *E. coli* 153(23.4%), *E. coli* 149(22.8%), and *K. pneumoniae* 89(13.6%) respectively. The highest number of virulent genes recorded in NICU from *K. pneumoniae* were fimH 18(20.2%), iutA 14(15.7%), traT 15(16.9%), mrkA:ABW83989 12(13.5%), clpK1 11(12.4%), fyuA 7(7.9%), and irp2 7(7.9%) (Figure 44).

The predominant gene found in wards showed B6 140(21.4%) and the predominant organism was *K. pneumoniae* 89(13.6%). The second one was D7 accounted for 93(14.2%) and the majority was *E. coli* 88(13.5%). The third one was C8 59(9%) and the most common bacteria was *E. coli* (Figure 45).



AmpC: Among 17 third-generation cephalosporin and cephamycin-resistant bacteria 136 virulent genes were identified. The predominant virulent genes were *terC*, 10(7.4%), *nlpI*, 8(5.9%), *gad*, 7(5.1%), *hlyE*, 7(5.1%), and *yehB*, 7(5.1%) (Figure 46).

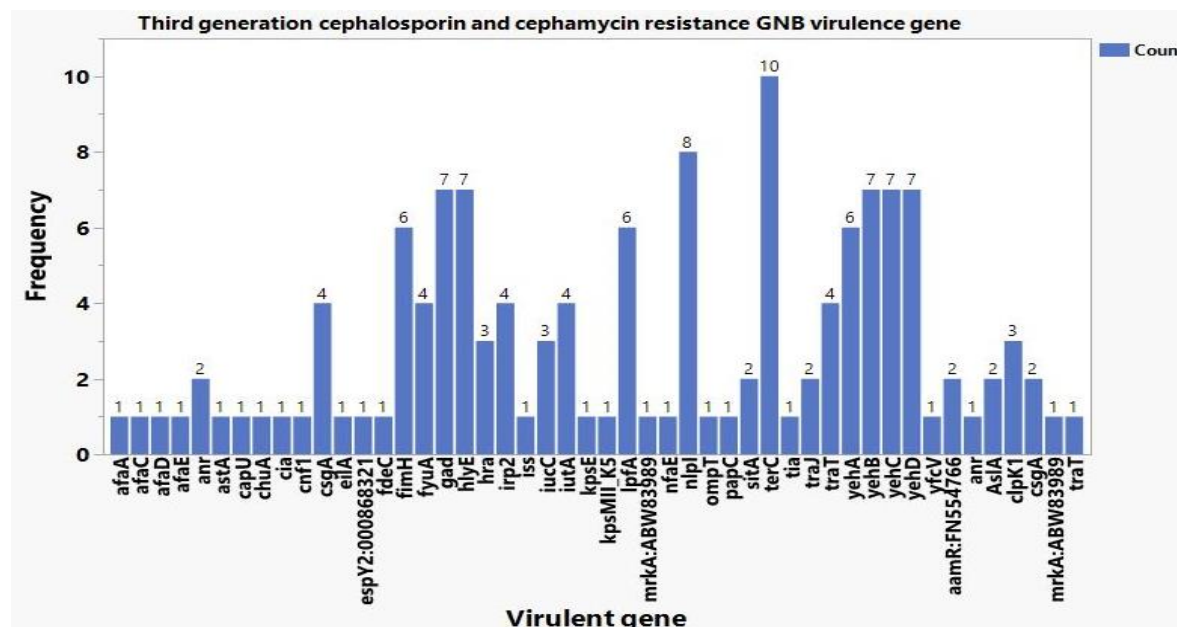


Figure 46: AmpC beta-lactam drug resistance bacteria virulent genes.

The higher prevalence of virulence gene identified in AmpC producer *Escherichia coli* 121(89.0%) in which the predominant were *terC*, *nlpI*, *gad*, *hlyE*, *yehB*, *yehC*, and *yehD* each accounted for 7(5.1%), respectively. Among *Enterobacter cloacae* 7(5.1%) the higher prevalence gene were *terC* 3(2.2%), *clpK1* 2(1.5%), *nlpI* 1(0.7%), and *mrkA:ABW83989* 1(0.7%). The higher prevalence of virulence gene identified in *Klebsiella pneumoniae* 7(5.1%) were *clpK1*, *fimH*, *fyuA*, *irp2*, *iutA*, *traT*, and *mrkA:ABW83989* each accounted for 1(0.7%), respectively. In *Klebsiella oxytoca* 1(0.7%) one *traT* 1(0.7%) genes were identified whereas no virulence genes were identified *A. baumannii*. Among AmpC beta-lactam-resistant bacteria high burden of virulent genes caused by *E. coli* was found in the Medical, Emergency, and ICU departments. However, *Klebsiella pneumoniae* was abundant in General surgery (Figure 47). The most abundant virulence genes were found in D7 38(27.9%), B4 36(26.5%), C8 29 (21.3%), and EOPD 21(15.4%) wards. The red color on the graph indicates the higher load of Virulence gene availability (Figure 48).

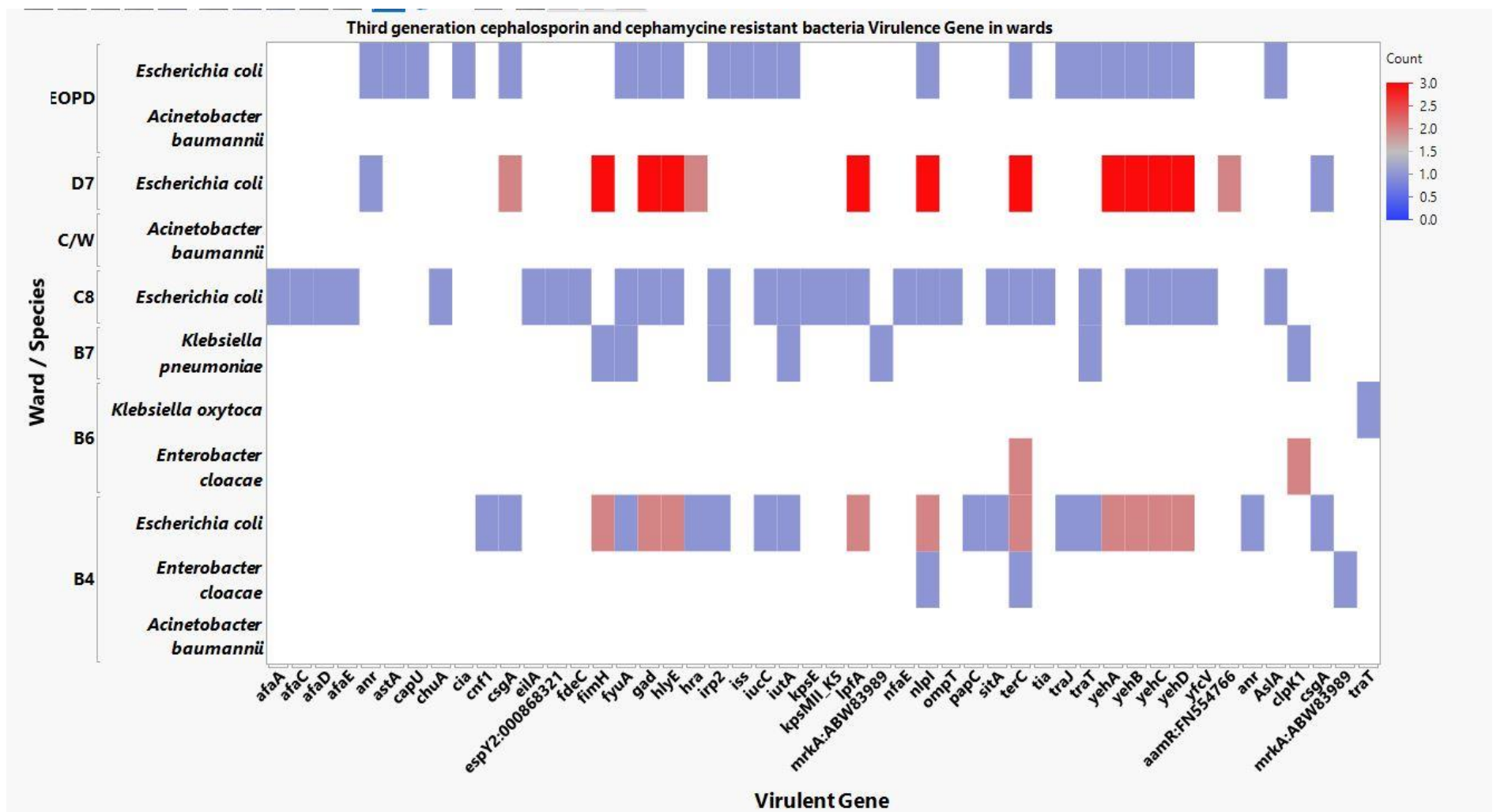


Figure 48: AmpC beta-lactam resistant gram-negative bacteria virulence genes in wards

Carbapenemase: Among these carbapenem-resistant gram-negative bacteria, 265 virulent genes were identified. The most predominant virulence factor causing virulence genes were iutA, fimH, fyuA, irp2, mrkA:ABW83989, and traT that accounted for 14(5.3%), 13(4.9%), 13(4.9%), 13(4.9%), 12(4.5%), and 12(4.5%), respectively (Figure 49).

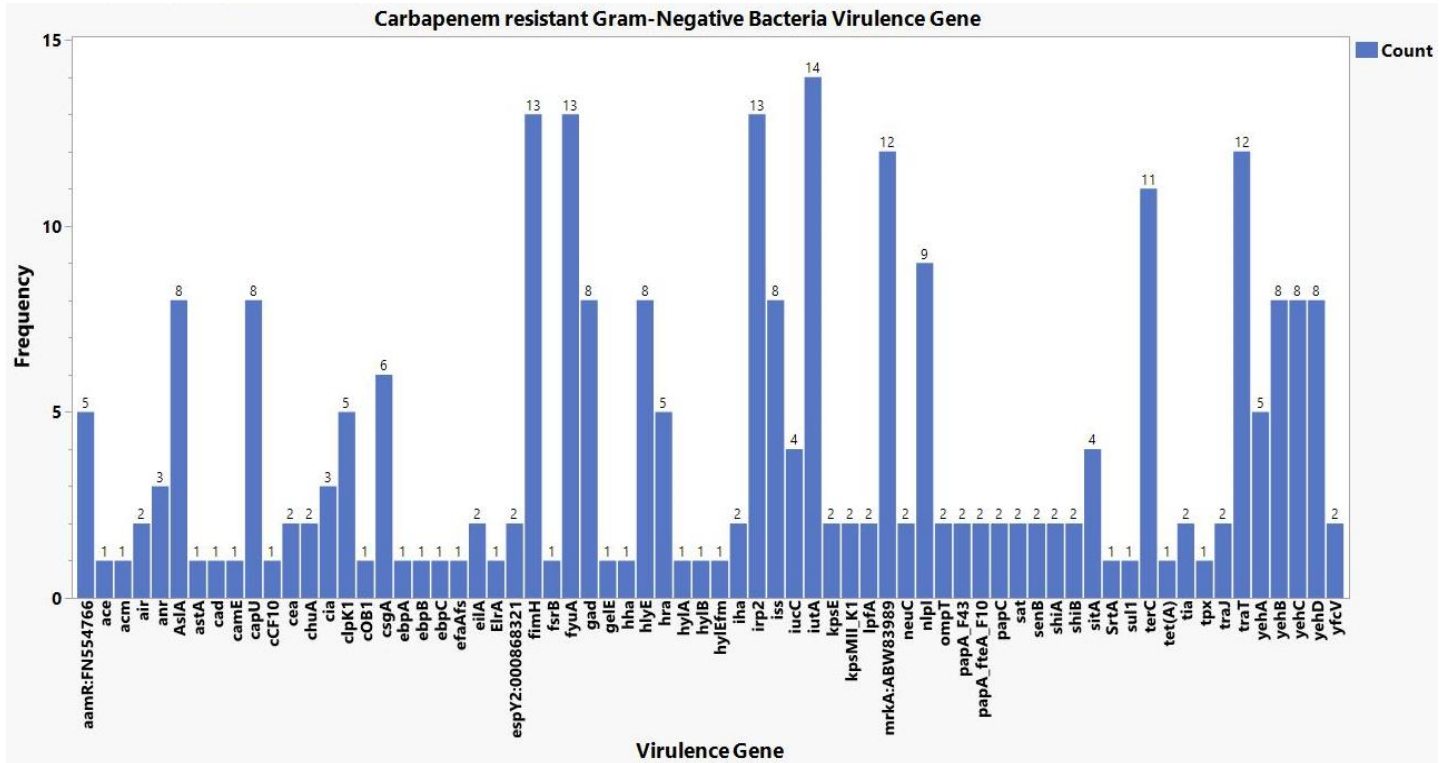


Figure 49: Virulent genes among carbapenem drug-resistant gram-negative bacteria

The predominant virulence gene identified in carbapenem resistance *K. pneumoniae* were fimH, mrkA:ABW83989, iutA, and fyuA, irp2 accounted for 11(4.2%), 11(4.2%), 10(3.8%), 8(3.0%), and 8(3.0%), respectively. The predominant virulence gene identified in *E. coli* terC, nlpI, AslA, capU, gad, hlyE, iss, yehB, yehC, and yehD each accounted for 8(3.0%), respectively. The virulence gene identified in *A. baumannii* were Ace, cad, came, cCF10, cOB1, ebpA, ebpB, ebpC, efaAfs, ElrA, fsrB, gelE, hlyA, hlyB, SrtA, Tpx, each accounted for 1(0.4%), respectively. Whereas three virulence genes identified in carbapenem resistance *E. cloacae* were mrkA:ABW83989, terC, and nlpI accounted for 1(0.40%), respectively.

The average virulent gene per carbapenem-resistant gram-negative bacteria was higher in *E. coli* followed by *K. pneumoniae*, and *E. cloacae* which account for 22.88%, 5.73%, and 3.0% respectively. *A. baumannii* accounted for 0.57 and the rest don't have virulence genes. Among carbapenem-resistant gram-negative bacteria in the Hematology department had a higher prevalence of carbapenem virulence genes 86 (32.5%). These virulent genes identified from *E. coli* 4(8.0%), and *K. pneumoniae* 2(4.0%), accounted for 75(28.3%), and 11(4.2%), respectively. However, from carbapenem resistance, *A. baumannii* 5(10%) and *Stenotrophomonas maltophilia* 1(2.0%) virulence genes were not identified. The second higher prevalence of virulence gene 76(28.7%) were found in MICU department from *A. baumannii* 1(2.0%) and *E. coli* 2(4.0%) accounted for 0(0.0%), and 76(28.7%). The third higher virulence gene 61(23.0%) was identified from the medical department from *A. baumannii* 6(12.0%), *E. coli* 2(4.0%), *K. pneumoniae* 2(4.0%), and *Pseudomonas putida* 1(2.0%) accounted for 16(6.0%), 32(12.1%), 13(4.9%), and 0(0.0%), respectively (Appendix 14) (Figure 50).

Among wards the higher virulence was recorded in B4 83(31.3%), B5 28(10.6%), and B6 24(9.1%). From B4 *A. baumannii* 7(14.0%), *E. coli* 2(4.0%), and *K. pneumoniae* 1(2.0%), accounted for 0(0.0%), 762(8.7%), 72(.6%), virulent gene respectively. Among B5 *E. coli* 1(2.0%), and *K. pneumoniae* 1(2.0%), accounted for 23(8.7%), and 5(1.9%), respectively. Among B6 *K. pneumoniae* 4(8.0%) accounted for 24(9.1%) (Appendix 14) (Figure 51).

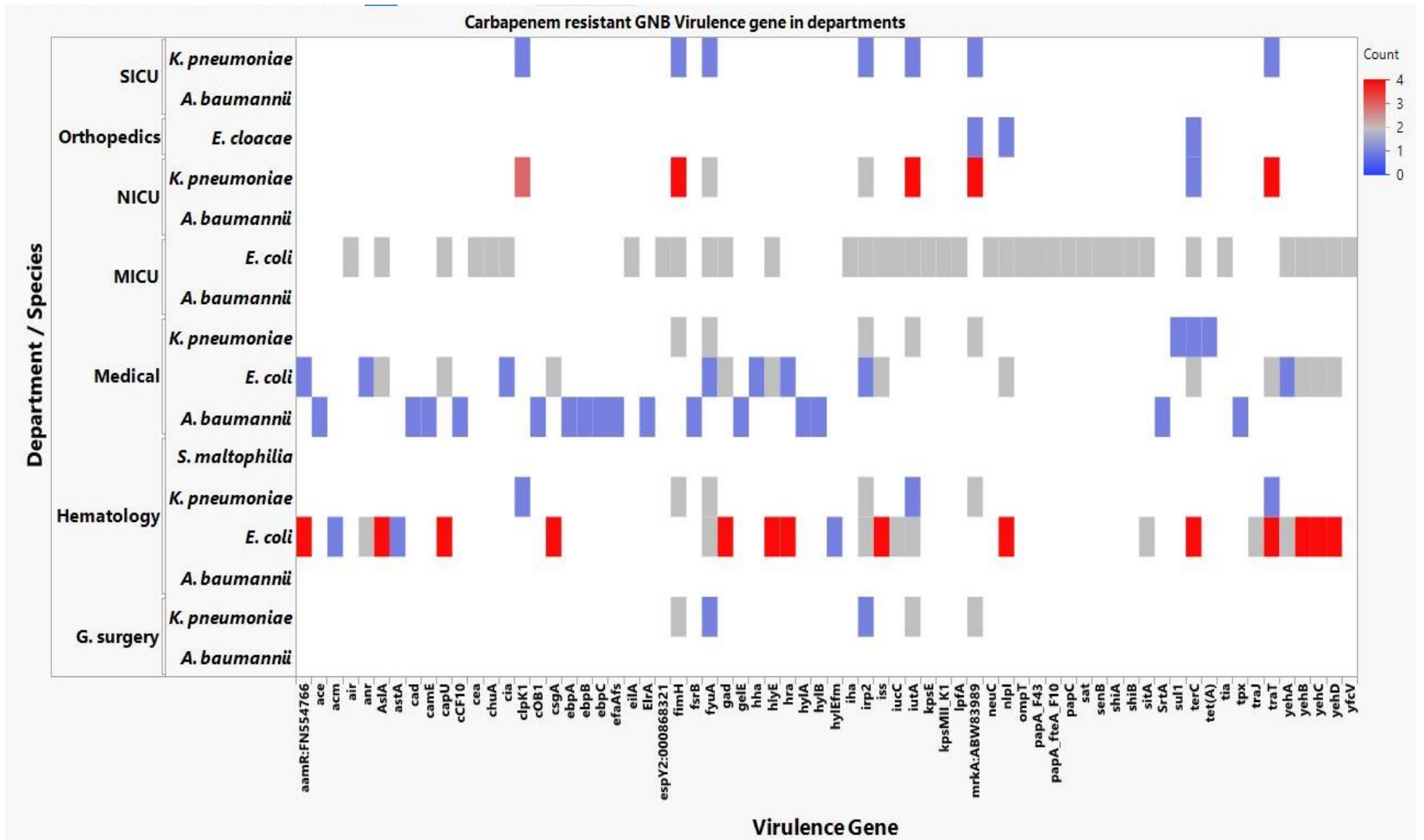


Figure 50: Carbapenem resistance gram-negative bacteria virulence genes in departments

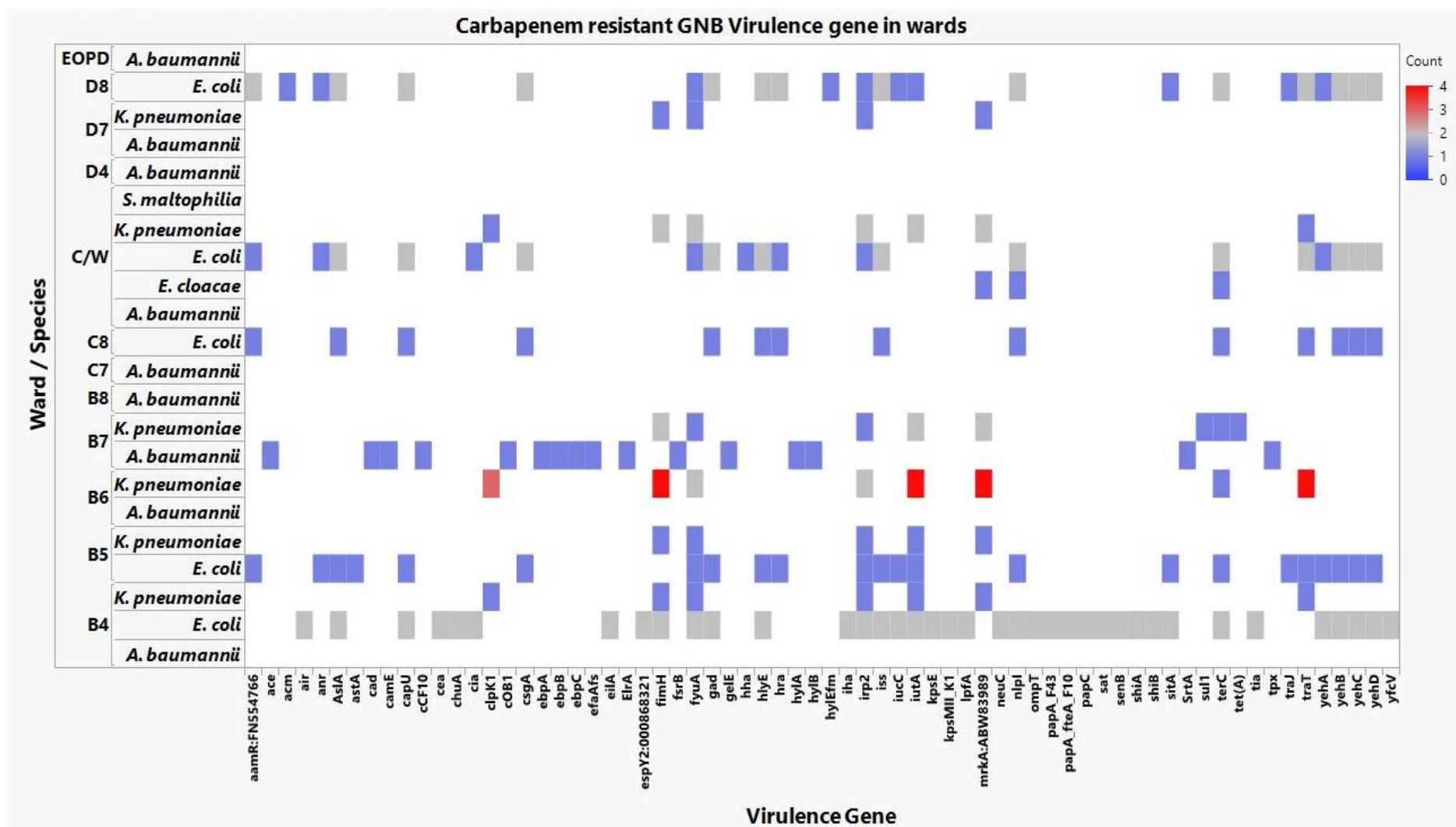


Figure 51: Carbapenem resistance gram-negative bacteria virulence factor among wards

4.5.8. Mutations in ESBL, AmpC, Carbapenemase, and MBLs

4.5.8.1. Mutations of Single Nucleotide Polymorphisms

In this study, there were 83/142(57.7%) GNB having antimicrobial resistance gene point mutation. Eight types ompK37 45(20%), acrR 43(19%), ompK36 43(19%), parC 30(14%), gyrA 30(14%), parE 26(12%) ramR 2(1%), and rpoB 2(1%), of a total of 220 antimicrobial gene mutations, were recorded from 83(58.5%) bacterial strains (Table 30).

Among *K. pneumoniae* 45/46 strains had 125(56.8%) gene mutation identified; mutations on ompK37, acrR, ompK36, and ramR accounted for 45(36%), 43(34.4%), 35(28%), and 2(1.6%), respectively. *E. coli*, 30/36, strains had 88 (40%) gene mutations; mutations on gyrA, parC, parE, and rpoB accounted for 30(34.1%), 30(34.1%), 26(29.5%), and 2(2.3%), respectively. Seven out of eleven strains of *K. oxytoca* contained a total of 7(3.2%) genes, all of which are ompK36 genes (Table 30).

Table 30: Gene mutations involved in AMR gram-negative bacteria n=142.

Species	gram-negative bacteria		Antimicrobial resistance gene mutation									
	Total	positive	ompK37	acrR	ompK36	gyrA	parC	parE	ramR	rpoB	Total	%
<i>K. pneumoniae</i>	46	45	45	43	35	0	0	0	2	0	125	56.8%
<i>E. coli</i>	36	30	0	0	0	30	30	26	0	2	88	40.0%
<i>K. oxytoca</i>	11	7	0	0	7	0	0	0	0	0	7	3.2%
<i>A. baumannii</i>	35	0	0	0	0	0	0	0	0	0	0	0.0%
<i>E. cloacae</i>	9	0	0	0	0	0	0	0	0	0	0	0.0%
<i>S. maltophilia</i>	2	0	0	0	0	0	0	0	0	0	0	0.0%
<i>P. monteilii</i>	1	0	0	0	0	0	0	0	0	0	0	0.0%
<i>P. rettgeri</i>	1	0	0	0	0	0	0	0	0	0	0	0.0%
<i>C. shandongense</i>	1	0	0	0	0	0	0	0	0	0	0	0.0%
Total	142	83	45	43	42	30	30	26	2	2	220	100%
%		58.5%	20%	20%	19%	14%	14%	12%	1%	1%	100%	

Mutations on ompK37, acrR, and ramR genes were found in *K. pneumoniae*. The ompK36 gene was found in *K. pneumoniae* and *K. oxytoca*. Mutations on gyrA, parC, and parE genes were found in *E. coli* (Figure 52).

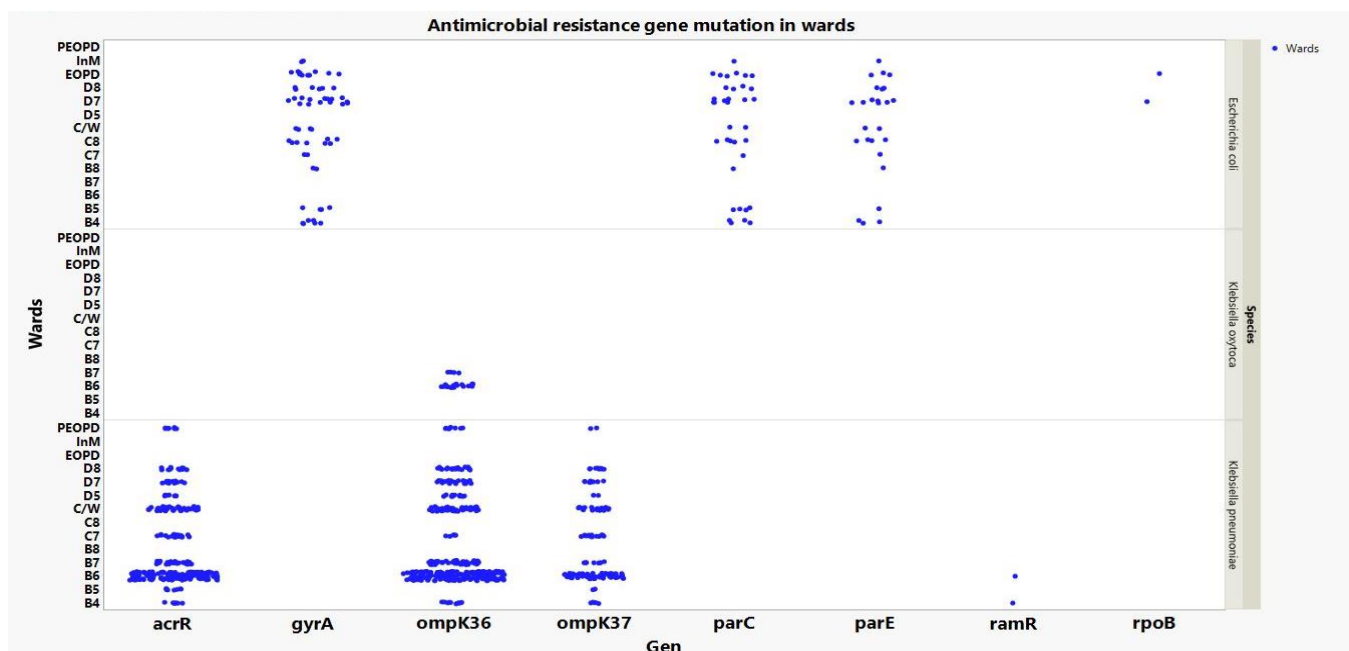
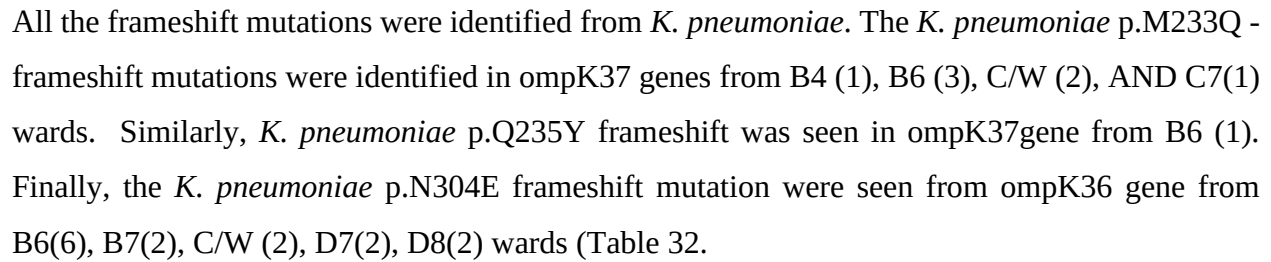


Figure 52: Mutations in antimicrobial resistance genes of Gram-negative bacteria in admission wards

Among eight genes, 956-point mutations were recorded. However, 22(2.3%) of the mutations were frameshift mutations and the rest 934 were point mutations. One or more gene mutations were observed on the antimicrobial resistance genes. Table 31 shows the prevalence of antimicrobial resistance gene mutations; the average number of point mutations on ompK36, acrR, ompK37, gyrA, parC, pare, ramR, and rpoB accounted for 9.5, 7.0, 3.1, 2.0, 1.2, 1.0, 1.0, and 1.0, respectively. ompK37, acrR and ompK36 were the most prevalent antimicrobial resistance mutations (Table 31).

Table 31: Different kinds of mutations prevalent in AMR gram-negative bacteria n=142.

Genes	Gene mutation		Point mutation		Frameshift mutation		Ave p-mutation
	N	%	N	%	N	%	N
ompK37	45	20.5%	140	14.6%	8	36.4%	3.1
acrR	43	19.5%	301	31.5%			7.0
ompK36	42	19.1%	390	40.8%	14	63.6%	9.3
gyrA	30	13.6%	59	6.2%			2.0
parC	30	13.6%	36	3.8%			1.2
parE	26	11.8%	26	2.7%			1.0
ramR	2	0.9%	2	0.2%			1.0
rpoB	2	0.9%	2	0%			1.0
Total	220	100%	956	100%	22		4.3



Highest gene mutation was observed in NICU 60 (27.3%), Medical 60 (27.3%), and Hematology 59 (26.8%) (Table 33).

Table 33: Antibiotic resistance gene mutation of gram-negative bacteria bloodstream infections in departments n=142.

Department of Follow-up	Antimicrobial resistance gene mutation								Total	Total %
	ompK37	acrR	ompK36	gyrA	parC	parE	ramR	rpoB		
NICU	20	18	21	0	0	0	1	0	60	27.3%
Medical	12	12	11	9	9	7	0	0	60	27.3%
Hematology	6	6	5	14	14	13	0	1	59	26.8%
G. Surgery	6	6	4	0	0	0	0	0	16	7.3%
SICU	1	1	1	1	1	1	1	0	7	3.2%
Emergency	0	0	0	2	2	1	0	1	6	2.7%
MICU	0	0	0	2	2	2	0	0	6	2.7%
Orthopedics	0	0	0	1	1	1	0	0	3	1.4%
ENT Unit	0	0	0	1	1	1	0	0	3	1.4%
Total	45	43	42	30	30	26	2	2	220	100 %

The most affected departments were NICU, medical, hematology, and general surgery with ompK36, acrR, and ompK37 genes (Figure 54).

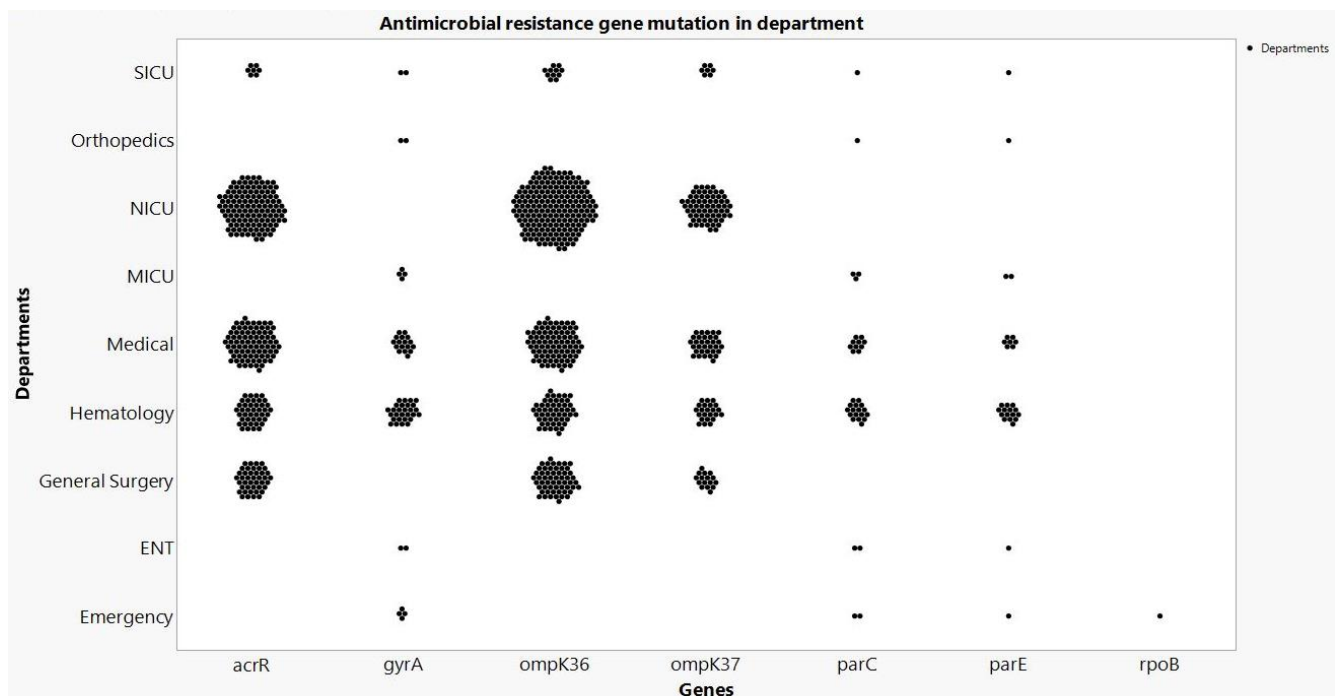


Figure 54: Antimicrobial resistance gene mutations in gram-negative bacteria isolated from bloodstream infections in departments

4.5.8.2. ESBL, AmpC, Carbapenemase, and MBLs Drug Resistance Due to Antimicrobial Resistance Gene Mutation of *E. coli*, *K. Pneumoniae*, and *K. Oxytoca*

ESBL: Among 83 strains, all 17 third-generation cephalosporin resistance and ESBL producer *E. coli* have gyrA (p.D87N, p.S83L), parC (p.S80I), and parE (p.L416F) or (p.I529L) or (p.S458A) or (p.S458T) mutations. But, 4 of them have parC (p.E84V, p.S80I) instead of parC (p.S80I). Among six third-generation cephalosporin resistance and ESBL producer *K. oxytoca*, all have ompK36 (p.N49S, p.T184P) except the one having ompK36 (p.A183S, p.A190W, p.A217S, p.E232R, p.F198Y, p.F207W, p.L191S, p.L228V, p.N218H, p.N49S) many point mutations. Among all 33 third-generation cephalosporin resistance and ESBL producer *K. pneumoniae*, six of them (ST1799*, Stnov, ST45*, Stnov, ST973*, Stnov, ST973*, Stnov, ST973*, Stnov, and ST973*, Stnov) are novel drug-resistant *K. pneumoniae*. All have acrR (p.F172S, p.F197I, p.G164A, p.K201M, p.L195V, p.P161R, p.R173G), ompK36 (different kinds of point mutation), and ompK37 (p.I128M, p.I70M) mutations. But seven of them didn't have the ompK36 gene and one didn't have the acrR mutation. Among *K. pneumoniae* twelve had additional different types of point mutations on the ompK37 gene and five of them had frameshift mutation on the ompK37 (p.M233Q) gene. Among 56 third-generation cephalosporin resistance and ESBL producers, 22 *Klebsiella spp* 18 *K. pneumoniae*, and 4 *K. oxytoca* were found in the NICU. That means there were ESBL producer *Klebsiella species* with higher prevalence in NICU (Sup Table Excel).

AmpC: Seven *E. coli*, one *K. pneumoniae*, and one *K. oxytoca* were AmpC and ESBL drug-resistant. All AmpC resistant bacteria have gyrA (p.D87N, p.S83L), parC (p.S80I), parE (p.S458A) point mutations except FF02_1478 *E. coli* ST410. ST692 had additional rpoB (p.Q513L) and FF02_2927 *E. coli* ST167 and ST877 have rpoB (p.Q513L) instead of parE (p.S458A) mutated gene. This indicated that gyrA (p.D87N, p.S83L), parC (p.S80I), and parE (p.S458A) genes have an association with AmpC and ESBL drug resistance (Sup Table). The AmpC, ESBL FF02_5807 *K. oxytoca* ST91 had an ompK36 (p.N49S, p.T184P) mutated gene. The FF02_6025 *K. pneumoniae* ST711 had acrR, ompK36, and ompK37 genes (Sup Table Excel).

Carbapenem: All carbapenem-resistant *K. pneumoniae* had acrR (p.F172S, p.F197I, p.G164A, p.K201M, p.L195V, p.P161R, p.R173G), ompK36 with different point mutations, and ompK37

(p.I128M, p.I70M) antimicrobial resistance mutation. Two FF02_291 and FF02_3884 *K. pneumoniae* ST147 lack the ompK36 antimicrobial resistance mutation gene. However, FF02_2840 *K. pneumoniae* ST4407 had only ompK37 (p.I128M, p.I70M) mutation. Among carbapenem resistance *E. coli* all have ST167, ST2 multilocus sequencing type except FF02_ had additional ST741 multilocus sequencing type. All have gyrA (p.D87N, p.S83L), parC (p.S80I), and pare (p.S458A) except FF02_3913 which has gyrA (p.D87N, p.S83L), and parC (p.E84G, p.S80I) mutations (Sup Table Excel).

4.5.9. Molecular Epidemiology of Plasmid MLST and Plasmid Gene in Gram-Negative Bacteria from Bloodstream Infection

4.5.9.1. Plasmid MLST and Plasmid Gene in Gram-Negative Bacteria from Bloodstream Infections

Plasmid multilocus sequence (PlasmidMLST) and drug resistance plasmid gene were identified using plasmidMLST and PlasmidFinder in order to comprehend the dynamics of drug resistance gene transmission. The PlasmidFinder outputs from 142 isolates showed that 103 (72.54%) of the gram-negative bacteria had drug-resistant plasmid multilocus sequencing and 108 (76%) had drug-resistant plasmid genes. Among all strains, 133 (100%) plasmidMLST and 451 (100%) plasmid genes were identified. Five isolates had drug resistance genes carrying plasmids without plasmid multilocus identification. Among isolates that had drug-resistant plasmid multilocus sequence type were *K. pneumoniae*, *K. oxytoca*, *E. coli*, and *E. cloacae* accounting for 46(100%), 11 (100%), 34 (94.4%), and 8(88.9%), respectively. All *K. pneumoniae*, *K. oxytoca*, *E. coli*, and *E. cloacae* isolates carried plasmids containing drug-resistant genes. Out of 35 *A. baumannii* isolates, plasmid genes were identified from 5 isolates (14.3%) and plasmidMLST from 3 isolates (8.6%) were identified. (Table 34).

Table 34: Plasmid MLST and plasmid gene in 142 gram-negative bacteria

Species	Species having Plasmid MLST				Species having Plasmid gene				T. Plasmid MLST		T. Plasmid Gene		Ave. P. Gene
	Neg	Pos	%	Total	Neg	Pos	%	Total	N	%	N	%	
<i>K. pneumoniae</i>	0	46	100%	46	0	46	100%	46	59	44.4%	171	37.9%	2.9
<i>E. coli</i>	2	34	94.4%	36	0	36	100%	36	37	27.8%	177	39.2%	4.8
<i>K. oxytoca</i>	0	11	100%	11	0	11	100%	11	20	15.0%	56	12.4%	2.8

<i>E. cloacae</i>	1	8	88.9%	9	0	9	100%	9	10	7.5%	29	6.4%	2.9
<i>A. baumannii</i>	32	3	8.6%	35	30	5	14.3%	35	6	4.5%	16	3.5%	2.7
<i>P. Rettgeri</i>	0	1	100%	1	0	1	100%	1	1	0.8%	2	0.4%	2.0
<i>S. maltophilia</i>	2	0	0.0%	2	2	0	0%	2	0	0.0%	0	0.0%	0.0
<i>P. monteilii</i>	1	0	0.0%	1	1	0	0%	1	0	0.0%	0	0.0%	0.0
<i>C. shandongense</i>	1	0	0.0%	1	1	0	0%	1	0	0.0%	0	0.0%	0.0
Total	39	103	72.5%	142	34	108	76%	142	133	100%	451	100%	3.4

Among 46(100%) *K. pneumoniae* a total of 59 (44.4%) plasmid MLST and 171(37.9%) plasmid genes were identified. Similarly, in 34/36 (94.4%) *E. coli* had 37 (27.8%) plasmid MLST and carried 177 (39.2%) plasmid genes. All of the 9 *K. oxytoca* isolates had 20(15%) plasmid MLST and carried 56(12.4%) plasmid replicons. In *E. cloacae* among 8(88.9%) isolates 9(100%) plasmid MLST were identified and all of them carried plasmid replicons. Among 32 *A. baumannii* 3 (8.6%) isolates had 6(4.5%) total plasmid MLST and 16(3.6%) plasmid replicons. There was no Plasmid MLST and plasmid gene identified in *P. monteilii*.

Among 133 drug-resistant plasmid incompatibility (Inc) groups IncF RST, 98 (74.8%), IncHI 23, (17.6%), IncA/C 5, (3.8%), and IncN, 5 (3.8%), were identified. Among the top 5 plasmid multilocus sequence types IncF RST[K7:A-B-], IncHI2 DLST1, IncFRST[F-A-B-], IncHI1 MLST Unknown, and IncF RST[K4:A-B-] accounted for 22(16.5%), 14(10.5%), 10(7.5%), 9(6.8%), and 8(6.0%), respectively. Among the top 5 plasmid genes IncFII(K), IncFIB(K), IncFIB(AP001918), IncFIA, and Col(pHAD28) accounted for 37(8.2%), 35(7.8%), 29(6.5%), 28(6.2%), and 23(5.1%), respectively (Figure 55a,b).

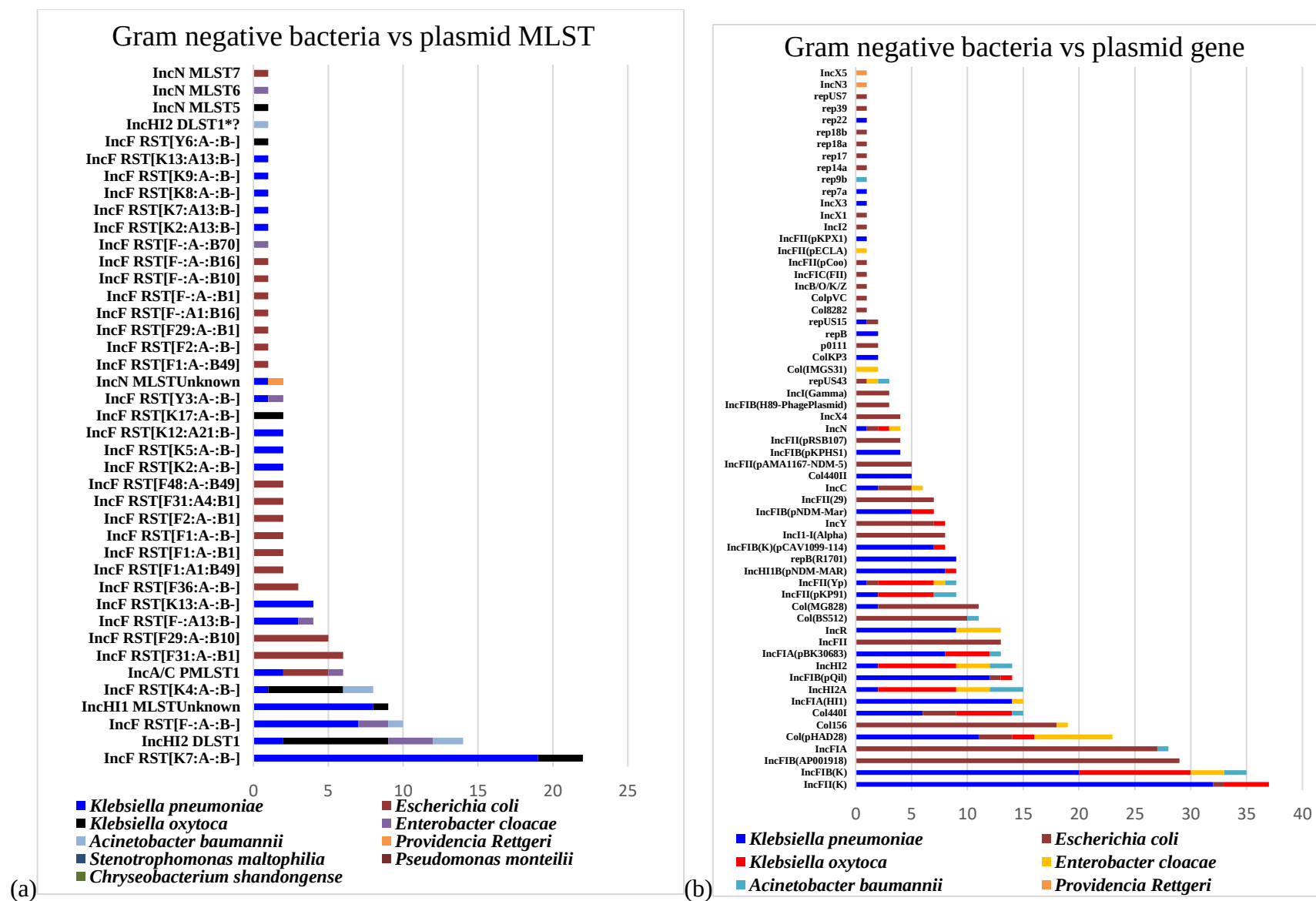


Figure 55: Plasmid MLST(a) and plasmid gene(b) of gram-negative bacteria isolated from bloodstream infections.

Among wards B6, D7, B4 and C/W were the most affected areas of Plasmid MLST and plasmid-containing drug-resistant gene transmission (Figure 56).

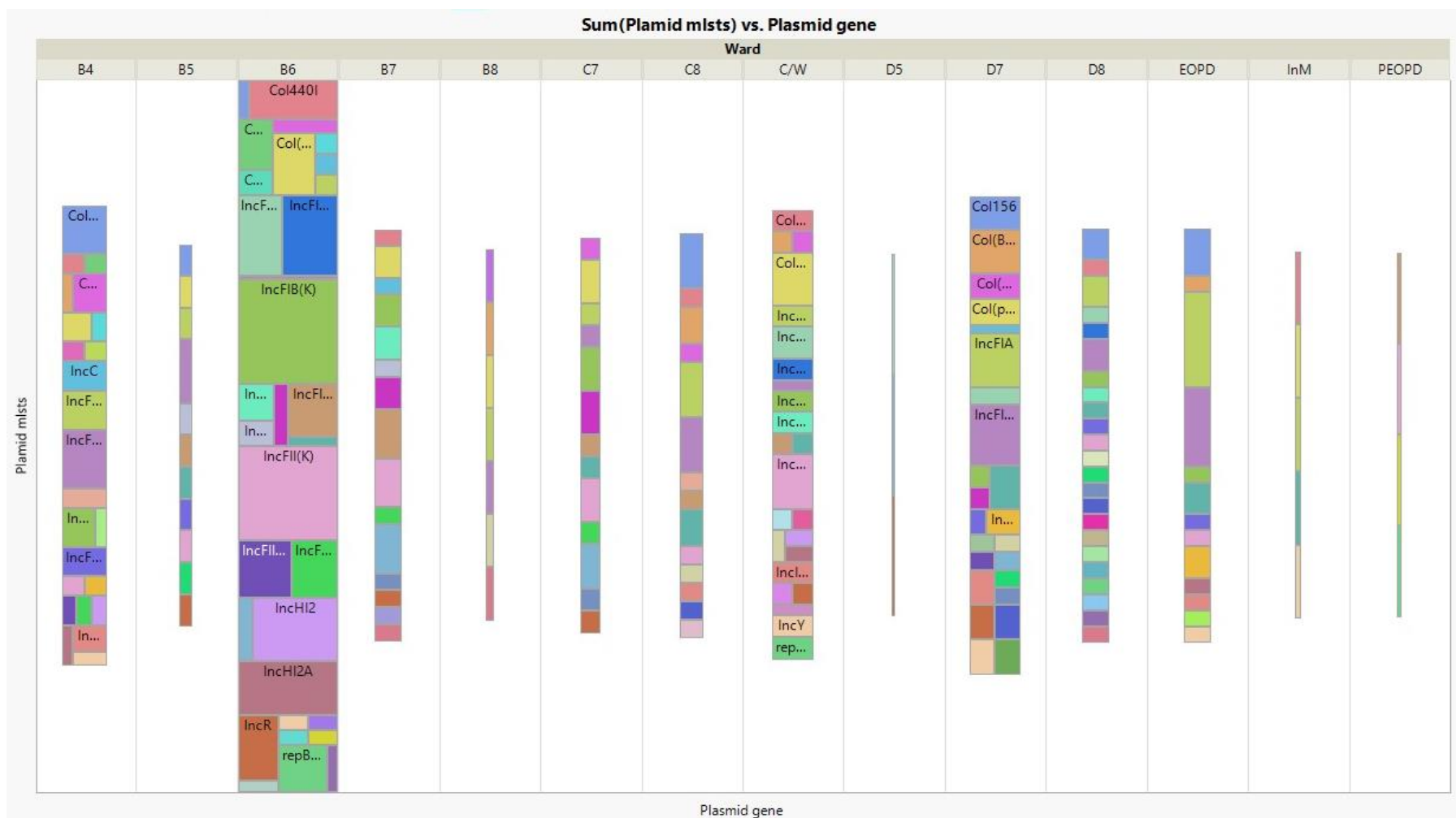


Figure 56: Plasmid MLST versus plasmid genes in their respective wards among gram-negative bacteria isolated from bloodstream infections

4.5.9.2. Plasmid MLST and Plasmid Genes Identified in ESBL, AmpC, MBLs, and Carbapenem carrying Gram-Negative Bacteria

ESBL: Among 74 ESBL-positive isolates 67(90.5%) PMLST and 70(94.6%) AMR plasmid genes were identified. Three *A. baumannii* ST1089*Stnov, ST1351, ST699, and ST193 were ESBL-producing but didn't have PlasmidMLST and antimicrobial-resistant plasmid genes.

All ESBL producer *E. coli* had IncF RST Plasmid incompatibility (Inc) groups with different types of plasmid genes, except two *E. coli* multilocus sequence typing of ST540/ST716 which didn't have plasmid MLST and they had IncY plasmid gene. One *E. coli* multilocus sequence typing of ST131/ST43 and IncF RST[F2:A-B1] plasmid 1st had IncFIB (AP001918), IncFIB (H89-PhagePlasmid), IncFIB (pQil), IncFII, IncFII(K), and IncI1-I(Alpha) genes. Two ESBL producer *E. coli* isolates had carbapenem genes. These *E. coli* ST224/ST479 and *E. coli* ST609 had similar IncF RST[F48:A-B49] plasmid MLST and had Col156, IncFIA, IncFIB(AP001918), IncFII(29), IncFII(pAMA1167-NDM-5), IncX1, and IncFIA, IncFIB(AP001918), IncFII(pAMA1167-NDM-5) genes, respectively.

All ESBL Producer *K. oxytoca* had plasmid MLST and plasmid genes. All *K. oxytoca* had IncF RST[K4:A-B-], IncHI2 DLST1 plasmid MLST except 3 had IncF RST[K4:A-B-], IncN MLST5, and IncF RST[K4:A-B-], IncHI1 MLST Unknown. Three *K. oxytoca* (ST137) had IncF RST[K4:A-B-], IncHI2 DLST1 plasmid MLST and Col440I, IncFIA(pBK30683), IncFIB(K), IncFII(Yp), IncFII(pKP91), IncHI2, IncHI2A plasmid gene. In *K. oxytoca* *K. oxytoca* (ST305) IncF RST[Y6:A-B-], IncHI1 MLST Unknown and IncF RST[K17:A-B-], IncHI2 DLST1 plasmid MLST had carbapenem gene Col440I, IncFIB(K)(pCAV1099-114), IncFIB(pNDM-Mar), IncFII(Yp), IncHI1B(pNDM-MAR) and IncFIB(K), IncFIB(pNDM-Mar), IncHI2, IncHI2A gene, respectively (Supp table Excel).

All ESBL producer *K. pneumoniae* had IncF RST plasmid incompatibility group but six of them had additional IncHI1 MLST Unknown and two of them had additional IncN MLST Unknown and IncHI2 DLST1. Six ESBL producer *K. pneumoniae* had IncF RST and IncHI1 plasmid incompatibility group had NDM (IncFIB(pNDM-Mar), and IncHI1B(pNDM-MAR)) carbapenem-resistant gene; however, the bacteria were phenotypically and PCR confirmed 3rd generation

cephalosporin-resistant and ESBL producer. The 3rd generation cephalosporin-resistant and ESBL producer *P. rettgeri* had IncN MLST Unknown plasmid MLST and IncN3, IncX5 plasmid gene; however, *S. maltophilia* didn't have plasmid gene (Supp table Excel).

AmpC: Among AmpC beta-lactamase 76.5% had plasmid carrying antimicrobial resistant genes. Among AmpC beta-lactamase resistance *A. baumannii* sequence types ST1397, ST1998, ST2018, ST2814, and ST1850 didn't have plasmid genes. Two *E. cloacae* sequence type ST252 had IncF RST[F-: A-:B-], and IncHI2 DLST1 plasmid MLST and Col(IMGS31), IncFIB(K), IncHI2, IncHI2A, and IncR genes. But, one ST148 sequence type had IncF RST[Y3:A-:B-] and Col(pHAD28), Col156, IncFIB(K), IncFII(Yp) AMR plasmid gene. In *E. coli* three strains had ST410, ST692 and IncF RST[F1:A1:B49] Plasmid MLST and Col(BS512), Col(MG828), IncFIA, IncFIB(AP001918), and IncFII(pAMA1167-NDM-5) AMR plasmid genes. The *E. coli* sequence type ST167/ST877 having IncF RST[F31:A4:B1] plasmid MLST showed Col(BS512), IncFIA, IncFIB(AP001918), IncFII, and IncI1-I(Alpha) AMR plasmid genes. Among *Klebsiella spp K. pneumoniae* and *K. oxytoca* ST711/ST91 had IncF RST[K7:A-:B-] plasmid MLST and IncFIB(pQil), IncFIB(K), IncFII(K) and Col(pHAD28), IncFIB(K), IncFII(K) plasmid AMR genes (Sup Table Excel).

Carbapenem: Among 28 carbapenem-resistant *A. baumannii* two of them multilocus sequence type ST1809/ST451/ST2 and ST1837/ST369/ST2 had plasmid MLST IncF RST[F-:A-:B-], IncHI2 DLST1* and IncHI2 DLST1* had IncFIA, IncHI2A, and IncHI2 plasmid gene. The rest two multilocus sequence types ST1806/ST208/ST2, and ST2323/ST405/ST1 didn't have plasmid MLST but had plasmid gene Col(BS512) and rep9b, repUS43. The rest 22 carbapenem and 2 MBLs-resistant *A. baumannii* didn't have plasmid MLST and plasmid genes. One MBLs-resistant *S. maltophilia* multilocus sequence type ST249 and Carbapenem-resistant *P. putida* multilocus sequence type ST46 didn't have plasmid MLST and plasmid gene. One carbapenem resistance *E. cloacae* multilocus sequence type ST310 had IncF RST[F-:A-:B70] plasmid MLST and Col(pHAD28), IncFII(pECLA) plasmid gene.

Of the 8 carbapenem-resistant *E. coli* isolates sequenced, one multilocus sequence type ST617/ST2/ST741 had IncF RST[F1:A-:B-] plasmid MLST and Col(MG828), Col(pHAD28), IncFIA, IncFIB(AP001918), IncFII(pRSB107), IncI1-I(Alpha), IncY plasmid genes. Two

carbapenem-resistant *E. coli* multilocus sequence type ST8915/ST757 had IncFRST [F29:A:-B10], IncA/C PMLST1 plasmid MLST and Col(MG828), Col156, IncC, IncFIB(AP001918), IncFIB(H89-PhagePlasmid), IncFII(29), IncI1-I(Alpha) plasmid genes (Figure 57) and (Supp table Excel).

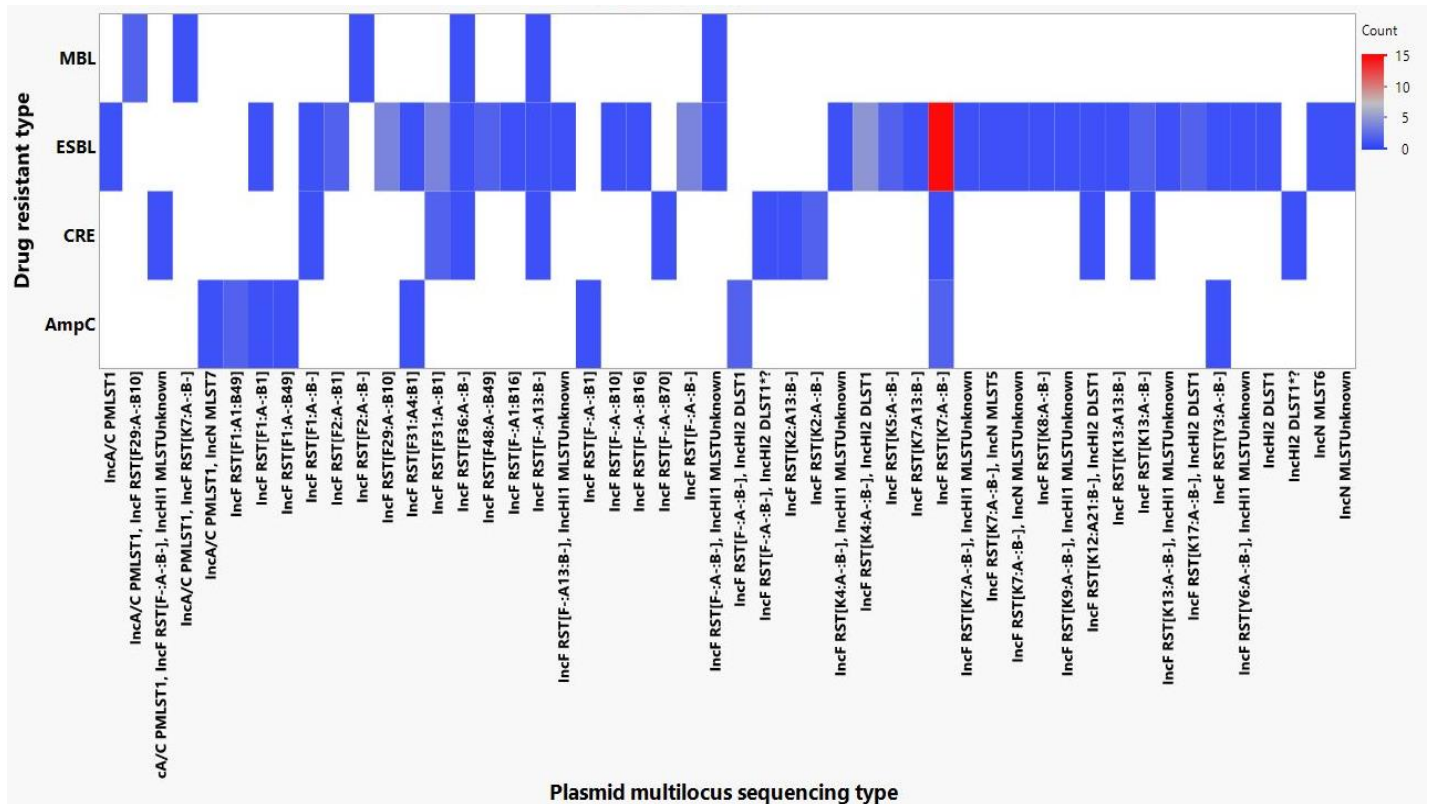


Figure 57: Plasmid MLST in different beta-lactamase gram-negative bacteria

Two *E. coli* multilocus sequence types ST167/ST2 had IncF RST[F36:A:-B-] plasmid MLST and Col156, Col440I, IncFIA, IncFII, IncX4 plasmid genes. Other two *E. coli* multilocus sequence types ST167/ST2 had IncF RST[F31:A:-B1] plasmid MLST and IncFIA, IncFIB(AP001918), IncFII, IncI(Gamma) plasmid genes; but the one had additional rep14a, rep17, rep18a, rep18b, repUS15, repUS43, repUS7 plasmid genes. Finally from carbapenem-resistant one *E. coli* multilocus sequence type ST167, ST2 had IncF RST[F2:A:-B-] plasmid MLST and Col(BS512), IncFIA, IncFII, IncI1-I(Alpha) plasmid genes. Surprisingly except for one *K. pneumoniae* and 2 *A. baumannii* 13 PDR isolates didn't have a plasmid gene. This result showed that it is a chromosomal drug-resistant character (Figure 57) and (Supp table Excel).

4.5.10. Molecular Epidemiology of Hospital Disinfectant Resistant Gram-Negative Bacteria and Responsible Genes in Bloodstream Infections

4.5.10.1. Disinfectant Resistant Bacteria and Disinfectant Resistance Genes

Among whole genome sequenced GNB 90(63.4%) some of them were resistant to disinfectants like Benzalkonium chloride 77(52.2%), Cetylpyridinium chloride 77(52.2%) and had OqxA 41(28.9), OqxB 41(28.9), and qacE 49(34.5) disinfectant-resistant genes. In this study, Benzalkonium chloride, Cetylpyridinium chloride, Chlorhexidine, and Hydrogen peroxide resistance genes were identified however other disinfectant-resistant genes were not identified. These disinfectants include alcohol, glutaraldehyde, hydrogen peroxide, sodium hypochlorite (bleach), iodine, peracetic acid, phenol, and silver. As a result, the disinfectants mentioned here are not resisted by gram-negative bacteria and can be used based on our purpose. Among disinfectant-resistant GNB *E. coli*, *K. pneumoniae*, *E. cloacae*, and *A. baumannii* accounted for 35(97.20%), 42(91.30%), 4(44.40%), and 7(20%), respectively. Among disinfectant-resistant bacteria *A. baumannii*, 7(20%), *E. cloacae*, 4(44.40%), and *P. rettgeri*, 1(100%), species were resistant to benzalkonium chloride, cetylpyridinium chloride, chlorhexidine disinfectant and had qacE disinfectant resistant genes. All, 42(91.3%), disinfectant-resistant *K. pneumoniae* were resistant to benzalkonium chloride and cetylpyridinium chloride and possessed OqxA and OqxB disinfectant-resistant genes; but 14 of them had additional chlorhexidine disinfectant-resistant genes. Twenty 22 *E. coli* had benzalkonium chloride, cetylpyridinium chloride, and chlorhexidine, and 21 of them had hydrogen peroxide resistance genes (Table 35).

Table 35: Identification of chemicals disinfectant resistance and their gene profile of gram-negative bacteria from bloodstream infection n=142.

Gram-Negative bacteria	Chemical disinfectant resistant								Dis. resistance gene			
	N	Neg	Pos.	%	Benzalkonium chloride	Hydrogen peroxide	Cetylpyridinium chloride	Chlorhexidine	OqxA	OqxB	qacE	Sit-ABCD
<i>K. pneumoniae</i>	46	4	42	91.3%	42	0	42	14	41	41	14	0
<i>E. coli</i>	36	1	35	97.2%	22	21	22	22	0	0	22	21
<i>A. baumannii</i>	35	28	7	20.0%	7	0	7	7	0	0	7	0
<i>K. oxytoca</i>	11	10	1	9.1%	1	0	1	1	0	0	1	0

<i>E. cloacae</i>	9	5	4	44.4%	4	0	4	4	0	0	4	0
<i>S. maltophilia</i>	2	2	0	0.0%	0	0	0	0	0	0	0	0
<i>P. Rettgeri</i>	1	0	1	100%	1	0	1	1	0	0	1	0
<i>P. monteilii</i>	1	1	0	0.0%	0	0	0	0	0	0	0	0
<i>C. shandongense</i>	1	1	0	0.0%	0	0	0	0	0	0	0	0
Total	142	52	90	63.4%	77	13	77	49	41	41	49	21
		142										

In this study 13 hydrogen peroxide disinfectant-resistant *E. coli* strains had sitABCD gene. The benzalkonium chloride, cetylpyridinium chloride, chlorhexidine, and hydrogen-peroxide-resistant *E. coli* had qacE and SitABCD genes. The benzalkonium chloride, cetylpyridinium chloride, and chlorhexidine resistance were associated with the qacE gene in all GNB except *K. pneumoniae* having OqxA and OqxB genes. Like other antimicrobial resistance genes, B6 (29) still had the leading prevalence of disinfectant resistance genes followed by C/W (12) and D7 (10) (Figure 58) (Sup Table Excel). Hydrogen peroxide may be helpful for NICU because it can disinfect commonly found gram-negative bacteria in the department (Figure 59).

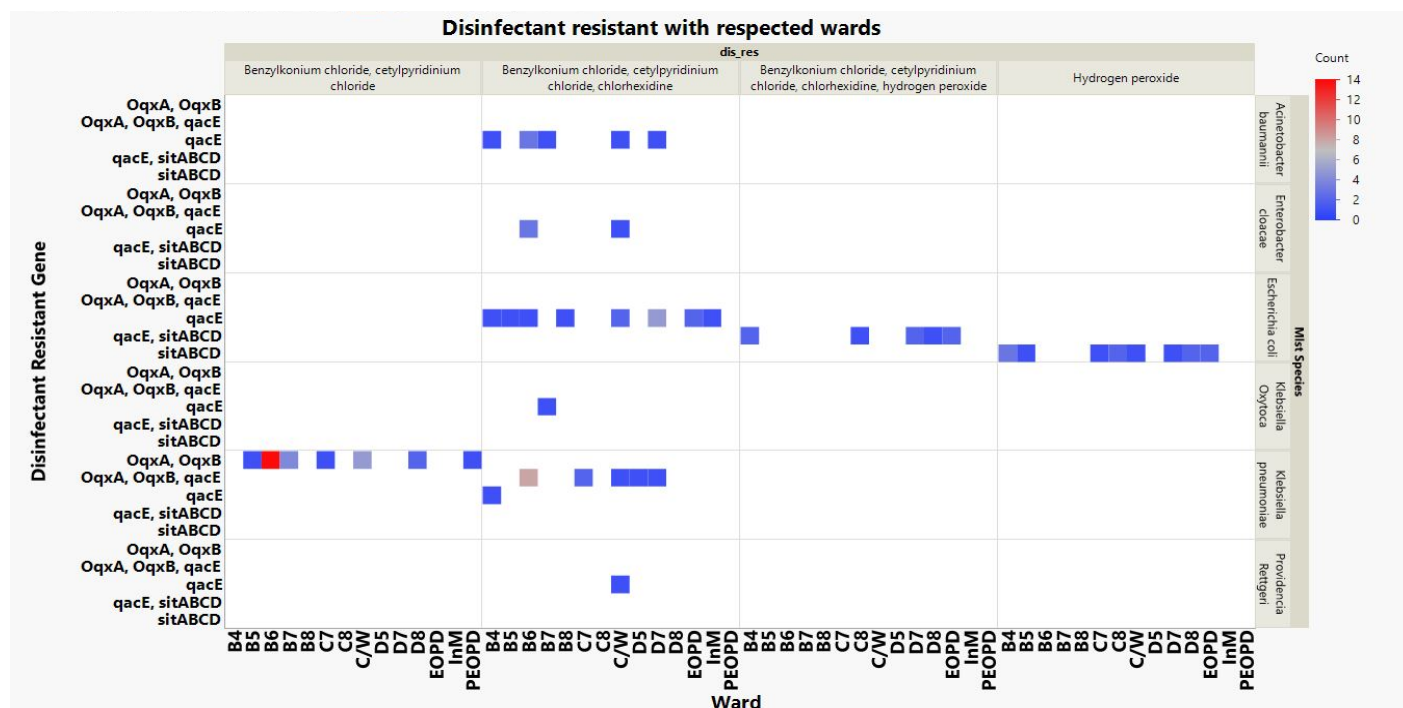


Figure 58: Disinfectant-resistant genes in gram-negative bacteria isolated from bloodstream infections in wards

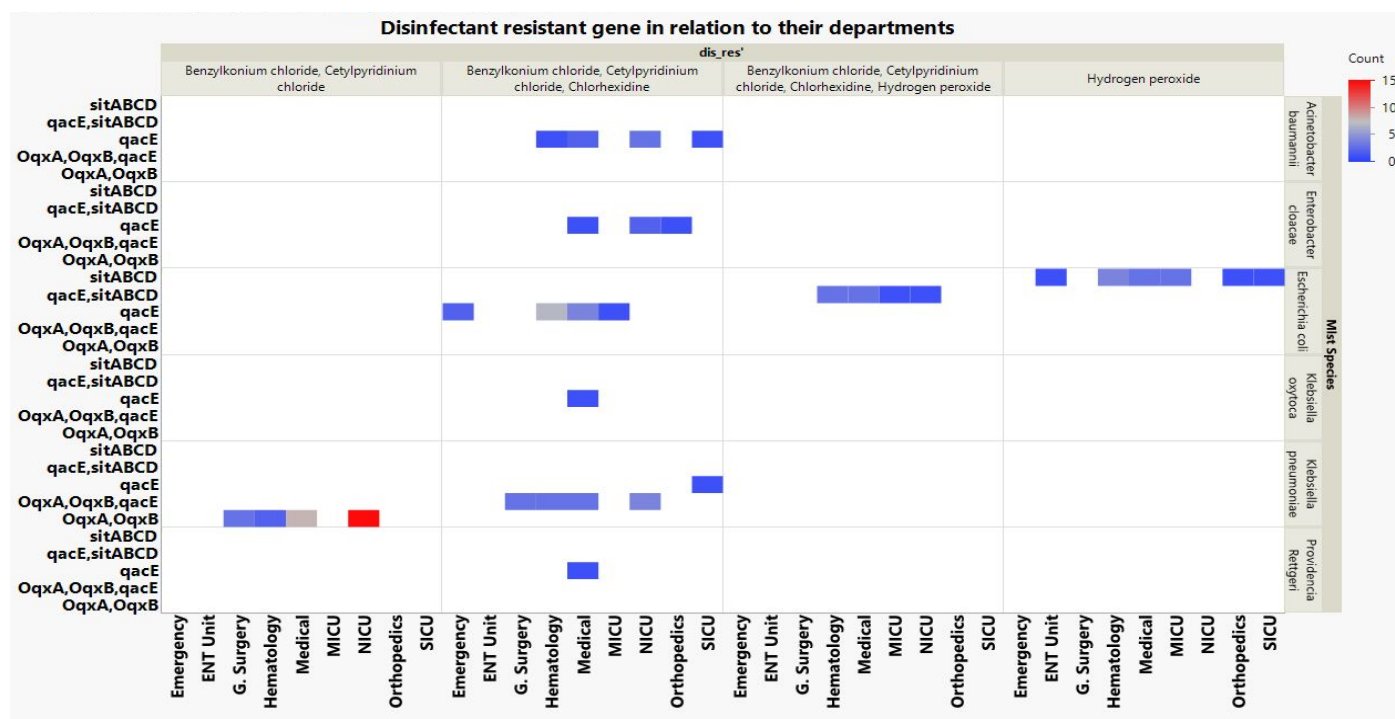


Figure 59: Disinfectant-resistant genes in gram-negative bacteria isolated from bloodstream infection in departments

4.5.10.2. Disinfectant with ESBL, AmpC, Carbapenemase, and MBLs Drug Resistant Gram-Negative Bacteria

There are disinfectant chemical resistance and disinfectant chemical resistance genes indirectly associated with drug resistance type of ESBL, AmpC, Carbapenemase, and MBLs drug resistance in GNB. In this study *E. coli* strains regardless of drug-resistant type (ESBL, AmpC, Carbapenem, and MBL) are resistant to hydrogen peroxide disinfectant and were associated with the sitABCD gene. The benzalkonium chloride, cetylpyridinium chloride, and chlorhexidine resistance were associated with the qacE gene in Third-generation cephalosporin resistance and mainly in Carbapenem-resistant *A. baumannii*. In *K. pneumoniae* having OqxA, and OqxB genes were associated with benzalkonium chloride and cetylpyridinium chloride disinfectant resistance in the majority of 3rd generation cephalosporin and Carbapenem resistance. Almost all bacterial species that are resistant to benzalkonium chloride, cetylpyridinium chloride, and chlorhexidine were associated with the qacE gene (Figure 60).

during multilocus sequence typing. The rest FF02_5528 *K. pneumoniae* sequence types were ST101.

4.5.11.2. Antimicrobial Resistance in PDR Bacteria

The antimicrobial resistance cluster identified for the ten *A. baumannii* ST1837+ST369/ST2, and the two ST1809+ST451/ST2 were aminoglycoside, beta-lactam, carbapenem, folate pathway antagonist, macrolide, streptogramin b, and tetracycline. Whereas, the two different *A. baumannii* FF02_4067-A ST1697/ST2535/ST2 and FF02_6351 ST1697/ST2535/ST2 had antimicrobial resistance clusters for the Aminoglycoside, Amphenicol, Beta-Lactam, Carbapenem, folate pathway antagonist, Macrolide, and Streptogramin B cluster, in addition to Aminocyclitol resistance gene for FF02_6351 ST1697+ST2535/ST2. The FF02_5528 *K. pneumoniae* ST101 was resistant to Aminocyclitol, Aminoglycoside, Amphenicol, Beta-Lactam, Carbapenem, Folate pathway antagonist, Fosfomycin, and Quinolone antimicrobial clusters.

Regarding antimicrobial resistance, the ResFinder analysis showed sample numbers FF02_1726, FF02_1726.6, FF02_1961.2, FF02_3274, FF02_2606, FF02_3004, FF02_3557, FF02_3985, FF02_5289, and FF02_5292 have ST1837/ST369/ST2 sequence type. *A. baumannii* had antimicrobial resistance gene for Amikacin, Amoxicillin, Ampicillin, Azithromycin, Cephalothin, Doxycycline, Erythromycin, Gentamicin, Imipenem, Isepamicin, Kanamycin, Lividomycin, Meropenem, Minocycline, Neomycin, Netilmicin, Paromomycin, Piperacillin, Pristinamycin, Quinupristin, Ribostamycin, Streptomycin, Sulfamethoxazole, Tetracycline, Ticarcillin, Tobramycin, Virginiamycin. But for the sample number FF02_5292 ST1837+ST369/ST2 *A. baumannii*, additional drug resistance genes for Amoxicillin + Clavulanic acid, Ampicillin + Clavulanic acid, Astromicin, Cefepime, Cefixime, Cefotaxime, Cefoxitin, Ceftazidime, Ertapenem, Fortimicin, Piperacillin + Tazobactam, and Temocillin were identified. The sample number and sequence type FF02_4067-A ST1697+ST2535/ST2 and sample number and sequence type FF02_6351 ST1697/ST2535/ST2 *A. baumannii* were identified as having resistance genes for Amikacin, Amoxicillin, Amoxicillin+ Clavulanic Acid, Ampicillin, Ampicillin+ Clavulanic Acid, Azithromycin, Cefepime, Cefixime, Cefotaxime, Cefoxitin, Ceftazidime, Chloramphenicol, Ertapenem, Erythromycin, Gentamicin, Imipenem, Isepamicin, Kanamycin, Lividomycin, Meropenem, Neomycin, Netilmicin, Paromomycin, Piperacillin, Piperacillin+ tazobactam,

Pristinamycin, Quinupristin, Ribostamycin, Streptomycin, Sulfamethoxazole, Temocillin, Tobramycin, and Virginiamycin. The sample number and sequence type of FF02_1573 ST1809+ST451/ST2, and FF02_3205 ST1809+ST451/ST2 were identified as having resistance genes for Amikacin, Azithromycin, Doxycycline, Erythromycin, Gentamicin, Imipenem, Isepamicin, Meropenem, Minocycline, Netilmicin, Pristinamycin, Quinupristin, Streptomycin, Sulfamethoxazole, Tetracycline, Tobramycin, and Virginiamycin. The FF02_5528 sequence type ST101 *K. pneumoniae* was identified as having a resistance gene against Amikacin, Amoxicillin, Amoxicillin+ Clavulanic Acid, Ampicillin, Ampicillin+ Clavulanic Acid, Aztreonam, Carbapenem, Cefepime, Cefixime, Cefotaxime, Cefoxitin, Ceftazidime, Ceftriaxone, Cephalosporins, Cephalothin, Chloramphenicol, Ciprofloxacin, Dibekacin, Ertapenem, Fluoroquinolone, Fosfomycin, Gentamicin, Imipenem, Meropenem, Netilmicin, Piperacillin, Piperacillin+ Tazobactam, Sisomicin, Spectinomycin, Streptomycin, Sulfamethoxazole, Temocillin, Ticarcillin, Tobramycin, and Trimethoprim.

4.5.11.3. Antimicrobial Resistance Genes in PDR Bacteria

The above-identified antimicrobial resistance was due to the bacterial antimicrobial resistance genes. ResFinder result showed sample number FF02_1726, FF02_1726.6, FF02_1961.2, FF02_3274, FF02_2606, FF02_3004, FF02_3557, FF02_3985, FF02_5289 and FF02_5292 *A. baumannii* sequence type ST1837/ST369/ST2 had aph(3')-Ia, aph(6)-Id, armA, blaADC-25, blaOXA-23, blaOXA-66, blaTEM-1D, mph(E), msr(E), sul2, and tet(B) drug-resistance genes. However, FF02_5292 ST1837+ST369/ST2 did not have aph(3'')-Ib, gene and it has additional aac(3)-Ia, ant(3'')-Ia, aph(3'')-Ib, blaNDM-1, and sul1 drug resistance genes.

The sample number FF02_1573 and FF02_3205 sequence type ST1809/ST451/ST2 *A. baumannii* had aph(3'')-Ib, aph(6)-Id, armA, blaADC-25, blaOXA-23, blaOXA-66, mph(E), msr(E), sul2, and tet(B) drug resistance genes. The sample number FF02_4067-A and FF02_6351 sequence type ST1697/ST2535/ST2 *A. baumannii* had ant(3'')-Ia, aph(3'')-Ib, aph(6)-Id, armA, blaADC-25, blaNDM-1, blaOXA-23, blaOXA-66, catB8, mph(E), msr(E), sul1, and sul2 drug resistance gene in addition to aph(3')-Ia in FF02_4067-A and aadA1 in FF02_6351. The one FF02_5528 *K. pneumoniae* ST101 had drug resistance gene of aac(3)-IIa, aac(6')-Ib, aac(6')-Ib-cr, aadA1,

aph(3'')-Ib, aph(6)-Id, *bla*CTX-M-15, *bla*NDM-1, *bla*OXA-1, *bla*OXA-9, *bla*TEM-1A, catA2, dfrA14, fosA, qnrS1, sul2.

4.5.11.4. Antimicrobial Resistance Mutation in PDR Bacteria

Among 15 PDR GNB, PointFinder analysis showed only sample number FF02_5528 sequence type ST101 *K. pneumoniae* had drug resistance mutation on *acrR*(p.F172S, p.F197I, p.G164A, p.K201M, p.L195V, p.P161R, p.R173G), *ompK36*(p.A190W, p.A217S, p.E232R, p.F207W, p.L191S, p.L59V, p.N218H, p.N304E - Frameshift, p.N49S, p.T254S), and *ompK37*(p.I128M, and p.I70M) genes.

4.5.11.5. Virulence Factors in PDR Bacteria

Only sample number FF02_5528 *K. pneumoniae* sequence type ST101 had virulence genes of *fimH*, *fyuA*, *irp2*, *mrkA*:ABW83989; whereas, the rest of *A. baumannii* did not.

4.5.11.6. Plasmid MLST in PDR Bacteria

The FF02_5528 *K. pneumoniae* ST101 had plasmid multilocus IncF RST[F-:A13:B-] and plasmid gene of Col(pHAD28), IncFIA(HI1), and IncR. The FF02_3205 *A. baumannii* ST1809, ST451, ST2 had plasmid multilocus IncF RST[F-:A-:B-], and IncHI2 DLST1*? Plasmid gene IncFIA, and IncHI2A, respectively. The FF02_1726 *A. baumannii* ST1837/ST369/ST2 had plasmid multilocus IncHI2 DLST1* and plasmid gene of IncHI2.

4.5.11.7. Disinfectant Resistance and Disinfectant Resistance Gene

The sample number FF02_4067-A and FF02_6351 sequence type ST1697/ST2535/ST2 *A. baumannii* ha disinfectant resistance genes for benzalkonium chloride, cetylpyridinium chloride, chlorhexidine. FF02_4067-A and FF02_6351 *A. baumannii* sequence type ST1697+ST2535/ST2 had *qacE* disinfectant resistant gene.

5. CHAPTER FIVE: DISCUSSION

5.1. Epidemiology of Bloodstream Infection

The study result showed microbial profile, antimicrobial susceptibility pattern, and associated risk factors of bloodstream infections caused by gram-negative bacteria in a relatively large number of patients. This may help in the management of potentially life-threatening infections caused by MDR organisms in health care services.

5.1.1. Blood Culture

The overall prevalence of culture-positive results in this study, 28.06%, was comparable with the study done in Mekelle, Ethiopia, 28% (Wasihun *et al.*, 2015), in Addis Ababa Ethiopia, 27.9%, (Negussie *et al.*, 2015), in Weldia, Ethiopia, 25.78% (Alemnew *et al.*, 2020), and Athens, Greece, 29% (Apostolopoulou *et al.*, 2014). However, the current prevalence is lower than the study done in Addis Ababa, Ethiopia, 40.2% (Mitiku *et al.*, 2019), in Bahirdar, Ethiopia, 39.2% (Hailu *et al.*, 2016a), and in Addis Ababa, Ethiopia, 32.8% (Kitila *et al.*, 2018). On the other hand, the current finding is higher than the study done in Addis Ababa, Ethiopia, 20.8% (Endalafer *et al.*, 2011), Gonder, Ethiopia, 18.2% (Dagnew *et al.*, 2013), and Malawi 30 % (Archibald *et al.*, 2000). It is also higher compared to reports from other countries such as Nepal 16.2% (Aryal *et al.*, 2020), Ghana, 13.08% (Deku *et al.*, 2019), and Iran 11.9% (Behmadi *et al.*, 2016). Meta-analysis data shows in Africa and Asia 19.1 (95 CI: 12.0–27.5); in Africa 15.5 (8.4– 24.4) and in Asia 28.0 (13.2–45.8) (Droz *et al.*, 2019). The possible explanation for the lower and higher prevalence compared with other studies could be due to differences in the study population, study design, geographical location, etiological agents, patient clinical case, seasonal variations, and differences in infection prevention policies and implementation between countries and institutions.

The higher involvement of gram-negative bacteria 54.57%, than gram-positive bacteria 45.43%, in bloodstream infection, detected in the current study is similar to previous reports from Addis Ababa, Ethiopia (Mitiku *et al.*, 2019; Negussie *et al.*, 2015), and Bahidar, Ethiopia (Endalafer *et al.*, 2011; Hailu *et al.*, 2016a). The difference could be due to the greater tendency of Gram-negative organisms to become a major drug-resistant cause of nosocomial infections (Brusselsaers *et al.*, 2011). Unlike this study, a lower prevalence was recorded for gram-negative bacteria than

for gram-positive bacteria (Kitila et al., 2018; Kumalo et al., 2016; Wasihun et al., 2015). Among the different gram-negative bacteria, *K. pneumoniae* accounted for 75(17.56%), *Acinetobacter spp* 47(11.01%), *E. coli* 38(8.9%), *K. oxytoca* 27(6.32%), and *E. coli* 11(2.58%) were the predominant isolates. This study has similar findings to a study done in Addis Ababa, Ethiopia, (Hailu et al., 2016a; Mitiku et al., 2019).

5.1.2. Factors Associated with Positive Blood Culture

In this study, the significant association of bacterial growth with patient age < 6 years $p=0.002$ has similar findings to studies done in Ethiopia (Dagnew et al., 2013; Endris et al., 2014; Kitila et al., 2018; Negussie et al., 2015). In Africa Ghana shows under 5 ages, the highest bacteremia was recorded (Deku et al., 2019). Again, in the US severe sepsis occurs more frequently in infants and elderly individuals than in other age groups (Martinez and Wolk, 2016). Similar findings were observed in the study of all age groups in Tanzania (Manyahi *et al.*, 2020). From this study, we can understand that small age groups are more affected by bacterial bloodstream infections. These predominant bacteria are known to cause nosocomial infection in children less than two years (Labib *et al.*, 2018). The more we go from preschool to neonates in the TASH setting the infection rate increases, probably this could be due to the immaturity of their immune system, contamination due to high workload, length of admission, loosened IPC protocol, and the optimal temperature for bacterial growth along with crowdedness of neonatal ICU, were the possible reasons. Therefore, due to their susceptibility, healthcare institutions serving pediatric patients should give special attention to nosocomial infections, and a strong infection prevention protocol should be implemented.

Among admission wards, NICU, MICU, and PEOPD have a high association with bacterial growth. A study done on TASH showed a high rate (44.7%) of positive blood cultures reported in the NICU (Ghiorghis, 1997; Negussie et al., 2015) and MICU (Mitiku *et al.*, 2019). Identification of bacteria in admission wards shows that *K. pneumoniae* has been identified abundantly from NICU, C/W, and MICU. *CoNS* were identified from MICU, C/W, and EOPD. The need to report *CoNS* or not has been a discussion point and in our settings, we Report *CoNS* only when their isolation is consistent with the clinical findings. *Acinetobacter spp* were identified from NICU, C/W, and B4 (McKay and Bamford, 2015). In this regard, NICU, and C/W, are highly affected by

K. pneumoniae, *Klebsiella Oxytoca*, and *Acinetobacter species* (Ghiorghis, 1997; Zarrilli et al., 2018)

Like wards, we analyzed the association of culture-positive results with departments where the medical service is given. Univariate and multivariate logistic regression analysis show NICU $p=0.002$, G. surgery $p=0.044$, SICU $p= <0.001$, and both Pedi and adult Emergency $p=0.024$ show strong statistical association with the culture-positive result. Several factors could account for this, the patients attending ICUs $p=<0.001$ have a higher risk of acquiring such infections because of their being immune-compromised, higher incidence of outbreaks, and use of invasive devices that provide access for pathogen entry to bloodstream infection. A study done in Tanzania shows patients admitted to ICU had a higher incidence of bloodstream infection and surgical wards and ICUs have an increased risk of mortality (Manyahi *et al.*, 2020). Prolonged hospital stays greater than five days have an association with positive blood culture. Similarly, a study in Egypt showed a stay greater than one week (Hassan *et al.*, 2017), in Ghana greater than one week (Adatara *et al.*, 2019) in Turkey (Özmeral Odabaşı and Bülbül, 2020) and in Italy, longer ICU stay >19.5 days has been associated with Nosocomial infection (Grasselli *et al.*, 2017). The variation could be their healthcare IPC protocol and implementation.

Among departments, bacterial identification in NICU *K. pneumoniae*, *K. oxytoca*, and *Acinetobacter spp* was found in high numbers compared with other bacteria whereas, in G. Surgery *K. pneumoniae*, CoNS, *Acinetobacter spp*, and *Staphylococcus lugdunensis* and Emergency CoNS, *Staphylococcus aureus*, and *E. coli* were found abundantly. In SICU and MICU *Acinetobacter spp*, *K. pneumoniae*, CoNS, and *E. coli* were found abundantly in addition to *S. aureus* in SICU. In general, *K. pneumoniae*, CoNS, and *Acinetobacter spp* were found predominantly in ICU and surgery (McKay and Bamford, 2015) and except for CoNS similar results were reported (Leal *et al.*, 2019). Therefore, intervention in fumigation, minimizing patient load, and strong infection prevention protocol should be implemented focusing on the above-mentioned wards and departments.

Among the total culture-positive patients, 67.6% of them have acquired bloodstream infections after three days of admission, due to admission other than bloodstream infection clinical diagnosis. Length of admission greater than five days in General Surgery, SICU, and MICU showed 71.7%,

67.6%, and 67.1% percentage culture-positive results respectively. The similarity of organisms identified from EOPD, MICU, NICU, SICU, and general surgery shows that nosocomial infection is the major source of bloodstream infection in the hospital. Due to the different mentioned factors, ICU patients are vulnerable to nosocomial infection (Brusselaers et al., 2011; Grasselli et al., 2017; Hassan et al., 2017; Tauriainen et al., 2017).

In this study Body temperature greater than 38⁰c $p= 0.013$ has an association with positive blood culture. This finding is in line with a study in Thailand (Phungoen *et al.*, 2021). The usage of medical instrumentation or indwelling medical devices during medical care $p= <0.001$ has an association with positive blood culture. Studies were done on the peripheral intravenous devices and any surgery in Arba Minch (Birru *et al.*, 2021), on catheters and central venous lines in Gonder (Endris *et al.*, 2014), mechanical ventilation, and extracorporeal membrane oxygenation in Italy (Grasselli *et al.*, 2017) intravenous line in Egypt (Hassan *et al.*, 2017) invasive procedures in Turkey (Özmeral Odabaşı and Bülbül, 2020) have statistically significant for bloodstream infection.

Having a history of chronic illness $p= 0.027$ is one of the factors of bloodstream infection. Similar results were found in the study in Ethiopia (Endris *et al.*, 2014). Among chronic illnesses; wound infection, ureteral tract infection, and respiratory tract infection were associated factors for bloodstream infection. These results are in line with the study done in Finland on wound infection (Tauriainen *et al.*, 2017). In this case, chronic illness causes depletion of the patient's immune status, increased length of admission, and exposure to different medical devices can cause a high chance of acquiring nosocomial infection.

Among neonates, a history of incubation in the NICU $p=0.013$ was a factor associated with positive blood culture. Review result in developing countries shows neonatal infections were 3–20 times higher in hospital-borne babies (Zaidi *et al.*, 2005). Similarly in the US NICU was a risk factor for neonatal bloodstream infection (Perlman *et al.*, 2007). This is because sharing limited resources of medical instruments, crowdedness, humidity, and optimal temperature for bacterial growth in NICU wards were aggravating factors for nosocomial infection. In this study educational level of the family, place of delivery, mode of delivery, Prolonged rupture of membranes, history of

maternal fever, and neonatal body weight during delivery do not have an association in logistic regression with positive blood culture.

5.1.3. Antimicrobial Resistance

Among all selected antibiotics for gram-negative bacteria antimicrobial susceptibility test, the isolates were resistant to 57.9% of the drug tested, and more than half of the bacteria resisted the antibiotics above 72.7%. In terms of the pathogens isolated *S. marcescens* 52/76(68.4%) and *S. typhi* 0/25(0.0%), the highest and lowest bacteria resistant to the drugs tested even though their rate of isolation is lower $n < 5$ compared to other pathogens. Whereas among the common pathogens isolated, *Acinetobacter spp*, *K. pneumoniae*, and *E. coli* were resistant to commonly used antibiotics at the rate of 65.2%, 64.1%, and 60.2% respectively. The top drug resistance was observed against Ampicillin 88(94.6%) followed by Tetracycline 94.3%, and Ampicillin–Sulbactam 94.1%. and the list was against Amikacin 25(10.8%) followed by Ertapenem 21.2%, and Cefotetan 23.8%. Almost similar resistance findings were reported from Jimma against Ampicillin, cephalothin, and tetracycline accounting for 85.7%, 71.4%, and 71.4% respectively (Kumalo *et al.*, 2016). In our country, Amikacin is one of the Anti TB treatments. It is reserved for RR/MDR-TB treatment and not used for routine purposes, it is still one of the most effective drugs against similar pathogens reported by (Worku, 2017). Another study done in Ethiopia also showed Amikacin has similar efficacy against *Enterobacteriaceae* 13.8% and *K. pneumoniae* 21.3% (Teklu *et al.*, 2019; Tola *et al.*, 2021). Our study and similar findings by others show the importance of the implementation of antibiotic stewardship in Ethiopia. The highest number of resisted drugs by non-fermenter bacteria were Cefotaxime 91.5%, Ceftriaxone 89.4%, Doxycycline 87.2%, and the lowest number of resisted drugs by non-fermenter bacteria were Amikacin 34.6%, Levofloxacin 36.5%, and Imipenem 46.2%. Among the total gram-negative bacteria, the highest Amikacin-resistant bacteria were *Acinetobacter spp* accounts 38.3% but still less resistance compared with other non-fermenter drugs. Study done In India, on ICU instruments all *Acinetobacter baumannii*, were resistant to Amikacin (Khan *et al.*, 2017). Highly susceptible drug Cefotetan was resistant 25.3% to *K. pneumoniae* and 31.6% to *E. coli* other bacteria were below 18.2%.

Among gram-negative bacteria, *S. marcescens*, *K. pneumoniae*, *E. coli*, *Acinetobacter spp*, *K. oxytoca*, and *E. coli* were on average resistant to 93.8%, 93.0%, 81.6%, 81.4%, 78.8%, and 69.8%

for cephalosporin (Cefotaxime, Ceftriaxone, Cefepime, Ceftazidime) drugs. *K. pneumoniae* had the highest number of carbapenem-resistant bacteria with a rate of resistance to Doripenem at 26.7%, Meropenem at 20% Imipenem at 22.7% next to *Acinetobacter*, with Doripenem at 63.8%, Meropenem 59.6%, and Imipenem 46.8%. These organisms are the known cause of nosocomial infection and acquire drug-resistant genes from surrounding organisms by different mechanisms. A study done in India shows that >80% of *K. pneumoniae* and *E. coli* were resistant to commonly used antibiotics (Khan *et al.*, 2017). Therefore, reducing bacterial colonization in the healthcare setup means reducing the occurrence of nosocomial infection and drug-resistant organisms (Bond *et al.*, 2017; Hassan *et al.*, 2017; Khan *et al.*, 2017).

5.2. Multidrug Resistance Bacteria

In this study, MDR organisms were predominant. Among gram-negative bacteria, 95.3% were multi-drug resistant and only 4.7% were not. Among the total drug-resistant gram-negative organisms MDR, XDR, and PDR accounted for 55.8%, 32.2%, and 7.3% respectively. A study done in Jimma also showed similar findings among gram-negative bacteria 80% p=0.438 of them were MDR and above (Kumalo *et al.*, 2016), and in Gaza, Palestine, and Addis Ababa, 92.7% were MDR and above (Elamreen, 2007; Negussie *et al.*, 2015). This is worrisome to patients treating physicians. A reduced rate was observed in Tanzania from nosocomial infection 70.5% of isolates were MDR (Manyahi *et al.*, 2020). In Italy, 56% of MDR (Grasselli *et al.*, 2017), and in Egypt 52% of MDR was identified (Hassan *et al.*, 2017). Among the 17 total PDR bacteria isolated, *Acinetobacter spp* accounts for 76.47%, *K. pneumoniae* accounts for 11.76%, and the rest of each account for 5.88%. Among 13 PDR *Acinetobacter*, 9(52.9%) of them were isolated from B4 (MICU) and B6 (NICU) account for 5(29.4%) and 4(23.53%) respectively. Other studies showed similar findings neonatal and pediatric ICUs were more affected by PDR *Acinetobacter species* (Coskun and Atici, 2020; Gainey *et al.*, 2020).

K. pneumoniae, *Acinetobacter spp*, *E. coli*, *K. oxytoca*, and *E. coli* were the most prevalent multi-drug resistance bacteria isolates in our study. Drug resistance with age category shows that the highest percentage of drug resistance is found in neonates and children. Among the total 222 drug-resistant bacteria, 66.67% of them were identified in patients below six years. The highest amount of the pathogens isolated in the age group of birth to < 1 month 81(36.49%), >1-<24 months

33(14.86%), and 02-<06 years 34(15.32%). Of the total 17 PDR, 35.29% of them were found at the age of 1 day - <28 days. This finding shows children were highly affected by drug-resistance bacterial and bloodstream infections in our study and similar findings were also reported elsewhere (Oishi *et al.*, 2021).

MDR distribution in the admission ward shows that B6(NICU), C/W, and B4, were the top three most affected wards that account for 75(32.19%), 36(15.45%) 25(10.73%) by drug-resistant bacteria. This result demonstrates that hospital pediatric patients are more affected by drug-resistant bacterial bloodstream infections (Chukwumeze *et al.*, 2021). Usage of Indwelling medical devices has exposure to multidrug resistance bloodstream infection. History of incubation has exposure to MDR bacterial infection.

5.2.1. Multi-Drug Resistance in Gram-Negative Bacteria

Our result 95.3% prevalence of MDR and above MDR bacteria was similar to the study done in Addis Ababa Ethiopia 95.11% (Hailu *et al.*, 2016b), 94.5% (Beyene *et al.*, 2019), in Nigeria 93.6% (Adeyemi and Akinde, 2021) but the prevalence was higher than the studies done in Addis Ababa Ethiopia 89% (Arega *et al.*, 2017), in Jimma Ethiopia 71.4% (Kumalo *et al.*, 2016), 70.5% in Tanzania (Manyahi *et al.*, 2020) Mekelle Ethiopia 59% (Wasihun *et al.*, 2015), in Egypt 52.6% (Hassan *et al.*, 2017) in Europe 47.8 % (Tabah *et al.*, 2012) and in Thailand 48.8% (Chaisathaphol and Chayakulkeeree, 2014). A study done in Addis Ababa Ethiopia showed 94.5% MDR of which 8.8% and 0.8% were XDR and PDR, respectively (Beyene *et al.*, 2019). This implies tertiary level hospitals show a higher amount of XDR and PDR isolates probably due to higher workload, crowdedness in NICU, poor IPC protocol, advanced procedure, and usage of different types of supportive medical equipment in addition to medical tourism (Bond *et al.*, 2017; Hugonnet *et al.*, 2004; Jean *et al.*, 2015; Russotto *et al.*, 2015; Seboxa *et al.*, 2015; von Baum *et al.*, 2005). A study done in Kenya showed 88% MDR and 26% XDR (Wangai *et al.*, 2019). In Europe among the total 47.8 % MDR and above isolates, 47.8 % were MDR 20.5% were XDR, and 0.4 % were PDR (Tabah *et al.*, 2012).

Studies showed that the mortality rate in the MDR group was 34.8% compared to 13.7% in the non-MDR group ($p = 0.002$) (Patolia *et al.*, 2018). Therefore, tackling hospital-acquired infection

due to some emerging pathogens like *Klebsiella spp* 48.20%, *E. coli spp* 21.62%, and *Acinetobacter spp* 19.82% found in our hospital needs attention and a strong IPC protocol should be in place. A study done in TASH pediatrics and other hospitals showed *Klebsiella species* were the predominant MDR bacteria (Beyene et al., 2019; Seboxa et al., 2015). Similarly, *K. pneumoniae* was the most frequently observed causative agent of bloodstream infection and had the highest resistance level in Brazil (Leal et al., 2019; Snyder et al., 2019). The majority of bacteria among the total XDR were *K. pneumoniae* and the most impressive issue is the finding of PDR bacteria PDR were *Acinetobacter spp*, accounting for 37.33%, and 76.47% respectively. Letter on those all PDR *Acinetobacter spp* were confirmed by WGS that it is *Acinetobacter baumannii*. A study done in Thailand shows that 88.7% of *A. baumannii* were MDR and above MDR (Chaisathaphol and Chayakulkeeree, 2014). A study done in Korea, on some strains of *Acinetobacter baumannii*, has been detected as drug-resistant to all antibiotics (Park et al., 2009).

Rapidly emerging resistant bacteria, reflecting the worldwide overuse of these drugs, and the lack of development of new antibiotics cause PDR bacteria to become a healthcare challenge (Ventola, 2015). The development of new antibiotics is crucial in the present pan-drug-resistant era. The choice of treatment for PDR is a combination therapy of colistin with tigecycline or colistin with Amikacin or other antibiotics mainly imipenem (carbapenem) was the main therapeutic modality (Jean et al., 2015; Levy et al., 2003; Villegas et al., 2016). Based on in vitro studies, the use of combinations of colistin with rifampicin is effective (Falagas and Bliziotis, 2007). However, in our country, Colistin and its combination therapy are not included in the list of the Drug Administration and Control Authority (DACA) of Ethiopia (EDITION, 2010). Therefore, colistin and a combination drug of colistin should be included in the DACA list to save those hopeless patients who are infected with this PDR bacteria.

5.2.2. Factors Associated with MDR

Age less than 28 days $p= 0.007$ was the most significant factor for MDR infection. Our result agrees with the study done in Vellore, South India, Ghana, and Australia tertiary care hospitals showed neonatal ICU admission has an association with MDR Infection (Deku et al., 2019; Prowle et al., 2011; Snyder et al., 2019). Contradicting results were found from Brazil that bloodstream

infection studies done from all age groups ≥ 60 years were associated with bloodstream infection in addition to the male sex which was not associated in our case (Leal *et al.*, 2019).

The highest MDR and above MDR infections were recorded in the ICU department. Multivariate analysis confirmed that NICU $p=0.027$ and SICU $p=0.001$ departments were the most susceptible to MDR gram-negative bacteria. Univariate analysis of all ICUs $p= <0.001$ shows a strong association with MDR bacterial infection. This result agrees with the univariate analysis of B4 ward (pediatrics & adult SICU, MICU) MDR prevalence. A study done in the Australian ICU showed that bloodstream infection incidence was high and independently associated with a close to three-fold increased risk of death from the time of positive blood culture (Prowle *et al.*, 2011). Another study in the USA, Australia, and India showed that MDR-gram-negative bacteria were typically isolated in ICUs (Di Franco *et al.*, 2021; Jarvis *et al.*, 2009; Snyder *et al.*, 2019). Among the total 17 PDR, the top two wards B6 and B4 account for 29.4% and 29.4% which is a total of 58.8%. This indicates that in ICU both pediatrics and adult were affected by pan-drug-resistant strains. The study done in Greece showed that PDR gram-negative bacteria s were typically isolated in ICUs (Karakonstantis *et al.*, 2020). Therefore, the medical equipment used for medical service, the workload of staff, experiences of staff, and infection prevention protocol should be revised in both departments

Next to ICU patients, the surgery department in General surgery and C/S delivered patients were highly exposed to XDR and PDR infection. Patients who pass through a surgical procedure in medical-surgical and neonates delivered with cesarian section were highly associated with XDR and PDR infection. In neonates, the cesarian section has an association with MDR prevalence AOR $P=0.04$. These results were similar to the association of MDR in the B7(pediatrics medical & surgical) ward. Surgery and surgical admission were independent risk factors in America with $P=0.04$ and in Australia with $P=0.037$ for MDR- bloodstream infection (de Campos *et al.*, 2022; Prowle *et al.*, 2011). Therefore, the medical equipment used for the ICU and surgical procedure should be sterilized, and the workload of staff, experiences of staff, and infection prevention protocol should be revised in both departments.

The prevalence of MDR and PDR increased with an increase in the length of admission of greater than five days. CDC guidelines showed that bloodstream infection acquired after 48-72 hours of

admission is a nosocomial infection. This is because healthcare-associated infections are frequently MDR strains (Timsit *et al.*, 2020). A study done in Australia on patients admitted to ICU shows positive blood culture associated with 7 days and above (Prowle *et al.*, 2011). Prolonged in-hospital stays and Previous hospitalizations are the risk factors for MDR infection (Di Franco *et al.*, 2021; Fram *et al.*, 2015). A study done in Italy showed hospitalization for >2 days in the preceding 90 days was an independent risk factor for acquiring MDR infection (Aliberti *et al.*, 2012) whereas a study in Malaysia hospital stays longer than 8 days have an association with MDR infection (Haque *et al.*, 2018). A study in Brazil predicted that the previous hospitalization had a 6.6-fold risk for MDR bloodstream infection (Fram *et al.*, 2015). The study done in Greece demonstrated wider outbreak potential, including dissemination to long-term care facilities (Karakonstantis *et al.*, 2020). Antibiotic treatment before ten days of sample collection has an association with the prevalence of MDR. A similar study in Brazil and Thailand showed the previous antimicrobial as a risk factor for MDR bacteremia (Chaisathaphol and Chayakulkeeree, 2014; Fram *et al.*, 2015; Leal *et al.*, 2019), and a study done in South India tertiary care hospital showed prior carbapenem use (OR 8.4) have an association with MDR bloodstream infection (Snyder *et al.*, 2019). A study in the USA showed previous antibiotic use had three-fold and inappropriate empiric antibiotics had 7.6 fold MDR gram-negative bacteria infection (Patolia *et al.*, 2018). Medical instrument usage during medical care has more than two-fold exposure for MDR infection than not used. Among nosocomial infections causing suspected medical devices IV lines, drainage tubes, and NG tubes have been associated with MDR prevalence. An IV line and drainage tube users have more than a double chance of acquiring MDR and NG tube users have nearly double the chance of acquiring MDR bacteria. A study was done in a South India tertiary care hospital (Snyder *et al.*, 2019) and cohort studies in different countries (Tabah *et al.*, 2012) showed that IV line and CVC (OR 4.8) were predicted for MDR infection. There was the same study finding on CVC in Brazil 11.2-fold, and in the USA and Australia indicated it was a predictor of MDR Infection (Fram *et al.*, 2015; Jarvis *et al.*, 2009). A study done in Australia on patients admitted to ICU shows intravascular catheter-associated bloodstream infection remained associated with a significant risk of death $P = 0.002$ (Prowle *et al.*, 2011). Among chronic illnesses, the suspected predisposing factors, ureteral tract infection (UTI), wound infection (WI), and respiratory tract infection (RTI) showed more than five, three, and two-fold acquiring MDR bloodstream infection. A study done in Thailand showed the significant risk factors of overall

MDR gram-negative bacteria were respiratory tract infection $p = 0.005$, wound infection $p = 0.007$, and hospital-onset infection $p < 0.001$ (Chaisathaphol and Chayakulkeeree, 2014). A study done in the USA on bloodstream infection showed that urinary catheter sources of UTI infection were significant risk factors 5.96 fold compared with other risk factors for the development of MDR gram-negative bacteria (Patolia *et al.*, 2018). A study in Malaysia showed wound infections or SSIs are still one of the most common adverse events causing between 2% - 36% of patients may develop SSIs in hospitalized patients (Haque *et al.*, 2018). Study in different European countries showed that RTI was a risk factor for MDR infection (Tabah *et al.*, 2012).

Among neonatal bloodstream infections neonatal incubation mode of delivery by C/S had three and two-fold exposure to multidrug resistance bloodstream infection. In the United States, 32% of all deliveries were done by C/S 2-7% experienced wound infections, and 2-16% developed endometritis (Kawakita and Landy, 2017). Neonates after cesarean delivery developed 2.8% confirmed wound infection (Martens *et al.*, 1995). Among factors associated with MDR bacterial infection premature rupture of membrane >18 hours and <18 hours do not have an association with MDR- bloodstream infection, and no rupture of the membrane due to cesarian section showed nearly four-fold exposure of MDR bloodstream infection. A study done in Iran on the comparison of vaginal delivery and C-section concluded that surgical intervention is associated with decreased labor pain and is a life-saving procedure, but it was a procedure associated with future complications (Zakerihamidi *et al.*, 2015). However, the PDR result of not having neonates' history of incubation was more than 5 times higher than having neonatal incubation. This might be due to most of the cesarean delivery neonates not using incubation. MDR association with patient attachment or admission to the general surgery department has a strong association with MDR prevalence. Surgical procedures were done in the gynecology department and the general surgery department has a strong association with MDR infection. Disinfection, fumigation of procedure rooms, sterilization technique for equipment, and appropriate surgical practice should be implemented.

5.3. Phenotyping Characterization

5.3.1. Culture Result

Comprehending the so-called "nightmare" of MDR bacteria is key to the successful treatment and control of hospital-acquired infection (Raphaely *et al.*, 2015). Therefore, this study was intended to identify the magnitude of MDR and their phenotypic drug-resistance mechanisms including the production of ESBL, AmpC-BL, MBL, and Carbapenemase along with their associated factors among patients with gram-negative bacteria bloodstream infection in Tertiary Care Hospitals in Ethiopia. A high percentage of ESBL + AmpC-BL bacteria (67.1%) were identified meanwhile carbapenemase + MBL was found to be 30.7%. In this study, 28.1% of bloodstream infection prevalence was identified, which is comparable to other related studies conducted in Ethiopia with prevalence rates of 28.0% (Wasihun *et al.*, 2015), 27.9% (Negussie *et al.*, 2015), and 25.8% (Alemnew *et al.*, 2020). However, the current result is higher than 18.2% (Dagnew *et al.*, 2013), 15.8% (Kumalo *et al.*, 2016), and lower than other studies conducted in Ethiopia which were 32.8% (Kitila *et al.*, 2018), and 39.2% (Hailu *et al.*, 2016a). A lower percentage was also reported from Bangladesh 13.6% (Ahmed *et al.*, 2017). These differences could be due to different confounding factors like high workload, crowdedness of the hospital, and poor infection prevention implementation of TASH as compared to other countries. The differences from earlier Ethiopian studies mentioned above may be due to the study's design, study area, patient type, clinical situation, etiological factors, and seasonal variations.

The higher prevalence of gram-negative bacteria (54.2%) was also reported in the present study, which accords with earlier Ethiopian studies 52.3% (Hailu *et al.*, 2016a), 51.8% (Negussie *et al.*, 2015); however, it was higher than other reports from the country such as 31% (Dagnew *et al.*, 2013), 22.6% (Kitila *et al.*, 2018). The gram-negative bacteria becomes a major problem in bloodstream infection and hospitalized patients (CDC, 2011a; Hailu *et al.*, 2016a; Oliveira and Reygaert, 2023). In this study, *K. pneumoniae*, *Acinetobacter spp*, and *E. coli* were the three most common isolates. This was similar to a study from Paris and CDC reports that also identified these three pathogens as the most common isolates (CDC, 2011b; Zarrilli *et al.*, 2018). Again similar findings were observed in Ethiopia, *K. pneumoniae* and *E. coli* were the highest (Seman *et al.*, 2022), and in Thailand *E. coli* (34.1%), *Klebsiella pneumoniae* (25.6%), and *Acinetobacter spp*. (22.7%) were the highest (Yungyuen *et al.*, 2021). This could be due to the sharing of drug-

resistant genetic materials that can cause a rapid increase of ESBL, AmpC-BL, and Carbapenemase-producing genes (CDC, 2011a; Hailu *et al.*, 2016a; Oliveira and Reygaert, 2023; Timsit *et al.*, 2014)

5.3.2. Antimicrobial Susceptibility Test

5.3.2.1. Screening and Confirmatory Test for ESBL & AmpC

Among all gram-negative bacteria extended-spectrum cephalosporin-resistant strains for Cefotaxime, Ceftazidime, Ceftriaxone, and Cefepime accounted for 81.4%, 72.7%, 80.5%, and 75.8% respectively. The higher percentage was reported in earlier studies in Ethiopia, 88.5% (Seman *et al.*, 2022) and 86% (Abdeta *et al.*, 2022). Similar findings were reported from the Gaza Strip with 79.5%, 78.9%, and 79.5% cefotaxime, ceftriaxone, and ceftazidime resistance, respectively (El Aila *et al.*, 2023). Phenotypic screening of extended-spectrum cephalosporin and cephamycin-resistant strains for Cefoxitin and Cefotetan accounted for 35.1% and 35.5% in this study. However, during the E-test of combined drug confirmatory test, 81.4% and 35.5% suspected ESBL and AmpC-BLs producers of gram-negative bacteria were lowered to 57.1% and 15.6%, respectively. Our finding of 57.1% ESBL producers was lower than the study done in Ethiopia with 67.3% (Seman *et al.*, 2022), 65% (Abdeta *et al.*, 2022), 75.5% (Legese *et al.*, 2022a), in Taiwan with 71.9 % (Chen *et al.*, 2021), in India with 82.5% (Dalela *et al.*, 2015), in Egypt with 78% (Abdulall *et al.*, 2012). This finding was higher than other studies conducted in Nigeria with 53.9% (Adeyemi and Akinde, 2021), and in Gaza with 51.6% (El Aila *et al.*, 2023). The 15.6 % of AmpC results were comparable with other studies 14% in Ethiopia (Legese *et al.*, 2022a) and 20.9% in Nigeria (Adeyemi and Akinde, 2021). A higher percentage, 26.9% in India (Dalela *et al.*, 2015), 29% in Egypt (Abdulall *et al.*, 2012), and 37.7 % in Taiwan (Chen *et al.*, 2021) were reported. The reduction of drug resistance during confirmatory tests because carbapenem drug-resistant bacteria were resistant to extended-spectrum cephalosporin and cephamycin drugs in addition to the efflux pump and changes in the binding site on membrane structure or microbial organs like ribosomes (Liwa and Jaka, 2015).

5.3.2.2. Confirmatory E- test (CDT)

The *K. pneumoniae* were the highest extended-spectrum cephalosporin-resistant strain bacteria, which showed 94.7% resistance to Ceftriaxone $n \geq 5$. In confirmatory results, the highest ratio of bacterial ESBL prevalence had a little bit of difference from primary screening in which *K. oxytoca* came first and *K. pneumoniae* followed which accounted for 77.8%, and 77.3% respectively. *Acinetobacter spp* were 83.0% resistant to Cepharmycin (Cefotetan) drugs on primary screening. However, during the double drug confirmatory E-test, *E. coli* came first and *Acinetobacter spp* followed which accounted for 21.1% and 19.1% respectively in AmpC-BLs production. A study done in Egypt shows similar findings of *K. pneumoniae* and *E. coli* were predominant ESBL and AmpC-BLs producers (Abdulall *et al.*, 2012). This implies that 66% of *Acinetobacter* were cephamycin-resistant without hydrolyzing enzyme production. Except for *Salmonella typhi*, extended-spectrum cephalosporin-resistant bacteria were identified from all species in the phenotyping screen but only 10/17 11/17 species were positive for extended-spectrum cephalosporin and cephamycin-resistant. However, in confirmatory of CDT ESBL were identified in 11/17 and AmpC-BLs producer was 7/17 9/17 species.

After the double drug confirmatory test, *K. oxytoca* (85.2%), *K. pneumoniae* (74.7%), and *E. coli* (63.6%) were the leading ESBL producers and among AmpC-BLs *E. coli* (26.3%), *Acinetobacter spp* (17.0%), and *K. oxytoca* (14.8%) were the top three but *E. coli* were not AmpC producer. On the contrary, *K. rhinoscleromatis*, *E. cloacae*, *M. morganii*, and *S. typhi* were not ESBL and AmpC producers.

5.3.2.3. Carbapenemase and MBLs Screening and Confirmatory Test

Carbapenemase-producing bacteria are spreading worldwide and causing life-threatening illnesses. (Tzouvelekis *et al.*, 2014). During the primary screening, 32.0% of gram-negative bacteria were resistant to carbapenem drugs. Regarding each drug 26.4%, 24.2%, 16.5%, and 32.0% of gram-negative bacteria were resistant to Meropenem, Imipenem, Ertapenem, and Doripenem on average 24.8% were resistant. Lower results were observed in Ethiopia Meropenem, 16.3%, and Imipenem 14.4% were resistant (Seman *et al.*, 2022). However, confirmatory tests, of carbapenem-resistant Carbapenemase and MBLs-producing strains were 25.1% and 5.6% together

30.7%, which reduced from 32.0% to 30.7% due to resistance other than enzyme production. This study was similar to the study done in Nigeria 28% (Olowo-okere *et al.*, 2019) and higher than the study done in Nigeria 25.2% (Adeyemi and Akinde, 2021), in Ethiopia, 17.3% were non-susceptibility to carbapenem drugs were confirmed by mCIM become 8(7.7%) (Seman *et al.*, 2022), from screening 14.2% to confirmatory 6.7% from urine sample (Seman *et al.*, 2021). The MBL-production test revealed that 19 (38.0%) of the carbapenem-resistant gram-negative bacteria or (17.2%) gram-negative bacteria were MBL-positive (Olowo-okere *et al.*, 2019). Our study is lower than the study done in Egypt ICU patients at 36.7% (Eldegla *et al.*, 2021). in Egypt 17% had MBL and only 6% were KPC producers (Abdulall *et al.*, 2012).

The highest drug-resistant in Carbapenemase and MBLs were recorded in *Acinetobacter spp* accounting for 53.2%, and 10.6%, and *K. pneumoniae* accounted for 20%, and 5.3% $n \geq 5$. A study done in Saudi shows carbapenem resistance was increasing among the non-fermenters (Yezli *et al.*, 2015). double combination meropenem + colistin and triple combination meropenem + colistin + tigecycline against CP-gram-negative bacteria can improve the prognosis of the treatment (Tsala *et al.*, 2019; Zhou *et al.*, 2020). Among all carbapenemase producers, *Acinetobacter spp* 63.8%, *K. pneumoniae* 25.3%, and *E. coli* 15.8% were the top three, and *C. diversus*, *K. ozaenae*, *E. agglomerans*, *P. rettgeri*, *E. aerogenes*, and *S. typhi* were not Carbapenemase and Metallo beta-lactamase producer.

5.3.3. ESBL, AmpC, MBL, Carbapenemase

The percentage (84.4%) of bacteria in the current study that produced carbapenemase, MBL, ESBL, and AmpC-BL was higher than the study done in Addis Ababa Ethiopia (70%) (Beyene *et al.*, 2019) and lower than the study conducted in Nigeria (98.8%) (Adeyemi and Akinde, 2021). Of 195 drug resistance enzyme-producing bacteria, we found 15.9% that produced two or more enzymes which is lower than the Nigerian report showing two enzyme co-production from ESBL, AmpC-BL, or Carbapenemase in 69.6% of isolates (Adeyemi and Akinde, 2021).

Our study came up with an ESBL (52.8%), carbapenem producer gram-negative bacteria (25.1%), AmpC-BL (14.3%), and MBL (5.6%) which is found to have comparable results with the study in Nigeria for ESBL (53.9%) and AmpC-BL (20.9%). The carbapenemase-producing bacteria in this

study were 30.7%, which is higher than 25.2% in recent studies in Nigeria (Adeyemi and Akinde, 2021) and much higher than 2% (Beyene *et al.*, 2019) and 12.12% (Legese *et al.*, 2017) in other studies. These indicate that the prevalence of gram-negative bacteria that produce drug-hydrolyzing enzymes has increased in recent studies. Carbapenemases, with versatile hydrolytic capacity against β -lactams, are now an important cause of resistance in gram-negative bacteria (Jean *et al.*, 2015). In this study, 30.7% of carbapenemase-producing gram-negative bacteria was higher than that of a study done in Latin America with 20.8% (Villegas *et al.*, 2016). This could be due to the high percentage of *A. baumannii* which accounts for 76.5% of the total possible PDR and 63.8% of *Acinetobacter spp* isolates which is carbapenem drug-resistant in this study. The genes encoding for the acquired carbapenemase production were associated with a high potential for dissemination (Jean *et al.*, 2015). Carbapenem-resistant *Acinetobacter spp* can carry mobile genetic elements that help with nosocomial infection and some of them are becoming pan-drug-resistant (Kadri, 2020). Therefore, special attention must be for all types of *K. pneumoniae* and possible PDR *Acinetobacter spp* transmission dynamics, drug resistance genes, and virulence factors as per the findings of this research.

The prevalence of ESBL and AmpC-BL producer bacteria was 67.1%, comparable to the study conducted in Addis Ababa, which had reported 67% (Beyene *et al.*, 2019) and 78.6% (Legese *et al.*, 2017). In our study, the percentage (52.8%) of producing gram-negative bacteria isolated from patients with bloodstream infection was similar to the nine-year retrospective study conducted in Ghana which accounts for 50.2% (Deku *et al.*, 2019). A lower result was also found in previous similar studies conducted in Ethiopia (38.6%) (Tekele *et al.*, 2020), Asia (47%) (Kang *et al.*, 2004), and Thailand (37.8%) (Chaisathaphol and Chayakulkeeree, 2014). The difference could account for differences in the research methodology, sample type, geographical variation, and hospital infection prevention practice.

In our study, *K. pneumoniae* was the highest drug-resistance enzyme-producing bacteria accounting for 36.7% followed by *Acinetobacter* (21.7%). *Acinetobacter spp* was the high producer of carbapenemase and MBL producers with 53.2% and 10.6% prevalence, respectively. According to a study conducted on urine samples in Nigeria, the percentage of *K. pneumoniae* strains resistant to cephalosporins due to ESBL production increased from 18.2% in 2010 to 29.4% in 2011 and further to 34.1% in 2012 (Lucero-Prisno *et al.*, 2017). A study conducted in Kenya

revealed *K. pneumoniae*, *E. coli*, and *A. baumannii* had resistance to cephalosporins 57-84%, 16-43%, and 81%, respectively (Wangai *et al.*, 2019). The systematic review of research conducted in China for 20 years on bloodstream infection showed that hospitalized non-ICU patients for carbapenem-resistant *K. pneumoniae* was below 5% in 1998–2012 but this figure has increased to 34.9% during 2013–2017. In contrast, sadly, the detection rate for carbapenem-resistant *K. pneumoniae* in ICU patients has increased from 0% in 2013 to 75% in 2016 (Tian *et al.*, 2019).

Having a double drug resistance enzyme-producing bacteria implies having a high drug resistance capacity for the bacteria and this in turn worsens the success rate of the treatment to the worst level. In this study, about 15.9% of the isolated bacteria produced more than one drug hydrolyzing enzyme that was higher than that of China, which reported 7% of ESBL-producing *K. pneumoniae* isolates manifested “cross-resistance to carbapenems (Lucero-Prisno *et al.*, 2017). In this research, *K. pneumoniae*, *Acinetobacter spp*, and *K. oxytoca* were the three dominant bacteria that produced double-drug hydrolyzing enzymes. This implies that *K. pneumoniae*, *Acinetobacter spp*, and *E. coli* are becoming aggressive drug resistance bacteria. In addition to this, having more than one drug-hydrolyzing enzyme strengthens the bacterial pathogens to be more resistant to antibiotics, increases treatment failure rate, longer hospital stays, high economic burden, and unpredicted emotional costs to patients.

The higher percentage of Carbapenemase and MBL producer gram-negative bacteria reported in general surgery and SICU department were almost five times more likely to be contagious than other admission departments. Neonates delivered by caesarean section were almost 10 times more likely to acquire carbapenemase and MBL producer bacteria than those delivered by normal procedure. In recent years, the rate of caesarean section has been increasing, and whether that is indicated or not is an issue but the fact that the procedure is a risk factor that exposes the neonates to MDR bacteria calls for serious attention in the national maternal health programs. Patients who pass through surgical procedures including caesarean section had an association with carbapenem infections. These results will be directly associated with a lack of infection prevention protocol implementation in the caesarean section room and medical instruments used during surgical procedures. Fumigation of the room, proper sterilization technique for the instruments, and standard surgical procedure should be implemented. These factors were also found in another

study conducted in Latin America where ICU admission and general surgery have an association with carbapenem drug-resistant bacterial infection (Villegas *et al.*, 2016).

The other major factors associated with carbapenem resistance bloodstream infection in the current study were the usage of instruments especially inserted devices during medical care were almost four times more likely to be infectious for carbapenemase and MBL producer bacteria. Usage of antibiotic treatment ten days before sample collection was also associated with carbapenemase and MBL producer bacteria. The misuse of antibiotics is one of the causes of this problem. From chronic illness, patients with wound infection on the skin and UTI were almost 4 times more likely to acquire carbapenemase and MBL producer bacteria than those that do not have a wound and UTI. Wound infection on the skin was one of the exposing factors for nosocomial infection due to the lack of mechanical barrier of the skin. The association between wound infection and UTI with Carbapenemase producer gram-negative bacteria bloodstream infection was also reported in another study (Villegas *et al.*, 2016). The instruments used for medical care like a suction machine, an intravenous line, and a drainage tube were associated with Carbapenemase and MBL-producing bacterial infection. Therefore, surgical materials and indwelling medical equipment during medical care should be monitored for proper sterilization.

5.3.4. Factors Associated with ESBL, AmpC, CARBA, and MBLs

The NICU wards were almost 15 times more likely to acquire ESBL and AmpC-BL producer gram-negative bacteria in this study. The room temperature which is favorable for microbial growth, high workload, and crowdedness could be a major problem in NICU bloodstream infection. In addition, plasmid transfer genes that play a great role in nosocomial infection are common in gram-negative bacteria, especially in *E. coli* and *Klebsiella species* found in these wards. As per the research conducted in 11 African countries among neonates, gram-negative bacteria was the predominant cause of early-onset neonatal sepsis, with a high prevalence of ESBL-producing bacteria (Williams *et al.*, 2018). This implies that the NICU was more affected by third-generation cephalosporin-resistant bacteria meanwhile, the caesarean section delivery room was more affected by Carbapenem-resistant bacteria.

Among chronic illness patients those having UTI were almost 4 times more likely to acquire ESBL and AmpC-BL producer bacteria than those who don't have UTI. The RTI and tissue sarcomas were also two times more likely to acquire ESBL and AmpC-BL producers than the others. Immune suppression and prolonged admission in chronic illness patients could be the exposing factors for hospital-acquired infection.

Here the advanced stage of Carbapenemase and MBLs producer CR gram-negative bacteria association factors were analyzed. Analysis in the department showed carbapenemase producer carbapenem drug-resistant infections were highly associated with G. Surgery and SICU. ICU admission in which MICU, Ped MICU, and SICU have an association with carbapenem drug-resistant bacterial infection. A study done in India shows ICU admission (OR 3.0) was significantly associated with bloodstream infection with NDM-1-producing MDR strains (Snyder *et al.*, 2019). A study done by Villegas in Latin American countries shows ICU admission $P < 0.001$ and general surgery 49% $p=0.008$ have an association with carbapenem drug-resistant bacterial infection (Villegas *et al.*, 2016).

Third-generation cephalosporin resistance ESBLs and AmpC-BLs producer bacterial infections were more prevalent in neonates, and pediatrics (NICU, Pediatrics Medical and Surgical, and Pediatrics Hematology) but carbapenem resistance Carbapenemase producer gram-negative bacteria was more prevalent in general surgery and SICU for both pediatrics and adult. Surgical ICU was strongly associated with carbapenem resistance bacterial infection. Therefore, Surgical materials and indwelling medical equipment during medical care should be monitored.

Unlike ESBLs and AmpC-BLs producers Carbapenemase producer gram-negative bacteria bloodstream infection was highly associated with the usage of antibiotic treatment ten days before sample collection and a length of admission, greater than 3 days. The major factor associated with carbapenem resistance bloodstream infection was instrument usage during med care. Those instruments associated with medical care were a suction machine, an intravenous line, and a drainage tube. A study in 7 Latin American countries shows Empirical treatment and Hospital-acquired infection due to length of admission have an association with CPE (Villegas *et al.*, 2016). About 4% of U.S. short-stay hospitals had at least one patient with a serious carbapenem-resistant Enterobacteriaceae infection during the first half of 2012. About 18% of long-term acute care

hospitals had one Carbapenem-Resistant *Klebsiella spp.* 11% Carbapenem-resistant *E. coli* 2% (CDC, 2013a).

In the case of chronic illness, wound infection on the skin, and ureteral tract infection, were associated with Carbapenemase producer gram-negative bacteria bloodstream infection but chronic illness, ureteral tract infection respiratory tract infection, and tissue sarcomas were associated with ESBLs and AmpC-BLs producer gram-negative bacteria bloodstream infection. The same study shows that wound infection and urethral tract infection were associated with carbapenem resistance gram-negative bacteria bloodstream infection $p=0.005$ (Villegas *et al.*, 2016).

Regarding neonatal bloodstream infection mode of delivery, premature rupture of the membrane (C/S) was associated with Carbapenemase gram-negative bacteria, and a history of neonatal incubation had a strong association with ESBLs and AmpC-BLs. This implies NICU was more affected by third-generation cephalosporin-resistant bacteria and the C/S delivery room was more affected by Carbapenem-resistant bacteria.

Regarding age groups, neonates acquired gram-negative bacteria having all types of drug resistance mechanisms 5-fold than compared to all other age groups. Patients of NICU, SICU, and G. surgery were infected by all enzyme-producing gram-negative bacteria bloodstream infection. Again, admission to the ICU has a four-fold chance to acquire all ESBL, AmpC, CARBA, and MBL drug-resistant types. Patients admitted for greater than 5 days, were one of the factors in acquiring all DRT bloodstream infections, and taking antibiotics ten days before sample collection has an association with acquiring all enzyme-producing drug resistance mechanisms. Supportive instrumentation usage during medical care has threefold exposure than those who are not used. From this medical instrumentation using a urinary catheter, IV line, and drainage tube has a double-fold higher chance of acquiring DRT bloodstream infection than not-used instruments during medical care. Having a chronic illness has greater than two-fold exposure to DRT than those who have no chronic illness. Among chronic illnesses, UTI and RTI have fivefold, and two-fold chances of acquiring DRT infection. A study done in India shows CVC placement (OR 4.8), prior carbapenem use (OR 8.4), and ICU admission (OR 3.0) were significantly associated with bloodstream infection with NDM-1 producing and other MDR strains (Snyder *et al.*, 2019).

Neonates delivered by cesarean section (C/S) showed a high prevalence of all DRT. The bivariate and multivariate logistic regression analysis showed that neonates who are admitted to the NICU incubation room have five-fold exposure than other patients.

5.4. Molecular Characterization

5.4.1. Sociodemographic Characteristics

In this study, the prevalence of gram-negative bacteria was 53.7% with 1.7% of polymicrobial growth. 84.4% of the gram-negative bacteria had one or more enzyme-producing drug resistance mechanisms, higher than the study done in Ethiopia 75% (Seman *et al.*, 2022), and in Pakistan 72% (Saghir *et al.*, 2009). The neonate age group showed higher (30.8%) drug resistance mechanisms, similar findings were identified from Ethiopia for < 28 days at 38.5% (Seman *et al.*, 2022).

5.4.2. PCR Identification

Out of 84.4% of phenotypically confirmed drug resistance mechanisms, 76.2% had PCR-confirmed drug resistance enzyme encoding genes. This is comparable to the study done in Ethiopia with 75% having drug-resistance genes (Seman *et al.*, 2022) and lower than the study done in Asian Pacific regions 86.2% (Chen *et al.*, 2021). Whereas non-drug resistance mechanisms were increased from phenotypic screening and confirmatory results of 36(15.6%) to 55(23.8%) PCR-negative gram-negative bacteria. In the first case, drug resistance mechanisms were due to other than beta-lactamase and carbapenemase producers. Nineteen strains which it was drug-resistant in the second confirmatory test were found to be negative in PCR molecular tests. This might be due to other genes that were not included in this study. Among PCR-confirmed drug-resistance beta-lactamase producers, ESBL, MBLs, Carbapenemase, and AmpC accounted for 52.8%, 21.6%, 19.9%, and 9.5% respectively.

5.4.2.1. ESBL Gene Identification

In this study, the highest prevalence of PCR-confirmed ESBL encoding genes in gram-negative bacteria was *bla*CTX-M and *bla*TEM accounting for 78.8% and 73.5%, respectively. Similar results were found in Ethiopia *bla*CTX-M and *bla*TEM genes accounted for 73% and 63%,

respectively (Legese *et al.*, 2022a), in Burkina Faso, *bla*CTX-M was highest (65.5%) followed by *bla*TEM (25.7%) (Zongo *et al.*, 2015), in Egypt *bla*CTX-M and *bla*TEM accounted for 80.8% and 48.5%, respectively (El-Kholy *et al.*, 2020). Contrary, a study conducted in Vietnam showed that *bla*TEM and CTX-M accounted for 76.3% and 38.1%, respectively (Cao *et al.*, 2002). In the Gaza Strip *bla*CTX-M and *bla*TEM, accounted for 60% and 57.6%, respectively (El Aila *et al.*, 2023). However, among minor ESBL genes *bla*BEL 15.9% was highly prevalent than the commonest *bla*SHV 9.1%. This indicates that the distribution and prevalent drug-resistant genes changed from time to time (Bogaerts *et al.*, 2013). The previous result shows in Africa similar patterns were observed in the prevalence of drug-resistance genes in ESBL. In this study, ESBL was the highest (53.2%) beta-lactamase producer lower than the study conducted in Ethiopia (67.3%) (Seman *et al.*, 2022), 75.5% (Legese *et al.*, 2022a) in the Gaza Strip (59%) (El Aila *et al.*, 2023), in Asia-Pacific regions (71.9 %) (Chen *et al.*, 2021). In this study, the highest prevalence of PCR-confirmed ESBL encoding gene in gram-negative bacteria was *bla*CTX-M at 78.8%, which was higher than the study done in Ethiopia (67.3%) (Seman *et al.*, 2022) and 73.3% in Thailand (Yungyuen *et al.*, 2021) 60% in the Gaza strip (El Aila *et al.*, 2023). The next highly prevalent ESBL gene *bla*TEM 73.5% was also higher than the study done in Ethiopia (64%) (Yungyuen *et al.*, 2021), and in Vietnam (76.3%) (Cao *et al.*, 2002).

The *E. coli*, *K. pneumoniae*, *K. oxytoca*, and *E. coli* showed the highest percentage of the *bla*CTXM gene accounting for 100%, 91.1%, 87.0%, and 72.7%, respectively. A study in Sudan shows CTXM was the most frequent gene (Basheer *et al.*, 2020). The *E. coli* showed 100% *bla*TEM gene followed by *K. pneumoniae* (91.1%), and *K. oxytoca* (87.0%) but *Acinetobacter* does not express this type of Gene. The *Acinetobacter spp*, *E. coli*, and *K. pneumoniae* produced the *bla*BEL gene with 41.7%, 22.7 and 10.7%, respectively. *K. pneumoniae* accounted for 8(14.3%) of the *bla*SHV gene, also in another study SHV is mainly found in *K. pneumoniae* (Yezli *et al.*, 2015). The *K. pneumoniae* and *E. coli* accounted for 7.1% and 22.7% of the *bla*GES gene, respectively. The *bla*VEB gene was found in *Acinetobacter spp* and *E. coli* with 16.7% and 14.3%, respectively.

5.4.2.2. AmpC-BLs Gene Identification

The AmpC-BLs producer accounted for 9.5% of this study. Similar findings were found in Egypt (9.7%) (El-Kholy *et al.*, 2020) and lower than the study conducted in Ethiopia (14%) (Legese *et al.*, 2022a) and in the Asia Pacific region (37.7 %) (Chen *et al.*, 2021). The highest AmpC beta-lactamases encoding gene was *blaFOX*M (36.1%), and *blaCIT*M (33.3%). In South Africa, 12.9% of *blaCIT* and 9.7% of *blaFOX* genes were reported (Fadare and Okoh, 2021). In India, *blaCIT* accounted for 26.7%. In this study, *E. coli*(23.7%) and *K. pneumoniae* (9.3%) were the highest AmpC-BLs gene producer (Rameshkumar *et al.*, 2021). This result was in agreement with the study conducted in the Asia Pacific region *E. coli* (42.3 %) followed by *E. Cloacae* (22.0 %) and *K. pneumoniae* (21.0 %) (Chen *et al.*, 2021). In Iran, *K. pneumoniae* was the highest and *E. coli* were the second 24.4% and 13.8%, respectively (Kazemian *et al.*, 2019). *Pseudomonas spp* were positive for AmpC confirmatory E-test and do not express AmpC-BLs genes. In addition, ten bacterial species do not express the AmpC-BLs producer gene.

5.4.2.3. Carbapenemase and MBL gene identification

Molecular characterization of Carbapenemase and MBLs genes 27.3% of all gram-negative bacteria were higher than the study done in Ethiopia 7.7% (Seman *et al.*, 2022), 16% (Legese *et al.*, 2022a) in Sudan 3.8 % (Basheer *et al.*, 2020) and in the Asia-Pacific region 14.7% (Chen *et al.*, 2021). This carbapenemase gene's prevalent result was higher than other similar study results. In this study the six highest prevalence of carbapenemase and MBLs genes were *blaOXA* -23, *blaNDM*, *blaVIM*, *blaKPC*, *blaOXA*48, and *blaOXA*-58 accounted for 28(40.6%), 27(39.1%), 24(34.8%), 10 (14.5%), 7(10.1%), and 7(10.1%) respectively and the least prevalent were *blaDIM* 0(%). A study done in Tanzania shows IMP (21.6%), VIM (12.3%), OXA-48 (4.9%), KPC (3.5%), and NDM (3.1%) (Sahle, 2021). In South Africa, *blaNDM*-1, *blaKPC*, and *blaOXA*- 48, were accounted for 42%, 10%, and 6 % respectively were predominant (Sahle, 2021). A study done in Saudi-Arabia Carbapenem resistance in the latter is mainly due to OXA-48 and NDM-1. (Yezli *et al.*, 2015), in Egypt, the carbapenemase gene detection was *blaKPC* 59.6%, *blaVIM* 26.3%, *blaIPM* 22.8%, *blaOXA* 15.8, and *blaNDM* 1.75% (Eldeglia *et al.*, 2021). in Saudi Arabia, OXA-48 and NDM-1 are the dominant carbapenemases among Enterobacteriaceae with a low prevalence of *blaVIM*. (Memish *et al.*, 2015). In Sudan, the most commonly encountered gene

was *bla*SPM 22.5%, *bla*KPC 19.7%, and *bla*OXA 48 19.7% (Basheer *et al.*, 2020). The higher *bla*OXA -23 in Ethiopia is a new experience compared with other African and Asian countries in Sudan *bla*SPM in South Africa *bla*NDM-1, in Tanzania IMP, in Egypt *bla*KPC, in two studies of Saudi-Arabia OXA-48. The other carbapenemase-encoding genes were highly prevalent in other countries.

5.4.2.4. Carbapenemase Gene Identification

In this study, 19.9% of Carbapenemase producers were found, which was higher than prior study conducted in Ethiopia (7.7%) (Seman *et al.*, 2022) and 16% (Legese *et al.*, 2022a), 3.8 % in Sudan (Basheer *et al.*, 2020), and 14.7% in Asia-Pacific region (Chen *et al.*, 2021). Among carbapenemase-encoding genes *bla*OXA-23 28(40.6%) was the highest followed by KPC 10(14.5%), *bla*OXA48 (10.1%), and *bla*OXA-58 7(10.1%). A similar finding was found in Taiwan and Iraqe showed that *bla*OXA-23 was the predominant gene (Chen *et al.*, 2021). The *Acinetobacter spp* (42.6%) and *K. pneumoniae* (16.0%) were the most prevalent gram-negative bacteria-producing Carbapenemase genes. A study done in Iran on *Enterobacteriaceae* isolates showed *K. pneumoniae* and *E. coli* were the highest and accounted for 37.8% and 20%, respectively (Kazemian *et al.*, 2019). The *Acinetobacter spp* express *bla*OXA23 (55.2%), *bla*OXA-58 (13.8%), *bla*OXA48 (6.9%), *bla*KPC (3.4%), and *bla*OXA-24/143 (3.4%). The *K. pneumoniae* expresses *bla*KPC (33.3%), *bla*OXA23 (27.8%), *bla*OXA48 (5.6%), *bla*OXA-24/143 (0.0%), and *bla*OXA-58 (0.0%) genes. In Egypt, *K. pneumoniae* is dominated by *bla*OXA-48 (40.6%) (El-Kholy *et al.*, 2020). Among five gram-negative bacteria species *Klebsiella Ozaenae*, *Citrobacter Diversus*, *P. rettgeri*, *E. agglomerans*, and *S. typhi* do not express Carbapenemase-producing genes.

5.4.2.5. MBLs Gene Identification

In this study, the second-highest beta-lactamase prevalence was recorded by MBLs at 21.6%. Among MBLs gram-negative bacteria, *Acinetobacter spp* 46.8%, and *K pneumoniae* 17.3% accounted for the highest prevalence. *K. ozaenae*, *C. diversus*, *P. rettgeri*, *E. agglomerans*, and *S. typhi* don't express MBLs genes. *E. aerogenes* and *P. mirabilis* were positive for MBLs confirmatory test and negative for Carbapenemase-producing genes. The most prevalent MBL

producers were *bla*NDM (39.1%) and *bla*VIM (34.8%). The highest prevalence of *bla*NDM in our study showed agreement with the study done in Taiwan (Chen *et al.*, 2021). The higher *bla*NDM was recorded in *K. pneumoniae* 13(72.2%) in this study. A study done in Addis Ababa TASH suggested carbapenem resistance to *K. pneumoniae* may be associated with medical tourism in Taiwan (Seboxa *et al.*, 2015). A study done in Iraque showed the highest prevalence was *bla*VIM in (54.7%), and *bla*NDM in (4.76%) of genes among MBLs-producing gram-negative bacteria (Hasoon and Hamed, 2019). The *K. pneumoniae*, *bla*OXA-48 dominated (40.6%), followed by *bla*NDM-1 (23.7%) and *bla*OXA-232 (4.5%) in this study. In *E. coli*, the most frequent genes were *bla*NDM-5 (9.6%), *bla*OXA-181 (5.5%), *bla*OXA-244 (3.7%), and *bla*NDM-1 (3.7%). Notably, 32.3% of isolates carried more than one resistance gene. Notably, 32.3% of isolates carried more than one resistance gene (El-Kholy *et al.*, 2020).

5.4.3. Summary of PCR Result

The gram-negative bacteria with the highest prevalence were *K. pneumoniae*, *K. oxytoca*, and *E. coli* whereas in terms of percentage prevalence of drug resistance mechanisms, *K. pneumoniae*, *Acinetobacter spp.*, and *E. coli* had uppermost prevalence. Ninety-three percent of the 75 *K. pneumoniae* have drug-resistance mechanisms. PCR detected seven more drug-resistance mechanisms than the AST confirmatory test did with a maximum of seven drug-resistant genes and three drug-resistance mechanisms. Any bacterium on average typically has more than two drug-resistance genes. This implies that > 90% of *K. pneumoniae* were enzyme-producer drug-resistant bacteria and some of the bacteria were loaded with high amounts of DR genes. The 68.09% of 47 *Acinetobacter spp* have PCR-confirmed drug resistance mechanisms but 19.14% of them don't and 12.8% of them do have drug resistance mechanisms in the phenotyping test but don't have drug-resistant gene during molecular characterization. Possibly it might be due to other drug resistance mechanisms like efflux pump, membrane alteration, or impermeability and target modification. In addition, we do not do (mCIM) for *Acinetobacter spp* because the guideline does not allow us to do it. There were 69 drug-resistant genes with an average of 2.16 identified for each gram-negative bacteria, the highest drug-resistant gene was 5 indicating a higher load of drug-resistant genes in each bacterium with a majority of Carbapenemase and MBLs genes. The whole genome sequence showed that among all *Acinetobacter spp*, 32(68.1%) of them were *Acinetobacter baumannii*. It will be discussed in the next paper. Sixty-three percent of 38 *E. coli*

were positive by PCR method. Three ESBL-positive by phenotyping detection were negative by PCR test due to other than enzyme production. A high load of more than 2.5 on average with a maximum of 6 genes was identified from each drug-resistant bacteria. Eighty-two percent of 11 *E. coli* were positive for drug resistance mechanisms. All positive for phenotyping were positive for the PCR test.

5.4.4. Associated Factor

The ESBL and AmpC BLs-producing genes were more prevalent in neonates while Carbapenemase and MBLs genes were in NICU and surgery-associated departments like general surgery, SICU, and C/S unit. The instruments used medical care suction machine and drainage tube were associated with Carbapenemase and MBLs producers' genes. The UTI and RTI were associated with ESBL and AmpC BLs-producing genes and SWI and UTI were associated with carbapenemase production. Length of admission greater than 3 days was associated with Carbapenemase and MBLs producer genes. This means most of the community-acquire drug resistance mechanisms were associated with ESBL and AmpC-BLs producers and hospital-acquired infections were associated with Carbapenemase and MBLs producers of gram-negative bacteria.

5.5. Whole Genome Sequence

5.5.1. Whole Genome Sequencing and In Silico Data Analysis

Three q-scores or higher on the quality assessment indicator comprised the WGS data used in this investigation (Parks et al., 2015). The bacterial complete genome assembly achieved an overall performance of 99.97%, Median, 99.74% Mean, 0.54 Standard Deviation, and 96.35% Minimum and 100.0% Maximum value which is the best within our method acceptance range > 90% (Parks et al., 2015). Our mean and median contamination check values were 0.62% and 0.7%, respectively, falling on the border excellent expected value range (Parks et al., 2015). In specifics, there was 0.70 percent and 0.10% contamination with vector and human DNA, respectively, which is acceptable at less than 4% (Kneaddata, 2023). The WGS required quantity was fully filled by the average G-C content, N50, and nucleotide read values for all bacteria, which were 50.7%,

113751.8, and 4028944.1, respectively. 39 out of 965 contigs assembled satisfied the full requirement for quantity (Andrews, 2017).

K. pneumoniae mean completeness of sequence, mean G-C content, and contamination check, accounted for 99.63%, 57%, and 0.74%, respectively fulfilling the requirement of expected value (Andrews, 2017). The mean nucleotides read 4000450 and mean total nucleotides read 593358946, were a little bit higher 5,159,649 and 73,951,923 bp than the study done in the U.S.A. (Kumar et al., 2011). The *E. coli* mean value of completeness of sequence, average G-C content, and contamination check were 99.86%, 50.6%, and 0.39%, respectively were acceptable. Our mean total nucleotides read were 58,917,423, have higher result than 11,996,490 in the study done in Korea (Song et al., 2015) and lower than 381,590,477 the study done in Malaysia (Mat-Sharani et al., 2021). The *A. baumannii* mean completeness of sequence, and contamination check accounted for 99.93%, and 0.51%, respectively as per the standard. The mean total nucleotides read 645968164 were higher >9 million than the study done in China (Zhu et al., 2013). The *K. oxytoca* mean completeness of sequence was 99.73, contamination check was 1.9%, and G-C content was 55.2% fulfilling the standard. The mean nucleotides read were 4122133 similar to 5,423,834 in the study done in Russia (Giliazeva et al., 2019)

5.5.2. Whole Genome Sequence-based Multilocus Sequence Typing Novel Allelic Species Identification Gram-Negative Bacteria

Core genome MLST (cgMLST), a high-resolution molecular typing technique based on whole genome sequencing, has been used for precise bacterial identification (Yan *et al.*, 2021). To characterize isolates of different microbial species, DNA sequences of internal fragments from seven well-conserved housekeeping genes or loci within the microbes were used (Ibarz Pavón and Maiden, 2009; Maiden *et al.*, 1998).

Among the six *Klebsiella* species, there were three novel drug-resistant *K. pneumoniae* strains FF02_1686 ST45 infB:1*, FF02_3799 ST1799 tonB:34*, and FF02_5876 ST973 gapA:16*, representing three distinct allelic types of drug-resistant bacteria. The next FF02_5971, FF02_6480, and FF02_6568 three strains of *K. pneumoniae* share similar ST973 gapA:16*, allelic profiles while being novel. Among the *Acinetobacter* species, FF02_824 *A. baumannii* ST85

Pas_rplB:3* and FF02_5055 *A. baumannii* ST63 Pas_pyrG:10* had novel allelic profiles. In addition, one of the FF02_3862 *E. coli* ST131 adk:53* had a novel allelic profile.

These four novel *K. pneumoniae* species have the same antimicrobial resistance cluster and antimicrobial resistance. The genes have similar antimicrobial resistance genes OqxA, OqxB, aac(3)-IIa, aac(6')-Ib-cr, aph(3'')-Ib, aph(6)-Id, blaCTX-M-15, blaLEN16, blaOXA-1, blaTEM-1B, dfrA14, qnrB1, sul2, tet(A), except FF02_6568 which have additional cat(pC221), and tet(K) virulence gene. The cat(pC221) gene, found in Gram-negative bacteria, confers resistance to chloramphenicol; it is a chloramphenicol acetyltransferase gene (Schnellmann *et al.*, 2006). is. The gene has been found in several resistance plasmids in *Pseudomonas putida*, *Acinetobacter species*, and *K. pneumoniae* (Schwarz *et al.*, 2004; Silva *et al.*, 2022). The tet(K) gene is a tetracycline and doxycycline resistance gene (Eliopoulos *et al.*, 2003; Hedayatianfard *et al.*, 2014; Milanović *et al.*, 2023) but it has also been detected in a few gram-negative isolates (Eliopoulos *et al.*, 2003; Hedayatianfard *et al.*, 2014; Milanović *et al.*, 2023). This gene encodes for the tetracycline efflux protein, which actively pumps tetracycline out of the bacterial cell (Milanović *et al.*, 2023).

All novels have the same virulent gene of clpK1, fimH, and traT except FF02_5876 which does have additional nlpI, csgA gene, and FF02_6568 has iutA gene. The clpK1, fimH, and traT genes are virulent genes found in gram-negative bacteria, particularly in *E. coli* (Boll *et al.*, 2017). The clpK1 gene encodes for the Clp ATPase, ClpK, which confers heat resistance. The ClpK protein is associated with fimH, hlyA, and crl and is involved in the breakdown of misfolded proteins. It is also necessary for *E. coli* to survive in heat-stressed environments (Boll *et al.*, 2017; Cepas and Soto, 2020).

The fimH gene encodes for the type 1 adhesin FimH, which binds to mannosylated glycoproteins on the surface of human and murine bladder cells, facilitating bacterial colonization, invasion, and biofilm formation (Hojati *et al.*, 2015; Schwartz *et al.*, 2013). The fimH gene is considered to be the most prevalent virulence factor (Hojati *et al.*, 2015). The fimH gene is often found in conjunction with other virulence genes such as sfa, papA, papC, and iutA (Sarowska *et al.*, 2019; Schwartz *et al.*, 2013). The traT gene encodes for a transfer protein that inhibits the classical pathway of complement activation, leading to serum survival (Montenegro *et al.*, 1985). The traT gene is part of the tra operon of the F-like conjugative plasmids (Rezatofighi *et al.*, 2021). The

fimH gene has been detected in *K. pneumoniae* isolates from nosocomial infections (Kuş *et al.*, 2017; Stahlhut *et al.*, 2009), and the iutA gene has been detected in *K. pneumoniae* isolates from teaching hospitals (Ferreira *et al.*, 2018). However, we did not get information that mentioned the detection of clpK1, traT, nlpI, and csgA genes in *K. pneumoniae* isolates. These bacteria have the same plasmid MLST IncF RST[K7:A:-B-] and plasmids IncFIB(K), IncFII(K), except in FF02_6568 have found additional rep22, rep7a plasmid genes in which their responsibility is unknown.

The nlpI, csgA, and iutA genes are virulence genes found in gram-negative bacteria. The nlpI gene is essential for the pathogenesis of neonatal meningitis and bacteremia causing *E. coli* (NMEC) in blood and the penetration of the blood-brain barrier (BBB) (Cole *et al.*, 2019; Wijetunge *et al.*, 2015). The loss of NlpI enhances the production of outer membrane vesicles (OMVs), which carry virulence factors and can stimulate immune responses (Cole *et al.*, 2019; Tiku and Tan, 2021). The csgA gene is a virulence gene found in *E. coli* and *Acinetobacter baumannii*. The gene is involved in the production of curli fibers, which are extracellular matrix components that contribute to biofilm formation and adhesion to host cells (Anchana *et al.*, 2021; Iswarya Jaisankar *et al.*, 2020; Shadan *et al.*, 2023). The csgA gene has been detected in carbapenem-resistant *A. baumannii* strains and is considered highly virulent due to its role in biofilm formation (Anchana *et al.*, 2021). The iutA gene is a virulence gene found in uropathogenic *E. coli* (UPEC). The gene encodes for the iron uptake receptor protein, which is involved in the acquisition of iron from host cells (Chouikha *et al.*, 2008; López-Banda *et al.*, 2014).

The rest two novels FF02_1686 ST45 and FF02_3799 ST1799 *K. pneumoniae* have significant differences in their sequence type and their conserved housekeeping genes which are infB:1* and tonB:34*. The housekeeping gene translation initiation factor 2 infB:1* is involved in fundamental metabolic processes necessary for cell maintenance. It encodes translation initiation factor II, which is necessary for the start of bacterial protein synthesis (Saha *et al.*, 2019).

The tonB:34 housekeeping gene encodes the TonB-dependent transporters (TBDTs) of bacterial outer membrane proteins, which bind and transport ferric chelates known as siderophores as well as vitamin B12 are controlled by the TonB energy transduction system (Noinaj *et al.*, 2010). The FF02_1686 ST45 infB:1*, have ARR-3, OqxA, OqxB, aac(3)-IIa, aac(6')-Ib-cr, aadA16, blaCTX-

M-15, *bla*SHV-148, *bla*TEM-1B, *dfr*A27, *fos*A, and *sul*1 and the FF02_3799 ST1799 *ton*B:34*, have *OqxA*, *OqxB*, *aac*(3)-IIa, *aph*(3'')-Ib, *aph*(6)-Id, *bla*CTX-M-15, *bla*SHV-28, *bla*TEM-1B, *cat*A2, *dfr*A14, *fos*A6, *sul*2, *tet*(D) different type of drug resistance genes.

The two *K. pneumoniae* have *fimH*, *fyuA*, *irp2*, *iutA*, and *mrkA*:ABW83989 virulent factor in common and FF02_3799 ST1799 have additional *clpK1*, and *traT* virulent genes. Both bacteria also have exceptional *mrkA*:ABW83989 virulent gene in common. A virulence gene called *mrkA* is present in gram-negative bacteria, especially *K. pneumoniae*. The type 3 fimbrial adhesion protein MrkA, which is encoded by this gene, is involved in the formation of biofilms and adhesion to host cells, thereby aggravating infections (Cepas and Soto, 2020; Huang *et al.*, 2009; Mirzaie and Ranjbar, 2021). In addition to adhesion, this virulence gene is involved in various functions such as adhesion, invasion, immune evasion, and toxin production (Cepas and Soto, 2020). The *mrkA* gene has been detected in *K. pneumoniae* isolates from hospitals in Tehran, Iran, and has been associated with antibiotic resistance (Bakhtiari *et al.*, 2021).

Again, both FF02_1686 and FF02_3799 *K. pneumoniae* have frameshift antimicrobial mutation on *ompK37*(p.M233Q - Frameshift,) and *ompK36*(p.N304E – Frameshift), respectively. A genetic mutation affecting the outer membrane protein K37 (*OmpK37*) that is present in carbapenem-resistant *K. pneumoniae* is known as *ompK37*(p.M233Q - Frameshift). At position 233, it causes a frameshift mutation that causes glutamine (Q) to replace methionine (M), and it also causes the outer membrane to become more permeable, which in turn reduces the susceptibility of *K. pneumoniae* isolates to carbapenem (Simner *et al.*, 2022). A genetic mutation known as *ompK36*(p.N304E – Frameshift) affects the outer membrane protein K36 (*OmpK36*), changing the amino acid (N) at position 304 to glutamic acid (E). This mutation is linked to the permeability of carbapenems (Wong *et al.*, 2019). One of the mutations that have been found to contribute to *K. pneumoniae* resistance to carbapenem is *ompK36*(p.N304E – Frameshift) (Özad Düzgün, 2021).

The novel FF02_824 ST85* and FF02_5055 ST63* *A. baumannii* have *Pas_rplB*:3* and *Pas_pyrG*:10* unique loci respectively. ST85* *A. baumannii* were resistant to aminoglycoside, amphenicol, beta-lactam, carbapenem, folate pathway antagonist, macrolide, streptogramin b, and ST63* were resistant to beta-lactam and carbapenem antibiotic classes. The ST85* *A. baumannii*

are beta lactam resistance gene and consisting ant(2'')-Ia, aph(3')-VI, *bla*ADC-25, *bla*NDM-1, *bla*OXA-94, *floR*, *mph*(E), *msr*(E), and *sul2*, and the ST63* *A. baumannii* have *bla*OXA-272 carbapenem antimicrobial resistance genes. It is phenotypically confirmed carbapenem resistance. The *bla*OXA-272 gene is a class D β -lactamase gene that has been identified in *Acinetobacter* spp. The gene is homologous to *bla*OXA-273 and has been detected in the genomes of *Acinetobacter pittii* strains (Périchon *et al.*, 2014) The *bla*OXA-272-like gene has also been detected in *Acinetobacter nosocomialis* isolates in Taiwan (Chen *et al.*, 2019). The function of the *bla*OXA-272 gene is to produce an enzyme that can hydrolyze β -lactam antibiotics, including carbapenems (Sharma *et al.*, 2022).

The rest novel bacteria is FF02_3862 ST131 *E. coli* have unique loci of *adk*:53*. Which is resistant to benzalkonium chloride, cetylpyridinium chloride, chlorhexidine, and hydrogen peroxide disinfectants. This organism resists all tested disinfectants and it has a high ability to cause nosocomial infection. This bacteria is resistant to all disinfectants and has a wide range of antimicrobial resistance genes that are *aac*(3)-IIa, *aadA*5, *aph*(3'')-Ib, *aph*(6)-Id, *bla*CTX-M-15, *bla*TEM-1B, *dfrA*17, *mph*(A), *sul1*, and *sul2*. These drug-resistance genes resist aminoglycoside (Bellaaj *et al.*, 2003; Zhang *et al.*, 2023), third-generation cephalosporin (Mazumder *et al.*, 2020; Zhang *et al.*, 2013), macrolides, trimethoprim (White *et al.*, 2000), and Sulfonamide (Frank *et al.*, 2007)

The vast range of virulence genes that are *aslA*, *aamR*:FN554766, *afaA*, *afaB*, *afaC*, *afaD*, *chuA*, *fimH*, *fyuA*, *gad*, *hha*, *hra*, *iha*, *irp2*, *iucC*, *iutA*, *kpsE*, *kpsMII*, *nlpI*, *ompT*, *papA_F43*, *sat*, *senB*, *shiA*, *sitA*, *terC*, *traJ*, *traT*, *yehA*, *yehB*, *yehC*, and *yehD* identified in the novel bacteria.

The adhesins *AfaA*, *AfaB*, *AfaC*, and *AfaD* support *E. coli* attachment to host cells. These belong to the *Afa/Dr* family of adhesins, which is linked to recurrent cystitis and pyelonephritis (Sarowska *et al.*, 2019). *FimA* to *FimI* are genes that are involved in the synthesis of type 1 fimbriae, which are adhesins that cause UTI (Kaper *et al.*, 2004). *sitABCD*: *SitABCD* is an ABC transporter that is involved in the transport of iron and manganese in *E. coli* (Yao *et al.*, 2006). A study conducted on broilers in Ilorin, Nigeria, found that two genes, *qacE* and *sitABCD*, are linked to disinfectant resistance in extensively drug-resistant *E. coli* (Al-Mustapha *et al.*, 2022). The efflux pumps *qacE* and *sitABCD* encode for resistance to disinfectants, such as hydrogen peroxide and quaternary

ammonium compounds (QACs), respectively (Al-Mustapha *et al.*, 2022; Boyce, 2023). A correlation has been found between *qacE* and ampicillin, ciprofloxacin, cotrimoxazole, and cephalothin resistance, while *sitABCD* is associated with fluoroquinolones, Fosfomycin, sulfamethoxazole, ampicillin, cephalosporins, trimethoprim, aminoglycosides, chloramphenicol, tetracycline, and macrolides (Al-Mustapha *et al.*, 2022; Shafaati *et al.*, 2016). *SitABCD* was found to be correlated with resistance to hydrogen peroxide, a disinfectant (Sabri *et al.*, 2006). Therefore, these disinfectant-resistance genes are also drug-resistance genes.

There were two antimicrobial resistance mutations *gyrA*(p.S83L), and *parC*(p.S80I). Specifically, quinolone resistance in *E. coli* is correlated with the *gyrA*(p.S83L) and *parC*(p.S80I) mutations, are in the *gyrA* and *parC* genes, respectively (Bagel *et al.*, 1999; Johnning *et al.*, 2015; Tchesnokova *et al.*, 2023)

5.5.3. Molecular Epidemiology of Antimicrobial Resistance

In this study, 95.8% of all types of beta-lactam cluster drug resistance were identified from 142 MDR bacteria. This study was lower than the study conducted in Nigeria (98.8%) (Adeyemi and Akinde, 2021) and higher than the study done in Addis Ababa Ethiopia (70%) (Beyene *et al.*, 2019). The rest could be due to the efflux pump and binding site alteration (Beshah *et al.*, 2023). The ResFinder tool of antimicrobial resistance identification result was coherent with phenotyping identification. The highest resistance of Ampicillin 124 (87.32%) in ResFinder is similar to the highest resistance of Ampicillin 85.7%, and 94.6% in the Ethiopian phenotyping test (Beshah *et al.*, 2022). However dissimilar finding was observed in minimal drug resistance in tigecycline, 0.7%, instead of Amikacin, 10.8%, in the previous phenotyping study. Among gram-negative bacteria *K. pneumoniae*, *E. coli*, and *K. oxytoca* were the predominant drug-resistance bacteria (Beshah *et al.*, 2022; Beshah *et al.*, 2023). The WGS result of ESBL was 75.4%, and Carbapenemase was 21.8%, aligned with phenotyping drug combination test counted for 69%, and 35.1%, respectively; 48.6% carbapenem-resistant *A. baumannii* coherent with 53.2% of previous phenotyping studies (Beshah *et al.*, 2023).

5.5.4. Comparison of Phenotyping Combination Drug Test, Genotyping PCR Test, and Whole Genome Sequencing Result

We compare enzyme-producing drug resistance types detection method by PCR and WGS analysis with the baseline of the combination disk method, in gram-negative bacteria. Regarding cumulative results compared with the phenotyping detection method, we observed that the WGS 86.8% identification method was a better approach to the phenotyping CDT method than the 85.1% identification of the PCR method. This might be due to the low coverage of PCR primers for the identification of drug-resistance types. Comparing each drug-resistant type ESBL PCR 94.9% and WGS detection method 96.9% is better to approach phenotyping identification result. Whereas in the AmpC detection, PCR 65.4% and WGS 61.5% results are comparable but different from the phenotyping method result. The reason may be the plasmid genes disappeared during resub or there may be new genes not included in PCR and these new genes are not recognized in WGS analysis or there were false negative WGS-based AMR predictions as the study done in Singapore (Zwe et al., 2020). This needs further study. In the case of MBL, 100.0% PCR identification was aligned with phenotyping and better than 80.0% identification of the WGS method. Finally, in the case of carbapenemase detection, 82.5% of WGS detection was better than 70.0% of PCR detection compared with combination drug tests. As mentioned earlier the reason may be the coverage of primer.

Comparison of drug resistance types identification in a combination drug test with PCR method taking the higher rank shows phenotyping ESBL 74 (52.1%), lower than PCR test ESBL 78(54.9%), and higher than ESBL WGS 32(22.5%). The reason is that AmpC detection in PCR is minimal and the higher-grade selection will be affected and ESBL result increases. But in the case of carbapenemase unexpressed gene identification causes higher in carbapenemase and it lowers ESBL prevalence because of higher rank selection. In the case of AmpC identification phenotyping 17 (12.0%), was higher than PCR 9(6.3%) and WGS 10(7.0%) detection. However, the PCR and WGS results were similar. The phenotyping test might be raised by other factors like losing some plasmid genes during resub. Comparison of MBL drug resistance types identification in phenotyping 10(7.0%), is lower than PCR 13(9.2%), and higher than WGS MBL 6(4.2%) identification. This indicates that PCR identification is better for MBL than a combination drug test. Comparison of Carbapenemase drug resistance types identification in phenotyping

40(28.2%), is higher than PCR 33(23.2%), and lower than WGS Carbapenemase 88(62.0%), identification due to unexpressed or silent gene in the bacteria. Therefore, in the case of carbapenemase gene detection, WGS is better considering all bacteria are not exposed to carbapenem PCR detection. These indicate that WGS is not appropriate for patient treatment (Fauzia et al., 2023; Yee et al., 2021) (4,5). It may lead patients to unnecessary drug exposure. A study done in the USA shows a genotype-to-phenotype dilemma, ensuring more accurate and reliable phenotyping AMR identification (Yee et al., 2021). However, WGS helps for scientific community to predict host response, to understand mutation, interactions with other microorganisms, and the possibility of drug resistant that comes from changed unexpressed genes (Fauzia et al., 2023). These unexpressed genes can still play important roles in regulating gene expression, and the development of AMR gen (Mugnier et al., 2010). For both cases, the combined approach is more illuminating than a phenotype test alone because it also affords information about the resistance mechanism and geographical distribution (Fauzia et al., 2023).

In terms of ESBL drug resistance type identification, 69.0% of combination drug tests outperformed 65.5% of PCR tests. However, it is lower when compared to 85.2% WGS identification. Phenotypical identification of 18.3% AmpC-BL drug resistance types was higher than PCR 12.0% but lower than AmpC 33.8% WGS identification. In both cases, WGS identification is higher because it was identified from all bacteria including unexpressed genes. Unlikely phenotypical identification of 7.0% MBL drug resistance type was lower than 26.8% PCR detection and 14.1% WGS identification. This needs further study of why the MBL gene was lower in WGS than PCR detection.

The comparison of the combination drug test of carbapenemase 28.2% is higher than 23.2% PCR identification and lower than 62.0% WGS result. However, regarding total carbapenemase identification of phenotyping method 50 (35.2%) is lesser than PCR 71 (50.0%) and WGS 107(76.1%). Phenotyping combination drug tests identified the active drug-resistant type of the isolates which is important for patient treatment. PCR tests have limitations on the primer we used. Whereas the WGS identified silent genes that have the potential to be drug resistance type but not the active drug resistance type (Fauzia et al., 2023; Mugnier et al., 2010). If we use WGS for patient treatment it may lead to unnecessary cost and antibiotic load on patients. So, for clinical

purposes, CDT is better than the rest while the research purposes whole genome sequence is the best method (Fauzia et al., 2023; Yee et al., 2021).

5.5.5. Genetic Diversity of Antimicrobial Resistance Gene in Gram-Negative Bacteria From, Bloodstream Infection

5.5.5.1. Antimicrobial Resistance Gene

A total of 97.1% AMR genes were identified. For every gram-negative bacteria, an average of 12 AMR genes were identified which is similarly the highest AMR load observed in other studies (Moghnia and Al-Sweih, 2022; Wang et al., 2018a). The highest AMR genes were observed in *K. pneumoniae* (39.8%), followed by *E. coli* (23.0%), and *A. baumannii* (20.2%) were in line with the previous studies except for *A. baumannii* (Legese et al., 2022b; Mbelle et al., 2020). The higher prevalence of the AMR gene was *sul2* (5.7%), followed by *blaCTX-M-15* (5.2%), and *aph(3'')-Ib* (5.1%) were similar to the study done in South Africa (Mbelle et al., 2020). Whereas, the highest ten beta-lactamase genes were *blaCTX-M-15* (21.5%), followed by *blaTEM-1B* (16.1%), and *blaOXA-1* (13.3%) genes have similarity with the Russian study (Mbelle et al., 2020; Punina et al., 2015).

5.5.5.2. Antimicrobial Resistance Gene in ESBL, AmpC, MBL, and Carbapenemase-producing Gram-Negative Bacteria

ESBL: The whole genome sequencing result was aligned with phenotyping and molecular characterization. In both cases, *blaCTX-M-15* and *blaTEM-1B* were the majority of ESBL-producer genes in third-generation cephalosporin-resistant gram-negative bacteria (Beshah et al., 2023; Mbelle et al., 2020). Even though, the prevalence is low 14 variants of *blaSHV* genes were identified from third-generation cephalosporin resistance gram-negative bacteria. In this study 223 beta-lactamase genes identified, including *blaACT*, *blaADC*, *blaCMY*, *blaCTX-M*, *blaLEN*, *blaPLA1a*, *blaSCO*, *blaSHV*, and *blaTEM* variants, that are associated with resistance to beta-lactam antibiotics and are commonly found in clinical isolates of Gram-negative bacteria (Cherubini et al., 2022b; Mbelle et al., 2020; Pitout et al., 2019; Sadeghi et al., 2016).

The genes *aac(3)-IIa*, *aac(6')-Ib-cr*, *aadA16*, *aadA2*, *aadA5*, *ant(2'')-Ia*, *ant(3'')-Ia*, *aph(3')-Ia*, *aph(3'')-Ib*, *aph(3')-VI*, and *aph(6)-Id* are all associated with aminoglycoside resistance (Ho et al.,

2010; Mbelle et al., 2020; Soleimani et al., 2014) accounting for 251(28.1%) which is the highest prevalence in third-generation cephalosporin resistance bacteria. The genes *dfrA12*, *dfrA14*, *dfrA15*, *dfrA16*, *dfrA17*, *dfrA19*, *dfrA27*, *dfrA5*, *dfrA7*, and *dfrG* were all associated with dihydrofolate reductase (*dfr*) genes and were linked to trimethoprim resistance (Faltyn *et al.*, 2019; Padungtod *et al.*, 2011). The *qnrB1*, *qnrB6*, and *qnrS1* genes are part of the quinolone resistance gene family, which encodes proteins that confer resistance to quinolone antibiotics (Xia *et al.*, 2013). The *tet(39)*, *tet(A)*, *tet(B)*, *tet(D)*, *tet(K)*, and *tet(M)* genes, which are associated with tetracycline resistance have been identified in various bacterial species (Adelowo and Fagade, 2009). Our findings revealed one *blaNDM-1* and one *blaCARB-2*, as well as the OXA families of different variants *blaOXA-1*, *blaOXA-10*, *blaOXA-120*, *blaOXA-232*, *blaOXA-58*, and *blaOXA-94* were identified. These variants are associated with carbapenem resistance in various bacterial species. These genes belong to the oxacillinase (OXA) family of beta-lactamase genes, which are known for their ability to hydrolyze carbapenem antibiotics (Chowdhury et al., 2022; Legese et al., 2022b; Li et al., 2023; Meng et al., 2023; Weber et al., 2019; Xiang et al., 2020). However, the strains are not carbapenem-resistant which indicates that these are silent genes.

Our previous study shows that NICU, Medical, Hematology, General Surgery, and MICU were affected by extended-spectrum beta-lactam (ESBL) drug-resistant bacteria (Beshah *et al.*, 2023; Hailu *et al.*, 2016a; Legese *et al.*, 2022a). The whole genome sequence result confirms that these departments have a higher prevalence of beta-lactam and other AMR genes. Like departments, wards of B6 (34.6%), C/W (12.3%), D7 (7.9%), and C7 (6.8%) were affected with similar AMR genes. With the most common prevalence of *K. pneumoniae*, *E. coli*, and *K. oxytoca* AMR bacteria. Carbapenem drug resistance associated with ICU and SICU with the abundant prevalence of *Acinetobacter spp* whereas ESBL drug-resistant bacteria associated with NICU, ICU, SICU, and MICU with *K. pneumoniae*, *E. coli*, and *K. oxytoca* (Beshah *et al.*, 2022; Beshah *et al.*, 2023; Legese *et al.*, 2022a).

Similar studies showed *sul2*, *blaTEM-1B*, *aph(3'')-Ib*, *aph(6)-Id*, and *blaCTX-M-15* among AMR genes in third-generation cephalosporin and cephamycin-resistant gram-negative bacteria (Beshah *et al.*, 2023; Pitout *et al.*, 2019; Sadeghi *et al.*, 2016). Our result showed that *blaCMY-2* (1.9%), and *blaCMY-6* (0.6%) genes were identified from third-generation cephalosporin and cephamycin-resistant gram-negative bacteria similar with (Heider et al., 2009; Islam et al., 2022;

Zhao et al., 2001). Except for three, all fourteen AmpC producer gram-negative bacteria were ESBL producers which have *bla*CTX-M-15(5.6%), *bla*SHV-12(1.9%), *bla*SHV-187(0.6%), *bla*TEM-1B (6.3%), *bla*VEB-1(0.6%), and *bla*OXA-1(5.0%) genes. These genes are highly prevalent in AmpC beta-lactam-resistant gram-negative bacteria (Beshah et al., 2023; Pitout et al., 2019; Sadeghi et al., 2016). The rest beta-lactamase families of *bla*OXA-120, *bla*OXY-5-1, *bla*CARB-5, *bla*OXA-1, *bla*ADC-25, and *bla*ACT-3 were identified (Chowdhury et al., 2022; Meng et al., 2023; Weber et al., 2019; Xiang et al., 2020). However, few carbapenemase genes were identified among third-generation cephalosporin and cephamycin-resistant gram-negative bacteria. These genes are *bla*NDM-1 (1.3%), and *bla*OXA-58 (0.6) (Chowdhury et al., 2022; Weber et al., 2019; Xiang et al., 2020). These discordant results were due to the carbapenemase producer genes being silent genes.

AmpC-BL: Among AmpC beta-lactam-resistant gram-negative bacteria, *Escherichia coli* (53.1%) and *E. cloacae* (19.4%) have the highest AMR genes (Beshah et al., 2023). The general surgery department was the site of almost all AmpC beta-lactam-resistant *K. pneumoniae*. According to a study conducted in a Saudi Arabian tertiary care hospital, the surgical department had a higher frequency of AmpC β -lactamase producers (34.8%) than the medicine department (29.2%) (Ibrahim et al., 2019). *E. cloacae* and *K. oxytoca*, two AmpC beta-lactam resistant bacteria, were discovered in the NICU (Beshah et al., 2023). The AmpC beta-lactam-resistant gram-negative bacteria are most abundantly found in B4, D7, and B6 wards and Hematology, General Surgery, and NICU departments.

Carbapenem: The most prevalent carbapenem-resistant gram-negative bacteria were *A. baumannii* (56.0%), *K. pneumoniae* (22.0%), and *E. coli* (16.0%) (Beshah et al., 2023). The most predominant AMR genes were *sul2* followed by *aph(3'')-Ib*, *aph(6)-Id*, *bla*ADC-25, *sul1*, *tet(B)* (Cherubini et al., 2022b; Mbelle et al., 2020; Punina et al., 2015).

Among all antimicrobial-resistant genes, the beta-lactamase was (30.2%). Nevertheless, specific carbapenem resistance genes were (20.2%) genes. Among the species carbapenemase producer genes *bla*OXA-23, *bla*OXA-66, *bla*NDM-1, *bla*OXA-1, *bla*OXA-69, *bla*OXA-9, *bla*CARB-5, *bla*NDM-5, *bla*OXA-120, *bla*OXA-232, *bla*OXA-272, and *bla*OXA-83 have direct association with carbapenem resistance (Alamri et al., 2020; Cherubini et al., 2022b; Legese et al., 2022b;

Merhi et al., 2023). However, some genes *blaACT-5*, *blaL1*, *blaCARB-10*, *blaADC-25*, and *blaSCO-1* are not frequently linked to resistance to carbapenems (Papagiannitsis et al., 2007; Warjri et al., 2015). Nonetheless, in certain instances, these genes have been discovered in bacteria resistant to carbapenem. A study in China showed a chromosomal metallo-beta-lactamase gene called *blaL1* in *Stenotrophomonas maltophilia*. Despite not being a carbapenemase gene, it is associated with high levels of carbapenem resistance in *Stenotrophomonas maltophilia* isolates (Han et al., 2020). A study conducted in Kenya discovered *blaACT-5* in isolates of *A. baumannii* that were resistant to carbapenems (Musila et al., 2021). The *blaACT-5* gene has been identified in Enterobacteriaceae that produce AmpC, including *K. pneumoniae*, *E. coli*, and *E. cloacae* (Musila et al., 2021). A study done in India reported *A. baumannii* isolates that possessed *blaCARB-10* and *blaADC-25* genes to be resistant to carbapenems (Vijayakumar et al., 2022). A study done in Canada showed that the isolates of *E. coli* that were resistant to carbapenems carried the *blaSCO-1* gene (Cooper et al., 2021). These genes have been identified in carbapenem-resistant bacteria, suggesting that they may rarely contribute to carbapenem resistance even though they are not normally linked to enzyme production.

The one novel strain of *A. baumannii* ST1030/ST63* has *blaOXA-272* carbapenemase gene. A study shows *blaOXA-272* identified in *Acinetobacter nosocomialis* carbapenem hydrolyzing class D β -lactamase that contributes to carbapenem resistance in *Acinetobacter nosocomialis* strains (Chen et al., 2019). This species of *A. baumannii* is identified as novel and this AMR gene *blaOXA-272* can hydrolyze carbapenem drug in *Acinetobacter baumannii*. This gene is previously identified in *E. cloacae* and *Acinetobacter nosocomialis* but not in *Acinetobacter baumannii* (Chen et al., 2019; Hu et al., 2022).

In this study, *blaADC-25* genes were identified in all carbapenem resistance *A. baumannii* except one novel carbapenem resistance bacteria. This gene was identified only in *Acinetobacter baumannii*. A study in China reported cephalosporins-encoding *blaADC-25* gene carrying *A. baumannii* which is a carbapenem-resistant strain (Zong et al., 2008). Our study shows that *blaOXA-23* and *blaOXA-66* each accounted for 34% of *Acinetobacter baumannii*. A similar study shows *blaADC-25* along with other resistance genes like *blaOXA-23* and *blaOXA-66* found in carbapenem-resistant strains, despite not being a carbapenem gene (Zong et al., 2008). The carbapenemase-producer genes which is not identified in other carbapenem-resistant gram-

negative bacteria but found in *A. baumannii* were *blaOXA-23*, *blaOXA-66*, *blaOXA-69*, *blaOXA-83*, and *blaOXA-120*. Several studies have reported that the presence of these genes in *A. baumannii* strains contributes to carbapenem resistance in *A. baumannii* (Bonnin *et al.*, 2020; Ganjo *et al.*, 2016; Mugnier *et al.*, 2010; Musila *et al.*, 2021; Rafei *et al.*, 2015; Vijayakumar *et al.*, 2022). The study in India showed that the *blaOXA-23*, *blaOXA-66*, *blaOXA-69*, *blaOXA-83*, and *blaOXA-120* genes of *A. baumannii* do contain carbapenem-resistant genes. These genes code for class D β -lactamases, which are serine-dependent enzymes that hydrolyze a variety of β -lactams, including carbapenems (Gupta *et al.*, 2022). Therefore, these *blaOXA-23*, *blaOXA-66*, *blaOXA-69*, *blaOXA-83*, and *blaOXA-120* genes are associated with class D β -lactamases carbapenem resistance.

The most common genes found in carbapenem-resistant *K. pneumoniae* (22.0%) were *blaNDM-1*, *blaOXA-9*, *blaSCO-1*, and *blaOXA-232*. The genes *blaNDM-1*, *blaOXA-9*, *blaSCO-1*, and *blaOXA-232* are associated with carbapenem resistance *Klebsiella pneumoniae*. However, the *blaNDM-1* gene is a well-known carbapenemase gene and is associated with carbapenem resistance (Moghadampour *et al.*, 2018; Musila *et al.*, 2021; Shahid *et al.*, 2022). In this study *blaOXA-9*, *blaSCO-1*, and *blaOXA-232* genes were found only in carbapenem-resistant *Klebsiella pneumoniae*. According to studies it has been found in a variety of carbapenem-resistant bacteria, including *K. pneumoniae* and *E. coli* (Moghadampour *et al.*, 2018; Shahid *et al.*, 2022). Studies reported that *blaOXA-9* is not as common as other carbapenemase genes and is not as frequently linked to carbapenem resistance as *blaNDM-1*; it has been occasionally detected in bacteria that are resistant to carbapenems (Musila *et al.*, 2021). Bacteria resistant to carbapenem have been found to carry the *blaSCO-1* gene, which contributes to their resistance phenotype. Bacteria resistant to carbapenem have been found to harbor the *blaOXA-232* gene, which is linked to carbapenem resistance (Lee *et al.*, 2020). Therefore, all *blaOXA-9*, *blaSCO-1*, and *blaOXA-232* of these genes are associated with carbapenem resistance.

The most common carbapenem-resistant genes in *E. coli* (16.0%) were *blaNDM-5* and *blaNDM-1*; however, in this study, the *blaNDM-5* gene is unique to *E. coli*. The *blaNDM-1* and *blaNDM-5* genes were detected in carbapenemase producer *E. coli* and have also been discovered in other gram-negative bacteria from various nations (Moghadampour *et al.*, 2018; Shahid *et al.*, 2022; Tian *et al.*, 2020). The *blaNDM-1* and *blaNDM-5* genes encode for the New Delhi Metallo- β -

lactamase-1 (NDM-1) and New Delhi Metallo- β -lactamase-5 (NDM-5) enzyme, which is a carbapenemase that hydrolyzes carbapenems and other β -lactam antibiotics (Chowdhury *et al.*, 2022). High levels of carbapenem resistance have been linked to the presence of *bla*NDM-5 (Chowdhury *et al.*, 2022; Yang *et al.*, 2020; Zou *et al.*, 2020). These strains often exhibit resistance to multiple antibiotic classes, including aminoglycosides, fluoroquinolones, trimethoprim-sulfamethoxazole, and beta-lactamase (Linkevicius *et al.*, 2023). The spread of *E. coli* strains carrying the *bla*NDM-5 gene is a growing concern due to the high prevalence of multidrug-resistant strains and their potential to transfer the gene to other enteric pathogens (Chowdhury *et al.*, 2022; Linkevicius *et al.*, 2023).

In this study, the only genes found in *Stenotrophomonas maltophilia* were *bla*L1 and *bla*CARB-10. Studies in the USA and China showed both the *bla*L1 and *bla*CARB-10 genes to be associated with carbapenem resistance in *Stenotrophomonas maltophilia*. It is known that the *bla*L1 gene confers carbapenem resistance in *Stenotrophomonas maltophilia*. It encodes a metallo- β -lactamase with a broad substrate spectrum, including carbapenems (Yang *et al.*, 2014). Comparably, the *bla*CARB-10 gene produces an enzyme called carbapenemase, which hydrolyzes carbapenem antibiotics and causes *Stenotrophomonas maltophilia* strains to become resistant (Brooke, 2012; Wang *et al.*, 2018b). Therefore, both *bla*L1 and *bla*CARB-10 contribute to the carbapenem resistance phenotype in *Stenotrophomonas maltophilia*.

The departments with the highest percentage of gram-negative bacteria resistant to carbapenem were NICU (24.0%), followed by Hematology (24.0%), Medical (22.0%), and SICU (14.0%). The wards with the highest percentages of gram-negative bacteria resistant to carbapenem were B6 (24.0%), B4 (20%), C/W (16%), and B7 (10%) (Beshah *et al.*, 2023; Legese *et al.*, 2022a; Legese *et al.*, 2017). However, the medical (20.9%), hematology (20.0%), and neonatal (25.8%) departments, as well as the particular service-giving wards B6 (25.8%), B4 (21.1%), and B7 (13.6%), showed comparatively higher AMR gene levels (Beshah *et al.*, 2023; Legese *et al.*, 2022a; Legese *et al.*, 2017).

In NICU, higher AMR gene levels in carbapenem-resistant gram-negative bacteria were attributed to *A. baumannii* (15.8%) and *K. pneumoniae* (10.0%). In the hematology department of gram-negative bacteria, AMR genes are more common in *E. coli* (5.5%), *A. baumannii* (5.1%), and *K.*

pneumoniae (9.1%). The medical department's carbapenem-resistant gram-negative bacteria, namely *A. baumannii* (10.9%), *K. pneumoniae* (3.8%), and *E. coli* (6.2%), were the main carriers of the AMR gene. Among SICU patients with carbapenem resistance gram-negative bacteria, *A. baumannii* (12.9%) and *K. pneumoniae* (2.7%) had higher AMR genes (Beshah et al., 2023; Legese et al., 2022a).

The AMR gene for carbapenem resistance gram-negative bacteria was found to be present in *A. baumannii*, *K. pneumoniae*, and *E. coli* in B6, B4, and B7 wards.

5.5.6. Genetic Diversity of Antimicrobial Resistance Gene, Virulence (Factor) Genes, in Gram-Negative Bacteria From, Bloodstream Infection

5.5.6.1. Virulent Factors in Gram-Negative Bacteria

A total of 71.13% of gram-negative bacteria carry virulent genes. For every gram-negative bacterium, an average of 10.44 virulence genes were identified. Similarly, the highest virulence load was observed in other studies (Moghnia and Al-Sweih, 2022; Wang et al., 2018a). Each gram-negative bacteria shares *E. coli* (70.8%), *K. pneumoniae* (22.1%), and *E. cloacae* (2.9%) virulence genes. With a higher prevalence of *fimH* (6.0%), *iutA* (5.5%), and *traT* (5.1%) (Wang et al., 2018a).

The virulence factors of *Escherichia coli* are numerous and have a wide range of activities, including adhesins, toxins, iron acquisition factors, lipopolysaccharides, polysaccharide capsules, and invasins, which are usually encoded on pathogenicity islands (PAIs), plasmids, and other mobile genetic elements (Desvaux et al., 2020; Sarowska et al., 2019). The important virulence factors of *Escherichia coli* can broadly be divided into two groups: bacterial cell surface and secreted virulence factors (Shah et al., 2019). Some of the virulence factors of *Escherichia coli* include:

Adhesins: These are proteins that help *Escherichia coli* attach to host cells and tissues (Shah et al., 2019). **Toxins:** *Escherichia coli* can produce different types of toxins that can damage host cells and tissues (Johnson, 1991). **Iron acquisition factors:** *Escherichia coli* needs iron to survive and

grow, and it can produce proteins that help it acquire iron from host tissues. Examples of iron acquisition factors include siderophores (Sarowska *et al.*, 2019). Capsules: *Escherichia coli* can produce capsules that help it evade the host immune system. Invasins: These are proteins that help *Escherichia coli* invade host cells and tissues (Kaper *et al.*, 2004). Each pathotype of *Escherichia coli* has its characteristic pathogenicity mechanisms and a specific profile of virulence factors encoded by specific gene clusters (Pakbin *et al.*, 2021).

Adhesin

The majority of yehA, yehB, yehC, and yehD genes are virulence factors found in some strains of gram-negative bacteria, including *Escherichia coli* (Ravan and Amandadi, 2015). The yehA and yehB genes have been identified in studies on *Escherichia coli* pathotypes, uropathogenic *Escherichia coli* (UPEC), and *Escherichia coli* isolated from the cerebrospinal fluid of patients with meningitis (Ffrench-Constant *et al.*, 2000; García *et al.*, 2021; Schwechheimer and Kuehn, 2015; Spurbeck *et al.*, 2011; Szczepaniak *et al.*, 2020; Werneburg and Thanassi, 2018). The yehC gene has been identified in uropathogenic *E. coli* (UPEC) and the yehD gene has been identified in enteroaggregative *E. coli*, and *E. coli* isolated from municipal biosolids (Schwechheimer and Kuehn, 2015; Werneburg and Thanassi, 2018). The yehA, yehB, and yehC gene is part of the yeh fimbrial gene cluster, which is responsible for adhesion to some abiotic surfaces (Lu *et al.*, 2021; Schwechheimer and Kuehn, 2015; Werneburg and Thanassi, 2018). The yehC gene is a putative periplasmic chaperone found in some strains of *Escherichia coli* (García *et al.*, 2021). The yehD gene is a predicted fimbrial-like adhesion protein found in some strains of *Escherichia coli* during the study of bile salts inducing resistance to polymyxin (carbapenem-resistant) in enterohemorrhagic *Escherichia coli* O157:H7 (Kus *et al.*, 2011; Ligowska-Marzeta *et al.*, 2019).

As far as I know, the specific role of the yehA, yehB, yehC yehD genes in the virulence of gram-negative bacteria is yet not clear. However, the yehA gene has been associated with adhesion and has been upregulated in response to autoinducer 2-stimulated quorum sensing in *Escherichia coli*. The yehB gene has been identified in studies on *Escherichia coli* pathotypes, uropathogenic *Escherichia coli*, and *Escherichia coli* isolated from the cerebrospinal fluid of patients with meningitis (Miranda *et al.*, 2018; Werneburg and Thanassi, 2018). The yehB gene has also been associated with fimbrial activity, along with other genes such as lfhA, ycgR, and fimCDF (Miranda

et al., 2018). The *yehC* gene has been associated with fimbrial activity and has been identified as a fimbrial gene in *Escherichia coli* (Werneburg and Thanassi, 2018). The study shows *yehC* gene was identified as encoding an outer membrane protein carrying a lipoprotein (Wang *et al.*, 2016). It encodes an outer membrane protein carrying a lipoprotein and has been down-regulated in response to high levels of salinity (Li *et al.*, 2021). The *yehD* gene has been identified as a gene that is differentially expressed in response to oxygen levels and after chlorhexidine treatment. This *yehD* gene has been identified as a gene in the genome of *Shigella flexneri* (Ligowska-Marzeta *et al.*, 2019; Melson and Kendall, 2019).

The adhesin virulent gene of YehA (Pakbin *et al.*, 2021; Vanstokstraeten *et al.*, 2022), *papA_F43* (Santos *et al.*, 2021), *FdeC* (Nesta *et al.*, 2012), *papC* (Ghazvini *et al.*, 2019) and *AfaB* (Sarowska *et al.*, 2019; Sora *et al.*, 2021) have single virulent factor. However, some of the adhesin virulent genes have additional virulent factors. Apart from *csgA* and *fimH* encode Biofilm formation (Arafa *et al.*, 2022; Connell *et al.*, 1996; Luna-Pineda *et al.*, 2019), *lpfA* and *papA_fteA_F10* encode colonization of their host cell (Li *et al.*, 2018; Li *et al.*, 2019b; Shrikhande *et al.*, 1999).

Iron acquisition factors

The majority of iron acquisition factors encoding virulent genes are *fyuA* 20(2.6%), *iroN* 1(0.1%), *irp2* 20(2.6%), and *sitA* 20(2.6%). The Iron acquisition factors *fyuA* gene by encoding the production of siderophores (Rezatofighi *et al.*, 2021) can cause additional virulence factors that are biofilm formation (Hancock *et al.*, 2008), UTI association (Sarowska *et al.*, 2019), and pathogenicity island association virulence factor (Sora *et al.*, 2021). The *irp2* iron acquisition factors gene is additionally responsible for drug/imipenem/ resistance. This gene can be a target for therapeutic interventions (Beerepoot *et al.*, 2013; Yazdanpour *et al.*, 2020 Rakin, 1995 #143). The *iroN* gene is a virulent gene of Iron acquisition factors encoding the production of Siderophores (Khasheii *et al.*, 2021; Litwin and Calderwood, 1993). The *sitA* gene is a virulence factor involved in iron acquisition in gram-negative bacteria, including *Escherichia coli*. It is associated with the virulence of these bacteria and has been linked to various other virulence factors 1,4. The *sitA* gene encodes a receptor for the SitABCD transporter, which is involved in the uptake of iron from the host environment (D'Onofrio *et al.*, 2023; Kim *et al.*, 2022).

Toxin

Regarding the toxin virulent factor encoding gene there are 10 types and a total of 54(7.0%) genes. The highest prevalence in toxins were hlyE 29(3.80%), sat 11(1.40%), cnf1 3(0.40%), astA 3(0.40%), hha 2(0.30%), and hlyA 2(0.30%), recorded in this study. The hlyE encodes the hemolysin E protein, which is a pore-forming toxin that can lyse red blood cells and other host cells (Ahsan *et al.*, 2016; Murase, 2022). hlyF gene causes hemolytic-uremic syndrome and uropathogenic *E. coli* (UPEC) (Murase *et al.*, 2016) but The hlyF gene is associated with an increased production of outer membrane vesicles, which may contribute to the release of cytolethal distending toxin and other virulence factors (Murase *et al.*, 2016). The hlyA gene is a virulent factor found in gram-negative bacteria, specifically in uropathogenic *Escherichia coli* (UPEC) strains causing urinary tract infections (UTIs)(Ghazvini *et al.*, 2019; Moeinizadeh and Shaheli, 2021). The hlyA gene encodes for alpha-hemolysin (HlyA), which is a pore-forming toxin that lyses erythrocytes, induces kidney inflammation and other host cells (Koronakis and Hughes, 2002; Schwidder *et al.*, 2019; Wang *et al.*, 2020).

The sat gene encodes the secreted autotransporter toxin (Sat), which is a member of the serine protease autotransporters of Enterobacteriaceae (SPATEs) family of toxins (Guyer *et al.*, 2002). Sat is a large protein that is secreted by UPEC and can cause damage to host cells (Vieira *et al.*, 2020). The astA gene encodes the EAST1 toxin, which is a heat-stable enterotoxin of EPEC, EAEC, and ExPEC that can cause diarrhea in humans (Alkeskas *et al.*, 2015; Gomes *et al.*, 2016; Sweeney *et al.*, 1996; Yatsuyanagi *et al.*, 2003). The cnf1 gene has the virulent factor of Cytotoxicity and Adhesin in ExPEC & UPEC (Hofman *et al.*, 2000; Knust and Schmidt, 2010). The cnf1 gene encodes the cytotoxic necrotizing factor 1 (CNF1), in gram-negative bacteria, including *Escherichia coli*, and its activation and maturation of human monocyte-derived dendritic cells (Gall-Mas *et al.*, 2018). The Hha/YmoA family of proteins, which includes the Hha gene, is involved in the thermoregulation of the expression of virulence factors in bacteria. For one of these proteins, the Hha protein, it has been shown that it interacts with H-NS, and both proteins form a nucleoid-protein complex responsible for the thermoregulation of *E. coli* hemolysin (Madrid *et al.*, 2002a). The interaction of Hha and H-NS proteins it modulat of the expression of the operon encoding the toxin α -hemolysin (Hly) (Madrid *et al.*, 2002b; Nieto *et al.*, 2000). The presence of genes coding for homologs of both proteins on some conjugative plasmids and their

relation to thermoregulation suggests that this complex could also play a role in the regulation of plasmid transfer (Madrid *et al.*, 2002a).

Increased serum survival

The *iss* gene is a virulence factor found in some strains of *Escherichia coli* (*E. coli*) (Biran *et al.*, 2021; Sarowska *et al.*, 2019). It is responsible for the increased survival of bacteria in the serum. The *iss* gene is located on plasmid ColV, which is a huge virulence plasmid typical of avian pathogenic *E. coli* strains (Sarowska *et al.*, 2019). The function of the *iss* gene is not fully understood, but it is known to contribute to serum survival in many virulent septicemic *E. coli* strains (Biran *et al.*, 2021). The *senB* gene is part of the *cjrABC-senB* gene cluster, which contributes to the virulence of extraintestinal pathogenic *E. coli* (ExPEC) in the mouse model of urinary tract infections. The *senB* gene is thought to hinder the survival of ExPEC in the bloodstream by triggering complement-mediated killing (Huang *et al.*, 2020a; Nüesch-Inderbinen *et al.*, 2021).

Lipopolysaccharides

The *traT* gene is a virulence factor found in some strains of gram-negative bacteria, particularly in *E. coli* (Montenegro *et al.*, 1985; Rimoldi *et al.*, 2017; Sora *et al.*, 2021). It is located on the F plasmid, which is a conjugative plasmid involved in the transfer of antibiotic-resistance genes (Sora *et al.*, 2021). The TraT protein is a cell-surface-exposed, outer membrane lipoprotein that mediates interactions between gram-negative bacteria and their environment (Sukupolvi and O'Connor, 1990). The *traT* gene has been shown to mediate resistance to bacterial killing by serum (Montenegro *et al.*, 1985). The *traT* gene and PAI markers were highly prevalent among UPEC strains isolated from hospitalized patients in Kashan; hence, they could be considered useful targets for prophylactic intervention (Neamati *et al.*, 2015). The TraT protein is also involved in serum resistance and pathogenicity-related factors in clinical isolates of *E. coli* and other gram-negative bacteria (Montenegro *et al.*, 1985; Nojoomi and Ghasemian, 2019).

The *nlpI* gene encodes for the NlpI protein, which is an outer-membrane-anchored lipoprotein that participates in cell division and regulates the activity of peptidoglycan hydrolases Spr and PBP4, which play an essential role in cell wall renewal (Avila-Calderón *et al.*, 2021; Schwechheimer and

Kuehn, 2015; Schwechheimer *et al.*, 2015). The *nlpI* gene has been identified in studies on pathotypes, uropathogenic *E. coli*, and *E. coli* isolated from animals and animal food products (Avila-Calderón *et al.*, 2021; Barcellini *et al.*, 2022; Barnich *et al.*, 2004). In a study on the virulence of adherent invasive *E. coli* strain LF82 isolated from a patient with Crohn's disease, the loss of the NlpI negative mutant resulted in a broad spectrum of virulence factors, including the lack of flagella and type 1 pili, and reduced bacterial uptake by J774-A1 (Barnich *et al.*, 2004). The *nlpI* gene has also been associated with hyper-vesiculation and abnormal cell division in *E. coli* (Avila-Calderón *et al.*, 2021; Schwechheimer and Kuehn, 2015).

Polysaccharide capsule synthesis

The polysaccharide capsule synthesis virulent gene *kpsE* 13(1.70%), *capU* 12(1.60%), *kpsMII_K5* 7 (0.90%) were the major in this study. The *kpsE* gene is a virulence factor found in gram-negative bacteria, specifically in the group III capsular polysaccharide genes (Johnson and Stell, 2000). It is involved in the biosynthesis of capsular polysaccharides, which are important for bacterial virulence. The KpsE protein of *Escherichia coli* K5 has been characterized and localized (Roberts, 1995). The *kpsE* gene has been identified, along with other genes such as *kpsC*, *kpsD*, *kpsF*, *kpsM*, *kpsU*, *kpsS*-like, *ompA*, *entA*-like, and *entB*, in Gram-negative bacteria isolated from chronic wounds in Ghana (Dekker *et al.*, 2021). In a study on *Escherichia coli* bloodstream infections, the *kpsE* gene was found in an extraintestinal isolate of *Escherichia coli* (CP9, O4) (Daga *et al.*, 2019).

The *capU* gene is a virulence factor found in some strains of enteroaggregative *Escherichia coli* (EAEC) (Borgersen *et al.*, 2018; Tanih *et al.*, 2019). It is part of the *cap* locus that encodes a protein that is 50% identical to the *rfbU*-encoded protein involved in the biosynthesis of the O-antigen of lipopolysaccharides (Tanih *et al.*, 2019). The *capU* gene is regulated by the master virulence regulator gene *aggR*, which is responsible for the expression of several virulence genes in EAEC (Tanih *et al.*, 2019). The *capU* gene is involved in the biosynthesis of the EAEC capsular polysaccharide, which is important for the adherence of EAEC to intestinal epithelial cells (Borgersen *et al.*, 2018; Tanih *et al.*, 2019). The *capU* gene is often found in conjunction with other virulence genes such as *aar*, *pic*, and *agg4A* (Borgersen *et al.*, 2018).

The *kpsMTII_K5* gene is a virulence factor-encoding gene found in gram-negative bacteria, specifically in *Escherichia coli*. This gene is responsible for the synthesis of the K5 capsule, which is a type of capsular polysaccharide that helps the bacteria evade the immune system response and protect it from unfavorable conditions (Arafa *et al.*, 2022). The K5 capsule is also known to be involved in biofilm formation, which is a key factor in the pathogenesis of many bacterial infections (Cepas and Soto, 2020). The K5 capsule is also known to be involved in biofilm formation, which is a key factor in the pathogenesis of many bacterial infections. The *kpsMII_K5* gene is one of the eight ExPEC-associated virulence genes identified in porcine isolates of *E. coli* (Wu *et al.*, 2007).

The *kpsMII_K1* and *kpsMII* gene is a virulent factor found in gram-negative bacteria, specifically in *Escherichia coli* (D'Onofrio *et al.*, 2023; Vanstokstraeten *et al.*, 2022). The *kpsMII_K1* gene is responsible for the synthesis of the K1 capsular polysaccharide, which protects UPEC from unfavorable conditions and helps the bacteria evade the immune system response, particularly in urinary tract infections (Vanstokstraeten *et al.*, 2022; Zhu *et al.*, 2021). The K1 capsule is a major virulence factor in neonatal meningitis caused by *E. coli* (Vanstokstraeten *et al.*, 2022; Zhu *et al.*, 2021). The *kpsMII* gene encodes the K2 capsule protein, which is a component of the bacterial capsule and is important for virulence (Sarowska *et al.*, 2019; Yun *et al.*, 2023). The K2 capsule protects *E. coli* from phagocytosis and complement-mediated death and is a characteristic feature of neonatal meningitis caused by *E. coli* (Yun *et al.*, 2023).

Invasins

The invasins' common genes were *AslA* 25 (3.30%), *cia* 5 (0.70%), and *eilA* 5 (0.70%). The *AslA* gene is a virulence factor found in some Gram-negative bacteria, including *Escherichia coli*. It contributes to the invasion of brain microvascular endothelial cells in vitro and in vivo (Hoffman *et al.*, 2000; Yao *et al.*, 2006). The *AslA* gene product shows homology to a well-characterized arylsulfatase family of enzymes found in eukaryotes, as well as prokaryotes (Hoffman *et al.*, 2000). *AslA* is one of several microbial factors, including the K1 capsule, OmpA, type I fimbriae, Ibe proteins, and CNF1, that are involved in *E. coli* meningitis (Yao *et al.*, 2006). *AslA* is also required for invasion in some *E. coli* strains (Kaper *et al.*, 2004).

The "cia" gene is a virulence factor-encoding gene found in *Campylobacter jejuni*, a gram-negative bacterium. The cia gene encodes for Campylobacter invasion antigens (Cia) proteins, which are secreted by the bacterium and are involved in the invasion of human intestinal epithelial cells (IECs) in vitro (Konkel *et al.*, 2004; Neal-McKinney and Konkel, 2012). Some of the Cia proteins, such as CiaC, play a role in invasion, while others, such as CiaI, are required for intracellular survival in human IECs (Elmi *et al.*, 2020). The Cia proteins are considered to be important virulence factors in *C. jejuni* and are potential targets for the development of new antimicrobial therapies (Elmi *et al.*, 2020).

The eilA gene is a virulence factor-encoding gene found in enteroaggregative *Escherichia coli* (EAEC), a gram-negative bacterium. The eilA gene encodes for a HilA-like regulator protein that is involved in the regulation of several virulence factors, including the type III secretion system (T3SS) and its effectors. The T3SS is an important virulence factor in many gram-negative pathogens, including EAEC, and is involved in the injection of bacterial effector proteins into host cells, leading to the disruption of host cell signaling pathways and the promotion of bacterial survival and replication (Sheikh *et al.*, 2006). The eilA gene is involved in the regulation of the T3SS, which is a virulence factor that promotes bacterial invasion and survival in host cells. The eilA gene is considered to be an important virulence factor in EAEC and is a potential target for the development of new antimicrobial therapies (Havt *et al.*, 2017; Petro *et al.*, 2020; Tanih *et al.*, 2019).

Not well Known

The higher prevalence in the unknown virulence genes were erC, anr, aamR: FN554766, and yfcV. The aamR: FN554766 or aamR virulent gene identified from *E. coli* is a new type and further study is needed to understand the virulence mechanism. The "terC" gene is a virulence factor found in some strains of gram-negative bacteria, including *Escherichia coli*. The terC gene encodes for the TerC protein, which is involved in resistance to tellurium (Calero-Cáceres *et al.*, 2022). The terC gene has been identified in studies on *Escherichia coli* pathotypes, uropathogenic *Escherichia coli*, and *Escherichia coli* isolated from animals and animal food products (Calero-Cáceres *et al.*, 2022; da Cruz Nizer *et al.*, 2020; Orth *et al.*, 2007). The terC gene has also been associated with surviving reactive chlorine stress in gram-negative bacteria. The terC gene is part of the OI-43/48-encoded

genes, which are considered suitable markers of virulence in STEC serotypes that are implicated in severe human diseases and outbreaks (da Cruz Nizer *et al.*, 2020). The **terC** gene is not well known but it encodes tellurite resistance (Vanstokstraeten *et al.*, 2022).

Based on the search results, the "anr" gene is a regulatory gene found in gram-negative bacteria that belongs to the AraC Negative Regulators family. The ANR family is a large family of small regulatory proteins produced by diverse clinically relevant bacteria, including enterohemorrhagic *Escherichia coli*, *Salmonella enterica*, and *Vibrio cholerae* (Ray and Williams, 1997; Winteler and Haas, 1996; Zimmermann *et al.*, 1991). The "anr" gene is involved in the anaerobic growth and cyanide synthesis of *Pseudomonas aeruginosa*, and it is homologous to the *fnr* gene of *Escherichia coli* (Winteler and Haas, 1996; Zimmermann *et al.*, 1991). The ANR family has been shown to modulate the activity of histone-like proteins in pathogenic bacteria and suppress transcriptional expression of virulence factors such as fimbriae, toxins, and the type VI secretion system (Zhang *et al.*, 2019; Zimmermann *et al.*, 1991). While the "anr" gene is not a virulence factor per se, it is involved in the regulation of virulence factors in gram-negative bacteria (Zhang *et al.*, 2019).

The "yfcV" gene is a virulence factor found in some strains of gram-negative bacteria, including *Escherichia coli*. The yfcV gene has been identified in studies on *Escherichia coli* pathotypes, uropathogenic *Escherichia coli*, and clinically isolated carbapenem-resistant *Escherichia coli* (Bunduki *et al.*, 2021; El-Baz *et al.*, 2022; Sora *et al.*, 2021; Spurbeck *et al.*, 2012). The yfcV gene is part of a multiplex of four virulence factor-encoding genes, including *vat*, *fyuA*, and *chuA*, that are highly associated with uropathogenic *Escherichia coli* strains that can efficiently colonize the urinary tract (Spurbeck *et al.*, 2012). The specific role of the yfcV gene in the virulence of gram-negative bacteria is not clear from the search results. However, the yfcV gene has been associated with efficient colonization of the urinary tract (Spurbeck *et al.*, 2012).

5.5.6.2. Virulent Factors in ESBL, AmpC, MBL, and Carbapenemase-producing Gram-Negative Bacteria

The virulence genes were more common in *E. coli* (67.70%), *K. pneumoniae* (24.90%), and *K. oxytoca* (4.10%); a similar study done in Addis Ababa and Jimma and Iran reported high prevalence of virulence genes in *E. coli* and *K. pneumoniae* (Dadi *et al.*, 2020; Malekzadegan *et*

al., 2018; Sewunet *et al.*, 2021). Of third-generation cephalosporin-resistant gram-negative bacteria few virulence genes were identified in *E. cloacae* (3.20%); whereas, no virulent gene was detected in *Acinetobacter baumannii*, *P. rettgeri*, *Stenotrophomonas maltophilia*. This result might be due to genetic factors. Like AMR genes higher burden of virulent genes was recorded in Medical (32.7%), Hematology (26.1%), and NICU (21.4%). Likewise, B6 (21.4%), D7 (14.2%), and EOPD (12.5%) have higher virulent genes. A study done in Iran reported a higher occurrence of virulence genes in ICU medical wards, Surgery, and Transplantation (Malekzadegan *et al.*, 2018). The burden of virulent genes in the admission room varies according to the strains identified in specific wards and departments. The highest prevalence of virulent gene in third-generation cephalosporin and cephamycin-resistant gram-negative bacteria, *terC*, *nlpI*, *gad*, *hlyE*, and *yehB* were similar to other studies (Pitout *et al.*, 2019; Sadeghi *et al.*, 2016).

E. coli had the highest average virulence gene per carbapenem-resistant gram-negative bacteria, followed by *K. pneumoniae* and *E. cloacae*. The Hematology Department has the highest prevalence of carbapenem-resistant gram-negative bacteria (32.5%), followed by the MICU (28.7%) and the medical department (23.0%). The virulence gene was highest in-service giving wards of B4 (31.3%), B5 (10.6%), and B6 (9.1%) (Beshah *et al.*, 2022; Beshah *et al.*, 2023; Legese *et al.*, 2022a). The virulence genes were most abundant in D7, B4, and C8 wards and Hematology, medical, and SICU departments. Whereas, the most predominant virulence genes were *iutA*, *fimH*, *fyuA*, *irp2*, *mrkA*:ABW83989, and *traT* (Rezatofghi *et al.*, 2021; Sengeruan *et al.*, 2022).

5.5.7. Antimicrobial Resistance Types of ESBL, AmpC, Carbapenemase, and MBL Due to Chromosomal Gene Mutations in Gram-Negative Bacteria.

5.5.7.1. Prevalence of Chromosomal Point Mutations

The highest prevalence of gene mutation was in the NICU admission department (27.3%) followed by Medical (27.3%), Hematology (26.8%), and General Surgery (7.3%) with the highest burden in wards B6 (31.8%), followed by D7 (13.6%), and C/W (11.4%). The mutation on the outer membrane porin *ompK36*, the transcriptional repressor operon of the multidrug efflux transporter *acrR*, and a porin protein of *ompK37* genes of *K. pneumoniae* are predominantly found in NICU departments (Adler *et al.*, 2016; David *et al.*, 2022; Özad Düzgün, 2021). The departments are ideal for the growth and spread of nosocomial bacteria, because of the convenient temperature for

bacteria, heavy workload, usage of medical equipment in common, and inadequate infection control procedures.

A high prevalence of 220 chromosomal gene mutations from 82 (57.7%) gram-negative bacteria was found in this study. The *ompK37* (20%) gene had the highest prevalence, followed by *acrR* (19%), and *ompK36* (19%). Similarly, a study conducted in Ethiopia and Portugal found the highest prevalence of *ompK37* and *ompK36* gene mutations (Legese *et al.*, 2022b; Mendes *et al.*, 2022). The higher prevalence of *gyrA* (14%) and *parE*(12%) were found only in *E. coli* in this study were similar to the other studies conducted in Iran (Mirzaei *et al.*, 2018; Pourahmad Jaktaji and Mohiti, 2010). The *parE*, and *gyrA* gene mutations are associated with quinolone resistance, which encodes DNA gyrase and topoisomerase IV targets of quinolone antibiotics like Ciprofloxacin and Norfloxacin (Mirzaei *et al.*, 2018).

In the current study, eight genes had 956 point mutations out of 82 gram-negative bacterial strains. Nonetheless, 97.7% of the mutations were chromosomal point mutations, and only 2.3% of the mutations were frameshift mutations. Another study also reported highest frequency of frameshift mutations in NICU 45.5% of *ompK36* (p.N304E – Frameshift) 27.3% and *ompK37* (p.M233Q – Frameshift) 13.6% genes (Legese *et al.*, 2022b). The *K. pneumoniae* antimicrobial gene Frameshift mutation of *ompK37* (p.M233Q, p.Q235Y), and *ompK36* (p.N304E) were identified in this study and were similar to another study (David *et al.*, 2022; Legese *et al.*, 2022b). The prevalence of gene mutation shows *ompK37* as the highest followed by *acrR*, and *ompK36*. However, the *ompK36* gene has the largest average number of chromosomal point mutations (9.3) per gene, followed by *acrR*, *ompK37*, and 7.0 and 3.1, respectively (Clancy *et al.*, 2013; David *et al.*, 2022).

5.5.7.2. Antimicrobial Resistance Gene Mutation of Single Nucleotide Polymorphisms in Gram-Negative Bacteria

Chromosomal point mutations are quite prevalent in *K. pneumoniae*, *E. coli*, and *K. oxytoca*. The higher percentage of chromosomal point mutations in *K. pneumoniae* was identified in *ompK37*, *acrR*, *ompK36*, and *ramR* genes and is similar to another finding (Legese *et al.*, 2022b). In agreement with other findings, *E. coli* strains have high *gyrA*, *parC*, *parE*, and *rpoB* genes chromosomal point mutation (Jee *et al.*, 2016).

5.5.7.3. ESBL, AmpC, Carbapenemase, And MBL Drug Resistance Due to Antimicrobial Resistance Gene Mutation of *E. coli*, *K. Pneumoniae*, and *K. Oxytoca*

ESBL: In this study, all 17 third-generation cephalosporin resistance and ESBL producer *E. coli* have gyrA (p.D87N, p.S83L), parC (p.S80I), and parE (p.L416F) or (p.I529L) or (p.S458A) or (p.S458T) antimicrobial resistance gene mutation except four out of 17 have parC (p.E84V, p.S80I) instead of parC (p.S80I). Other studies showed the gyrA, parC, and parE chromosomal point mutations were found in *E. coli* and associated with multidrug resistance (Clancy *et al.*, 2013; David *et al.*, 2022; Mirzaii *et al.*, 2018; Pourahmad Jaktaji and Mohiti, 2010).

Among the total 56 third-generation cephalosporin resistance and ESBL producers, 18 *K. pneumoniae* and 4 *K. oxytoca* were found in the NICU. Among six third-generation cephalosporin-resistant and ESBL producer *K. oxytoca*, all have ompK36 (p.N49S, p.T184P) except the one having ompK36 (p.A183S, p.A190W, p.A217S, p.E232R, p.F198Y, p.F207W, p.L191S, p.L228V, p.N218H, p.N49S) chromosomal point mutation resulting in the outer membrane porin mutation. In other research findings, ompK36 (p.N49S, p.T184P) chromosomal point mutation has been identified in *K. oxytoca* and has been associated with resistance to cefaclor, ceftriaxone, and oxytetracycline (Abdelwahab *et al.*, 2021; Clancy *et al.*, 2013).

Six novel third-generation cephalosporin-resistant and ESBL producer *Klebsiella pneumoniae* of multilocus sequence typing (ST1799*, Stnov, ST45*, Stnov, ST973*, Stnov, ST973*, Stnov, ST973*, Stnov, and ST973*, Stnov) were identified in this study. All *Klebsiella pneumoniae* have acrR (p.F172S, p.F197I, p.G164A, p.K201M, p.L195V, p.P161R, p.R173G), ompK36 (different kinds of point mutation), and ompK37 (p.I128M, p.I70M) antimicrobial resistance point mutation. But seven of them do not have the ompK36 gene and one does not have the acrR antimicrobial resistance mutation gene. Among *Klebsiella pneumoniae* twelve of them have additional different types of point mutation on the ompK37 gene and five of them have frameshift mutation on the ompK37 (p.M233Q) gene. The acrR gene encodes a transcriptional repressor that increases the expression of the AcrAB-TolC efflux pump, which is associated with multidrug resistance in *Klebsiella pneumoniae*, *Escherichia coli*, and other gram-negative bacteria (Adler *et al.*, 2016; Bruchmann *et al.*, 2013). A study conducted in China found ompK37 (p.I128M, p.I70M) mutations

resulting in a porin protein mutation in *Klebsiella pneumoniae* and have been associated with β -lactam and carbapenem resistance by combating permeability (Guo *et al.*, 2023). However, in our study, these genes are associated with 3rd generation cephalosporin resistance and ESBL producer *Klebsiella pneumoniae*.

The AmpC producers, consisting of seven *E. coli*, one *K. pneumoniae*, and one *K. oxytoca*, were both AmpC-BL and ESBL enzyme producers. The AmpC, ESBL FF02_5807 *K. oxytoca* ST91 had ompK36 (p.N49S, p.T184P) mutated gene, which has an association with cefaclor and ceftriaxone (Abdelwahab *et al.*, 2021; Clancy *et al.*, 2013). The FF02_6025 *K. pneumoniae* ST711 had acrR, ompK36, and ompK37 genes in this study. Among these genes, ompK36 (p.N304E – Frameshift), and ompK37(p.I128M, p.I70M) are expected to be carbapenem resistance genes (Chukamnerd *et al.*, 2022; Guo *et al.*, 2023). The p.N304E mutation has also been identified as a baseline mechanism of cefiderocol (third- and fourth-generation cephalosporins) resistance in the Enterobacteriaceae (Simner *et al.*, 2022) and the OmpK36 (p.N304E) frameshift mutation has been identified in clinical isolates of *K. pneumoniae* and has been associated with multidrug resistance (Özad Düzgün, 2021),(Simner *et al.*, 2022).

AmpC-BL: In our study all AmpC beta-lactamase resistance bacteria had gyrA (p.D87N, p.S83L), parC (p.S80I), parE (p.S458A) chromosomal point mutation except FF02_1478 *E. coli* ST410/ST692 which had additional rpoB (p.Q513L) and FF02_2927 *E. coli* ST167/ST877 that had rpoB (p.Q513L) instead of parE (p.S458A) mutated gene. The gyrA genes encode subunits A protein of DNA gyrase, parC, and parE genes encode subunits of topoisomerase IV enzymes involved in DNA replication and repair (Arabameri *et al.*, 2021; LOPARDO *et al.*, 2014). Mutations in these genes have been associated with resistance to fluoroquinolone antibiotics (Arabameri *et al.*, 2021; LOPARDO *et al.*, 2014). The rpoB gene encodes the beta subunit of RNA polymerase and is involved in the transcription of DNA into RNA (Cai *et al.*, 2017), and mutations in this gene have been associated with resistance to rifampicin antibiotics in *E. coli*, and other bacteria (Cai *et al.*, 2017). This indicated that gyrA (p.D87N, p.S83L), parC (p.S80I), and parE (p.S458A) genes have an association with AmpC and ESBL drug resistance (Sup Table Excel).

Carbapenemase: All carbapenem-resistant *K. pneumoniae* had *acrR* (p.F172S, p.F197I, p.G164A, p.K201M, p.L195V, p.P161R, p.R173G) gene and point mutation. Studies in different countries showed that the *acrR* gene encodes the AcrAB-TolC gene of an efflux pump that has been associated with multidrug resistance drug resistance in gram-negative bacteria (Adler *et al.*, 2016; Bruchmann *et al.*, 2013).

Again, all carbapenem-resistant *K. pneumoniae* had *ompK36* with different point mutations; however, *ompK37* had only (p.I128M, p.I70M) point mutation. The *ompK36* (p.A217S and p.N218H) gene mutation are known carbapenem resistance gene (Legese *et al.*, 2022b). Similarly, the *K. pneumoniae ompK37*(p.I128M, p.I70M) gene mutation has a known association with carbapenem resistance in *K. pneumoniae* (Chukamnerd *et al.*, 2022; Guo *et al.*, 2023; Legese *et al.*, 2022b). FF02_291 and FF02_3884 carbapenem-resistant *K. pneumoniae* ST147 lacked *ompK36* antimicrobial resistance gene mutation. However, FF02_2840 *K. pneumoniae* ST4407 had only *ompK37* (p.I128M, p.I70M) antimicrobial resistance mutation that conferred carbapenem resistance gene that was confirmed in other studies (Chukamnerd *et al.*, 2022; Guo *et al.*, 2023; Legese *et al.*, 2022b).

The *OmpK36* (p.N304E) frameshift mutation has been identified in clinical isolates of *K. pneumoniae* in this study and has been associated with multidrug resistance as similar findings (Özad Düzgün, 2021; Simner *et al.*, 2022). The *OmpK36* porin is a major membrane porin mutation in *K. pneumoniae* and plays an important role in mediating resistance to carbapenems, and cefiderocol (third- and fourth-generation cephalosporins) resistance in the *Enterobacteriaceae* (David *et al.*, 2022; Simner *et al.*, 2022). The *ompK36* gene mutations caused by membrane porin and promoter alteration have been shown to impact the response of *K. pneumoniae* strains to Doripenem and Doripenem-colistin permeability (Clancy *et al.*, 2013). Therefore, it is possible that the p.N304E - Frameshift mutation on the *ompK36* gene could impact the function of the *OmpK36* porin and its response to Third- and fourth-generation cephalosporins and carbapenemase resistance. Studies revealed that *ompK36* and *ompK37* genes encode porins, which are outer membrane proteins that play a role in the uptake of nutrients and antibiotics in bacteria (Tsai *et al.*, 2011). Mutations in these genes have been associated with decreased susceptibility to beta-lactam antibiotics and carbapenem antibiotics in *K. pneumoniae* and other bacteria (Huang *et al.*, 2020a; LOPARDO *et al.*, 2014; Tsai *et al.*, 2011). Another study showed that the *OmpK37*

porin gene in *K. pneumoniae* was identified as a third porin gene and functional characterization suggested a narrower pore for the OmpK37 porin (Huang *et al.*, 2020b). A *K. pneumoniae* strain expressing porin OmpK37 (p.M233Q and p.Q235Y), was less susceptible to beta-lactam antibiotics (Huang *et al.*, 2020b). Therefore, the p.M233Q and p.Q235Y frameshift mutation on the ompK37 gene could impact the function of the OmpK37 porin and its response to certain antibiotics like beta-lactam antibiotics and carbapenem.

Six carbapenem-resistant *E. coli* strains of MLST ST167/ST2 showed chromosomal point mutations on gyrA (p.D87N, p.S83L), parC (p.S80I), pare (p.S458A) genes. However, chromosomal mutations on gyrA, parC, and pare were associated with fluoroquinolone antibiotics such as ciprofloxacin in gram-negative bacteria (Arabameri *et al.*, 2021; LOPARDO *et al.*, 2014). The ramR(p.L111R) gene encodes a transcriptional regulator that controls the expression of genes. Mutations in this gene have been associated with increased expression of genes involved in virulence and antibiotic resistance including carbapenem resistance in *K. pneumoniae* (Chiu *et al.*, 2017; Mao *et al.*, 2020b; Tsai *et al.*, 2011).

Mutations in these genes have the potential to cause antibiotic resistance and virulence factors in a variety of bacteria, such as *Klebsiella pneumoniae*, *Escherichia coli*, and *Klebsiella oxytoca*. The ompK37, acrR, ompK36, and ramR gene mutations can cause carbapenem resistance; whereas, gyrA parC, and parE gene mutations and rpoB gene mutation can cause fluoroquinolone resistance and rifampicin resistance, respectively.

5.5.8. Molecular Epidemiology of Plasmid MLST and Resistance Gene in TASH

5.5.8.1. AMR Plasmid MLST and Plasmid Gene in Gram-Negative Bacterial Isolate from Bloodstream Infections

Plasmid MLST is a technique for identifying and describing plasmids in bacteria (Carattoli *et al.*, 2014). It is particularly useful for subtyping IncF, IncI1, IncN, IncHI2, and IncHI1 plasmids, and causes traits like antibiotic resistance (Carattoli and Hasman, 2020; Carattoli *et al.*, 2014). The most common type of plasmid MLST identified from 131 plasmid incompatibility (Inc) groups in this study was IncF, RST, and IncHI, which is consistent with other studies (Yang *et al.*, 2015). Similar to previous studies, the top five plasmid multilocus sequence types, IncF RST[K7:A-B-],

IncHI2 DLST1, IncFRST[F-:A-:B-], IncHI1 MLST Unknown, and IncF RST[K4:A-:B-], had the highest prevalence in our study (Legese *et al.*, 2022b). The study conducted in China also revealed similar findings with a higher occurrence of plasmid replicons, IncFII(K), IncFIB(K), IncFIB(AP001918), IncFIA, and Col(pHAD28) (Yang *et al.*, 2015).

In this study, a high burden of plasmid MLST, 72.54%, and plasmid gene, 76%, were identified from 142 sequenced gram-negative bacteria. All *K. pneumoniae* and *Klebsiella oxytoca* have drug resistance plasmid MLST and replicon. A previous study conducted in Ethiopia showed that *K. pneumoniae* carried multiple plasmid genes (Legese *et al.*, 2022b). The *E. coli* and *E. cloacae* carried the higher plasmid MLST at 94.4% and 88.9% and all carried multiple plasmid replicon (Citterio *et al.*, 2020). In the current study, high carbapenem resistance and PDR isolates of *Acinetobacter Species* lately confirmed *A. baumannii* shows the lowest percentage prevalence of Plasmid MLST (8.6%), plasmid replicon (14.3%), and no drug resistance gene mutation. This might be due to chromosomal drug resistance and the efflux pump gene found in the bacteria (Ruppé *et al.*, 2015).

Among 46 *Klebsiella pneumoniae*, 59 plasmids MLST were identified in this study. Some of the bacteria have more than one MLST with 171 plasmid replicons identified. On average 3 plasmid replicons were identified per single plasmid MLST (Wang *et al.*, 2021). In 36 *Escherichia coli*, 34 of them have 37 plasmid MLST and 177 plasmid genes. On average 5 plasmid replicons were identified per single plasmid MLS, and this is the highest antimicrobial-resistant plasmid gene in the strain (Citterio *et al.*, 2020). Non-fermenter bacteria have the least plasmid MLST and plasmid replicon in this study. In *Acinetobacter baumannii*, 3(8.6%) strains carried 6(4.5%) total PlasmidMLST and 16(3.5%) drug-resistant plasmid genes. On average 2.7 plasmid replicons were identified per single plasmid MLST (Ruppé *et al.*, 2015). This indicates that most *Enterobacteriaceae* have plasmid genes that can share drug resistance (Rozwandowicz *et al.*, 2018). The lower amount of drug-resistant plasmid genes in non-fermenter gram-negative bacteria could be due to sequential chromosomal mutations in *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Stenotrophomonas maltophilia*. These mutations can cause target modifications, hyper-expression of efflux pumps, overproduction of intrinsic beta-lactamases, and permeability defects (Ruppé *et al.*, 2015).

5.5.8.2. Plasmid MLST and Gene in ESBL, AmpC, MBL, And CARBA of Gram-Negative Bacteria

ESBL: Among third-generation cephalosporin-resistant and ESBL-producer gram-negative bacteria, 90.5% carried plasmid MLST and 94.6% carried plasmid replicons. All three ESBL producers including novel ST1089**Stnov Acinetobacter baumannii* don't have plasmid genes. Similar to another study, all *Escherichia coli* carried IncF RST plasmid incompatibility (Inc) groups in this study (Yang et al., 2015). Two ESBL producer *Escherichia coli* multilocus sequence typing of ST540/ST716 carried IncY plasmid replicon. Similar findings from Chinese pig farms have been linked to the spread of genes that confer resistance to antibiotics genes (Saidani *et al.*, 2019). The *mcr-1* gene, which is a member of the IncY incompatibility group is carried by this particular type of plasmid called IncY, and is present in *Escherichia coli* and confers plasmid-mediated resistance to colistin (Zhang *et al.*, 2017). However, this strain was third-generation cephalosporin-resistant; possibly this gene is not actively expressed. One *Escherichia coli* ST131/ST43 plasmid MLST and plasmid MLST scheme IncF RST [F2: A:-B1] involves the detection and typing of IncF plasmid groups of various replicons, including FII, FIA, FIB, and FIC. The IncFIB(H89-PhagePlasmid) plasmid is classified as phage-like since the majority of its sequences are phage-related sequences (Kurittu *et al.*, 2021; Timmermans *et al.*, 2021; Zhang *et al.*, 2017). Another study agreed with our result found the presence of IncFIB(H89-PhagePlasmid), IncFIB(AP001918), IncFIB(pQil), IncFII, IncFII(K), and IncI1-I(Alpha) genes in extended-spectrum beta-lactamase-producing *E. coli* isolates (Kurittu *et al.*, 2021).

The IncF RST [F48: A:- B49] plasmid MLST is similar in both ESBL-producing *Escherichia coli* MLST ST224/ST479 and *Escherichia coli* MLST ST609. *Escherichia coli* MLST ST609 additionally carried IncFIA, IncFIB (AP001918), and IncFII(pAMA1167-NDM-5) plasmid replicon whereas, *Escherichia coli* MLST ST224/ST479 carried Col156, IncFIA, IncFIB (AP001918), IncFII(29), IncFII(pAMA1167-NDM-5), and IncX1 plasmid replicon. The IncFIA and IncFIB (AP001918) replicons are known to contribute to the spread of resistance determinants and the emergence of antibiotic resistance (Kurittu et al., 2021; Zhang et al., 2017; Zhang et al., 2021). It has been suggested that the IncFIB(AP001918) plasmid carries genes for both antibiotic resistance and virulence (Khajanchi *et al.*, 2017). The *bla*NDM-5 gene, which confers resistance to carbapenem antibiotics, is linked to the carriage of the IncFII(pAMA1167-NDM-5) gene in

Escherichia coli (Roer *et al.*, 2018; Xu *et al.*, 2019). Furthermore, this gene has been discovered in *E. coli* strains that co-harbor *bla*NDM-5 on a multi-replicon plasmid along with ESBL-encoding genes, highlighting its connection to the spread of antimicrobial resistance determinants (Mohamed *et al.*, 2022; Zhang *et al.*, 2021). The functions of Col156, IncFII(29), and IncX1 are not well understood yet but they are associated with the dissemination of antimicrobial resistance determinants (Villa *et al.*, 2010).

In this study, all *K. pneumoniae* carried the IncF RST plasmid incompatibility group but six of them carried additional IncHI1 MLST-Unknown, IncN MLST-Unknown, and IncHI2 DLST1 plasmid incompatibility group. All third-generation cephalosporin and ESBL producer *K. oxytoca* carry plasmid MLST and plasmid replicon. The presence of IncF RST, IncN, IncHI2, and IncHI1 in *Klebsiella oxytoca* and *Klebsiella pneumoniae* indicates the possibility of antibiotic resistance (Singh *et al.*, 2016; Stewart *et al.*, 2022; Wang *et al.*, 2017b; Yang *et al.*, 2022). The third-generation cephalosporin and ESBL producer *K. pneumoniae* and *K. oxytoca* carried the following plasmid genes: (Col(pHAD28), Col440I, IncFIA(pBK30683), IncFIB(K), IncFIB(K)(pCAV1099-114), IncFIB(pNDM-Mar), IncFIB(pQil), IncFII(K), IncFII(pKP91), IncFII(Yp), IncHI1B(pNDM-MAR), IncHI2, IncHI2A, IncN, and IncY). The presence of Col(pHAD28) genes in *Klebsiella oxytoca*, and *Klebsiella pneumoniae* isolates may contribute to the bacterium's antimicrobial resistance and virulence characteristics (Lorenzin *et al.*, 2022). Due to their size and ability to spread resistance genes, the IncHI2 and IncHI2A plasmids have been referred to as "super plasmids and shown to co-harbor regional Carbapenemases and mcr-9.1, which furthers the global spread of antibiotic resistance (Cain and Hall, 2012; García-Fernández and Carattoli, 2010).

There were additional Col(MG828), Col440II, ColKP3, IncFIA(HI1), IncFIB(K)(pN109), IncFIB(pKPHS1), IncR, IncX3, rep22, rep7a, repB, and repB(R1701) plasmid replicons found in *K. pneumoniae* but not in *K. oxytoca*. The Col(MG828), Col440II, ColKP3, IncFIA(HI1), IncFIB(K)(pN109), and IncFIB(pKPHS1) genes have been detected in *K. pneumoniae* found on plasmids that carry antimicrobial resistance genes (Bhatia *et al.*, 2022; Dekker *et al.*, 2021; Martin *et al.*, 2017). A study in India showed that IncFIB(K) and IncFIB(pNDM-Mar) replicons were found in hypervirulent *K. pneumoniae* isolates. The study also highlighted the replicons' correlation with virulence traits and antimicrobial resistance (Shankar *et al.*, 2022). The IncR and IncX3 replicons encode multidrug resistance determinants, including β -lactam (*bla*DHA-1,

*bla*SHV-12) (Compain *et al.*, 2014; Zhu *et al.*, 2020). The IncFIA(HI1), IncFIB(pKPHS1), and IncFIB(K)(pN109) genes in *Klebsiella* strains have been associated with plasmids carrying antibiotic resistance genes, beta-lactamase and carbapenemase (Hussain *et al.*, 2023; Wyres and Holt, 2018). The presence of the plasmid replicons-associated genes *rep22*, *rep7a*, *repB*, and *repB*(R1701) in *K. pneumoniae* strains suggests the possibility of plasmid-mediated horizontal gene transfer, which could lead to the spread of virulence traits and antimicrobial resistance (Alkompoz *et al.*, 2023; Hudson *et al.*, 2014; Mirzaie and Ranjbar, 2021).

The current study found six ESBL producer *K. pneumoniae* multilocus sequences of ST307, ST14, ST2054, ST307, ST34, and ST3717 that carried IncF RST/IncHI1 MLSTUnknown plasmid incompatibility group, which had IncFIB(pNDM-Mar)/IncHI1B(pNDM-MAR) plasmid replicons. Additionally, *K. oxytoca* multilocus sequences of ST305 carried IncF RST[Y6:A-:B-]/IncHI1 MLSTUnknown incompatibility groups, carried IncFIB(pNDM-Mar), IncHI1B(pNDM-MAR) plasmid replicon. Another *K. oxytoca* carried IncF RST[K17:A-:B-]/IncHI2 DLST1 plasmid MLST and IncFIB(pNDM-Mar) plasmid replicon. In agreement with this study, these genes were frequently identified in the context of hybrid plasmids, such as IncFIB/IncHI1B(pNDM-MAR) backbones, which lead to the convergence of AMR and virulence traits on a single plasmid (Shankar *et al.*, 2022). The IncFIB(pNDM-Mar) and IncHI1B(pNDM-MAR) genes are remarkable genetic markers connected to carbapenemase encoding genes and linked to antimicrobial resistance and virulence in *K. pneumoniae* and *K. oxytoca* strains, especially in hypervirulent strains (Shankar *et al.*, 2022; Spadar *et al.*, 2022). Another study showed that these IncFIB(pNDM-Mar) and IncHI1B(pNDM-MAR) genes are also known to carry the *bla*NDM-1 gene, which encodes the NDM-1 carbapenemase, contributing to the resistance of bacteria to carbapenem antibiotics (Dolejska *et al.*, 2012). However, these strains are third-generation cephalosporin and ESBL Producer bacteria. The reason could be that these genes are silent or not expressed plasmid replicon (Dolejska *et al.*, 2012). These genes are linked to antimicrobial resistance in *K. pneumoniae* and *K. oxytoca* strains that underline the significance of surveillance and control measures to address the spread of such genes in clinical settings.

AmpC: Among AmpC beta-lactamase 76.5% had antimicrobial-resistant plasmid genes, and three *Acinetobacter baumannii* didn't have plasmid genes (Yang *et al.*, 2015). The *Enterobacter cloacae* sequence type ST252 had IncF RST[F-:A-:B-]/IncHI2 DLST1 plasmid MLST genes that indicates

the possibility of mediating antibiotic resistance (Singh *et al.*, 2016; Wang *et al.*, 2017b). There were Col (IMGS31), Col(pHAD28), Col156, IncFIB(K), IncFII(Yp) IncHI2, IncHI2A, and IncR plasmid replicon found in *Enterobacter cloacae*; however, we discussed all plasmid replicons in ESBL except Col(IMGS31), and Col156 plasmid replicons. Up to this writeup specific information about the presence of the Col(IMGS31) or Col156 plasmid replicons in *E. cloacae* was not available. The IncFII plasmid has been found to carry the *bla*NDM-5 gene, which encodes New Delhi Metallo-beta-lactamase (NDM-5) in *E. coli*. This plasmid is known to be widely distributed in Enterobacteriaceae and contributes to the carriage and spread of antimicrobial resistance genes (Flerlage *et al.*, 2020; Mohamed *et al.*, 2022; Zhang *et al.*, 2021).

Carbapenemase: In this study, 26 *Acinetobacter baumannii* did not have plasmid MLST and 24 didn't carry plasmid replicon. *Acinetobacter baumannii* multilocus sequence type ST1809/ST451/ST2 and ST1837/ST369/ST2 carried plasmids IncF RST [F-: A-:B-], IncHI2 DLST1* and IncHI2 DLST1*, respectively are Novel plasmid multilocus sequence and it carried IncFIA, IncHI2A and IncHI2 plasmid replicon, respectively. Another study found that IncF RST plasmids contain virulence and resistance determinants (Villa *et al.*, 2010). Whereas, in other studies IncHI2 DLST1* plasmid has double Locus Sequence Type DLST1 and has been linked to the spread of antimicrobial resistance genes, such as *bla*NDM-1 (Ai *et al.*, 2021; Umeda *et al.*, 2021). Other studies also showed the spread of IncFIA and IncHI2A plasmids in *Acinetobacter baumannii* and other bacteria, which has been associated with antimicrobial resistance genes, including those encoding beta-lactamases (Altayb *et al.*, 2022; Mohamed *et al.*, 2022; Mouftah *et al.*, 2019; Zhang *et al.*, 2021). It has been reported that these plasmids contain genes that encode resistance to aminoglycosides, carbapenems, and extended-spectrum beta-lactamases (ESBLs) (Altayb *et al.*, 2022). Furthermore, the spread of carbapenemase genes, such as *bla*NDM-5, in a variety of bacterial species has been linked to the IncHI2A plasmids (Mohamed *et al.*, 2022; Mouftah *et al.*, 2019).

The current study found one carbapenem resistance FF02_151 *E. cloacae* multilocus sequence type ST310 which had IncF RST[F-:A-:B70] plasmid MLST and carried Col(pHAD28), IncFII(pECLA) plasmid replicons. *E. cloacae* identified from orthopedics patients phenotypically confirmed carbapenem-intermediate, third-generation cephalosporin and cefepime resistant and PCR confirmed ESBL, AmpC, CPs, MBL genes which had *bla*TEM, *bla*CTXM, *bla*ACC,

blaOXA48, *blaOXA23*, *blaVIM*, and *blaSIM* genes. IncF RST plasmids contain virulence and resistance determinants, while Col(pHAD28) plasmid is known to carry genes encoding resistance to colistin, an antibiotic of last resort, and has been associated with the dissemination of colistin resistance in Enterobacteriaceae (Biggel *et al.*, 2022; Villa *et al.*, 2010). The plasmid replicon IncFII(pECLA) is associated with the spread of antimicrobial resistance genes that encode extended-spectrum beta-lactamases and carbapenemase (Kubota *et al.*, 2018; Piccirilli *et al.*, 2023; Zhou *et al.*, 2017). The presence of this plasmid replicon underscores the importance of continued surveillance and control measures to address antimicrobial resistance in *E. cloacae*.

Eight carbapenem-resistant *E. coli* were sequenced; of these strains sequence number FF02_704 of MICU Female HAI suspected patient treated with Vancomycin, and Cefepime identified XDR type of *E. coli* from bloodstream infection. The strains were phenotypically third-generation cephalosporins (ceftazidime, ceftriaxone cefoxitin), cephamycin, and meropenem resistance. Molecular characterization found AmpC, (*blaCIT*, and *blaEBC*), and MBL (*blaNDM*) genes. The *E. coli* strain MLST ST8915/ST757 was identified to have IncA/C PMLST1, IncF RST[F29:A-B10] plasmidMLST and found to have Col(MG828), Col156, IncC, IncFIB(AP001918), IncFIB(H89-PhagePlasmid), IncFII(29), and IncI1-I(Alpha) plasmid replicon. The Col(MG828), IncFIB(H89-PhagePlasmid) plasmid replicon has been identified in *E. coli* isolates and is associated with the dissemination of antimicrobial resistance genes, including those encoding extended-spectrum beta-lactamases and other resistance determinants including colistin resistance in IncFIB(H89-PhagePlasmid) plasmid replicon (Adator *et al.*, 2020; Cherubini *et al.*, 2022a; Kurittu *et al.*, 2021; Montelongo Hernandez, 2021; Schmidt *et al.*, 2022). The IncC replicon has also been identified in *E. coli* and has been associated with plasmid-mediated antimicrobial resistance (Schmidt *et al.*, 2022). Numerous studies have identified the IncFIB(AP001918), IncFII(29), and IncI1-I(Alpha) replicons in isolates of *E. coli*, and associated with extended-spectrum beta-lactamase and carbapenemase resistance, and they are linked to the spread of genes that confer antibiotic resistance (Kurittu *et al.*, 2021; Montealegre *et al.*, 2020). Our whole genome sequencing result showed that this bacterium acquired *blaCMY-6*, and *blaNDM-1* which are AmpC, and Carbapenem drug resistance genes (Sup Table Excel).

In this study patient sample number FF02_1412 was admitted to general surgery for a Colostomy procedure. The patient acquired sepsis after the procedure and identified XDR *K. pneumoniae*

from bloodstream infection. The bacteria were phenotypically and PCR confirmed AmpC, Carbapenemase, and MBL producer bacteria. The FF02_1412 multilocus sequence type ST37 *K. pneumoniae* carried IncA/C PMLST1, IncF RST[F:-A:-B-], IncHI1 MLSTUnknown plasmid MLST and carried IncC, IncFIB(K)(pCAV1099-114), IncHI1B(pNDM-MAR) plasmid replicon.

The other patient of sample number FF02_3884 admitted to ward was identified with XDR and confirmed MBL producer *K. pneumoniae* multilocus sequence type of ST147 with IncF RST[F:-A:-B-], IncHI1 MLSTUnknown plasmid MLST carried Col(pHAD28), IncFIB(pKPHS1), IncFIB(pNDM-Mar), IncFIB(pQil), IncHI1B(pNDM-MAR), IncR plasmid replicons. As was mentioned in *E. coli* the IncFIB(pNDM-Mar) and IncHI1B(pNDM-MAR) genes are remarkable genetic markers connected to carbapenemase encoding genes and linked to antimicrobial resistance and virulence in *K. pneumoniae* and *K. oxytoca* strains, especially in hypervirulent strains (Shankar *et al.*, 2022; Spadar *et al.*, 2022; Spadar *et al.*, 2023). Another study found that IncFIB(pNDM-Mar) and IncHI1B(pNDM-MAR) genes are also known to carry the *bla*NDM-1 gene, which encodes the NDM-1 carbapenemase, contributing to the resistance of bacteria to carbapenem antibiotics (Dolejska *et al.*, 2012). In this study, FF02_1412 have *bla*NDM-1, *bla*OXA-1 Carbapenem drug resistance genes and FF02_3884 have *bla*CTX-M-15, *bla*NDM-1, *bla*OXA-1, *bla*OXA-9, *bla*TEM-1A, ESBL and Carbapenem drug resistance genes. Unexpectedly, except for 1 *Klebsiella pneumoniae* and 2 *Acinetobacter baumannii*, 13 PDR isolates did not carry plasmid replicon. This finding shows that *Acinetobacter baumannii*'s drug-resistant mechanism is chromosomal point mutation (Supp Table Excell). One *Stenotrophomonas maltophilia* and *Pseudomonas monteilii* didn't carry plasmid MLST and plasmid replicon.

5.5.9. Molecular Epidemiology of Hospital Disinfectant Resistant and Responsible Genes in Gram-Negative Bacteria Isolated from Bloodstream Infections

5.5.9.1. Hospital Disinfectant-Resistant Bacteria

Benzalkonium chloride, cetylpyridinium chloride, chlorhexidine, and hydrogen peroxide are all disinfectants that are used in hospital settings to prevent the spread of infections (Choi *et al.*, 2018; Merchel Piovesan Pereira and Tagkopoulos, 2019; Russell and Day, 1993). Each of these disinfectants has unique properties and is used for specific purposes. Benzalkonium chloride is effective against a wide range of bacteria, viruses, and fungi and is widely used in healthcare

settings (Jones, 1997; Moshrefi, 2002). Cetylpyridinium chloride is typically found in oral care products and has broad-spectrum antiseptic properties (Mao *et al.*, 2020a; Pitten and Kramer, 2001). Chlorhexidine is effective against Gram-positive and Gram-negative organisms, facultative anaerobes, aerobes, and yeasts (Moshrefi, 2002). Hydrogen peroxide is a stable and effective disinfectant when used on inanimate surfaces and is commonly used for cleaning, disinfecting, and stain removal (Alfa and Jackson, 2001; Pitten and Kramer, 2001; Rutala and Weber, 2008). Resistance to disinfectants is complex and can be influenced by many factors such as formulation effects, presence of an organic load, synergy, temperature, dilution, and test method (Amereh *et al.*, 2023; Dehnamaki *et al.*, 2020; Fang *et al.*, 2022).

In this study, Benzalkonium chloride, Cetylpyridinium chloride, Chlorhexidine, and Hydrogen peroxide resistance genes were identified however other disinfectant-resistant genes were not identified. These disinfectants include alcohol, glutaraldehyde, hydrogen peroxide, sodium hypochlorite (bleach), iodine, peracetic acid, phenol, and silver. As a result, the disinfectants mentioned here are not resisted by gram-negative bacteria and can be used based on our purpose.

K. pneumoniae have OqxA, and OqxB, genes responsible for benzalkonium chloride and cetylpyridinium chloride resistance, and qacE gene responsible for Chlorhexidine resistance. The recommended disinfectant was H₂O₂ and others not resisted disinfectants. *E. coli* have Sit-ABCD resistant gene responsible for resistance to H₂O₂ and qacE resistant gene responsible for resistance to benzalkonium chloride cetylpyridinium chloride and Chlorhexidine. The recommended disinfectant was other than resisted disinfectants. *A. baumannii* & *E. cloacae* have qacE-resistant genes responsible for resistance to benzalkonium chloride, cetylpyridinium chloride, and chlorhexidine. The recommended disinfectant was H₂O₂ and others were not resisted. B6, B7, D5, B8, InM = don't use benzalkonium chloride, cetylpyridinium chloride, and Chlorhexidine and use H₂O₂ & other not resisted disinfectants. B5, C7, C/W, D7, D8, EOPD, B4, C8 don't use All, but use not resisted disinfectants.

5.5.9.2. Hospital Disinfectant-Resistant Genes

In this study, OqxA, OqxB, qacE, and sitABCD genes are associated with disinfectant resistance. Our result showed *E. coli* strains are resistant to hydrogen peroxide disinfectant that was associated

with the *sitABCD* gene. The 97.2% of *E. coli* were resistant to one or more disinfectants. The benzalkonium chloride, cetylpyridinium chloride, chlorhexidine, and hydrogen-peroxide-resistant *E. coli* have *qacE* and *SitABCD* genes. Whereas benzalkonium chloride, cetylpyridinium chloride, and chlorhexidine-resistant *E. coli* have *qacE*. This indicates that the *SitABCD* gene has an association with hydrogen peroxide disinfectant resistance. Other studies found that the *SitABCD* gene encodes for a manganese and iron transporter that is associated with resistance to several disinfectants, including hydrogen peroxide, benzalkonium chloride, and chlorhexidine (Abbood et al., 2023; Sabri et al., 2006).

The benzalkonium chloride, cetylpyridinium chloride, and chlorhexidine resistance are associated with *qacE* gene in 3rd generation cephalosporin resistance and mainly in Carbapenem-resistant *A. baumannii*. Almost all bacterial species were resistant to benzalkonium chloride, cetylpyridinium chloride, and chlorhexidine were associated with the *qacE* gene. In these studies, the *qacE* gene encodes for a multidrug efflux pump that confers resistance to several disinfectants (Abbood et al., 2023; Zou et al., 2014). The *OqxA* and *OqxB* disinfectant genes were found only in *K. pneumoniae* and associated with benzalkonium chloride and cetylpyridinium chloride disinfectant resistance. A study done by Li et al. showed that *OqxA* and *OqxB* genes encode for the *OqxA* and *OqxB* efflux pump, which confers low to intermediate resistance to several disinfectants. Its efflux pump has also been found to be associated with resistance to quinolones, tigecycline, nitrofurantoin, and olaquinox (Li et al., 2019a). Hydrogen peroxide is an effective disinfectant against all gram-negative bacteria in this study except *E. coli*. This implies hydrogen peroxide is an effective disinfectant for patients infected with *K. pneumoniae*, *K. oxytoca*, *E. cloacae*, and *A. baumannii* and may be helpful for the NICU department.

5.5.10. Pan-Drug Resistant *A. baumannii* and *K. pneumoniae*

5.5.10.1. MLST and antimicrobial resistance in PDR isolates

All 35 *Acinetobacter* species were confirmed that it was *Acinetobacter baumannii*. In a study done in Slovakia *A. baumannii* isolates were more than 85% resistant to at least 13 out of the 19 tested antibiotics (Jalali et al., 2023).

Based on the whole genome sequencing result, 10 of the 17 *Acinetobacter* species with carbapenem resistance and potential PDR in this study were identified as *A. baumannii* sequence type ST1837/ST369/ST2. In China, outbreaks have been linked primarily to *A. baumannii* of sequence types ST1837 and ST369 (Kong *et al.*, 2023; López-Durán *et al.*, 2020; You *et al.*, 2023). In certain parts of China, Latin America, and the Caribbean, ST2 *A. baumannii* has also been linked to outbreaks (Yu *et al.*, 2022). The study found that PDR had a significant association with the use of inappropriate antibiotic treatment, length of admission (more than three days), and the use of medical instruments during treatment. An intravenous line, a drainage tube, and a suction machine were the main instruments utilized during PDR transfer. According to a study conducted in Algeria, mechanical ventilators served as the main source of PDR transmission (Brahmi *et al.*, 2016). The chronic illness of WI on the skin was associated with carbapenemase production (Beshah *et al.*, 2023).

In this study, all PDR bacteria have ST2 multi-locus sequence typing. This sequence typing may have been associated with major drug resistance. According to another study, ST2 was linked to high risks of fatal infections, severe infections, and ineffective antibiotic use (Chuang *et al.*, 2019). Another study also showed the ST2 strain's ability to form a strong biofilm, high serum resistance, and high pathogenicity (Yu *et al.*, 2021). ST2 has been associated with multidrug resistance in some studies (Chatterjee *et al.*, 2020). Antimicrobial-resistant *A. baumannii* is known for its exceptional survival skills in an unfavorable environment and the ability to rapidly acquire resistance mechanisms (Nawfal Dagher *et al.*, 2019).

All PDR *Acinetobacter baumannii*, based on our PCR results, have the OXA 23 gene, and 12 have the VIM gene. However, the WGS result confirmed that the following AMR groups were identified: *armA*, *mph(E)*, *msr(E)*, *sul2*, *tet(B)*, and from beta-lactam genes: *blaADC-25*, *blaOXA-23*, *blaOXA-66*, *blaTEM-1D*. Other studies show carbapenem resistance is common in *Acinetobacter baumannii*, and some strains have carried this gene (Brahmi *et al.*, 2016; Hahm *et al.*, 2022; Yu *et al.*, 2022). Whole-genome sequencing has been used to characterize resistance mechanisms and epidemiology of colistin-resistant *Acinetobacter baumannii*. ST1837 has been identified as one of the sequence types in the global clone. But here the WGS analysis found no mutation on *lpxA*, *lpxC*, *lpx D*, *pmrA*, *pmrB*, and *mcr1*, the genes known to be associated with colistin resistance found in other studies (Hahm *et al.*, 2022). In this study, it resists all antibiotics

except colistin which is not tested. *A. baumannii* ST1837 and ST369 are often associated with nosocomial transmission and outbreaks of extensively drug-resistant (XDR) strains have been reported in Mexico tertiary-level hospitals (Kong *et al.*, 2023; López-Durán *et al.*, 2020).

In this study, there were two carbapenem-resistant and possible PDR *A. baumannii* sequence type ST1809/ST451/ST2. There was no available information about *A. baumannii* Sequence type ST1809 during this study. It needs further study as it is possible for PDR bacteria. However, *A. baumannii* sequence type ST451 has been identified as a new sequence type in other studies (Kumar *et al.*, 2015; Lee *et al.*, 2018; You *et al.*, 2023). A study done in Daegu, South Korea reported *A. baumannii* strain KAB03 belonging to ST451 was extensively drug-resistant (Jun *et al.*, 2023; Lee *et al.*, 2018). According to another study, *Acinetobacter baumannii* strain 10441_14 belonging to ST451 was carbapenem-resistant (Kumar *et al.*, 2015). ST2 has been associated with multidrug resistance high rates of inappropriate antimicrobial therapy, severe infection, and mortality (Chuang *et al.*, 2019);(Chatterjee *et al.*, 2020).

Two PDR *A. baumannii* with carbapenem resistance, sample numbers FF02_4067-A and FF02_6351, and sequence types ST1697/ST2535/ST2 were identified in the present study. However, no available information was found on the characteristics of *A. baumannii* sequence types ST1697 and ST2535 during our study. The PCR test showed that both strains had OXA48 and KPC in sample number FF02_6351, along with NDM, VIM of carbapenem, and metallo-beta-lactam resistance genes. According to molecular characterization, both strains possess blaADC-25, blaNDM-1, blaOXA-23, and blaOXA-66 for AmpC, as well as drug-resistance genes against carbapenem and metallo-beta-lactamase. They are also resistant to ESBL, AmpC, and carbapenem.

This study found the carbapenem-resistant and potentially PDR FF02_5528 *K. pneumoniae* sequencing types ST101, a new high-risk clone of the bacteria with extensive antibiotic resistance. ST101 has been associated with carbapenem resistance and colistin resistance in other studies (De Koster *et al.*, 2022; Mammina *et al.*, 2012). *K. pneumoniae* strains producing extended-spectrum beta-lactamases or Carbapenemases are considered global priority pathogens (Wyres *et al.*, 2020). This strain was variable and encompasses plentiful chromosomal and plasmid-encoded antibiotic-resistance genes (Navon-Venezia *et al.*, 2017). According to other studies, ST101 strains have

been found in humans, animals, and the environment, and they showed significant genetic diversity (De Koster *et al.*, 2022; Mammina *et al.*, 2012).

5.5.10.2. Antimicrobial Resistance Gene in PDR Isolates

The sample numbers FF02_1726, FF02_1726.6, FF02_1961.2, FF02_3274, FF02_2606, FF02_3004, FF02_3557, FF02_3985, FF02_5289, and FF02_5292 were displayed in the ResFinder result for this study. The drug-resistant genes *aph(3')-Ia*, *aph(6)-Id*, *armA*, *blaADC-25*, *blaOXA-23*, *blaOXA-66*, *blaTEM-1D*, *mph(E)*, *msr(E)*, *sul2*, and *tet(B)* were found in *A. baumannii* sequence type ST1837/ST369/ST2. On the other hand, FF02_5292 ST1837/ST369/ST2 lacks the *aph(3'')-Ib* gene and possesses extra drug resistance genes, including *sul1*, *blaNDM-1*, *ant(3'')-Ia*, and *aac(3)-Ia*. Other studies have also found that resistance to a variety of antibiotic classes, including beta-lactams, aminoglycosides, macrolides, tetracyclines, and sulfonamides, is associated with antimicrobial resistance genes, including *aph(3'')-Ib*, *aph(3')-Ia*, *aph(6)-Id*, *armA*, *blaADC-25*, *blaOXA-23*, *blaOXA-66*, *blaTEM-1D*, *mph(E)*, *msr(E)*, *sul2*, and *tet(B)* (Kyriakidis *et al.*, 2021; Lin and Lan, 2014; Navon-Venezia *et al.*, 2017; Salih and Shafeek, 2019; Vijayakumar *et al.*, 2022).

The *aph(3'')-Ib*, *aph(3')-Ia*, and *aph(6)-Id* genes are aminoglycoside phosphotransferases that confer resistance to aminoglycoside antibiotics (Aruhomukama *et al.*, 2020; Kyriakidis *et al.*, 2021; McGann *et al.*, 2014; Rashvand *et al.*, 2021; Taitt *et al.*, 2014). These genes are associated with antibiotic inactivation and are classified as antibiotic inactivation enzymes, aminoglycoside modifying enzymes, phosphorylation of antibiotic conferring resistance, and aminoglycoside phosphotransferases (Ababneh *et al.*, 2023; Taitt *et al.*, 2014). The gene *armA* is classified as an aminoglycoside resistance methylase and is associated with resistance to aminoglycosides (Ayad *et al.*, 2016; Bogaerts *et al.*, 2007; González-Zorn *et al.*, 2005).

The *blaADC-25* gene encodes beta-lactam resistance and is associated with resistance to third-generation cephalosporins (Hwang *et al.*, 2021; Li *et al.*, 2017). It is often found in association with other resistance genes, such as *blaTEM-1D*, and is a significant concern in the treatment of infections caused by multidrug-resistant gram-negative bacteria (Li *et al.*, 2017).

The blaOXA-66 gene, which is associated with other resistance genes including blaOXA-23 and blaADC-25, is responsible for encoding beta-lactam resistance (Hwang *et al.*, 2021; Karah *et al.*, 2016; Vijayakumar *et al.*, 2022). The most common carbapenemase gene in *Acinetobacter baumannii*, blaOXA-23, encodes beta-lactam resistance. It is found on integrons, transposons, plasmids, and resistant islands, among other mobile genetic elements. It is also found in combination with other resistance genes, such as blaOXA-66 and blaADC-25 (Karah *et al.*, 2016; Vijayakumar *et al.*, 2022).

The msr(E) gene, which codes for the macrolide efflux pump, has been linked to *A. baumannii* and resistance to macrolide antibiotics like azithromycin (Hamed *et al.*, 2022; Wang *et al.*, 2017a). The tet(B) gene encodes tetracycline resistance while the sul2 gene encodes sulfonamide resistance. The gene has been detected in strains of *A. baumannii* from Egypt and the Czech Republic, among other countries (Hamed *et al.*, 2022; Mlynarcik *et al.*, 2021).

A. baumannii sequence type ST1697/ST2535/ST2 in this study include aph(3'')-Ib, aph(3')-Ia, aph(6)-Id, armA, blaADC-25, blaOXA-23, blaOXA-66, mph(E), msr(E), sul1, sul2, aadA1, ant(3'')-Ia, blaNDM-1, and catB8 drug resistance genes. The genes aadA1, ant(3'')-Ia, blaNDM-1, and catB8 are distinct from most of the genes, which were similar to the sequence type indicated above (Chen *et al.*, 2015).

The aadA1 and ant(3'')-Ia gene encodes aminoglycoside adenylyl transferase and is associated with resistance to aminoglycoside antibiotics (Aruhomukama *et al.*, 2020; Hamed *et al.*, 2022; Ramirez and Tolmasky, 2010). This gene has been found in *A. baumannii* strains from Egypt and Uganda but ant(3'')-Ia found in China and Singapore. The aadA1 catB8 genes are found with the sul1 gene. (Aruhomukama *et al.*, 2020; Hamed *et al.*, 2022; Ramirez and Tolmasky, 2010).

The catB8 gene encodes chloramphenicol acetyltransferase and is associated with resistance to chloramphenicol antibiotics. This gene has been found in *A. baumannii* strains from Singapore and Egypt, and found in association with the sul1 gene (Pagano *et al.*, 2016; Zeng and Jin, 2003)).

The blaNDM-1 gene encodes carbapenemase and is associated with resistance to carbapenem antibiotics. The gene has been found in *A. baumannii* strains from India and Egypt and is associated with the sul2 gene (Lin and Lan, 2014; Pagano *et al.*, 2016).

In the current study, the following *K. pneumoniae* of the ST101 type genes are found: blaCTX-M-15, blaNDM-1, blaOXA-1, blaOXA-9, blaTEM-1A, catA2, dfrA14, fosA, qnrS1, and sul2. In contrast to possible PDR *K. pneumoniae* ST101, *A. baumannii* possesses the genes blaCTX-M-15, blaOXA-1, blaOXA-9, blaTEM-1A, catA2, dfrA14, and fosA. The aac(3)-IIa, aac(6')-Ib, and aac(6')-Ib-cr, genes encode aminoglycoside acetyltransferase; whereas, the gene aadA1 encodes aminoglycoside adenyltransferase (McGann *et al.*, 2014; Ramirez and Tolmasky, 2010) and aph(3'')-Ib: and aph(6)-Id: genes encodes aminoglycoside phosphotransferase. All the above six genes are associated with resistance to aminoglycoside antibiotics (McGann *et al.*, 2014; Zeng and Jin, 2003)).

The extended-spectrum beta-lactamase gene, blaCTX-M-15, is associated with resistance to third-generation cephalosporins. The beta-lactamase-encoding blaTEM-1A gene has been associated with antibiotic resistance to penicillin. The gene is often found in association with each other (Osei Sekyere *et al.*, 2020; Thomson, 2010).

The blaOXA-1 and blaOXA-9 genes encode beta-lactamase and are associated with resistance to penicillin antibiotics. The genes have been found in various gram-negative bacteria, including *K. pneumoniae* (Osei Sekyere *et al.*, 2020; Pagano *et al.*, 2016). The blaNDM-1 gene encodes carbapenemase and is associated with resistance to carbapenem antibiotics (Hamed *et al.*, 2022; Pagano *et al.*, 2016).

The catA2 gene encodes chloramphenicol acetyltransferase (Bale *et al.*, 2023), the dfrA14: gene encodes dihydrofolate reductase (Partridge, 2011), the fosA gene encodes fosfomycin resistance protein (Adekanmbi *et al.*, 2020; Partridge, 2011), the qnrS1 gene encodes quinolone resistance protein (Adekanmbi *et al.*, 2020; Partridge, 2011), and the sul2 gene encodes sulfonamide resistance protein (Bale *et al.*, 2023; McArthur *et al.*, 2013). The drug resistance genes of catA2, dfrA14, fosA, qnrS1, and sul2 in *K. pneumoniae* are associated with resistance to chloramphenicol, trimethoprim, fosfomycin, quinolone, and sulfonamide antibiotics, respectively (Anderson *et al.*, 2023; Moghimi *et al.*, 2021; Schneiders *et al.*, 2003). Overall, these genes play a significant role in the development of antibiotic resistance in gram-negative bacteria and pose a challenge in the treatment of infections caused by these pathogens.

5.5.10.3. Antimicrobial Resistance Mutation in PDR Isolates

The FF02_5528 sequence type ST101 *K. pneumoniae* gene mutation on *acrR*, *ompK36*, and *ompK37* in this study suggests that this organism may have resisted a variety of antibiotics, such as carbapenem, doripenem-colistin, meropenem-vaborbactam, and fluoroquinolones (Adler *et al.*, 2016; David *et al.*, 2022; Dulyayangkul *et al.*, 2020; Padilla *et al.*, 2010; Wang *et al.*, 2001; Wong *et al.*, 2019).

According to our results, the *acrR* gene mutation in *K. pneumoniae* confers the highest antibiotic resistance point mutation also indicated in Padilla (Padilla *et al.*, 2010) study. In *K. pneumoniae*, the *acrR* gene has the following mutations: p.F172S, p.F197I, p.G164A, p.K201M, p.L195V, p.P161R, and p.R173G. These findings were consistent with another study (Schneiders *et al.*, 2003). Mutations in *AcrR*, a gene that represses the *AcrAB-TolC* multidrug efflux complex, can cause the efflux pump to be overexpressed, which can lead to high levels of resistance to several antibiotics (Adler *et al.*, 2016; Padilla *et al.*, 2010). Resistance to the antibiotic class fluoroquinolones, which is used to treat a range of bacterial infections, has been linked to mutations in the *acrR* gene (Wang *et al.*, 2001). Additionally, mutations in the *acrR* gene have been shown to cause high-level resistance to carbapenem antibiotics, a key class of antibiotics used to treat life-threatening infections (Adler *et al.*, 2016). This suggests that mutations in the *acrR* gene can impact the response of *K. pneumoniae* strains to Doripenem and Doripenem-colistin (Clancy *et al.*, 2013).

The drug resistance mutations identified in our study *ompK36* gene of *K. pneumoniae*: with p.A190W, p.A217S, p.E232R, p.F207W, p.L191S, p.L59V, p.N218H, p.N49S, and p.T254S point mutation and p.N304E – Frameshift in line with the following studies (David *et al.*, 2022; Faleti and Ibisani, 2023; Wong *et al.*, 2019). The mutation p.N304E - Frameshift in the *ompK36* gene of *K. pneumoniae* was observed in another study (Clancy *et al.*, 2013) and is associated with the emergence of multidrug-resistant clones and confer resistance to carbapenem antibiotics (David *et al.*, 2022; Wong *et al.*, 2019). The mutations mediate pore constriction and consist of amino acid insertions in extracellular loop 3 (L3) of *OmpK36*, a motif that plays an important role in mediating resistance to carbapenems (David *et al.*, 2022).

5.5.10.4. Virulence Factor in PDR Isolates

K. pneumoniae with sequence type ST101 (FF02_5528) was shown to be one of the virulence factor-causing genes among the ten potential pan-drug resistant bacteria. Type 1 and type 3 fimbriae, which are significant virulence factors in *K. pneumoniae* and aid in bacterial adherence to host cells, are encoded by the *fimH* and *mrkA* virulence genes. Fimbrial adhesins (de Cássia Andrade Melo *et al.*, 2014; Gomes AÉ *et al.*, 2020; Maleki *et al.*, 2023). The *fyuA* and *irp2* encode the yersiniabactin receptor and the iron-regulated protein involved in iron uptake in *K. pneumoniae* that has been associated with siderophores (de Cássia Andrade Melo *et al.*, 2014; Maleki *et al.*, 2023).

5.5.10.5. Plasmid MLST In PDR Isolates

The plasmid multilocus IncF RST[F-:A13:B-] and the plasmid genes of Col(pHAD28), IncFIA(HI1), and IncR are found in the FF02_5528 *K. pneumoniae* ST101 type investigated in the present study. The IncF plasmids are a diverse group of plasmids that are commonly found in Enterobacteriaceae, including *K. pneumoniae*, and are associated with antibiotic resistance and virulence factors (Ramirez *et al.*, 2019; Ramirez *et al.*, 2014). The IncF RST scheme uses DNA fragments containing the FII, FIA, FIB, and FIC replicons to identify the plasmid type (Villa *et al.*, 2010). The IncFII(K) replicon plasmid has been shown to carry genes coding for resistance to antibiotics and virulence factors, including the *bla*CTX-M-17, *aac*(6')-Ib, and *ant*(3'')-Ia genes, which confer resistance to extended-spectrum beta-lactam and aminoglycoside antibiotics (Ramirez *et al.*, 2014; Sumbana *et al.*, 2023). Overall, the function of the *K. pneumoniae* plasmid IncF RST[F-:A13:B-] is likely to be associated with antibiotic resistance and virulence factors (Hendrickx *et al.*, 2020).

Col(pHAD28) plasmid has been identified in *K. pneumoniae* isolates in this study and is associated with the IncFII(K) replicon plasmid family (Shelenkov *et al.*, 2023). Other findings suggest that the Col(pHAD28) plasmid is associated with resistance to quinolones in *Salmonella species* (Tanmoy *et al.*, 2021). The plasmid has also been shown to carry the single AMR gene *aac*(6')-Ib, providing resistance to aminoglycosides (Shelenkov *et al.*, 2023).

The IncFIA(HI1) plasmid is identified in *K. pneumoniae* and associated with resistance to extended-spectrum beta-lactam antibiotics (Ares-Arroyo et al., 2021; McMillan et al., 2020; Shelenkov et al., 2023). IncR plasmid has been identified in *K. pneumoniae* associated with resistance to aminoglycoside antibiotics (Qian et al., 2020). The IncR plasmids have also been shown to carry genes coding for resistance to other antibiotics, such as tetracycline, sulfonamides, and beta-lactams (Ramirez et al., 2019).

The other plasmid virulence gene in possible PDR bacteria identified were from FF02_3205 *A. baumannii* sequence type ST1809, ST451, ST2 of plasmid multilocus IncF RST[F-:A-:B-], and IncHI2 DLST1*? Plasmid gene IncFIA, and IncHI2A and from FF02_1726 sequence type ST1837/ST369/ST2 plasmid multilocus IncHI2 DLST1* and plasmid gene of IncHI2. The IncF RST[F-:A13:B-] was mentioned in *K. pneumoniae* IncHI2 DLST1* in the other study. This plasmid is a member of the IncHI2 incompatibility group and has been identified in *K. pneumoniae* and *E. coli* (Hancock et al., 2017). Other studies suggest that the IncHI2 plasmids are associated with resistance to extended-spectrum beta-lactam antibiotics (Hancock et al., 2017; McMillan et al., 2020.511). The IncHI2 plasmids have also been shown to carry genes coding for virulence factors (Hancock et al., 2017). Probably *A. baumannii* acquired IncF RST[F-:A-:B-], and IncHI2 DLST1*? gene from *K. pneumoniae* or *E. coli*.

The IncFIA plasmids are a diverse group of plasmids that are commonly found in *Enterobacteriaceae* and are associated with *bla*_{CTX-M}, *rmtB*, and *oqxB* resistance to extended-spectrum beta-lactam antibiotics, aminoglycoside and efflux pump fluoroquinolones, tetracyclines, and chloramphenicol antibiotics, respectively (Yang et al., 2015). The IncHI2A plasmids are a member of the IncHI2 incompatibility group and have been shown to carry genes coding for resistance to extended-spectrum beta-lactam antibiotics and aminoglycoside antibiotics, as well as virulence factors (Chen et al., 2016).

The elimination of IncHI2 plasmids correlated with the loss of β -lactamase genes (*bla*_{OXA-1} and *bla*_{TEM-1}) and PMQR genes (*qnrA* and *aac(6')-Ib-cr*) (Chen et al., 2016). The function of IncFIA and IncHI2A plasmid genes in *A. baumannii* confers resistance to colistin (Control and Prevention, 2016; Kalová et al., 2021). Another study identified IncHI2 plasmids carrying

*bla*NDM-like genes in *A. baumannii* (Poirel *et al.*, 2012). However, the specific function of IncFIA and IncHI2A plasmid genes in *A. baumannii* is not clear.

5.5.10.6. Disinfectant Resistance and Genes in PDR Isolates

The current study indicates the presence of disinfectant resistance genes *qacE* in *A. baumannii* sequence type ST1697/ST2535/ST2, which provides resistance to disinfectants used in hospitals for infection prevention (Babaei *et al.*, 2015; Gomaa *et al.*, 2017; Guo and Li, 2019; Mahzounieh *et al.*, 2014; Rajamohan *et al.*, 2010). Multiple drug-resistant *A. baumannii* clinical isolates have been found to contain the *qacE* gene (Babaei *et al.*, 2015), and is linked to efflux pump genes (Rajamohan *et al.*, 2010; Rostami *et al.*, 2021). The *qacE* gene has been linked to gram-negative bacteria such as *A. baumannii* (Ababneh *et al.*, 2023; Babaei *et al.*, 2015).

6. CHAPTER SIX: CONCLUSION AND RECOMMENDATION

6.1. Conclusion

In conclusion, this study determined the prevalence of bloodstream infection, MDR bacteria, AMR genes, virulent factors, plasmid transmission, and disinfectant-resistant bacteria and their respective genes among septicemic patients. Our findings showed higher gram-negative bacteria than gram-positive bacteria in bloodstream infection. Almost all of the isolates were MDR bacteria. The 7.4% Prevalence of possible PDR bacteria was an alarming result in this study. A large number of 84.4% gram-negative bacteria s had one or more phenotypically confirmed enzyme-producer drug-resistant bacteria, However, 76.2% of them had one or more PCR-confirmed drug-resistance genes. In AST confirmatory tests, Carbapenemase and MBLs-producing strains accounted for 29.9% and PCR-confirmed drug resistance mechanisms were 27.3%.

The most common bacteria isolated include *K. pneumoniae*, *Acinetobacter spp*, and *E. coli*. Like, in the other parts of the world, the isolates were highly resistant to commonly prescribed antibiotics and their rate of resistance against carbapenem is alarming. *K. pneumoniae* was the most prevalent XDR bacteria, and *Acinetobacter spp*. was the mainstream possible PDR bacteria. High carbapenem producer bacteria were found in our study compared with other studies. *K. pneumoniae*, *K. oxytoca*, and *E. coli* had the highest percentages of PCR-confirmed drug resistance mechanisms. The highest drug-resistant in Carbapenemase and MBLs were recorded in *A. baumannii* followed by *K. pneumoniae*. Among the factors associated with BIS infection age, ICU admission, indwelling medical devices, and chronic illness were the most important. NICU and SICU departments were most prone to all types of MDR, especially XDR and PDR gram-negative bacteria. While patients treated by surgical procedure at General surgery and caesarean section were heavily exposed to XDR and PDR infections. Phenotyping and molecular characterization showed NICUs have a high prevalence of ESBL and AmpC-BL-producing gram-negative bacteria; whereas, in general surgery, caesarean section delivery room, SICU, and NICU departments have a high prevalence of carbapenemase and MBL-producing gram-negative bacteria. Regarding the confounding variables length of stay, antibiotic use, and inserted instrument usage (IV lines, drainage tubes, and NG tubes) during medical care correlate with MDR bloodstream infection. Increasing length of admission was associated with Carbapenemase and MBLs nosocomial

infection. This means most of the community-acquired drug resistance mechanisms were associated with ESBL and AmpC-BLs producers and hospital-acquired infections were associated with Carbapenemase and MBLs producer's gram-negative bacteria. The suction machines, intravenous lines, and drainage tubes were the major cause of acquiring Carbapenemase and MBL producer bacteria. Among the confounding variables of chronic illness; UTI, WI, and RTI were correlated with MDR bloodstream infection. Wound infection and UTI were associated with Carbapenemase and MBL-producer bacteria, on the other hand, UTI, RTI, and tissue Sarcomas were associated with ESBL and AmpC-BL producer gram-negative bacteria in both Phenotyping and molecular characterization. Individuals below 6 years old were more vulnerable to bacterial bloodstream infection. Neonatal incubation and caesarean section delivery are both associated with MDR bloodstream infection. Among the total MDR bacteria, 35.8% of infections were in neonates. In this study, all of the newborns who acquired drug-resistant bloodstream infection infections were born in hospitals and 84.0% of them had a history of neonatal incubation. The most frequent potential cause of nosocomial infection with drug-resistance genes was the use of medical equipment (80.3%).

In this study, the highest prevalence of PCR-confirmed ESBL encoding genes in gram-negative bacteria was *bla*CTX-M, *bla*TEM, and *bla*BEL whereas *bla*FOX and *bla*CITM were the highest AmpC beta-lactamases expressed genes. Among Carbapenemase encoding genes *bla*OXA-23, *bla*KPC, and from MBLs, *bla*NDM, and *bla*VIM were the highest prevalent.

Nine novel AMR gram-negative bacteria were identified. Three of the nine novel drug-resistant bacteria shared the same allelic profiles, while the remaining six were unique. AMR genes with the highest frequency were *bla*CTX-M-15, and *aph*(3'')-Ib. On the other hand, *bla*CTX-M-15, *bla*TEM-1B, and *bla*OXA-1 were the top three of all beta-lactamase genes. *bla*CTX-M-15 and *bla*TEM-1B constituted the majority of ESBL-producing genes in both CDT and WGS. The one *bla*NDM-1 and one *bla*CARB-2 were identified in addition to the OXA families of *bla*OXA-1, *bla*OXA-10, *bla*OXA-120, *bla*OXA-232, *bla*OXA-58, and *bla*OXA-94 different variants in ESBL and AmpC beta-lactamase. However, the strains are not carbapenem-resistant which indicates that these genes were silent. AmpC beta-lactam-resistant gram-negative bacteria, *E. coli* (53.1%), and *E. cloacae* (19.4%) have the highest genes. According to our findings, *bla*CMY-2 (1.9%) and *bla*CMY-6 (0.6%) genes were identified from the AmpC beta-lactam resistant gram-negative

bacteria, in addition to *bla*CTX-M-15 (5.6%), *bla*SHV-12 (1.9%), *bla*SHV-187 (0.6%), *bla*TEM-1B (6.3%), *bla*VEB-1 (0.6%), and *bla*OXA-1 (5.0%) ESBL producer gene. The most prevalent carbapenem-resistant gram-negative bacteria were *Acinetobacter baumannii*, *K. pneumoniae*, and *E. coli*. The common genes that produced carbapenemase were *bla*OXA-23, *bla*OXA-66, *bla*NDM-1, and *bla*OXA-1. The virulent genes were found in 71.13% of the gram-negative bacteria, with an average of 10.44 virulence genes found in a single bacterium. The major virulence gene is shared by *E. coli* and *K. pneumoniae*. The virulence genes *fimH*, *iutA*, *traT*, *terC*, and *mrkA*:ABW83989 have a higher prevalence.

In 8 genes, 956 mutations were reported in 57.7% of the gram-negative bacteria. Despite this, 97.7% of the mutations were chromosomal point mutations, with only 2.3% being frameshift mutations. The most common gene was *ompK37*, followed by *acrR*, and *ompK36*. *K. pneumoniae* chromosomal point mutations were found in the *ompK37*, *acrR*, *ompK36*, and *ramR* genes, while *E. coli* strains had mutations in the *gyrA*, *parC*, *pare*, and *rpoB* genes.

A high burden of 72.54% plasmid MLST and plasmid replicon 76% were identified from 142 sequenced gram-negative bacteria. All *K. pneumoniae* and *K. oxytoca* have drug resistance plasmid MLST and plasmid replicon; whereas, *E. coli* and *E. cloacae* carried the higher plasmid MLST at 94.4% and multiple plasmid replicon at 88.9%. The most common plasmid multilocus sequence types were IncF RST[K7:A-B-], IncHI2 DLST1, and IncFRST[F-A-B-], and the most common plasmid replicon were IncFII(K), IncFIB(K), and IncFIB(AP001918).

Furthermore, our results showed that *E. coli* strains were resistant to hydrogen peroxide disinfectant and possessed the *sitABCD* gene. However, the rest gram-negative bacteria are sensitive to hydrogen peroxide. The *OqxA* and *OqxB* genes are associated with benzalkonium chloride and cetylpyridinium chloride disinfectant resistance in the majority of 3rd generation cephalosporin and Carbapenem resistance *K. pneumoniae*. Almost all bacterial species, resistant to benzalkonium chloride, cetylpyridinium chloride, and chlorhexidine were associated with the *qacE* gene. Our analysis shows Hydrogen peroxide disinfectant may be helpful for the NICU department.

The WGS result revealed that all 35 *Acinetobacter Species* were confirmed as it was *Acinetobacter baumannii*. We confirmed during this study, that there were *A. baumannii* epidemics in the hospital, especially in the NICU and medical and surgical ICU. The whole genome sequence result confirmed that among 17 carbapenem resistance and possible PDR ten of *Acinetobacter species* were *A. baumannii* sequence type ST1837/ST369/ST2. The sequence types ST1837 and ST369 *A. baumannii* have been identified as a dominant cause of outbreaks in China. The same is true for ST2 *Acinetobacter baumannii*, it was also found in some regions of China, Latin America, and Caribbean countries as a cause of hospital epidemics. This study showed that mechanical ventilators were the major cause of *A. baumannii* transmission. A study found that ST2 was associated with high rates of inappropriate antimicrobial therapy, severe infection, strong biofilm formation ability, high serum resistance, and high pathogenicity and mortality. There are two carbapenem resistance and possible PDR *A. baumannii* sequence type ST1809/ST451/ST2 and another two *A. baumannii* sequence type ST1697/ST2535/ST2. However, there is no available information about *A. baumannii* Sequence type ST1809 and ST1697/ST2535 during my study period. However, *A. baumannii* sequence type ST451 has been identified as a new sequence type in some studies. The *A. baumannii* sequence type ST1697/ST2535/ST2 has disinfectant resistance genes *qacE*. The *qacE* gene belongs to the quaternary ammonium compound (QAC) resistance gene family, and it confers resistance to disinfectants used in hospitals for infection control, such as benzalkonium chloride (BZK) and benzethonium chloride (BZT). The carbapenem resistance and possible PDR FF02_5528 *K. pneumoniae* sequence types ST101 is an emerging high-risk clone of *K. pneumoniae* that exhibits extensive drug resistance. ST101 has been associated with carbapenem resistance and colistin resistance in some studies.

6.2. Recommendation

- Special attention and intervention are needed for neonatal and pediatric patients who are more affected by drug-resistant bacterial bloodstream infections. The hospital management must extend their effort to address the concern of infection prevention protocols in the NICU department in particular and the hospital in general. Further study is needed for all types of *K. pneumoniae* and PDR *Acinetobacter spp* transmission dynamics.
- The hospital management and other stakeholders should work on infection prevention protocol implementation in the hospital, especially in NICU, ICU, general surgery

departments, and the caesarean section unit in the gynecology department. The sterilization method of the medical equipment used for medical service, the workload of staff, the experiences of staff, the fumigation of the room, and the infection prevention protocol in all procedures should be revised and controlled.

- Therefore, interventions using fumigation, minimizing patient load, and strong infection prevention protocol should be implemented focusing on the above-mentioned wards and departments.
- Due to their susceptibility, healthcare institutions that are serving pediatric patients should give special attention to nosocomial infections, and a strong infection prevention protocol should be implemented.
- Our analysis shows Hydrogen peroxide disinfectant may be helpful for the NICU department.
- The blind treatment should consider the antibiogram profile of the studies. In this study, ESBL and AmpC BLs-producing gram-negative bacterial prevalence in NICU is higher than carbapenemase, and MBL-producing gram-negative bacterial prevalence is higher in general surgery, C/S room, and ICU.
- The primary source of carbapenem-resistant bacteria is inserted medical equipment, such as a suction machine, drainage tubes, and intravenous lines, which should be properly sterilized and used in accordance with the IPC protocol's guidelines.
- Appropriate patient load reduces the crowdedness of the hospital and reduces nosocomial infection.
- Further study should be done to understand the transmission dynamics, drug resistance genes, and virulence factors of Carbapenemase producers and pan-drug resistance bacteria, especially *K. pneumoniae* and *Acinetobacter species*.

7. CHAPTER SEVEN: REFERENCES

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LIST OF PUBLICATIONS AND PAPERS

1. Beshah, D., Desta, A., Belay, G., Abebe, T., Gebreselasie, S., and Sisay Tessema, T. (2022). Antimicrobial Resistance and Associated Risk Factors of Gram-Negative Bacterial Bloodstream Infections in Tikur Anbessa Specialized Hospital, Addis Ababa. *Infect Drug Resist* 15, 5043-5059. <https://doi.org/10.2147/IDR.S371654>
2. Beshah D, Desta AF, Woldemichael GB, Belachew EB, Derese SG, Zelelie TZ, et al. (2023) High burden of ESBL and carbapenemase-producing gram-negative bacteria in bloodstream infection patients at a tertiary care hospital in Addis Ababa, Ethiopia. *PLoS ONE* 18(6): e0287453. <https://doi.org/10.1371/journal.pone.0287453>
3. Molecular characterization of ESBL, AmpC-BL, Carbapenemase, and MBL (beta-lactamase and carbapenemase) enzyme-producing genes in gram-negative bacteria among bloodstream infection Patients Admitted at Tikur Anbessa Specialized Hospital Addis Ababa Ethiopia. under process

Under preparation

4. Molecular epidemiology of antimicrobial resistance and virulence determinants in *Klebsiella spp* from hospitalized patients in TASH. under process. under process
5. Whole Genome Sequencing of XDR and PDR *Acinetobacter spp* in a Tertiary Care Hospital in TASH. under process
6. The highest virulence factor loads of *E. coli* found in isolates from patients in a TASH tertiary care facility. under process

LIST OF APPENDIXES

Appendix 1: English Version of the Participant Information Sheet, Consent Form, and Questionnaire 1 Month Below

1. Participant Information Sheet

Department of Microbial Cellular Molecular Biology (Infection Biology), Collage Of Computational and Natural Sciences, Addis Ababa University, Addis Ababa, Ethiopia

Title: Epidemiological and Molecular Characterization of Multi-Drug Resistant Bloodstream Infections of Gram-negative Bacteria Clinical Isolates Among Patients Admitted at Tikur Anbessa Specialized Hospital Addis Ababa Ethiopia.

First of all, we would like to thank you in advance for your cooperation and Consent to participate in this study. Please read or listen when it is read for you about the general information of the study. If you have any questions regarding the study please ask freely.

Background Information

bloodstream infections are a major cause of mortality in both developed and developing countries despite important progress in the treatment and prevention of infectious diseases. It is a common infectious presentation in hospital-acquired and community-acquired infections for a long time. gram-negative bacteria are the most causative agents of septicemia and from the family *Klebsiella*, *E. coli*, *Pseudomonas species*, *Enterobacter*, *Citrobacter*, *Proteus*, *Acinetobacter*, and *Salmonella* take the lead. In addition to their high prevalence the development of drug resistance is the challenge for controlling gram-negative bacteria nowadays. Many studies have been conducted in Ethiopia on bacterial isolation and drug susceptibility patterns from different clinical samples but to my knowledge, none of these studies address the prevalence and drug susceptibility patterns of gram-negative bacteria on blood cultures. Therefore assessing the prevalent Gram-negative bacteria and testing their antibiotic susceptibility will help clinicians provide safe and effective empirical therapies, develop rational prescription programs make policy decisions, and finally assess the effectiveness of all.

Aim of The Study

The purpose of the study is to determine the prevalence and antimicrobial resistance of bacterial isolates with special emphasis on gram-negative bacteria and associated risk factors among those suspected of septicemia in Tikur Anbessa University Hospital, Addis Ababa, Ethiopia.

Benefits for Participants

Study participants will not have any financial incentives or other inducements to participate in this study. However, results will be given to their physician for treatment or to get counseling. Most importantly, this study will contribute to providing information or data for future and further nationwide study and to develop health programs for health policymakers.

Risks and Complication

There is no considerable risk to the participants in participating in the study other than the possible minor bleeding from the site of vein puncture when they give the blood sample. Vein-puncture is a routine clinical practice for blood sample collection and has minimal risk, and the amount of blood collected will be 2- 5 ml for pediatrics and 5-10 ml for adults.

Confidentiality

To maintain the confidentiality of participants' information, the names will not be given and the samples will be coded. Participants will not be prohibited to stop or withdraw at any time from the study. No personal information will be disclosed to a third party or will not appear in any report from this study.

Assurance of Principal Investigator

I put my signature below to confirm that I take over the responsibility for the scientific ethical and technical conduct of the research project and provision of progress reports for all stakeholders of the research project. Daniel Beshah (PI): Signature: _____ Date: _____

Note: If you have any questions about this study, feel free to ask now or anytime throughout the study by contacting:

PI Address: Daniel Beshah: Department of Microbial Cellular Molecular Biology, College of Computational and Natural Science, Addis Ababa University, Addis Ababa, Ethiopia

E-mail: danibeshah@gmail.com; Tel.: +251911151317

Informed Consent

I have been informed about the study which plans to determine the prevalence and antimicrobial resistance of bacterial isolates with special emphasis on gram-negative bacteria and associated risk factors among patients suspected of bloodstream infection in Tikur Anbessa University Hospital, Addis Ababa, Ethiopia. The objective and the application of the study were briefly explained to me. Moreover, I have been well informed of my right to refuse information, decline to cooperate, and drop out of the study if I want and none of my actions will have any bearing at all on my overall health care.

It is therefore with a full understanding of the situation that I agreed to give informed consent voluntarily to the researcher to give my blood for the mentioned study. I agreed that the specimen would be tested for gram-negative bacteria. I have had the opportunity to ask questions about the project and received clarification to my satisfaction in a language I understand. I was also informed that results for the analysis of blood and/or urine isolates of gram-negative bacteria will be given to the health facility and that I may ask for the information if I want.

I **Guardian** _____ hereby give my Consent for giving of the requested information and specimen for this study. Signature: _____ Date: _____

Information was given by _____ Signature _____

2. Questionnaires

These questionnaires will be filled out by selected healthcare professionals in the hospital. That will help us to capture the information regarding drug resistance gram-negative bacteria. Please give us reliable information that helps us to generate reliable results.

Institution TASH_____ Address _____ Participant code No_____

Rejoin _____ zone _____ Wereda_____

Appendix 2: English Version of The Participant Information Sheet, Consent Form, and Questionnaire 1 Month Above

1. Participant Information Sheet

Department of Microbial Cellular Molecular Biology (Infection Biology), Collage Of Computational and Natural Sciences, Addis Ababa University, Addis Ababa, Ethiopia

Title: Epidemiological and Molecular Characterization of Multi-Drug Resistant Bloodstream Infections of Gram-negative Bacteria Clinical Isolates Among Patients Admitted at Tikur Anbessa Specialized Hospital Addis Ababa Ethiopia.

First of all, we would like to thank you in advance for your cooperation and Consent to participate in this study. Please read or listen when it is read for you about the general information of the study. If you have any questions regarding the study please ask freely.

Background Information

bloodstream infections are a major cause of mortality in both developed and developing countries despite important progress in the treatment and prevention of infectious diseases. It is a common infectious presentation in hospital-acquired and community-acquired infections for a long time. gram-negative bacteria are the most causative agents of septicemia and from the family *Klebsiella*, *E.coli*, *Pseudomonas species*, *Enterobacter*, *Citrobacter*, *Protues*, *Acinetobacter*, and *Salmonella* take the lead. In addition to their high prevalence the development of drug resistance is the

challenge for controlling gram-negative bacteria nowadays. Many studies have been conducted in Ethiopia on bacterial isolation and drug susceptibility patterns from different clinical samples but to my knowledge, none of these studies address the prevalence and drug susceptibility patterns of gram-negative bacteria on blood cultures. Therefore assessing the prevalent gram-negative bacteria and testing their antibiotic susceptibility will help clinicians provide safe and effective empirical therapies, develop rational prescription programs make policy decisions, and finally assess the effectiveness of all.

Aim of the Study

The purpose of the study is to determine the prevalence and antimicrobial resistance of bacterial isolates with special emphasis on gram-negative bacteria and associated risk factors suspected for septicemia in Tikur Anbessa University Hospital, Addis Ababa, Ethiopia.

Benefits for Participants

Study participants will not have any financial incentives or other inducements to participate in this study. However, results will be given to their physician for treatment or counseling. Most importantly, this study will contribute to providing information or data for future and further nationwide study and to develop health programs for health policymakers.

Risks and complication

There is no *CoNS*iderable risk to the participants in participating in the study other than the possible minor bleeding from the site of vein puncture when they blood give the sample. Vein-puncture is a routine clinical practice for blood sample collection and has minimal risk, and the amount of blood collected will be 2- 5 ml for pediatrics and 5-10 ml for adults.

Confidentiality

To maintain the confidentiality of participants' information, the names will not be given and the samples will be coded. Participants will not be prohibited to stop or withdraw at any time from the study. No personal information will be disclosed to a third party or will not appear in any report from this study.

Assurance of Principal Investigator

I put my signature below to confirm that I take over the responsibility for the scientific ethical and technical conduct of the research project and the provision of progress reports for all stakeholders of the research project. Daniel Beshah (PI): Signature: _____ Date: _____

Note: If you have any questions about this study, feel free to ask now or anytime throughout the study by contacting:

PI Address: Daniel Beshah: Department of Microbial Cellular Molecular Biology, College of Computational and Natural Science, Addis Ababa University, Addis Ababa, Ethiopia

E-mail: danibeshah@gmail.com; Tel.: +251911151317

Informed Consent

I have been informed about the study which plans to determine the prevalence and antimicrobial resistance of bacterial isolates with special emphasis on gram-negative bacteria and associated risk factors among patients suspected of septicemia in Tikur Anbessa University Hospital, Addis Ababa, Ethiopia. The objective and the application of the study were briefly explained to me. Moreover, I have been well informed of my right to refuse information, decline to cooperate, and drop out of the study if I want and none of my actions will have any bearing at all on my overall health care.

It is therefore with the full understanding of the situation that I agreed to give the informed Consent voluntarily to the researcher to give my blood for the mentioned study. I agreed that the specimen would be tested for gram-negative bacteria. I have had the opportunity to ask questions about the project and received clarification to my satisfaction in a language I understand. I was also informed that results for the analysis of blood and/or urine isolates of gram-negative bacteria will be given to the health facility and that I may ask for the information if I want.

I _____ hereby give my Consent for giving of the requested information and specimen for this study. Signature: _____ Date: _____

Information was given by _____ Signature _____

2. Questionnaires

These questionnaires will be filled out by selected healthcare professionals in the hospital. That will help us to capture the information regarding drug resistance gram-negative bacteria. Please give us reliable information that helps us to generate reliable results.

Institution _____ Address _____ Participant code No _____

Rejoin _____ zone _____ Woreda _____

Appendix 3: English Version of The Questionnaire for The Assessment of Bloodstream Infection Risk Factors

Co No	Res	Demography for all age groups				Answer	
San1.		Sample Number					
Con2.		Code Number					
Can3.		Card Number					
Age4.		Age of respondent	1 up to 1 month 2 >1-<24 months 3 02-<06 years	4 06-<13 years 5 13-<19 years 6 19-<45 years	7 45-<65 years 8 65-<80 years 9 >80 years		
Sex5.		Sex of respondent	☐ Male ☐ Female				
Cdx6.		Clinical diagnosis	1.				
Cdx7.	2.						
Cdx8.	3.						
Cdx9.	4.						
Cd10.	5. Other						
Io11.		Type of patient	☐ Inpatient ☐ Outpatient				
Wa12.		Ward of follow-up	1 PEOPD 2 C/W 3 EOPD	6 CTR 7 InM 8 C3	11 C4 12 D4 13 B5	16 A6 17 B6 18 C6	21 C7 22 D7 23 B8

			4 RT	9 D3	14 C5	19 D6	24 C8		
			5 R/C	10 B4	15 D5	20 B7	25 D8		
De13.		Depar of follow-up	1 Emergency	4 NICU	7 Obs &Gyn	10 Hemato	13 ENT		
			2 In Medic	5 Surgery	8 Gyne	11 Ortho	14 Others		
			3 MICU	6 SICU	9 Onco	12 Derma			
		Questioner for all age groups				Answer			
Qu14.		For inpatients, for how long do you admit?				_____days			
		1) 1-2d 2) 3-4d 3) 5-6d 4) 7-8d 5) >9 d							
Qu15.		For an inpatient not in ICU, have you been admitted to the ICU?				☐ Yes ☐ No			
Qu16.		From how many days before you were admitted to the ICU?				_____days			
Qu17.		For in/outpatient, have you been admitted to another hospital?				☐ Yes ☐ No			
Qu18.		If No 13 is yes, from how many days before?				_____days			
Qu19.		If No 13 is yes, have you been admitted to ICU?				☐ Yes ☐ No			
Qu20.		If No 13 is yes, from how many days before?				_____days			
Qu21.		What was the body temperature during sample collection?				_____°c			
Qu22.		What was the suspected cause of the high fever?		☐ Bacteremia ☐ Malaria ☐ Neutropenic fever ☐ Other_____					
Qu23.		Do you have a fever?				☐ Yes ☐ No			
Qu24.		Do you have chills?				☐ Yes ☐ No			
Qu25.		Do you have shivers?				☐ Yes ☐ No			
Qu26.		Do you have vomiting?				☐ Yes ☐ No			
Qu27.		Do you have a fast heartbeat?				☐ Yes ☐ No			
Qu28.		Have you taken any antibiotic treatment within 10 days?				☐ Yes ☐ No			
Qu29.		If yes please specify the antibiotic	1.						
Qu30.			2.						
Qu31.			3.						
Qu32.			4.						
Qu33.		Have you taken any antibiotic treatment before 10 days?				☐ Yes ☐ No			
Qu34.		If yes please specify the antibiotic	1.						
Qu35.			2.						
Qu36.			3.						
Qu37.			4.						
Qu38.		Did you have a history of instrumentation during medical care?				☐ Yes ☐ No			
Qu39.		Do you have a history of using mechanical ventilators?				☐ Yes ☐ No			
Qu40.		Did you have a history of using a central venous catheter?				☐ Yes ☐ No			
Qu41.		Did you have a history of using a urinary catheter?				☐ Yes ☐ No			
Qu42.		Did you have a history of using a suction machine?				☐ Yes ☐ No			

Qu43.	Did you have a history of using a tracheostomy?	☐ Yes ☐ No
Qu44.	Did you have a history of using intravenous lines?	☐ Yes ☐ No
Qu45.	Did you have a history of using a drainage tube?	☐ Yes ☐ No
Qu46.	Did you have a history of using shunt?	☐ Yes ☐ No
Qu47.	Did you have a history of using an NG tube?	☐ Yes ☐ No
Qu48.	Another instrument _____	
	For one month above	
Qu49.	Did you have a history of chronic illness?	☐ Yes ☐ No
Qu50.	Are you an RVI patient?	☐ Yes ☐ No
Qu51.	Are you a diabetic patient?	☐ Yes ☐ No
Qu52.	Do you have hypertension?	☐ Yes ☐ No
Qu53.	Do you have surgical wound infections?	☐ Yes ☐ No
Qu54.	Do you have any wound infections on the skin?	☐ Yes ☐ No
Qu55.	Do you have a gastrointestinal infection?	☐ Yes ☐ No
Qu56.	Do you have a ureteral tract infection?	☐ Yes ☐ No
Qu57.	Do you have a respiratory tract infection?	☐ Yes ☐ No
Qu58.	Do you have heart disease?	☐ Yes ☐ No
Qu59.	Do you have coronary artery disease?	☐ Yes ☐ No
Qu60.	Do you have a Stroke?	☐ Yes ☐ No
Qu61.	Do you have gangrene?	☐ Yes ☐ No
Qu62.	Do you have tuberculosis?	☐ Yes ☐ No
Qu63.	Do you have cirrhosis?	☐ Yes ☐ No
Qu64.	Do you have renal failure?	☐ Yes ☐ No
Qu65.	Do you have Hematologic Malignancy?	☐ Yes ☐ No
Qu66.	Do you have Tissue Sarcomas?	☐ Yes ☐ No
Qu67.	Another chronic disease _____	
	For neonate	
Qu68.	For neonates; the educational level of the family	☐ Not neo ☐ Illiterate ☐ Read & write ☐ Pri. Sch. ☐ Seco. SCH. ☐ Higher Edu.
Qu69.	Place of delivery	☐ In hospital ☐ At home
Qu70.	Does the neonate have a history of incubation?	☐ Yes ☐ No
Qu71.	Mode of delivery	☐ NVD ☐ C/S
Qu72.	Gestational age	☐ >37wks ☐ <37 wks
Qu73.	Premature rupture of membrane	☐ >18 hrs ☐ <18 hrs ☐ No rupture
Qu74.	Body weight (in grams) during delivery	_____ gm
Qu75.	History of maternal fever or infection during pregnancy	☐ Yes ☐ No

Data collector name _____ Date _____ Signature _____

Appendix 4: Amharic Version of the Participant Information Sheet, Consent form and Questioner 1 Month Below (የተሳታፊዎች መረጃ ቅጽ፤ የፈቃደኝነት ማረጋገጫ ና መጠይቅ)

1. የተሳታፊዎች መረጃ ቅጽ

የማይክሮብያል ሴሉላር ሞለኪዩላር ባዮሎጂ ዲፓርትመንት፣ የተፈጥሮ ሳይንስ ኮሌጅ፣ አዲስ አበባ ዩኒቨርሲቲ አዲስ አበባ፣ ኢትዮጵያ

አርሰት፡ በአዲስ አበባ ከተማ፣ በጥቁር አንበሳ ሆስፒታል በደም ውስጥ ጉብተው በሽታ የሚያመጡና ለመድሃኒት የማይበገሩ የባክቴሪያ ዘሮችን በተለይም ኢንተሮባክተርሴ የሚባሉትን ስርጭቱን ማወቅ፤ ለመድሃኒት ብግርነትና ለስርጭቱ መንስኤ የሆኑትን በራሂ ዘር/ ጂኑን ማሳየት፡፡

አጠቃላይ መረጃ

በጥናቱ በመሳተፍዎ ከልብ እያመሰገን ከመወሰንዎ በፊት፡- ይህንን ቅጽ በትክክል አንብቡ ወይም ሲነበብልዎ በትክክል ያድምጡ፤ እንዲሁም ግልፅ ያልሆነልዎትን ነገር በሙሉ በነፃነት ይጠይቁ፡፡

መግቢያ

ሰፒቲሴሚያ በሽታ በተለያዩ ተህዋስያን የሚመጡ የጤና ችግር ሲሆኑ በዓለም ላይ የሚያደርሱት የሞት እና የኢኮኖሚ ቀውስ ከፍተኛውን ደረጃ ይይዛል፡፡ በተለይም በታዳጊ ሀገሮች ውስጥ ለሚገኙ ህዝቦች የሞት እና ጉዳት ዋነኛ ምክንያት ነው፡፡ ለዚህም ዋነኛ መንስኤው ኢንተሮባክተሪሴ የተባለት ተህዋስያን የሚያመጡት በሽታ ምክንያት የሚሞቱት ሰዎች ከፍተኛውን ቁጥር ይይዛሉ፤ በተጨማሪም እነዚህ ባክቴሪያዎች ለመድከኒቶች በከፍተኛ ደረጃ የግትርነት ባህሪ እያሳዩ መሆኑ ችግሩን ውስብስብ አድረጎታል፡፡ ሥለሆነም ይህን በሽታ ከፍተኛ ጉዳት ከማስከተሉ በፊት በመከላከል የሕብረተሰቡን ሞት ለመቀነስ ያለውን ስርጭት እንዲሁም ለመድሃኒቶችም የለውን የግትርነት ማወቅ በጣም አስፈላጊ ነው፡፡

የጥናቱ አላማ

በአዲስ አበባ ከተማ፣ በ ጥቁር አንበሳ ሆስፒታል ውስጥ ባክቴሪያዎች በተለይም ኢንተሮባክተርሴ የሚባሉትን ለሴብቲሴሚያ በሽታ ከታከሙት ስርጭቱን ማወቅ ና ለመድከኒት ያለውን የግትርነት ደረጃ፤ እና መንስኤዎቹን ማሳየት፡፡

ለጥናቱ ተሳታፊዎች ያለው ልዩ ጥቅም

በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለውም፡፡ ነገር ግን በጥናቱ መሰረት ተህዋስያኑ ለተገኘባቸው ተሳታፊዎች ከሚመለከታቸው የሆስፒታሉ ባለሙያዎች ጋር በመነጋገር አስፈላጊ የሆነ ህክምና እንዲደረግላቸው ይደረጋል፡፡

በጥናቱ ተሳታፊዎች ላይ ያለው ጉዳት እና ተዛማጅ ችግር

በዚህ ጥናት ላይ በመሳተፍ ሊደርስብዎ የሚችል አንድም ጉዳት አይኖርም፡፡ ለዚህ ጥናት የምንጠቀምበት የደም ናሙና ከክንድዎ የሚወሰድ ሲሆን መጠኑም ከ 5 ሚሊ ሊትር (ከ 1_2 የሻይ ማንኪያ) ነው፡፡ ይህም ከመጠነኛ ስሜት በስተቀር በጤናዎ ላይ ምንም አይነት ጉዳት አያደርስም፡፡ ለሽንት ምርመራ የሚሆን ሽንት በሚያመጡበት ሰዓትም ቢሆን ምንም የተለየ ስሜት የለውም፡፡

የመረጃ ሚስጥራዊ አጠባበቅ

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

ያስታውሱ፤ ስለዚህ ጥናት ማንኛውም ጥያቄ ካልዎት በማንኛውም ጊዜ ከዚህ በታች በተጠቀሱት አድራሻዎች መጠየቅ ይችላሉ፡፡
ዳንኤል በሻህ (ዋተ) ፊርማ _____ ቀን _____

የዋና ተመራማሪው አድራሻ፤

 **ዳንኤል በሻህ ፤** የማይክሮ-ብያል ሴሉላር ሞለኪዩላር ባዮሎጂ ዲፓርትመንት፣ የተፈጥሮ ሳይንስ ኮሌጅ፣ አዲስ አበባ ዩኒቨርሲቲ_ አዲስ አበባ፣ ኢትዮጵያ
 **ኢ_ሜይል፡** danibeshah@gmail.com **ስልክ ፡** +251911151317
የፈቃደኝነት ማረጋገጫ ቅፅ

በአዲስ አበባ ከተማ፣ በ ጥቁር አንበሳ ሆስፒታል ውስጥ ባክቴሪያዎች በተለይም ኢንተርባክተርሴ የሚባሉትን ለሴብቲሴሚያ በሽታ ከታከሙት ስርጭቱን ማወቅ ና ለመድሐኒት ያለውን የግትርነት ደረጃ፤ እና መንስኤዎቹን ማሳየት በሚል ርዕስ ላይ ለማጥናት በተመለከተ በሚደረገው ጥናት ላይ ለመሳተፍ መሆኑ፤ የጥናቱ ዓላማና ጥቅም ተገልጾልኛል፡፡ በመጠይቁ ላይ የምሰጠው የእኔ ሙሉ መረጃም ሆነ የደም ናሙና ውጤቱ በሚሰጥ እንደሚያዝ ተነግሮኛል፡፡ በተጨማሪም ጥናቱ ውስጥ አለመሳተፍ መብቴ እደሆነና በማንኛውም ጊዜ ከጥናቱ በራሴ ወሳኔ መወጣት እንደምችልና በዚህም ምክንያት ምንም አይነት መጉላላት እንደማይደርስብኝ በሚገባ ተረድቻለሁ፡፡ ስለሆነም ሁኔታውን በሚገባ በማጤን በፈቃደኝነት በምርምሩ ላይ ለመሳተፍ ለተመራማሪው ፈቃደኝነቴን ሰጥቻለሁ፡፡ በተጨማሪም የምሰጠው የደም ናሙና ለኢንተርባክቴርሴ ባክቴሪያዎች ምርመራዎች ብቻ እንደሚወል ተነግሮኝ ተስማምቻለሁ፡፡ ማንኛውንም ያልገባኝን ነገር የመጠየቅ ዕድል ተሰጥቶኝ በሚገባኝ

ቋንቋ መልስ አግኝቻለሁ። በተጨማሪም የሁሉም የላብራቶሪ ምርመራ ውጤቶች ለተቋሞች እንደሚሰጥና ውጤቴን ማወቅ ከፈለኩ ማግኘት እንደምችል ተነግሮኛል።

እኔ አሳዳጊ ወይም ተንከባካቢ _____ የተባልኩ ግለሰብ ይህን ሁሉ በማገናዘብ ምርምሩ ላይ ስለኔ መረጃ እና የደም ናሙና ለመስጠት ተስማምቻለሁ። ፊርማ _____ ቀን _____

መረጃዉን ያስረዳዉ አካል _____ ፊርማ _____

2. መጠይቅ

በአዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ዲፓርትመንት

ይሕ የመጠየቅ ቅጽ ስለተሳታፊዎች አጠቃላይ መረጃ ና ለበሽታው (ለኢንተሮባክተርሴዎች) መንስኤ ሊሆኑ የሚችሉትን ምክንያቶች ለማወቅ የሚደረግ መጠይቅ ነው። መጠየቁን የሚያስሞላው በጥናቱ ጊዜ የተመረጠው(ችው) ባለሙያ ሲሆን መጠየቁ የሚሞላው ጥናቱ በሚከሄድበት ቦታ ነው። ለጥናቱ መሳካት ና ትክክለኛነት ያግዘን ዘንድ ጥያቄዎችን በጥንቃቄ አንብበዉ በታማኝነት ያለምንም ፍረቻ እንዲሞሉልን በትህትና እንጠይቃለን።

የተቋሙ ስም: ጥቁር አንበሳ ስፔሻላይዜድ ሆ/ል _____ አድራሻ (ከፍለ_ከተማ): ልደታ ከ/ከተማ _____

የተሳታፊው መለያ ቁጥር _____ አድራሻ
(ክልል) _____ ዞን _____ ወረዳ _____

Appendix 5: Amharic Version of the Participant Information Sheet, Consent form and Questioner 1 Month Above (የተሳታፊዎች መረጃ ቅጽ፣ የፈቃደኝነት ማረጋገጫ ና መጠይቅ)

3. የተሳታፊዎች መረጃ ቅጽ

የማይክሮብያል ሴሉላር ሞለኪዩላር ባዮሎጂ ዲፓርትመንት፣ የተፈጥሮ ሳይንስ ኮሌጅ፣ አዲስ አበባ ዩኒቨርሲቲ አዲስ አበባ፣ ኢትዮጵያ

አርሰት፡ በአዲስ አበባ ከተማ፣ በጥቁር አንበሳ ሆስፒታል በደም ውስጥ ጉብተው በሽታ የሚያመጡና ለመድሃኒት የማይበገሩ የባክቴሪያ ዘሮችን በተለይም ኢንተሮባክተርሴ የሚባሉትን ስርጭቱን ማወቅ፤ ለመድሃኒት ብግርነትና ለስርጭቱ መንስኤ የሆኑትን በራሂ ዘር/ ጂኑን ማሳየት፡፡

አጠቃላይ መረጃ

በጥናቱ በመሳተፍዎ ከልብ እያመሰገን ከመወሰንዎ በፊት፡- ይህንን ቅጽ በትክክል አንብቡ ወይም ሲነበብልዎ በትክክል ያድምጡ፤ እንዲሁም ግልፅ ያልሆነልዎትን ነገር በሙሉ በነፃነት ይጠይቁ፡፡

መግቢያ

ሰፒቲሴሚያ በሽታ በተለያዩ ተህዋስያን የሚመጡ የጤና ችግር ሲሆኑ በዓለም ላይ የሚያደርሱት የሞት እና የኢኮኖሚ ቀውስ ከፍተኛውን ደረጃ ይይዛል፡፡ በተለይም ቢታዳጊ ሀግሮች ውስጥ ለሚገኙ ህዝቦች የሞት እና ጉዳት ዋነኛ ምክንያት ነው፡፡ ለዚህም ዋነኛ መንስኤው ኢንተሮባክተሪስ የተባለት ተህዋስያን የሚያመጡት በሽታ ምክንያት የሚሞቱት ሰዎች ከፍተኛውን ቁጥር ይይዛሉ፤ በተጨማሪም እነዚህ ባክቴሪያዎች ለመድሃኒቶች በከፍተኛ ደረጃ የግትርነት ባህሪ እያሳዩ መሆኑ ችግሩን ውስብስብ አድረጎታል፡፡ ሥለሆነም ይህን በሽታ ከፍተኛ ጉዳት ከማስከተሉ በፊት በመከላከል የሕብረተሰቡን ሞት ለመቀነስ ያለውን ስርጭት እንዲሁም ለመድሃኒቶችም የለውን የግትርነት ማወቅ በጣም አስፈላጊ ነው፡፡

የጥናቱ አላማ

በአዲስ አበባ ከተማ፣ በ ጥቁር አንበሳ ሆስፒታል ውስጥ ባክቴሪያዎች በተለይም ኢንተሮባክተርሴ የሚባሉትን ለሴብቲሴሚያ በሽታ ከታከሙት ስርጭቱን ማወቅ ና ለመድሃኒት ያለውን የግትርነት ደረጃ፤ እና መንስኤዎቹን ማሳየት፡፡

ለጥናቱ ተሳታፊዎች ያለው ልዩ ጥቅም

በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለውም፡፡ ነገር ግን በጥናቱ መሰረት ተህዋስያኑ ለተገኘባቸው ተሳታፊዎች ከሚመለከታቸው የሆስፒታሉ ባለሙያዎች ጋር በመነጋገር አስፈላጊ የሆነ ህክምና እንዲደረግላቸው ይደረጋል፡፡

በጥናቱ ተሳታፊዎች ላይ ያለው ጉዳት እና ተዛማጅ ችግር

በዚህ ጥናት ላይ በመሳተፍዎ ሊደርስብዎ የሚችል አንድም ጉዳት አይኖርም፡፡ ለዚህ ጥናት የምንጠቀምበት የደም ናሙና ከክንድዎ የሚወሰድ ሲሆን መጠኑም ከ 5 ሚሊ ሊትር (ከ 1 _2 የሻይ ማንኪያ) ነው፡፡ ይህም ከመጠነኛ ስሜት በስተቀር በጤናዎ ላይ ምንም አይነት ጉዳት አያደርስም፡፡ ለሽንት ምርመራ የሚሆን ሽንት በሚያመጡበት ሰዓትም ቢሆን ምንም የተለየ ስሜት የለዎትም፡፡

የመረጃ ሚስጥራዊ አጠባበቅ

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

ያስታውሱ፤ ስለዚህ ጥናት ማንኛውም ጥያቄ ካልዎት በማንኛውም ጊዜ ከዚህ በታች በተጠቀሱት አድራሻዎች መጠየቅ ይችላሉ።
ዳንኤል በሻህ (ዋተ) ፊርማ _____ ቀን _____

የዋና ተመራማሪው አድራሻ፤

🌈 **ዳንኤል በሻህ ፤** የማይክሮብያል ሴሉላር ሞለኪዩላር ባዮሎጂ ዲፓርትመንት፣ የተፈጥሮ ሳይንስ ኮሌጅ፣ አዲስ አበባ ዩኒቨርሲቲ_ አዲስ አበባ፣ ኢትዮጵያ

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የፈቃደኝነት ማረጋገጫ ቅፅ

በአዲስ አበባ ከተማ፣ በ ጥቁር አንበሳ ሆስፒታል ውስጥ ባክቴሪያዎች በተለይም ኢንተሮባክተርሴ የሚባሉትን ለሴብቲሴሚያ በሽታ ከታከሙት ስርጭቱን ማወቅ ና ለመድሐኒት ያለውን የግትርነት ደረጃ፤ እና መንስኤዎቹን ማሳየት በሚል ርዕስ ላይ ለማጥናት በተመለከተ በሚደረገው ጥናት ላይ ለመሳተፍ መሆኔ፤ የጥናቱ ዓላማና ጥቅም ተገልጿል። በመጠይቁ ላይ የምሰጠው የእኔ ሙሉ መረጃም ሆነ የደም ናሙና ውጤቱ በሚስጥር እንደሚያዝ ተነግሮኛል። በተጨማሪም ጥናቱ ውስጥ አለመሳተፍ መብቴ እደሆነና በማንኛውም ጊዜ ከጥናቱ በራሴ ወሳኔ መወጣት እንደምችልና በዚህም ምክንያት ምንም ዓይነት መጉላላት እንደማይደርስብኝ በሚገባ ተረድቻለሁ። ስለሆነም ሁኔታውን በሚገባ በማጤን በፈቃደኝነት በምርምሩ ላይ ለመሳተፍ ለተመራማሪው ፈቃደኝነቴን ሰጥቻለሁ። በተጨማሪም የምሰጠው የደም ናሙና ለኢንተሮባክቴርሴ ባክቴሪያዎች ምርመራዎች ብቻ እንደሚወልድ ተነግሮኝ ተስማምቻለሁ። ማንኛውንም ያልገባኝን ነገር የመጠየቅ ዕድል ተሰጥቶኝ በሚገባኝ ቋንቋ መልስ አግኝቻለሁ። በተጨማሪም የሁሉም የላብራቶሪ ምርመራ ውጤቶች ለተቋሞች እንደሚሰጥና ውጤቴን ማወቅ ከፈለኩ ማግኘት እንደምችል ተነግሮኛል።

እኔ _____ የተባልኩ ግለሰብ ይህን ሁሉ በማገናዘብ ምርምሩ ላይ ስለኔ መረጃ እና የደም ናሙና ለመስጠት ተስማምቻለሁ። ፊርማ _____ ቀን _____

መረጃውን ያስረዳው አካል _____ ፊርማ _____

4. መጠይቅ

በአዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ዲፓርትመንት

ይሕ የመጠየቅ ቅጽ ስለተሳታፊዎች አጠቃላይ መረጃ ና ለበሽታው (ለኢንተሮባክተርሴዎች) መንስኤ ሊሆኑ የሚችሉትን ምክንያቶች ለማወቅ የሚደረግ መጠይቅ ነው። መጠየቁን የሚያስሞላው በጥናቱ ጊዜ የተመረጠው(ችው) ባለሙያ ሲሆን መጠየቁ የሚሞላው ጥናቱ በሚከሄድበት ቦታ ነው። ለጥናቱ መሳካት ና ትክክለኛነት ያግዘን ዘንድ ጥያቄዎችን በጥንቃቄ አንብበዉ በታማኝነት ያለምንም ፍረቻ እንዲሞሉልን በትህትና እንጠይቃለን።

የተቋሙ ስም: ጥቁር አንበሳ ስፔሻላይዜድ ሆ/ል አድራሻ (ክፍለ_ከተማ): ልደታ ክ/ከተማ

የተሳታፊው መለያ ቁጥር _____ አድራሻ (ክልል) _____ ዞን _____ ወረዳ _____

Appendix 6: Amharic Version of the Questionnaire for the Assessment of Bloodstream Infection Risk Factors

Co No	Res	Demography	Answer
San1.		የታካሚዉ ቁጥር	
Con2.		የለቦራቶሪ መዝገብ ቁጥር	
Can3.		ካርድ ቁጥር	
Age4.		እድሜ በአመት: በቀን:	1 up to 1 ወር 2 >1-<24 ወር 3 02-<06 አመት 4 06-<13 አመት 5 13-<19 አመት 6 19-<45 አመት 7 45-<65 አመት 8 65-<80 አመት 9 >80 አመት
Sex5.		ፆታ	☐ ወንድ ☐ ሴት
Cdx6.		የህመም አይነት ወይም	1.
Cdx7.		የታወቀው ህመም ስም	2.
Cdx8.			3.
Cdx9.			4.
Cd10.			5. ሌላ
Io11.		ታካሚው አይነት	☐ ተኝቶ ታካሚ ☐ ተመላላሽ ታካሚ
Wa12.		የተኛበት ክፍል	1 EOPD 2 C/W 3 EOPD 4 RT 5 R/C 6 CTR 7 InM 8 C3 9 D3 10 B4 11 C4 12 D4 13 B5 14 C5 15 D5 16 A6 17 B6 18 C6 19 D6 20 B7 21 C7 22 D7 23 B8 24 C8 25 D8
De13.		የሚታከምበት ዲፓርትመንት	1 Emergenc 2 In Medic 3 MICU 4 NICU 5 Surgery 6 SICU 7 Obs &Gyn 8 Gyne 9 Onco 10 Hemato 11 Ortho 12 Derma 13 ENT 14 ሌላ

		ጥያቄዎች ከ 1 ወር በላይ	መልስ
Qu14.		ተኝቶ ታካሚ ከሆኑ ለምን ያህል ቀን ተኝተው ነበር? ☐ 1-2 ቀናት፤ ☐ 3-4 ቀናት፤ ☐ 5-6 ቀናት ፤ ☐ ≥ 7 ቀናት	_____ ቀናት
Qu15.		ተኝቶ ታካሚ ከሆኑ የፅኑ ህመማን ክፍል ተኝተው ነበር? (ተኝተው ላሉ ታካሚዎች)	☐ አዎ ☐ አይደለም
Qu16.		ከስንት ቀን በፊት? _____	_____ ቀናት
Qu17.		ተመላላሽ ታካሚ ከሆኑ ከዚህ በፊት ሆስፒታል ተኝተው ነበር?	☐ አዎ ☐ አይደለም
Qu18.		ከስንት ቀን/ወር/አመት በፊት? _____	_____ ቀናት
Qu19.		ቁትር 11 አዎ ከሆነ አይ ሲዩ ህክምና ክፍል ተኝተው ነበር? (ለተመላላሽ ታካሚዎች)	☐ አዎ ☐ አይደለም
Qu20.		ከስንት ቀን/ወር/አመት በፊት? _____	_____ ቀናት
Qu21.		ምርመራው ሲታዘዝ የታካሚው የሙቀት መጠን ስንት ነበር?	_____ °C
Qu22.		በትኩሳቱ መጨመር የተጠረጠረው የህመም ☐ ከባክቴሪያ የሚመጣ ☐ ከወባ የሚመጣ ☐ ሌላ በሽታ አይነት ምንድን ነው? ከሆነ ጥቀስ _____	
Qu23.		ትኩሳት ፤ ☐	☐ አዎ ☐ አይደለም
Qu24.		የማላብ ምልክት ነበርዎት?	☐ አዎ ☐ አይደለም
Qu25.		የማንቀጥቀጥ ምልክት ነበርዎት?	☐ አዎ ☐ አይደለም
Qu26.		የማስመለስ ምልክት ነበርዎት?	☐ አዎ ☐ አይደለም
Qu27.		የልብ ምት መጨመር ምልክት ነበርዎት?	☐ አዎ ☐ አይደለም
Qu28.		በ አስር ቀን ጊዜ ውስጥ መድሃኒት ተጠቅመው ያውቃሉ?(ቀኑን በቁጥር ግለፅ)	☐ አዎ ☐ አይደለም
Qu29.	ከተጠቀሙ ምን አይነት መድሃኒት ነበር?	1.	
Qu30.		2.	
Qu31.		3.	
Qu32.		4.	
Qu33.		ከአስር ቀን በፊት መድሃኒት ተጠቅመው ያውቃሉ?(ቀኑን በቁጥር ግለፅ)	☐ አዎ ☐ አይደለም
Qu34.	ከተጠቀሙ ምን አይነት መድሃኒት ነበር?	1.	
Qu35.		2.	
Qu36.		3.	
Qu37.		4.	
Qu38.		የህክምና መሳሪያ በሰውነቶች ላይ ተገጥሞሎት ያውቃል?	☐ አዎ ☐ አይደለም
Qu39.		የመተንፈሻ መርጃ መሳሪያ?	☐ አዎ ☐ አይደለም
Qu40.		በደን ውስጥ የሚቀበር ቱቦ?	☐ አዎ ☐ አይደለም
Qu41.		ብልት ውስጥ የሚቀበር የመሽኛ ቱቦ?	☐ አዎ ☐ አይደለም
Qu42.		ከአፍና ጉሮሮ ፈሳሽ መምጠጫ ማሽን	☐ አዎ ☐ አይደለም
Qu43.		ጉሮሮ ላይ የሚቀበር የመተንፈሻ ቱቦ?	☐ አዎ ☐ አይደለም
Qu44.		በደምስር የሚወሰድ መድሃኒት ቱቦ?	☐ አዎ ☐ አይደለም
Qu45.		ከሰውነት ውስጥ ፈሳሽ ማስወገጃ ቱቦ?	☐ አዎ ☐ አይደለም
Qu46.		የጭንቅላት ፈሳሽ ማስወገጃ ቱቦ?	☐ አዎ ☐ አይደለም

Qu47.	በጉሮሮ የሚገባ መመገቢያ ቱቦ?	☐ አዎ ☐ አይደለም
Qu48.	ሌላ ካለ የግለፅ _____	
	ከአንድ ወር በላይ ለሆኑ ታካሚዎች	
Qu49.	የቆየ ህመም አለቦት?	☐ አዎ ☐ አይደለም
Qu50.	የኤች አይቪ ህመም አለብዎት?	☐ አዎ ☐ አይደለም
Qu51.	የስኳር በሽታ አለቦት?	☐ አዎ ☐ አይደለም
Qu52.	የደም ግፊት ህመም አለብዎት?	☐ አዎ ☐ አይደለም
Qu53.	ቀዶ ጥገና ተሰርቶሎታል?	☐ አዎ ☐ አይደለም
Qu54.	ቆዳ ላይ ያለ ቁስል አለቦት?	☐ አዎ ☐ አይደለም
Qu55.	የአንጀት ህመም አለብዎት?	☐ አዎ ☐ አይደለም
Qu56.	የሽንት መሽኛ ኢንፌክሽን አለቦት?	☐ አዎ ☐ አይደለም
Qu57.	የመተንፈሻ አካል ኢንፌክሽን አለቦት?	☐ አዎ ☐ አይደለም
Qu58.	የልብ ህመም አለብዎት?	☐ አዎ ☐ አይደለም
Qu59.	የልብ ቱቦ ህመም አለብዎት?	☐ አዎ ☐ አይደለም
Qu60.	ከደም ግፊት የተነሳ ደም በጭንቅላት መፍሰስ ህመም አለብዎት?	☐ አዎ ☐ አይደለም
Qu61.	የጋንግሪን ህመም አለብዎት?	☐ አዎ ☐ አይደለም
Qu62.	የሳንባ ህመም አለብዎት?	☐ አዎ ☐ አይደለም
Qu63.	የጉበት ህመም አለብዎት?	☐ አዎ ☐ አይደለም
Qu64.	የኩላሊት መድከም ህመም አለቦት?	☐ አዎ ☐ አይደለም
Qu65.	የደም ካንሰር ህመም አለቦት?	☐ አዎ ☐ አይደለም
Qu66.	የካንሰር ህመም አለቦት?	☐ አዎ ☐ አይደለም
Qu67.	ሌላ ካለ የግለፅ _____	
	ለጨቅላ ህፃናት ከ 30ቀን በታች	
Qu68.	የህፃኑ ቤተሰብ የት/ት ደረጃ ?	☐ ህፃን አይደለም ☐ ያልተማሩ ☐ መፃፍና ማንበብ ☐ አንደኛ ደረጃ ☐ ሁለተኛ ደረጃ ☐ ከፍተኛ ት/ት
Qu69.	የተወለደበት ቦታ የት ነው?	☐ ሆስፒታል ☐ በመኖሪ ቤት
Qu70.	ህፃኑ ማሞቂያ ክፍል ገብቶ ታከሞአል?	☐ አዎ ☐ አይደለም
Qu71.	የአወላለድ አይነት እንዴት ነው?	☐ በማህፀን ☐ በቀዶ ጥገና
Qu72.	የእርግዝና ጊዜ እርዝመት ስንት ነው?	☐ >37ሳምንት ☐ <37 ሳምንት
Qu73.	ያለ ሰአቱ የፅንሰ መሸፈኛ መቀደድ (Premature rupture of membrane)?	☐ >18 ሰአት ☐ <18 ሰአት ☐ አይደለም በቀዶ ጥገና የተዋለደ
Qu74.	የህፃኑ ክብደት ስንት ነው?	_____ ግራም
Qu75.	እናትየው የኢንፌክሽን ምልክት ነበራት?	☐ አዎ ☐ አይደለም

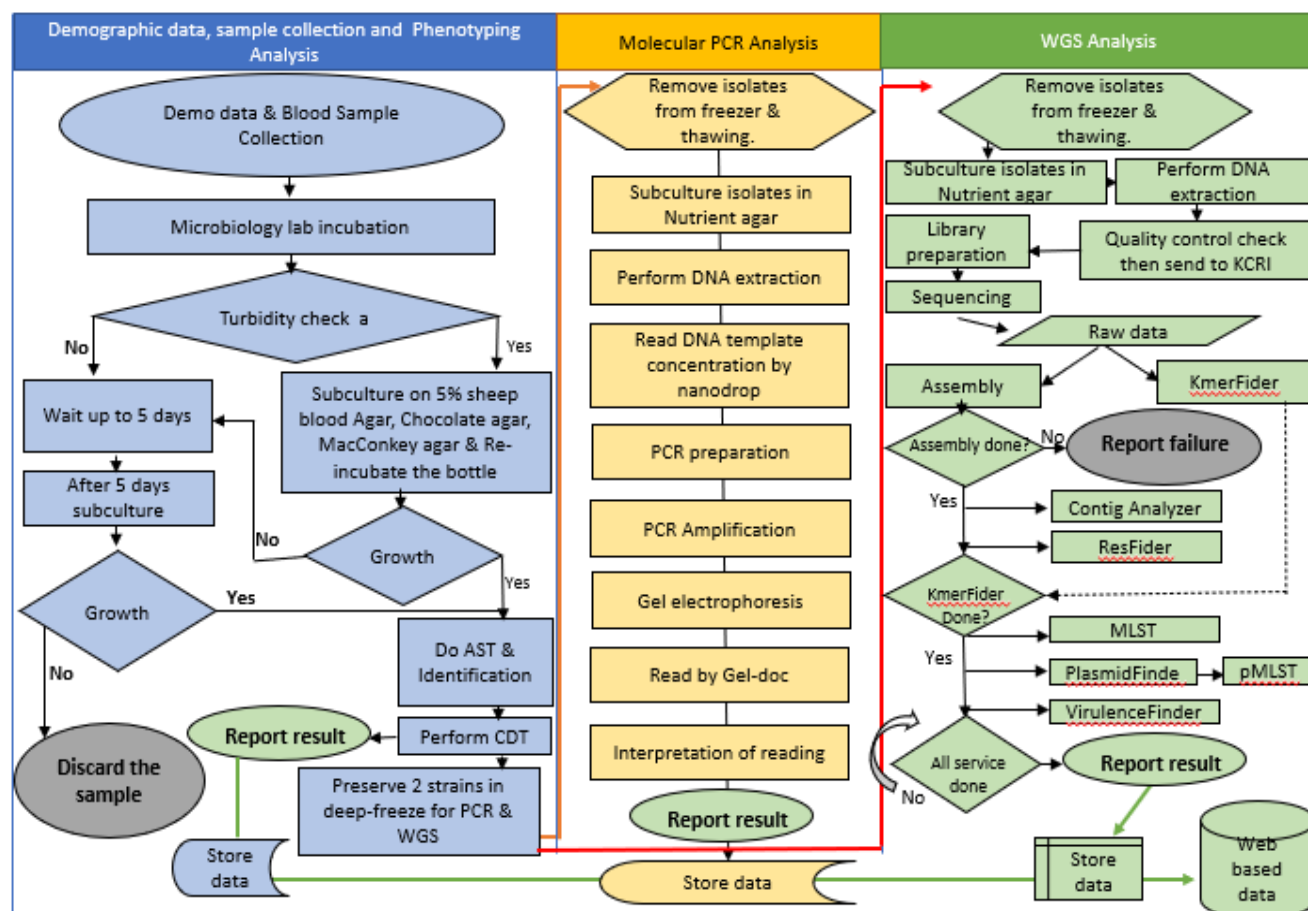
መረጃ ሰብሳቢ _____ ቀን _____ ፊርማ _____

Appendix 7: Laboratory AST Result in Recording Format

No	Date	Code Number	Card no	Age	Sex	War	Strain reserved	Strain identified	Drug resistance type	Amoxicillin+	Ampicillin	Pepracillin	Amikacin	Ampicillin	Aztreonam	Cefalotin	Cefazolin	Cefepime	Cefixime	Cefotaxime	Cefotetan	Cefoxitin	Ceftazidime

[illegible]

Appendix 9: The Workflow of Specimen Collection, Culture and Sensitivity, and Molecular Characterization and WGS of Drug-Resistant Gram-Negative Bacteria.



Appendix 10: Summary of PCR-Confirmed Drug Resistance Genes

Phenotyping and CDT				Genotyping PCR				
Isolate of Gram-negative bacteria	#	Enzyme producer for drug resistance	# Drug resistance type	Drug resistance enzyme producer gene identification	# Drug resistance type	# Gene	# Not Drug resistance type	Bac. have drug resistance type
<i>K. pneumoniae</i>	4	No	0	0	0	0	4	0
	1	CPs	1	0	0	0	1	0
	1	AmpC+CPs	2	CIT+FOX+OXA-48+NDM	3	4	0	1
	1	AmpC+MBL	2	CIT+FOX+KPC+NDM	3	4	0	1
	1	AmpC+MBL	2	FOX+NDM	2	2	0	1
	1	CPs	1	CIT+FOX+KPC+NDM+GIM+SIM	3	6	0	1
	1	CPs	1	EBC+KPC+NDM+IMP	3	4	0	1

	2	CPs	2	KPC	2	2	0	2
	1	CPs	1	NDM+IMP	1	2	0	1
	2	CPs	2	OXA-23	2	2	0	2
	2	CPs	2	OXA-23+NDM	4	4	0	2
	2	ESBL	2	CTX-M	2	2	0	2
	1	ESBL	1	CTX-M+BEL	1	2	0	1
	1	ESBL	1	GES	1	1	0	1
	3	ESBL	3	TEM	3	3	0	3
	32	ESBL	32	TEM+CTX-M	32	64	0	32
	1	ESBL	1	TEM+CTX-M+BEL	1	3	0	1
	1	ESBL	1	TEM+CTX-M+BEL+GES	1	4	0	1
	6	ESBL	6	TEM+CTX-M+SHV	6	18	0	6
	1	ESBL	1	TEM+CTX-M+VEB+BEL	1	4	0	1
	1	ESBL	1	VEB	1	1	0	1
	2	ESBL+Amp	4	TEM+CTX-M	2	4	0	2
		C						
	1	ESBL+Amp	2	TEM+CTX-M+SHV+BEL	1	5	0	1
		C		+GES				
	1	ESBL+Amp	2	TEM+CTX-M+SHV+BEL	2	7	0	1
		C		+GES+MOX+EBC				
	1	ESBL+Amp	3	TEM+CTX-M+CIT+FOX	3	5	0	1
		C + CPs		+NDM				
	1	ESBL+CPs	2	TEM+CTX-M+NDM	2	3	0	1
	1	ESBL+CPs	2	TEM+CTX-M+OXA-23+	3	5	0	1
				NDM+ IMP				
	1	MBL	1	KPC+NDM	2	2	0	1
	1	MBL	1	NDM	1	1	0	1
Total	75		82		88	164	5	70
				Average and % frequency	1.3	2.34	6.7%	93.3%
<i>K. oxytoca</i>	5	No	0	0	0	0	5	0
	1	ESBL+Amp	2	0	0	0	1	0
		C						
	1	ESBL	1	CTX-M	1	1	0	1
	1	ESBL	1	CTX-M+SHV	1	2	0	1
	1	CRE	1	KPC	1	1	0	1
	2	ESBL	2	TEM	2	2	0	2
	11	ESBL	11	TEM+CTX-M	11	22	0	11
	1	ESBL+Amp	2	TEM+CTX-M	1	2	0	1
		C						
	1	ESBL+Amp	3	TEM+CTX-M+ACC+OXA-48	4	7	0	1
		C +CPs		+OXA-23+VIM+SIM				
	2	ESBL	2	TEM+CTX-M+BEL	2	6	0	2
	1	ESBL	1	TEM+CTX-M+GES	1	3	0	1
Total	27		26		24	46	6	21
				Average and % frequency	1.14	2.19	22.2%	77.8%
	1	ESBL+CPs	2	TEM+CTX-M+KPC	2	3	0	1

<i>K. ozaenae</i>	1	ESBL+Amp C	2	TEM+CTX-M+MOX+ACC+FOX	2	5	0	1
	1	ESBL+Amp C	2	TEM+SHV+MOX+EBC	2	4	0	1
Total	3		6		6	12	0	3
				Average and % frequency	2	4	0%	100%
<i>K. rhinoscleromatis</i>	2	No	0	0	0	0	2	0
	1	MBL	1	OXA-58+NDM	2	2	0	1
	1	CPs	1	OXA-23	1	1	0	1
Total	4		2		3	3	2	2
				Average and % frequency	1.5	1.5	50%	50%
<i>Acinetobacter spp</i>	9	No	0	0	0	0	9	0
	1	AmpC	1	0	0	0	1	0
	1	CPs	1	0	0	0	1	0
	2	ESBL+Amp C	4	0	0	0	2	0
	1	ESBL+CPs	2	0	0	0	1	0
	1	ESBL+MBL	2	0	0	0	1	0
	1	CPs	1	CIT+FOX+VIM	2	3	0	1
	1	CPs	1	KPC+OXA-48+OXA-23+NDM +VIM	2	5	0	1
	1	CPs	1	NDM	1	1	0	1
	1	CPs	1	NDM+VIM	1	2	0	1
	2	CPs	2	OXA-23	2	2	0	2
	2	CPs	2	OXA-23+NDM+VIM	4	6	0	2
	1	CPs	1	OXA-23+OXA-58+VIM	2	3	0	1
	8	CPs	8	OXA-23+VIM	16	16	0	8
	1	CPs	1	OXA-24/143+GIM	2	2	0	1
	1	CPs	1	OXA-58+VIM+AIM+SIM	2	4	0	1
	1	CPs	1	VIM	1	1	0	1
	1	ESBL	1	BEL	1	1	0	1
	1	ESBL	1	CTX-M	1	1	0	1
	1	ESBL+Amp C	2	BEL	1	1	0	1
	1	ESBL+Amp C	2	BEL+MOX	2	2	0	1
	1	ESBL+Amp C	2	CTX-M+GES+DHA	2	3	0	1
	1	ESBL+Amp C	2	VEB+BEL	1	2	0	1
	1	ESBL+Amp C +CPs	3	BEL+GES+OXA-48	2	3	0	1
	1	ESBL+CPs	2	VEB	1	1	0	1
	1	MBL	1	NDM	1	1	0	1
	1	MBL	1	OXA-23+NDM+VIM	2	3	0	1
	1	MBL	1	OXA-23+OXA-58+VIM+GIM	2	4	0	1
	1	MBL	1	OXA-58+NDM	2	2	0	1
Total	47		49		53	69	15	32

				Average and % frequency	1.66	2.16	31.9%	68.1%
<i>E. coli</i>	11	No	0	0	0	0	11	0
	3	ESBL	3	0	0	0	3	0
	1	AmpC+MBL	2	CIT+EBC+NDM	2	3	0	1
	1	AmpC	1	TEM+CTX-M+MOX+ACC	2	4	0	1
	1	AmpC+CPs	2	CIT+FOX+AIM	2	3	0	1
	1	CRE	1	NDM+VIM+IMP	1	3	0	1
	1	CRE	1	OXA-23+VIM	2	2	0	1
	5	ESBL	5	CTX-M	5	5	0	5
	5	ESBL	5	TEM+CTX-M	5	10	0	5
	1	ESBL	1	TEM+CTX-M+BEL+GES	1	4	0	1
	1	ESBL	1	VEB+BEL+GES	1	3	0	1
	1	ESBL+Amp C	2	CTX-M	1	1	0	1
	2	ESBL+Amp C	4	TEM+BEL+GES+PER+CIT+FOX	4	12	0	2
	1	ESBL+Amp C	2	TEM+CTX-M+BEL+CIT+FOX	2	5	0	1
	1	ESBL+Amp C	2	TEM+CTX-M+CIT+FOX	2	4	0	1
	1	ESBL+Amp C+CPs	3	CTX-M+CIT+FOX+KPC	3	4	0	1
	1	MBL	1	OXA-58+NDM+VIM	2	3	0	1
Total	38		36		35	66	14	24
				Average and % frequency	1.5	2.75	36.8%	63.2%
<i>E. coli</i>	2	No	0	0	0	0	2	0
	1	CRE	1	OXA-48+OXA-23+GIM	2	3	0	1
	5	ESBL	5	TEM+CTX-M	5	10	0	5
	1	ESBL	1	TEM+CTX-M+BEL	1	3	0	1
	1	ESBL	1	TEM+CTX-M+VEB+BEL	1	4	0	1
	1	MBL	1	NDM+AIM	1	2	0	1
Total	11		9		10	22	2	9
				Average and % frequency		2.4	18.2%	81.8%
<i>Pseudomonas spp</i>	1	No	0	0	0	0	1	0
	1	CPs	1	0	0	0	1	0
	1	ESBL+Amp C	2	BEL	1	1	0	1
	1	ESBL+Amp C+CPs	3	TEM+CTX-M+SHV+GES+IMP	2	5	0	1
	1	CRE	1	AIM	1	1	0	1
Total	5		7		4	7	2	3
				Average and % f	1.3	2.3	40%	60%
<i>S. marcescens</i>	1	ESBL	1	0	0	0	1	0
	1	CPs	1	VIM	1	1	0	1
	1	CPs	1	OXA-23+VIM	2	2	0	1

	1	ESBL+Amp C	2	TEM+CTX-M+EBC	2	3	0	1
Total	4		5		5	6	1	3
				Average and % f	1.7	2	25%	75%
<i>P. rettgeri</i>	1	ESBL	1	TEM+CTX-M	1	2	0	1
	1	ESBL	1	TEM+CTX-M+VEB	1	3	0	1
Total	2		2		2	5	0	2
				Average and % f	1	2.5	0%	100%
<i>M. morganii</i>	1	No	0	0	0	0	1	0
	1	CRE	1	OXA-58+NDM	2	2	0	1
Total	2		1		2	2	1	1
				Average and % f			50%	50%
<i>P. mirabilis</i>	1	No	0	0	0	0	1	0
	1	ESBL+CPs	2	CTX-M+SHV+OXA-48+OXA-23	2	4	0	1
Total	2		2		2	4	1	1
				Average and % f	2	4	50%	50%
<i>Citrobacter diversus</i>	3	No	0	0	0	0	3	0
	2	ESBL	2	TEM+CTX-M	2	4	0	2
Total	5		2		2	4	3	2
				Average and % f	1	2	60%	40%
<i>E. agglomerans</i>	1	No	0	0	0	0	1	0
	1	ESBL	1	TEM+CTX-M	1	2	0	1
Total	2		1		1	2	1	1
				Average and % f	1	2	50%	50%
<i>E. cloacae</i>	1	No	0	0	0	0	1	0
	1	CRE	1	OXA-23+VIM	2	2	0	1
Total	2		1		2	2	1	1
				Average and % f	1	2	50%	50%
<i>E. aerogenes</i>	1	ESBL+CPs	2	VEB+OXA-48	2	2	0	1
Total	1		2		2	2	0	1
<i>S. typhi</i>	1	No	0	0	0	0	1	0
Total	1		0		0	0	1	0

Appendix 11: Quality Parameters Reference Range of The WGS Data from Bloodstream Infection Patients At TASH.

Quality score	Failed	Mixed spp	Bad	Acceptable	Good	Excellent
Checkm_ complete			<90%	>90%	98%	99.50%
Checkm_ contamination		>10%/>5%			< 2%	< 0.5%
Gunc_css		>0.45				
Contam_ human			>4%	<4%		
Contam_ vector			>4%	<4%		

Nucleotide read	<200,000				
Total nucleotide read					
Calculating phred quality scores					
Number of contigs in the assembly	>3500	>1000	<1000	< 480	< 160
Total number of contigs					
n1	>5000				
N50 (Sequence length in base pair)	<2000	<5000	>15 000	>15000	>30000
L50 (number of length)					
Avg_dp (Aver depth of coverage)	<15x				
q30_dp (quality and depth)	≥ 50%				
Reference length					
pct_gc (% G-C content)	30.8% to 70.90%				
Quality indicator	0	1	2	3	4
					5

Appendix 12: Quality Parameter of the WGS Data from Bloodstream Infection Patients at TASH.

Quality assessment result of all gram-negative bacteria	Median	Mean	Standard Deviation	Minimum	Maximum
Checkm _ complete	99.97	99.74	0.54	96.35	100.00
Checkm _ contamination	0.62	0.70	0.85	0.03	8.82
Gunc_css (lineage homogeneity)	0.00	0.12	0.26	0.00	1.00
Contamination with human	0.10	0.15	0.08	0.10	0.50
Contamination with vector	0.70	0.85	0.63	0.10	3.30
Nucleotide read	3935600	4028944	1354010	1425450	9757236
Total nucleotide read	586769178	597403045	200579590	211396814	1434240980
Calculating Phred Quality Scores	88.6	88.4	1.6	75.7	90.9
Number of contigs in the assembly	133	199	182	39	965
Total number of contigs	5082870	4990804	730399	3440462	6438312
N1	302511	321818	160966	59145	1159173
N50 (Sequence length in base pair)	104028	113752	70480	10460	368285
L50 (number of length)	15	24	23	5	127
Avg_dp (Average depth of coverage)	115	123	48	37	360
q30_dp (quality and depth)	102	109	43	33	316
pct_gc (% G-C content)	51.0	50.7	7.4	36.9	66.8

Appendix 13: The Summary Statistics of The Assembled Draft Genomes of Gram-Negative Bacteria Isolates.

MLST_ species		checkm_ complete	checkm_ contami	gunc_ css	contam_ human	contam_ vector	Nucleotide read	T. nucleotide read	Calcul. Phred	Number of contigs	Total Number of contigs	N1	N50	L50	avg_dp	q30_dp	ref_len	pct_gc
<i>A. baumannii</i>	Mean	99.93	0.51	0.03	0.13	0.27	4358872	645968164	89.1	97.4	3932646	323000	130755	12.8	164.1	146.3	3950040	39.2
	Median	99.98	0.63	0.00	0.10	0.30	4046338	604289339	89.0	94.0	3979338	288508	104028	13.0	161.0	142.0	3983069	38.9
	Std Devia	0.14	0.32	0.16	0.04	0.13	1516556	225916780	1.0	32.8	145816	124815	80985	5.0	56.2	50.1	176420	0.9
	Minimum	99.59	0.05	0.00	0.10	0.10	1553906	229263653	87.3	39.0	3440462	139851	52525	5.0	59.0	52.0	3198423	38.6
	Maximum	100.00	1.57	0.94	0.20	0.60	9757236	1434240980	90.9	189.0	4128817	762860	367993	23.0	360.0	316.0	4149444	42.6
<i>E. cloacae</i>	Mean	99.01	0.94	0.46	0.12	0.81	3440636	510507241	88.8	308.4	4801816	333014	93542	39.2	106.7	94.9	4887094	55.1
	Median	99.47	0.90	0.70	0.10	0.80	3219258	478685892	88.8	133.0	4765461	315619	88042	17.0	95.0	83.0	4876999	55.2
	Std Devia	1.15	0.61	0.44	0.04	0.26	1539111	225954931	1.0	305.6	151064	240060	76953	42.0	48.5	43.5	123235	0.3
	Minimum	96.39	0.14	0.00	0.10	0.40	1786424	265248532	87.2	59.0	4569639	79766	10460	7.0	56.0	49.0	4661849	54.6
	Maximum	99.97	1.83	0.89	0.20	1.20	6759002	991951212	90.2	835.0	5054144	752236	251354	127.0	212.0	188.0	5140725	55.5
<i>E. coli</i>	Mean	99.86	0.39	0.21	0.23	1.76	3945645	585917423	88.3	142.5	5049692	365000	137131	14.5	116.1	102.6	4956513	50.6
	Median	99.93	0.33	0.13	0.20	1.70	3983895	592496321	88.7	137.0	5037184	358074	133320	13.0	113.0	100.5	4891769	50.7
	Std Devia	0.13	0.30	0.28	0.10	0.47	1324163	196046163	2.5	49.8	225405	124652	49466	6.3	39.1	35.2	216288	0.2
	Minimum	99.67	0.07	0.01	0.10	1.00	1838886	272442205	75.7	82.0	4619307	125328	41529	7.0	55.0	48.0	4541147	50.1
	Maximum	99.97	1.05	1.00	0.50	3.30	8188636	1207363553	90.4	347.0	5500912	696085	224194	38.0	243.0	215.0	5434745	51.0
<i>K. oxytoca</i>	Mean	99.73	1.90	0.08	0.13	0.68	4122133	607875148	88.4	277.8	6143467	335208	95494	28.4	98.9	87.5	5828558	55.2
	Median	99.99	1.32	0.00	0.10	0.70	3950834	587924246	88.2	167.0	6160501	370565	98586	20.0	95.0	85.0	5845628	55.1
	Std Devia	0.46	2.39	0.12	0.05	0.13	967475	144448134	1.2	204.1	235960	155168	47722	21.5	23.3	20.9	236691	0.3
	Minimum	98.66	0.26	0.00	0.10	0.50	2122254	315799816	86.7	86.0	5810144	121145	24855	10.0	49.0	43.0	5508933	54.9
	Maximum	100.00	8.82	0.31	0.20	0.90	5646100	828037871	89.9	667.0	6438312	625178	161880	69.0	135.0	121.0	6149586	55.8
<i>K. pneumoniae</i>	Mean	99.63	0.74	0.06	0.11	0.68	4000450	593358946	88.0	274.4	5549217	268976	88976	33.2	107.1	94.3	5424630	57.0
	Median	99.85	0.68	0.00	0.10	0.70	3904024	579697946	87.7	164.0	5562123	272385	77897	23.0	105.0	92.0	5430436	57.1
	Std Devia	0.66	0.45	0.24	0.03	0.20	1333579	197010006	1.1	223.8	173787	127088	59479	26.6	35.8	31.8	145345	0.3
	Minimum	96.35	0.10	0.00	0.10	0.30	1425450	211396814	84.9	65.0	5069178	90954	10709	8.0	37.0	33.0	5159815	55.8
	Maximum	100.00	2.85	1.00	0.20	1.10	9471932	1409554143	90.1	965.0	6003203	531948	216350	126.0	255.0	227.0	5803733	57.4
<i>P. monteilii</i>	Mean	99.74	2.94	0.00	0.20	0.10	4976728	739030559	87.7	357.0	5963878	190291	31674	52.0	124.0	109.0	5801682	62.1

	Median	99.74	2.94	0.00	0.20	0.10	4976728	739030559	87.7	357.0	5963878	190291	31674	52.0	124.0	109.0	5801682	62.1
	Minimum	99.74	2.94	0.00	0.20	0.10	4976728	739030559	87.7	357.0	5963878	190291	31674	52.0	124.0	109.0	5801682	62.1
	Maximum	99.74	2.94	0.00	0.20	0.10	4976728	739030559	87.7	357.0	5963878	190291	31674	52.0	124.0	109.0	5801682	62.1
<i>S. maltophilia</i>	Mean	99.80	0.24	0.00	0.45	0.30	2908412	432189973	86.1	522.5	4508457	81316	15032	87.5	95.5	82.5	4578253	66.6
	Median	99.80	0.24	0.00	0.45	0.30	2908412	432189973	86.1	522.5	4508457	81316	15032	87.5	95.5	82.5	4578253	66.6
	Std Devia	0.29	0.14	0.00	0.07	0.00	687681	103361861	0.4	62.9	42563	31355	2948	16.3	21.9	19.1	20329	0.4
	Minimum	99.59	0.14	0.00	0.40	0.30	2422148	359102100	85.8	478.0	4478360	59145	12947	76.0	80.0	69.0	4563878	66.3
	Maximum	100.00	0.34	0.00	0.50	0.30	3394676	505277846	86.4	567.0	4538553	103487	17116	99.0	111.0	96.0	4592627	66.8
<i>P. rettgeri</i>	Mean	100.00	0.03	0.32	0.10	0.50	3361948	502206044	89.5	72.0	4841734	686766	368285	5.0	104.0	93.0	4050173	54.9
	Median	100.00	0.03	0.32	0.10	0.50	3361948	502206044	89.5	72.0	4841734	686766	368285	5.0	104.0	93.0	4050173	54.9
	Minimum	100.00	0.03	0.32	0.10	0.50	3361948	502206044	89.5	72.0	4841734	686766	368285	5.0	104.0	93.0	4050173	54.9
	Maximum	100.00	0.03	0.32	0.10	0.50	3361948	502206044	89.5	72.0	4841734	686766	368285	5.0	104.0	93.0	4050173	54.9
Chryseoba- cterium shandongense	Mean	100.00	0.49	0.00	0.20	0.20	3193612	475942342	88.6	66.0	4029238	265999	99665	14.0	118.0	105.0	4390955	36.9
	Median	100.00	0.49	0.00	0.20	0.20	3193612	475942342	88.6	66.0	4029238	265999	99665	14.0	118.0	105.0	4390955	36.9
	Minimum	100.00	0.49	0.00	0.20	0.20	3193612	475942342	88.6	66.0	4029238	265999	99665	14.0	118.0	105.0	4390955	36.9
	Maximum	100.00	0.49	0.00	0.20	0.20	3193612	475942342	88.6	66.0	4029238	265999	99665	14.0	118.0	105.0	4390955	36.9

Key:

Appendix 14: Summary of AMR Gene and Virulent Gene in Carbapenem Resistance Gram-Negative Bacteria

Department	Isolate		Carbapenem-resistant			AMR gene				Virulence Gene			
	N	%	isolate	N	%	N	%	Total	%	All	%	Total	
NICU	12	24%	<i>A. baumannii</i>	8	16.0%	87	15.8%			0	0.0%		
			<i>K. pneumoniae</i>	4	8.0%	55	10.0%	142	25.8%	24	9.1%	24	9.1%
Hematology	12	24%	<i>A. baumannii</i>	5	10.0%	28	5.1%			0	0.0%		
			<i>E. coli</i>	4	8.0%	30	5.5%			75	28.3%		
			<i>K. pneumoniae</i>	2	4.0%	50	9.1%			11	4.2%		
			<i>S. maltophilia</i>	1	2.0%	2	0.4%	110	20.0%	0	0.0%	86	32.5%
Medical	11	22%	<i>A. baumannii</i>	6	12.0%	60	10.9%			16	6.0%		
			<i>E. coli</i>	2	4.0%	34	6.2%			32	12.1%		
			<i>K. pneumoniae</i>	2	4.0%	21	3.8%			13	4.9%	61	23.0%
			<i>P. putida</i>	1	2.0%	0	0.0%	115	20.9%				
MICU	3	6%	<i>A. baumannii</i>	1	2.0%	0	0%			0	0.0%		
			<i>E. coli</i>	2	4.0%	30	5.5%	30	5.50%	76	28.7%	76	28.7%
SICU	7	14%	<i>A. baumannii</i>	6	12.0%	0	0%			0	0.0%		
			<i>K. pneumoniae</i>	1	2.0%	86	15.6%	86	15.60%	7	2.6%	7	2.6%
G. surgery	4	8%	<i>A. baumannii</i>	2	4.0%	0	0%			0	0.0%		
			<i>K. pneumoniae</i>	2	4.0%	59	10.7%	59	10.7%	8	3.0%	8	3.0%
Orthopedics	1	2%	<i>E. cloacae</i>	1	2.0%	8	1.5%	8	1.5%	3	1.1%	3	1.1%
All				50	100%	550	100%	550	100%	265	100%	265	100%
Ward	Isolate		Carbapenem-resistant			AMR gene				Vir gene			
	N	%	isolate	N	%	All	%	total	%	N	%	Total	%
B4	10	20.0%	<i>A. baumannii</i>	7	14.0%	83	15.1%			0	0.0%		
			<i>E. coli</i>	2	4.0%	18	3.3%			76	28.7%		
			<i>K. pneumoniae</i>	1	2.0%	15	2.7%	116	21.1%	7	2.6%	83	31.3%
B5	2	4.0%	<i>E. coli</i>	1	2.0%	10	1.8%			23	8.7%		
			<i>K. pneumoniae</i>	1	2.0%	18	3.3%	28	5.1%	5	1.9%	28	10.6%
B6	12	24.0%	<i>A. baumannii</i>	8	16.0%	87	15.8%			0	0.0%		
			<i>K. pneumoniae</i>	4	8.0%	55	10.0%	142	25.8%	24	9.1%	24	9.1%
B7	5	10.0%	<i>A. baumannii</i>	3	6.0%	37	6.7%			16	6.0%		
			<i>K. pneumoniae</i>	2	4.0%	38	6.9%	75	13.6%	11	4.2%	27	10.2%
B8	1	2.0%	<i>A. baumannii</i>	1	2.0%	6	1.1%	6	1.1%	0	0.0%	0	
C7	1	2.0%	<i>A. baumannii</i>	1	2.0%	10	1.8%	10	1.8%	0	0.0%	0	
C8	1	2.0%	<i>E. coli</i>	1	2.0%	10	1.8%	10	1.8%	14	5.3%	14	5.3%
C/W	8	16.0%	<i>A. baumannii</i>	2	4.0%	14	2.5%			0	0.0%		
			<i>E. cloacae</i>	1	2.0%	8	1.5%			3	1.1%		
			<i>E. coli</i>	2	4.0%	21	3.8%			32	12.1%		
			<i>K. pneumoniae</i>	2	4.0%	28	5.1%			12	4.5%		
			<i>S. maltophilia</i>	1	2.0%	2	0.4%	73	13.3%	0	0.0%	47	17.7%
D4	1	2.0%	<i>A. baumannii</i>	1	2.0%	2	0.4%	2	0.4%	0	0.0%	0	
D7	4	8.0%	<i>A. baumannii</i>	3	6.0%	24	4.4%			0	0.0%		
			<i>K. pneumoniae</i>	1	2.0%	17	3.1%	41	7.5%	4	1.5%	4	1.5%
D8	2	4.0%	<i>E. coli</i>	2	4.0%	30	5.5%	30	5.5%	38	14.3%	38	14.3%
EOPD	3	6.0%	<i>A. baumannii</i>	2	4.0%	17	3.1%	17	3.1%	0	0.0%		

			<i>P. putida</i>	1	2.0%	0	0.0%	0		0	0.0%	0	0.0%
	50	50		50	100%					265		265	100%

Appendix 15: *Escherichia coli* virulence factors; Adhesins, Toxins, Iron Acquisition Factors (Siderophore), Lipopolysaccharides, Polysaccharide Capsules, and Invasins

No	Virulence gene	N	%	Virulence factors; Adhesins, Toxins, Iron Acquisition Factors (Siderophore), Lipopolysaccharides, Polysaccharide Capsules, and Invasins	
				Mechanisms	Additional
1	gad	36	4.70%	Adhesins	Acid resistance
2	yehA	30	3.90%	Adhesins	
3	yehB	36	4.70%	Adhesins	Pathogenicity
4	yehC	34	4.40%	Adhesins	Pathogenicity
5	yehD	36	4.70%	Adhesins	Pathogenicity
6	csgA	25	3.30%	Adhesins	Biofilm formation
7	fimH	24	3.10%	Adhesins	Biofilm formation
8	hra	17	2.20%	Adhesins	heat-resistant agglutinin, adhesion, and biofilm formation
9	traJ	14	1.80%	Adhesins	Systemic dissemination
10	iha	12	1.60%	Adhesins	Iron acquisition
11	lpfA	12	1.60%	Adhesins	Colonization
12	papA_F43	12	1.60%	Adhesins	
13	ompT	12	1.60%	Adhesins	Invasins, Cleave and inactivate antimicrobial peptides
14	papC	6	0.80%	Adhesins	Encoding P fimbriae
15	fdeC	4	0.50%	Adhesins	
16	afaA	3	0.40%	Adhesins	Diffusely adherent. add toxins and iron acquisition system
17	afaC	3	0.40%	Adhesins	Diffusely adherent, diarrheal disease
18	afaD	3	0.40%	Adhesins	Diffusely adherent. toxins, and iron acquisition system
19	air	3	0.40%	Adhesins	Toxins
20	cea	2	0.30%	Adhesins	Toxins
21	papA_fteA_F10	2	0.30%	Adhesins	Colonization
22	acm	1	0.10%	Adhesins	Pathogenicity of <i>E. faecium</i> in endocarditis
23	afaB	1	0.10%	Adhesins	
24	afaE	1	0.10%	Adhesins	Binding and internalization
24		329			

1	iutA	23	3.00%	Iron uptake protein	Aerobactin, Invasive
2	iucC	23	3.00%	Iron chelating agent	Siderophore, Aerobactin
3	fyuA	20	2.60%	Iron acquisition factors	Siderophores
4	irp2	20	2.60%	Iron acquisition factors	Target for therapeutic interventions and antibiotic resistance
5	sitA	20	2.60%	Iron acquisition factors	Disease severity
6	chuA	12	1.60%	Iron uptake protein	Hemin uptake, Therapeutic interventions
7	iroN	1	0.10%	Iron acquisition factors	
7		119			
1	astA	3	0.40%	Toxins	encodes a heat-stable enterotoxin known as EAST-1
2	cnf1	3	0.40%	Toxins	Cytotoxicity and Adhesin
3	cvaC	1	0.10%	Toxins	
4	hha	2	0.30%	Toxins	Hemolysin/ Toxin
5	hlyA	2	0.30%	Toxins	Induce kidney inflammation & Encodes inactive precursor of hemolysin
6	hlyE	29	3.80%	Toxins	Toxin pore-forming
7	hlyF	1	0.10%	Toxins	hemolytic-uremic syndrome and uropathogenic <i>E. coli</i> (UPEC
8	vat	11	1.40%	Toxins	Secreted autotransporter toxin (Cytotoxin)
9	usp	1	0.10%	Toxins	Pathogenicity and the persistence
10	vat	1	0.10%	Toxins	Vacuolating Autotransporter Toxin
10		54			
1	iss	24	3.10%	Increased serum survival	Protection factor against phagocytosis
2	senB	9	1.20%	Increased serum survival	In the bloodstream by triggering complement-mediated killing
2		33			
3	nlpI	36	4.70%	Lipopolysaccharides	Virulence factor found in some plant pathogens.
3	traT	27	3.50%	Lipopolysaccharides	Surface exclusion protein
2		63			
1	AslA	25	3.30%	Invasins of brain	
2	cia	5	0.70%	Invasins	Invasion of host epithelial cells

3	eilA	5	0.70%	Invasins	Aggregative adherence regulator (aggR), protein dispersin, and heat-stable toxin EAST-1
4	tia	3	0.40%	Invasins	Adhesion
4		13			
1	kpsE	13	1.70%	Polysaccharide Capsules K5 gene	involved in the biosynthesis of capsular polysaccharides
2	capU	12	1.60%	Polysaccharide capsule synthesis	
3	kpsMII	2	0.30%	Polysaccharide capsule synthesis	
4	neuC	3	0.40%	Polysaccharide capsule synthesis	
5	kpsMII_K1	3	0.40%	Polysaccharide capsule synthesis	Strongly associated with neonatal meningitis
6	kpsMII_K5	7	0.90%	Polysaccharide Capsules K5	Adhesin (P fimbriae, S fimbriae) & toxins
6		27			
no	Virulence gene	N	%	Mechanisms	Additional
1	terC	36	4.70%	Not well known	Responsible for tellurite resistance
2	anr	13	1.70%	Not well known	Indirect contribution by regulating the expression of genes, Involved in anaerobic respiration
3	aamR: FN554766	12	1.60%	Not well known	Plasmid gene aggregative adherence fimbriae (AAF) and plasmid enteroaggregative <i>Escherichia coli</i> (EAEC)
4	yfcV	10	1.30%	Not well known	P fimbriae, S fimbriae, & hemolysin
5	shiB	5	0.70%	Not well known	Cause of hemolytic uremic syndrome (HUS) Shiga toxin-producing <i>E. coli</i> (STEC)
6	shiA	4	0.50%	Not well known	Which encodes several virulence factors
7	espY2: 000868321	4	0.50%	Not well known	Involved in the regulation of virulence genes, aggregative adherence regulator (aggR), protein dispersin, and heat-stable toxin EAST-1
8	nfaE	2	0.30%	Not well known	Piglets with diarrhea
9	clpK1	2	0.30%	Not well known	Stress response in bacteria
10	aap	1	0.10%	Not well known	
11	hylEfm	1	0.10%	Not well known	Hydrolyzes hyaluronic acid, found in vancomycin-resistant <i>E. faecium</i> and in HAI of <i>E. faecium</i> . biofilms, used for persistence and resistance to antibiotics.
12	mchF	1	0.10%	Not well known	Microcin H47 system

13	etsC	1	0.10%	Not well known
	13	92		
	Total	768	100%	

SUPPLEMENTARY

- Supplementary table Excel: for AMR profile, AMR gene, Virulence gene, AMR mutation, Plasmid MLST, Plasmid gene, Disinfectant resistant and Disinfectant resistant gene of 142 gram-negative bacteria from bloodstream infection.