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Polymyxin B and Tigecycline Susceptibility Profiles Among Carbapenem-Resistant Gram-Negative Bacilli Clinical Isolates at the Ethiopian Public Health Institute, Addis Ababa, Ethiopia

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This is to certify that the thesis prepared by **Bruke Gezhagene**, entitled "**Polymyxin B and Tigecycline Susceptibility Profiles Among Carbapenem-Resistant Gram-Negative Bacilli Isolates at the Ethiopian Public Health Institute, Addis Ababa, Ethiopia,"** and submitted in partial fulfillment of the requirements for a Master of Science degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology track) complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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Abbreviations

AST: Antimicrobial susceptibility test

ATCC: American Type Culture Collection

BLs/BLIs: beta-lactams/beta-lactamase inhibitors

CLSI: Clinical and Laboratory Standards Institute

CPGNB: Carbapenem-resistant Gram-negative bacilli

CRAB: Carbapenem-resistant Acinetobacter baumannii

CRE: Carbapenem-resistant Enterobacterales

CREC*:* Carbapenem-Resistant *Escherichia coli*

CRKP: Carbapenem-resistant Klebsiella pneumoniae

CRPA*:* Carbapenem-resistant Pseudomonas aeruginosa

eCIM: EDTA-carbapenem inactivation method

EEML: Ethiopian Essential Medicines List

EPHI: Ethiopian Public Health Institution

ESBL: Extended-spectrum beta-lactamase

EUCAST: European Committee on Antimicrobial Susceptibility Testing

MBL: Metallo-beta-lactamase

mCIM: Modified carbapenem inactivation method

MEM: Meropenem

MEV: Meropenem vabrobactam

MIC: Minimum inhibitory concentration

NFGNB: Non-fermenter Gram-negative bacteria/bacilli

PMB: Polymyxin B

TGC: Tigecycline

WHO: World Health Organization

Abstract

Background: Carbapenem-resistant Gram-negative bacilli (CRGNB) are a growing threat to clinical care because treatment options are increasingly limited. Polymyxin B and tigecycline remain among the few agents used against these pathogens, yet local susceptibility data in Addis Ababa, Ethiopia, are scarce.

Objective: To determine the susceptibility profiles of Polymyxin B and tigecycline among stored CRGNB isolates at the Ethiopian Public Health Institute, Addis Ababa, Ethiopia.

Methods: A A retrospective laboratory-based cross-sectional study was conducted on 130 non-duplicate stored CRGNB isolates collected from January 1, 2019, to December 28, 2023. Isolates were revived on Tryptic Soy Agar and MacConkey Agar. Carbapenem resistance was confirmed using meropenem, imipenem, and ertapenem disks according to CLSI M100, 35th edition, and EUCAST version 14 criteria. Phenotypic carbapenemase detection used the modified carbapenem inactivation method (mCIM) and EDTA-modified carbapenem inactivation method (eCIM). Polymyxin B activity was assessed by MIC testing, whereas tigecycline susceptibility was determined by disk diffusion. The data analysis was done using SPSS version 27 and pythone 13.5 version software.

Results: Among 130 isolates, carbapenem-resistant *Acinetobacter baumannii* 48 (36.9%) and carbapenem-resistant *Klebsiella pneumoniae* 33 (25.4%) predominated. Urine, pus, and blood were the main specimen sources. Of 130 isolates with complete confirmatory results, 44 (34.4%) were mCIM positive, 40 (31.3%) were eCIM positive, 3 (2.3%) showed a serine carbapenemase pattern, and 3.9% were ESBL positive. Tigecycline showed better overall activity than polymyxin B. Among 115 isolates with valid tigecycline results, 109 (93.1%) were susceptible and 8 (6.8%) were resistant. Polymyxin B demonstrated a predominantly intermediate interpretive range, with susceptibility in 120 (92.3%) and resistance observed in 10 (7.7%) of isolates. High resistance to cephalosporins,fluoroquinolones and newer β-lactam/β-lactamase inhibitors was common, most isolates met the MDR and XDR definitions.

Conclusion: CRGNB isolates were dominated by carbapenem-resistant *A. baumannii* and carbapenem-resistant *K. pneumoniae*. Tigecycline and polymyxin B retained the best in vitro activity, although polymyxin B showed a less favorable categorical profile but mostly low to moderate MIC values. Ongoing surveillance, susceptibility testing, carbapenemase detection, infection control, and antimicrobial stewardship are needed.

Keywords: Carbapenem-resistant Gram-negative bacilli, polymyxin B, tigecycline, susceptibility, Addis Ababa, Ethiopia

1. Introduction

Carbapenem-resistant Gram-negative bacilli (CR-GNB) are a clinically important group of pathogens that includes carbapenem-resistant *Enterobacterales* (CRE) like *Klebsiella pneumoniae* (CRKP) and *Escherichia coli* (CREC) and non-fermenters such as *Pseudomonas aeruginosa* (CRPA) and *Acinetobacter baumannii* (CRAB). These organisms are especially concerning because carbapenems are among the most potent β-lactam agents used for severe infection caused by multidrug-resistant Gram-negative bacteria, including extended-spectrum β-lactamases (ESBLs). Once carbapenem resistance emerges, treatment options become sharply restricted, and the risk of poor clinical outcome rises. Ethiopian studies already show that carbapenem resistance is no longer sporadic but is established in referral hospitals, intensive care settings, and national reference laboratory collections (1–4).

Carbapenems (CR), belonging to the β-lactam group alongside ertapenem, imipenem, meropenem, and doripenem, stand out as the most potent antibiotics for severe infections (5). Carbapenem resistance (CR) in Gram-negative bacilli is driven by diverse mechanisms. The most important include carbapenemase production, particularly serine beta-lactamases and metallo-beta-lactamases such as New Delhi metallo-beta-lactamase (NDM) and Verona integron-encoded metallo-β-lactamase (VIM), together with permeability defects, efflux activity, and other structural changes that reduce antibiotic entry or action. Ethiopian studies have repeatedly identified carbapenemase-associated resistance in *Enterobacterales*, while work on Acinetobacter and Pseudomonas has shown a more complex pattern involving blaNDM (New Delhi metallo-β-lactamase), blaVIM (Verona Integron-borne Metallo-β-lactamase), blaKPC-like (Klebsiella pneumoniae carbapenemase), and OXA-type (Oxacillinases) molecular class D enzymes that hydrolyze oxacillin and related penicillins (determinants 6–11). This mechanistic diversity is important because it affects both laboratory detection and likely responses to reserve antibiotics.

As carbapenem resistance increases, clinical attention shifts toward reserve agents such as polymyxin B (PMB) and tigecycline (TGC). Polymyxin B, including Polymyxin E (Colistin), acts mainly through interaction with the lipopolysaccharide-rich outer membrane of Gram-negative bacteria and is bactericidal, whereas tigecycline, a novel antibiotic of the glycylcycline class, is a bacteriostatic agent; it inhibits protein synthesis by binding to the 30S ribosomal subunit. Both drugs remain important options for difficult-to-treat infections, but neither can be assumed to retain

universal activity. Polymyxin resistance is increasingly linked to membrane modification and mobile resistance determinants such as mcr genes, while tigecycline resistance may arise through efflux mechanisms and other acquired determinants. In Ethiopia, genomic work has already detected mobile genes (*mcr-9)* among *Enterobacterales*, showing that resistance to last-resort polymyxins is no longer only a theoretical concern (8). Comparator studies also show that susceptibility to Polymyxins and tigecycline varies substantially by species and setting.

The Ethiopian Essential Medicines List (EEML), 2020, adds an important national stewardship context to this topic. The sixth edition of the EEML adopted the World Health Organization (WHO) AWaRe (Access, Watch, and Reserve) classification with local adaptation and grouped 33 prototype antibiotics into 16 access, 10 watch, and 7 reserve agents. The document states that Access antibiotics should account for at least 60% of institutional antimicrobial consumption, while Reserve agents should be protected for highly specific situations involving multidrug-resistant organisms. In the EEML-2020 AWaRe table, PMB and newer β-lactam/β-lactamase inhibitors (BLs/BLIs) like meropenem-vaborbactam and ceftazidime-avibactam are listed in the Reserve group, reinforcing their status as protected last-line antibiotics within Ethiopia's stewardship framework. This national policy context strengthens the rationale for ongoing local susceptibility surveillance and cautious use of PMB in clinical practice (12).

There is now substantial local literature on carbapenem resistance, carbapenemase production, and multidrug resistance in Gram-negative pathogens, but far less direct Ethiopian evidence on PMB and TGC susceptibility itself (1,2,8,9). In practice, this leaves clinicians and laboratories with few dependable local data on two of the most important reserve drugs for carbapenem-resistant infection. A thesis centered on Ethiopian Public Health Institute's National Bacteriological and Mycology Reference Referral Laboratory (EPHI-NCBMRL) isolates therefore has clear value for treatment decisions, antimicrobial stewardship, infection prevention, and laboratory surveillance.

1.1 Statement of the problem

Antimicrobial resistance (AMR) has become one of the most serious global public health threats and is increasingly affecting the treatment of severe bacterial infections in both high-income and low-income settings. The global burden analysis for 2019 estimated that bacterial antimicrobial resistance was associated with 4.95 million deaths and directly attributable to 1.27 million deaths worldwide, while the burden in the WHO African Region was among the highest reported globally (13, 14). In Ethiopia, the burden is also substantial, with recent regional estimates showing about 21,200 deaths directly attributable to antimicrobial resistance and about 85,300 deaths associated with drug resistant infections in 2019 (14, 15). These figures show that antimicrobial resistance is no longer only a future concern. It is already a major cause of illness, death, and pressure on health systems.

Among resistant organisms, CRGNB are of particular concern because they cause severe infections and leave very limited treatment options. These organisms include CRE, CRAB, and CRPA, all of which are associated with hospital-acquired infection, prolonged hospitalization, and high mortality (16, 17). In Ethiopia, the problem is already evident in both Enterobacterales and non-fermenters. At Jimma Medical Center, phenotypic carbapenem resistance was reported in 19.0% of Gram-negative isolates, and 69.0% of tested resistant isolates carried at least one carbapenemase gene, with blaNDM as the leading acquired gene (18). In a multicenter Ethiopian study of *Enterobacterales* from sepsis patients, reduced meropenem susceptibility was found in 9.0% of isolates, carbapenemase genes in 6.6%, and *mcr-9* in 0.7%, indicating that both carbapenem resistance and transferable Polymyxin resistance determinants are already present locally (19). In Addis Ababa, carbapenemase production was detected in 21.2% of *Klebsiella pneumoniae* isolates, and blaNDM accounted for 92.9% of gene positive isolates (20).

Treatment options for these infections are narrowing. Polymyxin B and tigecycline remain among the few agents that may retain activity against some carbapenem-resistant isolates, but their usefulness is constrained by toxicity, pharmacokinetic limitations, emerging resistance, and strong variation across organisms and settings (16). The WHO emphasizes the importance of monitoring Polymyxin resistance and other reserved antibiotics. International in vitro studies show that Polymyxin B and tigecycline do not perform uniformly. In Singapore, Polymyxin B plus tigecycline was bactericidal in only (29.0%) of carbapenem resistant *CRAB*isolates (21).

In China, dual heteroresistance to both agents was detected in 48.4% of carbapenem-resistant CRKP isolates, although the combination showed better bacterial killing than monotherapy in selected strains (22). In a multicenter clinical cohort, 48.9% of patients receiving the Polymyxin B plus tigecycline combination died within 28 days, compared with 28.3% in the Polymyxin B group, showing that the combination was not superior to appropriate Polymyxin B therapy (23). These findings indicate that the clinical effectiveness of PMB and TGC cannot be assumed and requires continuous local verification.

In Ethiopia, the evidence base remains incomplete. Most published studies describe the burden of carbapenem resistance and the distribution of carbapenemase genes, but few directly provide a dense local susceptibility profile for Polymyxin B and tigecycline in carbapenem-resistant clinical isolates (10, 18, 19). This evidence gap creates major clinical and laboratory challenges. First, clinicians are managing infections caused by organisms with very limited remaining treatment options. Second, laboratories and clinicians have insufficient local evidence to judge how active Polymyxin B and tigecycline still are against current isolates in the Ethiopian setting. This gap is made more important by laboratory methods because polymyxin susceptibility testing is technically challenging, and several studies have demonstrated poor agreement between routine methods and reference broth microdilution (24–26).

Another problem is that the arrival of newer beta-lactam beta-lactamase inhibitor combinations has not solved treatment selection in a uniform way. These drugs expanded options for selected CRE phenotypes, particularly KPC producers, but their activity depends strongly on the underlying carbapenemase genotype (27, 28). Ceftazidime avibactam (CAZ), imipenem relebactam, and meropenem vaborbactam (MEV) were all highly active against KPC producers, yet only ceftazidime avibactam remained highly active against OXA-48-like isolates, and none showed activity against metallo-beta-lactamase producers (29). In aztreonam avibactam outperformed ceftazidime avibactam against carbapenem-resistant CREC and CRKP in a setting where metallo-beta-lactamases were common (30). This means that newer agents do not make PMB and TGC irrelevant. Rather, they sharpen the need to know which resistance mechanisms and susceptibility profiles are actually present locally.

Therefore, generating local phenotypic data on the in vitro susceptibility of CRGNB to PMB and TGC is essential. Such data are needed to define the remaining therapeutic space, bridge the knowledge gap, improve laboratory reporting, support antimicrobial stewardship, and guide treatment decisions in Addis Ababa,Ethiopia and more broadly. For this reason, the present study was designed to determine the susceptibility profiles of PMB and TGC among CRGNB isolates at the Ethiopian Public Health Institute, Addis Ababa, Ethiopia.

1.2 Significance of the study

A study focused on local clinical isolates is therefore important for following significance.

- To bridge knowledge gaps regarding CR-GNB susceptibility, contributing to national and global treatment guidelines and antibiotic stewardship programs.
- To provide phenotypic data on the in vitro susceptibility of local CR-GNB strains to last-line drugs like Polymyxin B and the novel tigecycline, guiding healthcare professionals in selecting appropriate treatments.
- To offer critical baseline data on antibiotic resistance in Addis Ababa, Ethiopia, highlighting the prevalence of CR-GNB and pan-drug-resistant strains.
- To aid in identifying new treatment options and support clinicians in managing resistant infections, potentially reducing patient healthcare costs.
- To emphasize the need for enhanced surveillance, rational antibiotic use of alternative therapies and to combat rising CR-GNB organism.
- To highlight the importance of understanding local epidemiology and resistance mechanisms for developing effective treatment protocols against CR-GNB infections.
- To give current species-level susceptibility data for CRGNB isolated from routine patient care.
- To show whether PMB and TGC still remain viable laboratory options in the local setting.

2. Literature review

Carbapenems became the backbone for treatment of severe infections caused by multidrug-resistant Gram-negative bacilli because of their broad spectrum, stability against many beta-lactamases, and reliable activity in serious hospital-acquired infections (31) (32). What was once treated as a problem of isolated outbreaks is now an international epidemiologic pattern shaped by hospital transmission, clonal spread, plasmid transfer, and antibiotic selection pressure (33–35). The biology of this resistance is diverse but clinically coherent in its effect. In *Enterobacterales*, the most important carbapenemases include KPC, NDM, and OXA 48 like enzymes, with additional contribution from porin loss and efflux mechanisms (36–38).

In CRAB, OXA 23 and related oxacillinases dominate many settings, while in CRPA carbapenem resistance often reflects metallo beta lactamases together with OprD loss and efflux pump overexpression (39)(40). These mechanisms matter because they do not simply make carbapenems less active. They also shape cross resistance, alter the usefulness of companion drugs, and determine whether newer beta lactam beta lactamase inhibitor combinations have any practical value.

CRGNBs have become one of the most serious challenges in modern infectious disease care because they are associated with limited treatment options, hospital transmission, severe infection, and poor outcomes (16, 41). The epidemiology is now strongly regional. Global surveillance from ATLAS across 2018 to 2022 showed that detection of CRE increased in the Asia Pacific, Europe, Latin America, and the Middle East and Africa, while difficult-to-treat resistance increased in all regions studied (42, 43). KPC remained the dominant carbapenemase in North America and Europe, while NDM predominated in several other regions by 2022 (42, 43). Earlier global synthesis reached the same broad pattern, with KPC entrenched in the United States, Greece, Italy, and Israel, NDM strongly linked to the Indian subcontinent and surrounding spread, and OXA 48 like enzymes centered in Turkey and neighboring regions before broader international dissemination (44). This regional variation is not a background detail. It determines which isolates are likely to be encountered in a hospital and which drugs are likely to remain active.

The clinical burden is severe. Carbapenem resistance is associated with prolonged hospitalization, outbreaks in intensive care and long-term care settings, major infection control costs, and high mortality (45, 46). A meta-analysis of serious Enterobacterales infections found that carbapenem resistance was associated with substantially higher mortality than carbapenem susceptibility, with an odds ratio of 3.39 (45). Jean and colleagues also describe case fatality rates above 50 % for carbapenem resistant CRAB in critically ill patients and high fatality rates for CRPA as well (47). The problem therefore extends beyond microbiologic resistance profiles. It directly shapes patient survival, hospital burden, and stewardship priorities.

The global epidemiology literature shows that the most clinically important groups are carbapenem-resistant *Enterobacterales*, CRAB, and CRPA (16,17,48). These organisms are no longer rare or isolated findings. Rather, they are part of a broad international pattern driven by clonal spread, mobile resistance genes, antibiotic pressure, and healthcare-associated transmission (48,49). Clinical outcome studies reinforce the severity of this problem. A systematic review and meta analysis found that carbapenem resistance in serious *Enterobacterales* infections was associated with higher mortality than carbapenem susceptibility (50). For this reason, the literature repeatedly focuses on the few drugs that may still retain activity, especially Polymyxins and TGC (16,51).

Because therapeutic options are few, clinicians have long relied on PMB and TGC as salvage or backbone agents for many carbapenem-resistant infections (52, 53). Polymyxin B retained attention because of broad in vitro activity against many resistant Gram-negative pathogens, while TGC remained useful against a large subset of CRE and some CRABisolates (52,53), . Yet both drugs have persistent weaknesses. Polymyxins are limited by nephrotoxicity, neurotoxicity, and difficult pharmacokinetics, while TGC has low serum concentrations and uncertain utility in bloodstream infection unless the MIC is very low , (52,53). Even before resistance is considered, these are not ideal drugs.

TGC, a novel glycylcycline bacteriostatic agent, offers a broad spectrum of activity, inhibiting protein synthesis, and is designed to overcome tetracycline-resistant mechanisms (8). It is approved for human use and has a broad spectrum of activity against many classes of microorganisms, including multi-drug resistant nosocomial pathogens (9). Presently, TGC is approved as a standalone treatment for adults in three instances: complicated skin and skin

structure infections excluding diabetic foot infections, complicated intra-abdominal infections, and community-acquired bacterial pneumonia (10). Currently, it faces resistance mechanisms such as main tetracycline resistance genetic mechanisms, upregulation of efflux pumps, and plasmid-mediated genes (11, 12). 2 In recent years, PMB has made a comeback from its relatively obscure status as a less toxic alternative to drugs, alongside other similar polymyxins like colistin, also known as polymyxin E. PMB, among the five types of polymyxins (A to E), has re-entered the spotlight and is increasingly utilized for treating infections caused by multi-drug-resistant Gram-negative organisms (13). In contrast with TGC, polymyxins are bactericidal polypeptide antibiotics that disrupt bacterial cell wall synthesis and membrane integrity (14). Inherent resistance to polymyxins is observed, whereas they are not active against some gram-negative bacilli, while acquired resistance mechanisms include mutation, lipopolysaccharide (LPS) modification, and plasmid-mediated resistance (15).

Geographical variations in susceptibility profiles for PMB and TGC have been widely documented. At the global level, treatment reviews identify PMB and TGC as important fallback agents for carbapenem-resistant Gram-negative infections, especially when routine beta-lactams, cephalosporins, and fluoroquinolones are no longer reliable (16, 51). However, both drugs have important limits. Polymyxins are constrained by nephrotoxicity, neurotoxicity, and difficult pharmacokinetics, while TGC is less suitable for bloodstream infection because of low serum exposure despite favorable in vitro activity (16). Broader clinical reviews also emphasize that although newer agents such as ceftazidime avibactam, meropenem vaborbactam, and imipenem relebactam have improved treatment for some carbapenem-resistant organisms, their usefulness depends heavily on the carbapenemase type (54, 55). Molecular studies confirm that these newer combinations perform best in KPC dominant settings and much less well in NDM rich settings (56,57). This point is highly relevant when interpreting Ethiopian studies, where NDM linked resistance appears to be common.

The susceptibility literature reflects that fragility. In carbapenem-nonsusceptible *Enterobacterales*, PMB showed higher activity than TGC, but TGC susceptibility changed sharply when FDA and EUCAST breakpoints were compared (58, 59). In a multicenter CRE series from China, Henan, susceptibility remained high for both PMB and TGC, but TGC non susceptibility had already emerged in CRKP and was linked to mutations in ramR, rpsJ, and tetA (60). In CRAB, Polymyxin

susceptibility is often preserved, yet TGC MIC distributions are broader and clinical interpretation is less stable (61–63). These findings show that the two drugs cannot be treated as interchangeable or uniformly reliable across all carbapenem resistant Gram negative bacilli. In Ethiopia, genomic work has already detected mcr-9 among Enterobacterales, showing that resistance to last-resort Polymyxins is no longer only a theoretical concern (19).

Early worldwide laboratory and surveillance studies helped establish the importance of PMB and TGC in carbapenem-resistant infections. In one of the classic early reports, carbapenemase-producing *Klebsiella pneumoniae* isolates from Brooklyn remained susceptible to PMB in vitro, supporting its use as one of the few active salvage agents (64). Zhang and colleagues later showed that TGC and PMB both retained useful in vitro activity against *Enterobacterales* with decreased susceptibility to carbapenems, although susceptibility estimates depended on the interpretive criteria used (65). Global surveillance work also showed that Polymyxins retained strong activity across large collections of Gram negative bacilli, with low resistance rates and MIC90 values in the low range (66). These studies formed the early evidence base for the use of PMBand TGC in carbapenem resistant organisms before the newer beta lactam and beta lactamase inhibitor era.

Studies from Asia continued to support the in vitro value of these drugs but also showed that resistance was beginning to emerge. In Singapore, Lim and colleagues studied carbapenem-resistant CRAB and found PMB susceptibility of 100.0%, with MIC 50 of 1 mg per L and MIC 90 of 2 mg per L, whereas TGC showed a broader MIC distribution (15). Subsequent pharmacodynamic work also suggested that PMB and TGC may have useful roles against CRAB, particularly in combination, but neither drug alone was uniformly bactericidal (67). In China, a multicenter study of carbapenem-resistant *Enterobacterales* found TGC susceptibility of (97.0%) and PMBsusceptibility of 97.0% (68). At the same time, that study documented emergence of TGC non susceptible CRKP, showing that even in settings with high activity, resistance can evolve (68). Other Asian studies on CRAB and CRKP similarly found that TGC and Polymyxins often remained among the more active agents, but that susceptibility varied with clone and resistance mechanism (69–72).

European and multinational surveillance studies have also consistently supported TGC as one of the more active drugs against carbapenem-resistant Enterobacterales. In the SENTRY surveillance program across 18 European nations, TGC retained activity against a large proportion of

carbapenem-resistant *Enterobacterales* (73). In Africa and Middle East countries, Renteria and colleagues likewise found that TGC compared favorably with many other agents against carbapenem-resistant *Enterobacterales* (74). More recent surveillance from the ATLAS program across Africa and the Middle East reported TGC susceptibility among Enterobacterales at 96.0%, amikacin susceptibility at 96.7%, and ceftazidime avibactam susceptibility at 96.6%, while CRPA showed lower meropenem susceptibility at 73.1% (75). These findings confirm that TGC remains an important in vitro option in many regional datasets, especially for Enterobacterales.

Interpretation of PMB and TGC results depends not only on microbiology but also on test methods. Comparative studies of colistin and PMB susceptibility testing have shown that broth microdilution remains the reference method and that Etest and some automated systems may misclassify isolates (26, 76). Similar issues have been reported for TGC, where different susceptibility testing methods can give different results in carbapenem-resistant *Enterobacterales* (77, 78) This is important because some of the apparent differences between studies may reflect methodology as well as true geographic variation. For any thesis or manuscript discussion, this means that comparisons across studies should be made carefully, especially when the test platform and interpretive criteria differ.

Across Africa, the burden of carbapenem and Polymyxin resistance is increasingly well documented. A review of overlapping carbapenem and Polymyxin resistance in CREC and *Klebsiella* in Africa found crude carbapenem resistance proportions of 2.4% for CREC and 13.7% for *Klebsiella* species, while crude polymyxin resistance proportions were 2.4% and 1.8%, respectively (79). The same review documented overlap between carbapenem and Polymyxin resistance in multiple African countries, showing that Polymyxin activity cannot be assumed to remain intact everywhere (79). In South Africa, surveillance of carbapenem-resistant Enterobacterales bacteraemia found TGC susceptibility of 0.2%, amikacin susceptibility of 69.4%, ciprofloxacin susceptibility of 11.0%), and colistin resistance of 18.6%, with CRKP accounting for 79.8% of isolates (80). This suggests a narrower therapeutic space than that reported in some Asian and multinational surveillance studies.

Other African studies further show that the resistance profile and organism distribution vary across countries. In Kenya, a multicenter molecular epidemiology study of carbapenem non-susceptible isolates found that CRAB accounted for 56.3% of isolates and CRPA for 29.2%, indicating a

strong non-fermenter burden (81). In Ghana, a clinical source gram-negative study showed a different pattern, with CREC accounting for 46.0%, CRKP for 17.0%, CRPA for 4.4%, and *Acinetobacter* for 2.2%, while urine contributed 63.0% of specimens (82). Another Ghanaian study found phenotypic carbapenem resistance in 5.6% of MDR CREC and CRKP isolates, with carbapenemase genes in 3.5% and a predominance of *bla*OXA 48 over *bla*NDM (83). These differences show that African resistance ecology is not uniform and that local organism mix strongly shapes the clinical picture.

In Ethiopia, the literature consistently supports a substantial burden of carbapenem resistance, especially in referral and reference laboratory settings. At EPHI, a phenotypic study found carbapenem non-susceptibility in 15.4% of Gram-negative isolates and phenotypically confirmed carbapenemase production in 34.8% of the carbapenem non-susceptible subset (84). Males accounted for 54.3% of isolates, urine was the most frequent specimen source, and 82.6% of carbapenemase-positive isolates came from the ICU (84). These findings established a strong local baseline and showed that carbapenem resistance was already concentrated in high acuity healthcare settings before later studies focused more directly on non fermenters and molecular mechanisms.

Subsequent Ethiopian studies have reinforced the same general picture. At EPHI, carbapenem resistance in *Acinetobacter* species reached 61.0%, while CRPA showed 22.0% resistance, and carbapenem resistance in *Acinetobacter* increased from 50.0% to 76.2% over time (85). A molecular study from Jimma found phenotypic carbapenem resistance in 19.0% of Gram negative isolates and at least one carbapenemase gene in 69.0% of resistant isolates, with *bla*NDM 35.0%, *bla*VIM 24.0%, and *bla*KPC* 13.0% among the commonest acquired genes (86). Trend analysis of CREC and CRKP at EPHI showed that carbapenem resistance in *CRKP* rose from 0.0% to 38.0%, while CREC remained much lower at 5.0% (87). Together, these studies show that the Ethiopian burden is increasing, species specific, and genetically diverse.

In Addis Ababa, Ethiopia specific studies are especially important because they closely match the referral and case mix context of the present work. In Tikur Anbessa Hospital, carbapenemase positivity among CRKP reached 21.2%, and among gene-positive isolates *bla*NDM accounted for 92.9%, while *bla*KPC* accounted for only 3.6% (38). Urine 35.7% and blood 25.0% were the main specimen sources for carbapenemase-positive isolates (20). A bloodstream infection study

from Addis Ababa found carbapenemase prevalence of 25.1% among Gram negative isolates and showed strong concentration of resistant organisms in intensive care and surgical settings (88). These reports strongly suggest that Addis Ababa carbapenem resistance is shaped by hospital associated infection and by NDM linked *Enterobacterales* rather than the KPC dominated ecology reported in some other world regions.

When this literature is viewed as a whole, a clear global to local pattern emerges. International studies generally show that PMB and TGC remain among the most important salvage agents against carbapenem-resistant Gram-negative bacilli, with many studies reporting strong in vitro activity, especially for TGC (89–92). However, activity is not uniform, and both organism mix and testing method affect the reported percentages (24, 93). In Africa and Ethiopia, the problem is further complicated by the heavy burden of CRAB, the growing prominence of CRKP, and the importance of NDM linked carbapenemases (18, 20 , 94). This means that local susceptibility testing remains essential and that international data, while useful for context, cannot fully replace local evidence.

The arrival of newer beta-lactam beta-lactamase inhibitor combinations changed the treatment landscape, but not in a universal way. Ceftazidime avibactam, meropenem vaborbactam, and imipenem relebactam provide major advances for selected carbapenemase profiles, especially KPC producers (27, 28 , 95). Their activity, however, is genotype-dependent. In molecularly characterized CRE, ceftazidime avibactam (CAZ), imipenem relebactam, and meropenem vaborbactam (MEV) were each highly active against KPC producers, but only CAZ retained high activity against OXA-48-like producers, and none were active against metallo beta lactamase producers (29). A bloodstream isolate study from China similarly found aztreonam-avibactam superior to ceftazidime avibactam against CREC and CRKP where metallo beta lactamases were common (30). This means that newer combinations improve therapy for some resistance genotypes while leaving major gaps for others. For a this Study its centered on PMB and TGC susceptibility, this broader treatment context is essential. The emergence of genotype specific new drugs does not remove the need for careful local surveillance of older agents. Instead, it makes local microbiology even more important, because treatment choice increasingly depends on knowing both the regional resistance mechanisms and the actual susceptibility profile of the isolates recovered in a given hospital.

3. Objectives

3.1 General objective

To determine the susceptibility pattern of Polymyxin B and tigecycline among carbapenem-resistant Gram-negative bacilli stored isolates at the Ethiopian Public Health Institute, Addis Ababa, Ethiopia.

3.2 Specific objectives

- To determine the magnitude of carbapenemase enzyme and extended-spectrum β-lactamase production among stored carbapenem-resistant Gram-negative bacilli strains.
- To assess the antimicrobial susceptibility pattern of carbapenem-resistant Gram-negative bacilli strains to other classes of antibiotics.
- To determine the trends of resistance to Polymyxin B and tigecycline among carbapenem-resistant Gram-negative bacilli isolates.

4. Materials and methods

4.1 Study area

This study was conducted at the National Clinical Bacteriology and Mycology Reference and Referral Laboratory (NCBML) section of the Ethiopian Public Health Institute (EPHI), which is located in the Gulele sub-city of Addis Ababa, Ethiopia. Also, it serves as a confirmatory and critical repository for bacterial isolates, providing a unique opportunity to investigate the susceptibility profiles of PMB and TGC. The section has a referral laboratory, providing clinical testing services that come from health care facilities found throughout Ethiopia. The National Clinical Bacteriology and Mycology Reference Laboratory in Addis Ababa serves as the central hub for the collection, storage, analysis, and referral laboratory of clinical isolates from across the country. Also, this laboratory plays a critical role in bacterial culture and sensitivity testing and monitoring the prevalence and trends of antimicrobial resistance surveillance in Ethiopia.

4.2 Study design and period

This study was using a laboratory-based cross-sectional with retrospective method design to analyze CRGNB isolates stored from January 1, 2019, to December 31, 2023 , at the Ethiopian Public Health Institute's National Clinical Bacteriology and Mycology Reference Laboratory. This time frame spans five years and enables a thorough examination of resistance trends and patterns in CRGNB.

4.3 Sampling population

4.3.1 Source population/Isolates

All clinical patient specimens submitted to the National Clinical Bacteriology and Mycology Reference and Referral Laboratory (NCBML) for culture and susceptibility testing during the study period showed the recovery of all isolates tested to carbapenem drugs except PMB and TGC. All are tested and should be carbapenem-resistant for at least one carbapenem (meropenem, ertapenem, or imipenem) resistance isolate considered for this study.

4.3.2 Study population/isolates

All non-duplicate carbapenem-resistant Gram-negative bacilli isolated were manually identified from eligible clinical specimens during the study period. The study included all carbapenem-resistant Gram-negative bacteria (CRGNB) isolates resistant to at least one of meropenem, ertapenem, or imipenem, stored from January 1, 2019, to December 31, 2023. These isolates, obtained from clinical samples referred from private and government hospitals to the Ethiopian

Public Health Institute's National Clinical Bacteriology and Mycology Reference Laboratory (EPHI, NCBMRL) for routine culture and sensitivity tests during the study period, were included if they meet the inclusion criteria. Various clinical specimens, such as urine, wounds, blood, etc., were considered, and all isolate susceptibility testing follows EUCAST and CLSI (M100 2024 version 34, zone of inhibition is resistance for carbapenem disk); see details in **Appendix H.**

4.4 Inclusion and exclusion criteria

4.4.1 Inclusion criteria

- Clinically significant Gram-negative bacilli isolated from routine patient specimens.
- Isolates confirmed as carbapenem resistant by routine AST and confirmatory testing.
- Only the first isolate per patient per species during the study period.
- Samples are stored at appropriate temperatures. All isolates were stored at -80°C in a deep freezer with 10% glycerol to maintain their viability.

4.4.2 Exclusion criteria

- Duplicate isolates from the same patient with the same species and similar susceptibility pattern.
- Isolates that are not stored properly
- Isolates with incomplete identification or contaminated mixed growth unsuitable for reliable AST.
- Organisms are intrinsically polymyxin-resistant and TGC-resistant genera according to the CLSI and EUCAST guidelines.
- Isolates from unlabeled and mislabeled samples.

4.5 Study variables

4.5.1 Dependent variables

- Antimicrobial resistance pattern of each CRGNB isolate for TGC susceptibility results and PMB susceptibility results.
- The extent of carbapenemase-enzyme gram-negative bacilli in clinical isolates.
- Species of the CRGNB isolates

4.5.2 Independent variables

- Age
- Sex
- Bacterial species
- Specimen type
- Hospital unit asnd sites
- Patient age group and sex, if available in de identified form
- Carbapenem resistance phenotype
- Antimicrobial other drugs Susceptibility pattern of each CRGNB isolate.
- Type of CRGNB isolated from the stored clinical samples
- Species type ofthegram-negativeBacilliisolates

4.6 Measurement and data collection

Species identification may be performed by standard biochemical methods or an approved automated platform used in the study laboratory. Isolates meeting screening criteria for carbapenem resistance should undergo confirmatory AST. Where local capacity permits, organism grouping into CRE, CRAB, and CRPA should be preserved because the literature shows clear between-group differences in PMB and tigecycline activity.

4.6.1 Sample size determination

All eligible non-duplicate stored isolates collected between January 01, 2019, and December 31, 2023, A total of 130 isolate were eligibel based on prior routine laboratory evidence of resistance to at least one carbapenem agent. Isolates were selected by non-probability consecutive sampling from Addis Ababa's stored reference laboratory collection.

4.7 Sampling method

Non-probability consecutive sampling was used. Every eligible non-duplicate CRGNB isolate stored and recovered during the study period was enrolled until the period ends or the minimum analyzable sample is reached that indicated resistance to at least one carbapenem antibiotic.

4.8 Isolate handling and data collection

Each isolate was required to be properly labeled with the organism name, specimen type, date of identification, and unique laboratory identification number. Eligible isolates were stored at $-80^{\circ}C$ in Tryptic Soy Broth with 5% glycerol. Data on antimicrobial resistance profiles were

extracted from laboratory records, while patient demographic and specimen data were collected from request forms and registration books using a pre-developed data collection sheet. The collected variables included age, sex, specimen type, referring site, and the carbapenem drug class to which the isolate was resistant. Data collection was carried out by the principal investigator. For each isolate, a structured laboratory form will record patient code, age group, sex, ward, specimen type, date of isolation, bacterial species, carbapenem results, and PMB and TGC, AST results. Data was entered into a spreadsheet or statistical software package with regular cross checks against bench records. See (**Supplementary Appendix k**).

4.9 Laboratory analyses

4.9.1 Recovery and Identification of CRGNB

Previously, stored isolates were retrieved from cryopreservation storage, resuspended in Tryptic Soy Broth, and subcultured on sheep blood agar and MacConkey agar. A detailed media priparation procedure is provided in Supplementary **Appendix A,B,C.** Plates were incubated at 37°C for 18 to 24 hours, and recovered isolates were checked for purity before further analysis. Primary culture was done on routine bacteriologic media according to specimen type. After species identification, carbapenem resistance was screened using the routine laboratory method in place for meropenem, ertapenem, or imipenem. Isolates meeting resistance criteria were retained for confirmatory testing and final inclusion.

4.9.2 Antimicrobial susceptibility testing

Routine antibiotic susceptibility testing was performed by Kirby-Bauer disk diffusion using standard laboratory procedures aligned with CLSI-M100 and EUCAST recommendations. TGC susceptibility was interpreted using EUCAST criteria (96, 97). PMB also, minimum inhibitory concentration (MIC) testing was performed by E test on Mueller-Hinton agar. Carbapenemase production was assessed phenotypically using the modified carbapenem inactivation method. The literature strongly supports using BMD as the reference method for PMB testing and as the preferred comparator for TGC as well. For that reason and due to resource limitations and the core laboratory method, we prioritize MIC based testing rather than agar diffusion swhenever feasible. Consequently,interpretation of E-test results should be approached with caution,and further confirmation through BMD is advisable See thetest procedure in **Appendix D.**

4.9.2.1 Polymyxin B MIC testing

Polymyxin B susceptibility was determined using commercial E test® strips by Liofilchem® MIC (0.064 to 1024 µg/ml) on Mueller-Hinton agar (BBL-Becton Dickinson). A 0.5 McFarland suspension prepared from fresh colonies was spread to produce a confluent lawn, after which the strip was applied to the agar surface. Plates were incubated at 35 ± 2°C for 16 to 24 hours, and MIC values were read at the point where the inhibition ellipse intersected the strip. Where heteroresistant growth was seen within the ellipse, the highest visible MIC intersection was recorded. Because Polymyxin susceptibility testing is method sensitive, findings were interpreted cautiously and, where needed, considered for confirmation by broth microdilution.

PMB MIC should be determined by broth microdilution using cation-adjusted Mueller Hinton broth and standard quality control strains. Interpretation should follow the breakpoint system adopted by the laboratory, stated clearly in the thesis. This approach is favored because the E-test shows poor agreement with BMD for PMB in CRE and other Gram-negative bacilli. In this study, we utilized Liofilchem® Reseto (Italy) MIC (0.064 to 1024 mg/l) and then placed test strips on the Mueller Hinton agar (Oxoid,Uk) surface, adhering to Clinical and Laboratory Standards Institute (CLSI-M100) guidelines. Because E-test strips are used because of local resource constraints, the result should be treated as provisional, and any borderline or clinically important value should be confirmed by BMD. A detailed procedure is provided in Supplementary **Appendix E.**

4.9.2.2 Tigecycline and other disk diffusion testing

Testing for TGC susceptibility followed European Committee on Antimicrobial Susceptibility Testing standards using the Kirby-Bauer disk diffusion method. A bacterial inoculum equivalent to a 0.5 McFarland standard was evenly spread over Mueller-Hinton agar using a sterile cotton swab, after which tigecycline 15 μg, disks (Oxoid, UK) were placed on the agar surface for analysis. After incubation, zone diameters were measured and interpreted using the relevant EUCAST breakpoints. The same disk diffusion approach was used for other antimicrobials, including all antimicrobial susceptibility testing disks used in this study. They were obtained from Oxoid Ltd., United Kingdom (Thermo Fisher Scientific, Basingstoke, UK), and prepared according to standard Kirby–Bauer disk diffusion methodology. The antibiotic disks and their respective potencies were as follows: amikacin (30 µg), gentamicin (10 µg), cefazolin (30 µg), amoxicillin/clavulanic acid (20/10 µg), azithromycin (15 µg), cefepime (30 µg), cefoxitin (30 µg), ceftazidime (30 µg), cefuroxime (30 µg), cefixime (5 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), nitrofurantoin (300 µg), fosfomycin with glucose-6-phosphate (200 µg, BD formulation or equivalent standardized disk), ceftazidime-avibactam (30/20 µg), piperacillin/tazobactam (100/10 µg), meropenem-vaborbactam (10/10 µg, standardized combination disk where available), minocycline (30 µg), tigecycline (15 µg), tetracycline (30 µg), and Polymyxin B (300 units). All disks were used in accordance with manufacturer instructions and international CLSI for other classes. For tigcycline were used FDA and EUCAST guidelines were used to ensure accuracy and reproducibility of antimicrobial susceptibility. **Supplementary Appendix H.**

4.9.2.3 Phenotypic screening and confirmation of ESBL production

Phenotypic assessment of extended-spectrum β-lactamase production was performed for Enterobacterales isolates that showed reduced susceptibility to one or more indicator oxyimino-cephalosporins or aztreonam on routine disk diffusion testing. Initial screening was done on Mueller-Hinton agar using a bacterial suspension adjusted to the 0.5 McFarland turbidity standard. Zone diameters were interpreted using the disk diffusion framework and interpretive tables in CLSI M100-Ed34, the current edition published on January 26, 2026, which replaced Ed35 from 2025. The screening panel included cefotaxime, ceftazidime, ceftriaxone, cefpodoxime, and aztreonam because reduced susceptibility to these agents is classically used to identify isolates that may harbor ESBL enzymes (CLSI M100-Ed34). The phenotypic confirmation was then performed with a commercial BD BBL ESBL combination disk set using cefotaxime and ceftazidime disks tested alone and in combination with clavulanic acid.

After the surface of the Mueller-Hinton agar plate had been inoculated evenly with the standardized suspension, the paired disks were applied with adequate spacing to avoid overlapping inhibition zones. The plates were incubated aerobically at 35 ± 2°C for 16 to 18 hours. An isolate was interpreted as ESBL positive when the inhibition zone around either the cephalosporin-plus-clavulanic acid disk or the cephalosporin disk was at least 5 mm larger than the zone around the corresponding cephalosporin disk alone, which is the standard phenotypic principle for clavulanate-based ESBL confirmation. Initial screening was based on reduced susceptibility to third-generation cephalosporins or aztreonam, interpreted according to the current CLSI M100 standard. Isolates meeting the screening criteria were subjected to confirmatory testing using BD BBL™ Sensi-Disc™ combination disks. A detailed procedure is provided in Supplementary Appendix F.

Phenotypic confirmation of ESBL production was carried out with paired disks of cefotaxime and cefotaxime/clavulanic acid and ceftazidime and ceftazidime/clavulanic acid. After inoculation of the Mueller-Hinton agar plate, the disks were placed with adequate spacing, and the plates were incubated aerobically at 35 ± 2°C for 16 to 18 hours. An isolate was interpreted as ESBL-positive when the inhibition zone around either the cephalosporin-plus-clavulanic acid disk was at least 5

mm larger than the zone around the corresponding cephalosporin disk alone. Quality control for ESBL testing was performed with *Klebsiella pneumoniae* ATCC 700603 as the positive control and *Escherichia coli* ATCC 25922 as the negative control. All media preparation, inoculum standardization, disk application, incubation, and zone reading procedures were carried out according to the laboratory standard operating procedure and the relevant CLSI disk diffusion framework, CLSI M100-Ed36. Supplementary **Appendix F.**

4.9.2.4 Phenotypic carbapenemase detection

Phenotypic carbapenemase detection was performed using the modified carbapenem inactivation method and the EDTA-modified carbapenem inactivation method. The older modified Hodge test was not used in the rewritten method section because CLSI replaced it with mCIM and eCIM for phenotypic carbapenemase detection. Current CLSI guidance places mCIM and eCIM within the recommended phenotypic workflow for carbapenemase detection, whereas the modified Hodge test is now an archived method rather than the preferred contemporary approach (CLSI M100-Ed36).

For each isolate, a loopful of colonies from an overnight culture was emulsified in 2 mL of tryptic soy broth for mCIM testing. A 10-µg meropenem disk was added to the suspension, and the tube was incubated at 35 ± 2°C for 4 hours. For eCIM testing, a second tube containing 2 mL of tryptic soy broth plus 20 µL of 0.5 M EDTA was prepared in parallel and processed in the same manner. At the same time, a 0.5 McFarland suspension of *Escherichia coli* ATCC 25922 was used to prepare a lawn culture on Mueller-Hinton agar. After incubation of the meropenem disks in broth, the disks were removed and placed onto the inoculated indicator plate, which was then incubated at 35 ± 2°C for 18 to 24 hours.

For mCIM, a zone diameter of 6 to 15 mm, or pinpoint colonies within a 16 to 18 mm zone, was interpreted as positive for carbapenemase production, while a clear zone of 19 mm or greater was interpreted as negative. If mCIM was positive, eCIM was used to differentiate metallo-β-lactamase activity from a non-metallo-carbapenemase pattern. An increase of at least 5 mm in the eCIM inhibition zone compared with the corresponding mCIM zone was interpreted as metallo-β-lactamase production, whereas a smaller difference supported a serine carbapenemase pattern.

Quality control and interpretation were performed with the CLSI-recommended indicator strain and standard internal quality procedures. This approach aligns the methods section with the phenotypic carbapenemase categories already reported in the results and discussion, including mCIM positivity, eCIM-based metallo-β-lactamase detection, and serine carbapenemase differentiation. A detailed procedure is provided in Supplementary **Appendix G.**

4.9.2.5 Quality assurance and safety measures

Quality assurance procedures covered the pre-analytical, analytical, and post-analytical phases of the study. Standard American Type Culture Collection control strains were used to verify organism recovery, identification, and antimicrobial susceptibility testing performance. Control strains *included Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and additional control organisms where appropriate for carbapenemase-related procedures. Similarly, ATCC BAA-1705**:** A CRKP strain that harbors the bla_KPC gene (a KPC-positive carbapenemase producer). It is used as a positive control in phenotypic tests and molecular diagnostics and ATCC BAA-1706. A CRKP strain that is KPC-negative (does not carry the bla_KPC gene). It is often used alongside BAA-1705 as a negative control (96). Laboratory work was conducted using appropriate biosafety practices, including personal protective equipment, safe isolate handling, secure storage, routine decontamination, and proper waste disposal. In the pre-analytical phase, isolates and media were checked for integrity and contamination. In the analytical phase, susceptibility testing against PMB, TGC, and the routine antibiotic panel was performed under standardized conditions with control strains processed in parallel to ensure result reliability.

4.10 Data collection, analysis, and interpretation

Demographic and specimen data were extracted from request forms, registration books, and laboratory records using a pre-developed data collection Excel sheet and WHONET 2023. Data were entered and analyzed using SPSS version 27 (IBM Corp.) and Visual Studio with Python version 13.5. Results are presented as a count over a total with percentage. Data was analyzed using descriptive statistics. Species distribution, specimen sources, and hospital location of isolates were summarized using frequencies and percentages. Susceptibility to PMB and TGC was reported overall and by organism group. MIC distributions were calculated for major species groups when isolate numbers permit.

4.11 Ethical considerations

Ethical approval for this study was obtained from the Departmental Research and Ethics Review Committee (DRERC) of the School of Allied Health Sciences, College of Health Sciences, Addis Ababa University (DRERC/225/24). Additionally, permission was granted by the Ethiopian Public Health Institute's (EPHI) National Clinical Bacteriology and Mycology Reference Laboratory directorate (NCBMRL) by signing our support letter in MLST 111/16/24. Under the according to the NCBMRL.

4.12 Dissemination of Results

The finding of this study will be presented to the College of Health Sciences, Department of Medical Laboratory Sciences, Addis Ababa University, as the partial fulfillment of the master's program and then be accessible for healthcare communities, clinicians, policymakers, and local and international conferences. Moreover, the result of the study will be disseminated to the international scientific community through publication in a high-profile peer-reviewed clinical research journal.

4.13 Operational definitions

- **Carbapenem-resistant Gram-negative bacilli:** Gram-negative bacilli classified as resistant or non-susceptible to at least one carbapenem by the method and interpretive standard adopted in the study laboratory.
- **Non-duplicate isolate**: the first isolate of a given species recovered from one patient during the study period.
- **Multidrug resistant**: non-susceptibility to at least one agent in at least three antimicrobial classes.
- **Susceptible, intermediate, and resistant:** are AST categories assigned by the selected interpretive standard, stated explicitly in the thesis.
- **Multiple Drug Resistance (MDR):** indicates resistance of Gram-negative bacteria species to at least one antimicrobial drug in three or more antimicrobial categories.
- **Extensive Drug Resistance (XDR): is** the non-susceptibility of Gram-negative bacteria species to all antimicrobial agents except in two or fewer antimicrobial categories.
- **Pan Drug Resistance (PDR): indicates** Gram-negative bacteria species' resistance to all antimicrobial agents in all antimicrobial categories including PMB and colistin.

5. Results

5.1 Demographic characteristics of source patients

A total of 130 non-duplicate stored clinical isolates were confirmed as carbapenem resistant using CLSI-M100 V.34 breakpoint criteria with Oxoid UK, meropenem (10 µg), imipenem (10 µg), and ertapenem (10 µg) disks. All 130 isolates were successfully recovered on Tryptic Soy Agar and MacConkey agar. The isolates had originally been collected from a range of clinical specimens between January 1, 2019, and December 28, 2023.

The largest age group was 25 to 44 years, which accounted for 45 of 130 isolates, or 34.6%. This was followed by 45 to 64 years, with 23 isolates, or 17.7%. Infants younger than 1 year accounted for 17 isolates, or 13.1 %. Male patients accounted for 85 of 130 isolates, or 65.4%, while female patients accounted for 45 of 130 isolates, or 34.6%. In terms of age groups by WHO, see more in detail; more detailed age grouping has been presented in Supplementary Table 1 below.

Table 1: Age distribution among CRGNB isolates, January 1, 2019, and December 28, 2023.

Age group	Frequency	%
Less than 1 year	17	13.1
1 to 14 years	11	8.5
15 to 24 years	18	13.8
25 to 44 years	45	34.6
45 to 64 years	23	17.7
More than 65 years	16	12.3
Total	130	100.0

5.2 Distribution of carbapenem-resistant isolates

5.2.1 Distribution by year of collection

The annual distribution of stored isolates varied across the study period. The highest number of isolates was recorded in 2019, with 53 of 130 isolates, or 40.8%. This fell sharply in 2020 to 12 isolates, or 9.2%, then increased again in 2021 to 38 isolates, or 29.2%. A lower number of isolates was recorded in 2022 with 13 isolates, or 10.0%, followed by 14 isolates, or 10.8%, in 2023. Overall, the pattern suggests a marked reduction in isolate collection during 2020, with partial recovery in subsequent years The total number of isolates per year has been shown in Figure1 below.

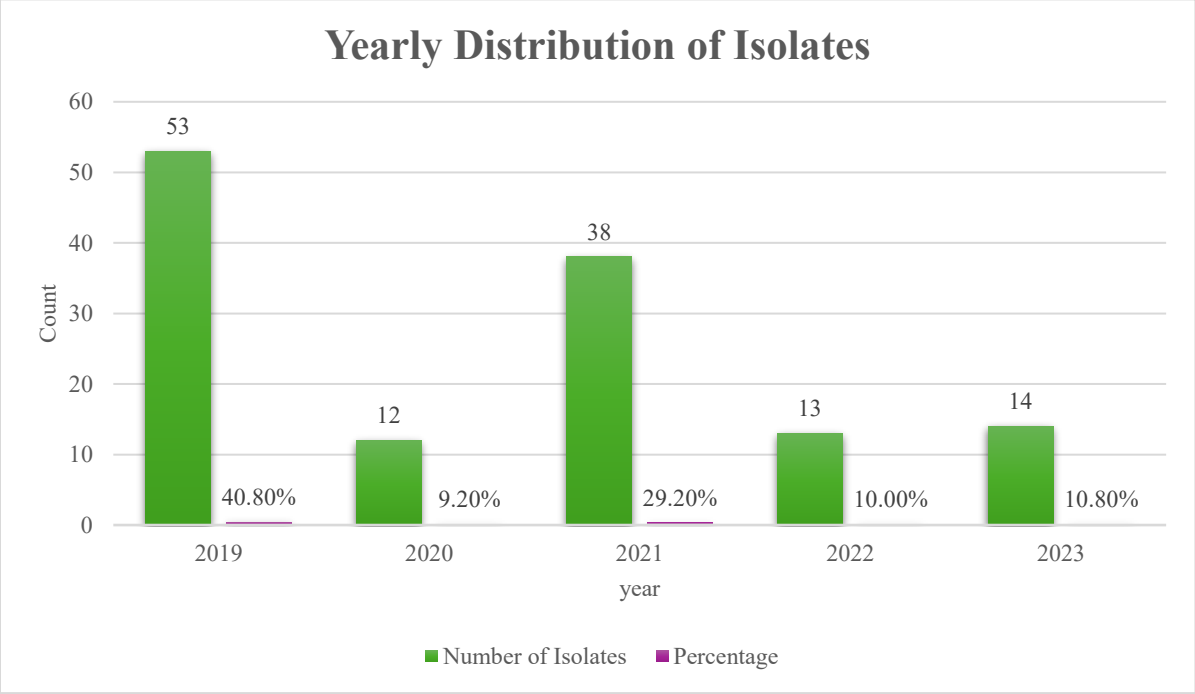


Figure 1: The yearly distribution of major CRGNB isolates from January 1, 2019, to December 28, 2023.

5.2.2 Species distribution

A total of 130 CRGNB isolates were included in the final analysis. Based on the species counts provided, Enterobacterales accounted for 65/130 isolates, or 50.0%, while non-fermenters accounted for 65/130 isolates, or 50%. CRAB was the single most common species, with 48 isolates, or 36.9%, followed by CRKP with 33 isolates, or 25.4%. CRPA accounted for 15 isolates, or 11.5%, and CREC accounted for 12 isolates, or 9.2%. The remaining isolates were distributed across *Enterobacter cloacae*, *Klebsiella oxytoca*, *Klebsiella ozaenae*, *Citrobacter diversus*, *Pantoea agglomerans*, *Alcaligenes faecalis*, and *Acinetobacter lwoffii*. Overall, the species distribution highlights the predominance of CRAB and *CRKP* among stored carbapenem-resistant isolates at EPHI. (Fig. 2) can be used to display the relative contribution of each bacterial species.

The most predominant organism across the study period was CRAB*,* accounting for the highest overall proportion (36.92%), followed by CRKP (25.38%). Other important isolates included CRPA (11.54%), CREC (9.23%), and CR-*Enterobacter cloacae* (4.62%). In 2019, CRAB was the dominant isolate, while in later years, particularly from 2020 to 2023, CRKP showed increased prevalence. The chart also demonstrates fluctuations in the distribution of different bacterial species over time, indicating changing epidemiological trends of multidrug-resistant pathogens in the study setting.

Overall, the findings highlight the significant burden of CRAB and CRKP as leading resistant pathogens, emphasizing the importance of continuous antimicrobial resistance surveillance, infection prevention strategies, and appropriate antibiotic stewardship programs. The total number of Species distribution from recoverd isolates in five year has been shown in Figure 2 below.

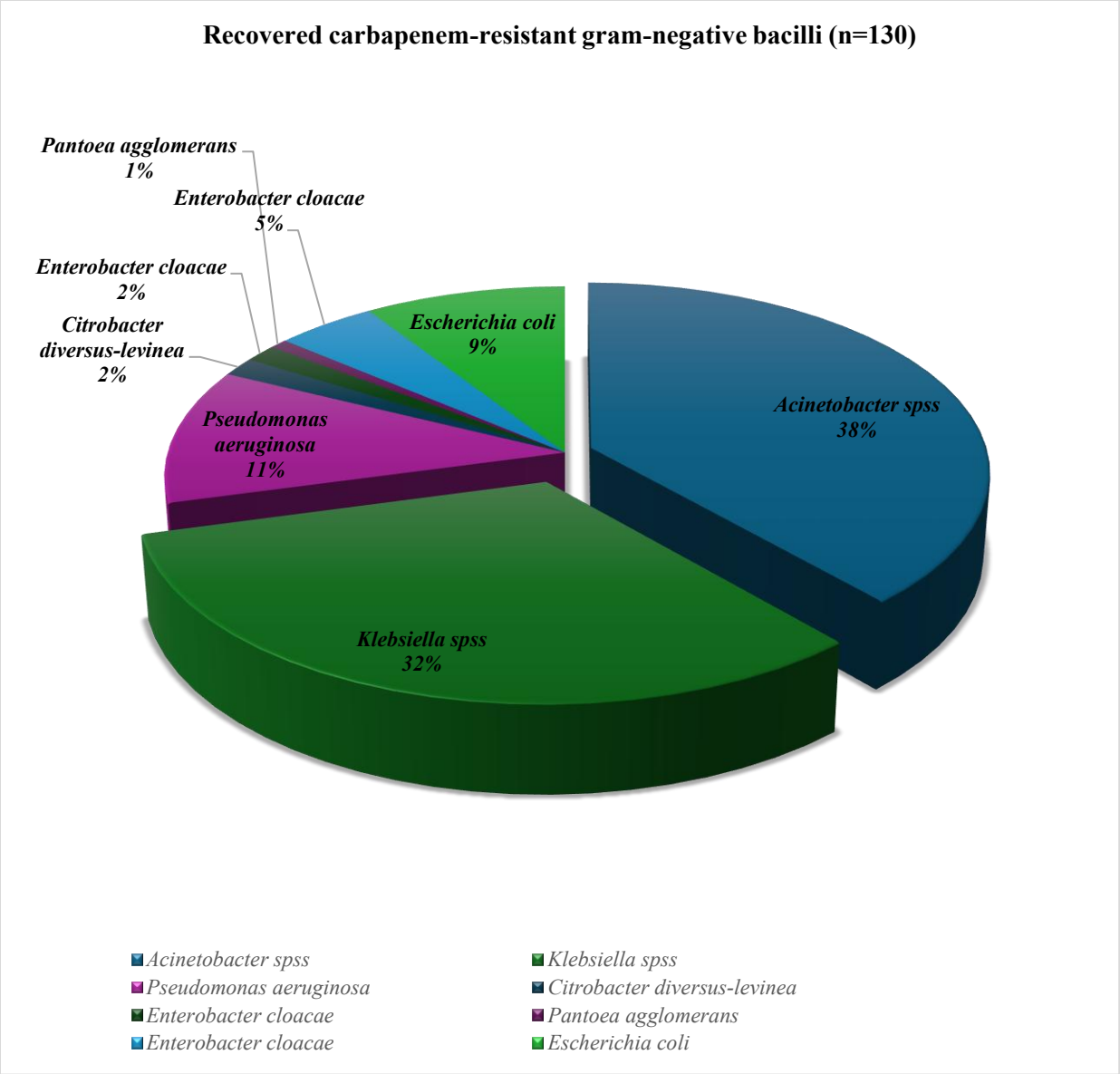


Figure 2: Species distribution of recovered CRGNB (***Acinetobacter sps.,*** *Acinetobacter lwoffii*, *Acinetobacter baumannii, and* ***Klebsiella sps.,*** including *Klebsiella pneumoniae, Klebsiella oxytoca, and Klebsiella ozaenae*).

5.2.3 Distribution by health facility

The highest number of isolates came from AeBET Hospital, which contributed 60 of 130 isolates, or 46.2%. This was followed by Minilik I Referral Hospital with 17 isolates, or 13.1%, and Ras Desta Referral Hospital with 12 isolates, or 9.2%. The Federal Police Referral Hospital contributed 10 isolates, or 7.7 %. The remaining isolates came from St. Peter Hospital, St. Paul Hospital, Yekatit 12 Hospital, Tikur Anbessa Specialized Hospital, private clinics, government health centers, and Bethezata Hospital. Department-level data also varied, with isolates reported most often from the intensive care unit and from records with unknown departments. These details can be summarized in Supplementary Table 2.

Table 2: Frequency of CRGNB isolates among Addis Ababa health facilities

Health facility	Frequency	%
AeBET Hospital	60	46.15
Minilik I Referral Hospital	17	13.08
Ras Desta Referral Hospital	12	9.23
Federal Police Referral Hospital	10	7.69
St. Peter Hospital	7	5.38
Government Health Center	7	5.38
St. Paul Hospital	6	4.62
Private Clinic	4	3.08
Yekatit 12 Hospital	3	2.31
Tikur Anbessa Specialized Hospital	3	2.31
Bethezata Hospital	1	0.77
Total	130	100.00

In this study, the Emergency Department (EME) contributed 9 isolates (6.9%), while the Intensive Care Unit (ICU) accounted for the highest number with 36 isolates (27.7%), reflecting the critical nature of infections in intensive care settings. Other departments, including Medical (MED) with 16 isolates (12.3%), Neonatal Unit (NEO) with 8 isolates (6.2%), and Outpatient Department (OUT) with 14 isolates (10.8%), showed significant contributions to the overall distribution.

Isolates from lesser-represented departments included Pediatrics (PED) and Surgery (SUR), each with 2 isolates (1.5%), and Neonatal ICU (NEOICU), Orthopedics (ORTH), Internal Medicine (IN), and Internal Treatment (INT), each contributing 1 isolate (0.8%). A considerable portion of the isolates (39, or 30.0%) were categorized as "Unknown" (UNK), highlighting gaps in fully written patient demographic data or the challenges of tracing the source of certain infections.

Additionally, isolates from different health facilities AeBET Hospitals contributed the majority of patients, 60, followed by Minilik II Referral Hospital with 17 and Ras Desta Referral Hospital with 12. Federal Police Referral Hospital reported 10 cases, while other clinics and hospitals provided fewer patients. Distribution of departments was also different, like intensive care unit (27.7%) and unknown (30.0%) The heatmap shows the distribution of CRGNB across different hospitals has been shown in Figure 3 below.

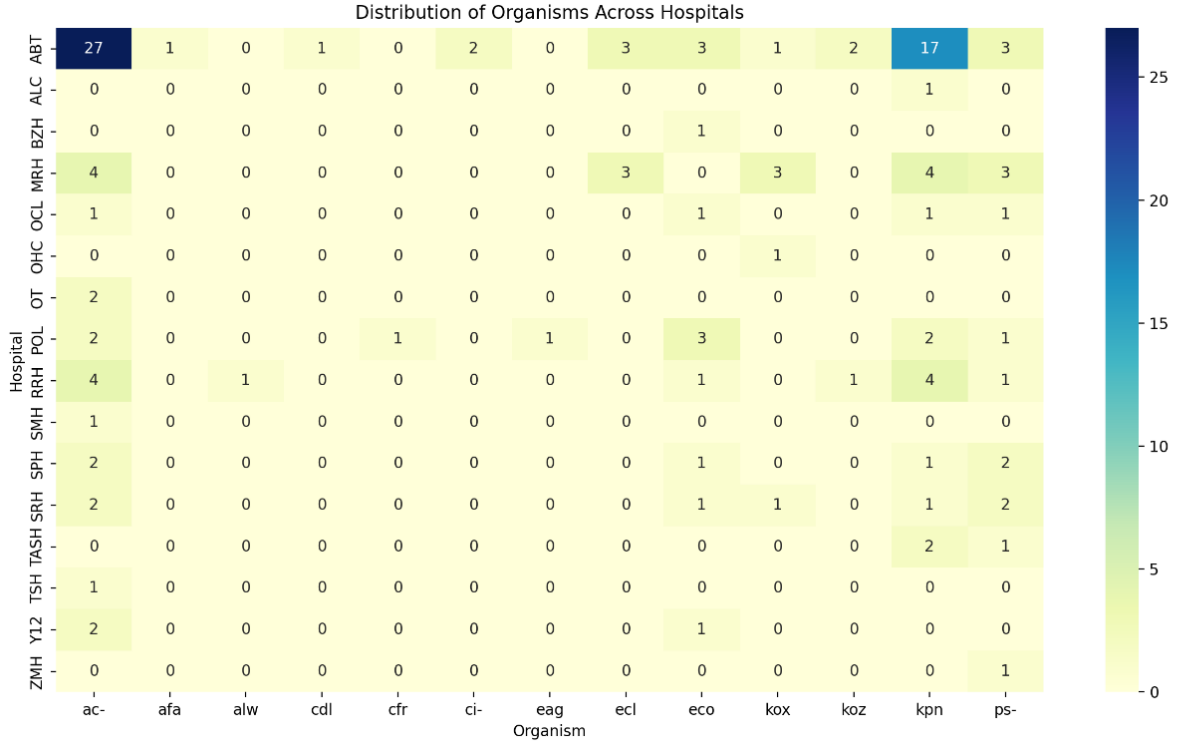


Figure 3: The heatmap shows the distribution of CRGNB across different hospitals. This heatmap indicates that *Acinetobacter spp*. and *Klebsiella pneumoniae* (kpn) were the most frequently isolated organisms in most healthcare facilities. A higher concentration of isolates was observed in AURH, particularly for *Acinetobacter spp. (ac)* and K. pnemonae (kpn), suggesting a greater burden of CRGNB infections in this hospital. Other hospitals showed relatively lower frequencies with occasional detection of organisms such as ac (*Acinetobacter spp*.), afa (*Alcaligenes/Aeromonas faecalis* group), cfr (*Citrobacter freundii*), kpn (*Klebsiella pneumoniae*), *Escherichia coli (eco)*, *Enterobacter cloacae (ecl)*, and *Pseudomonas spp. (ps)*. The hospital abbreviations used in the figure include ABT (Abebech Gobena/Alert Hospital), ALC (All African Leprosy Center), BZH (Betezatha Hospital), MRH (Menelik Referral Hospital), POL (Police Hospital), RRH (Ras Desta Referral Hospital), SMH (St. Michael Hospital), SPH (St. Paul's Hospital Millennium Medical College), SRH (St. Raphael Hospital), TASH (Tikur Anbessa Specialized Hospital), TSH (Tirunesh Beijing Hospital), Y12 (Yekatit 12 Hospital), and ZMH (Zewditu Memorial Hospital).

Overall, the findings demonstrate variability in CRGNB distribution among hospitals, highlighting the importance of continuous antimicrobial resistance surveillance and infection prevention strategies. CRGNB isolates were recovered from a wide range of specimen types. Urine was the most common source, accounting for 49 of 130 isolates, or 37.7%. Pus accounted for 29 isolates, or 22.3%, and blood accounted for 27 isolates, or 20.8%. Cerebrospinal fluid contributed 8 isolates, or 6.2%, while sputum accounted for 7 isolates, or 5.4%. Smaller numbers were recovered from other body fluids, tracheal aspirates, eye specimens, throat swabs, and other specimen types.

Species-specific analysis showed that CRKP was the most common CRE isolate and was most often recovered from blood and urine. CREC was recovered mainly from urine. CRAB was the most common isolate overall and was especially frequent in urine and pus specimens, suggesting an important contribution to urinary and wound-associated infections. Pseudomonas aeruginosa showed a broader distribution across sputum, blood, urine, pus, eye specimens, and other specimen types.

In this study, carbapenem-resistant organisms were identified across various sample types, indicating the widespread nature of resistance between the Enterobacterales family and non-fermenting bacteria. Amongst the CRE family (n=66), CRKP, n=33 (25.4%), showed the highest prevalence next to the non-fermenter bacteria CR-AB, accounting for 25.4% of cases. It was most frequently isolated from blood samples (10%) and urine (7.7%), with sporadic occurrences in pus, cerebrospinal fluid (CSF), sputum, throat, and ear samples.

Among fermenter CRE family such as CREC (n=12, 9.2%), carbapenem-resistant *Klebsiella oxytoca* (n=6, 4.6%), and carbapenem-resistant *Klebsiella ozaenae* (n=3, 2.3%); among opportunistic CRE infections, carbapenem-resistant *Enterobacter cloacae* (n=8, 6.2%), carbapenem-resistant *Pantoea agglomerans* (n=1, 0.8%), carbapenem-resistant *Citrobacter diversus* (n=1, 0.8%), and *Alcaligenes faecalis (n=1,* 0.8%) had lower occurrences of cases, with *CREC* showing the highest representation in urine samples (6.9%). Among the non-fermenting bacteria isolates counted as n=67, we found carbapenem-resistant *Acinetobacter baumannii* (CR-AB) n=48 (36.9%), which was predominant, representing 36.9% of the total isolates.

Similarly, CRAB was commonly found in urine (13.8%), pus (13.1%), blood (3.8%), and CSF (3.1%), suggesting its association with both urinary tract infection (UTI) and wound infections. In the same way, we find the other strain, *Acinetobacter lwoffii* (n=1, 0.8%), from a sterile site fluid, cerebrospinal fluid (CSF). Another non-fermenter, *CRPA* (n=15, 11.5%), was found in 11.5% of cases and displayed varied distribution, with a notable presence in eye (1.5%), blood (2.3%), and sputum samples (3.1%); The heatmap shows the distribution of CRGNB across different specimen types has been shown in Figure 4 below.

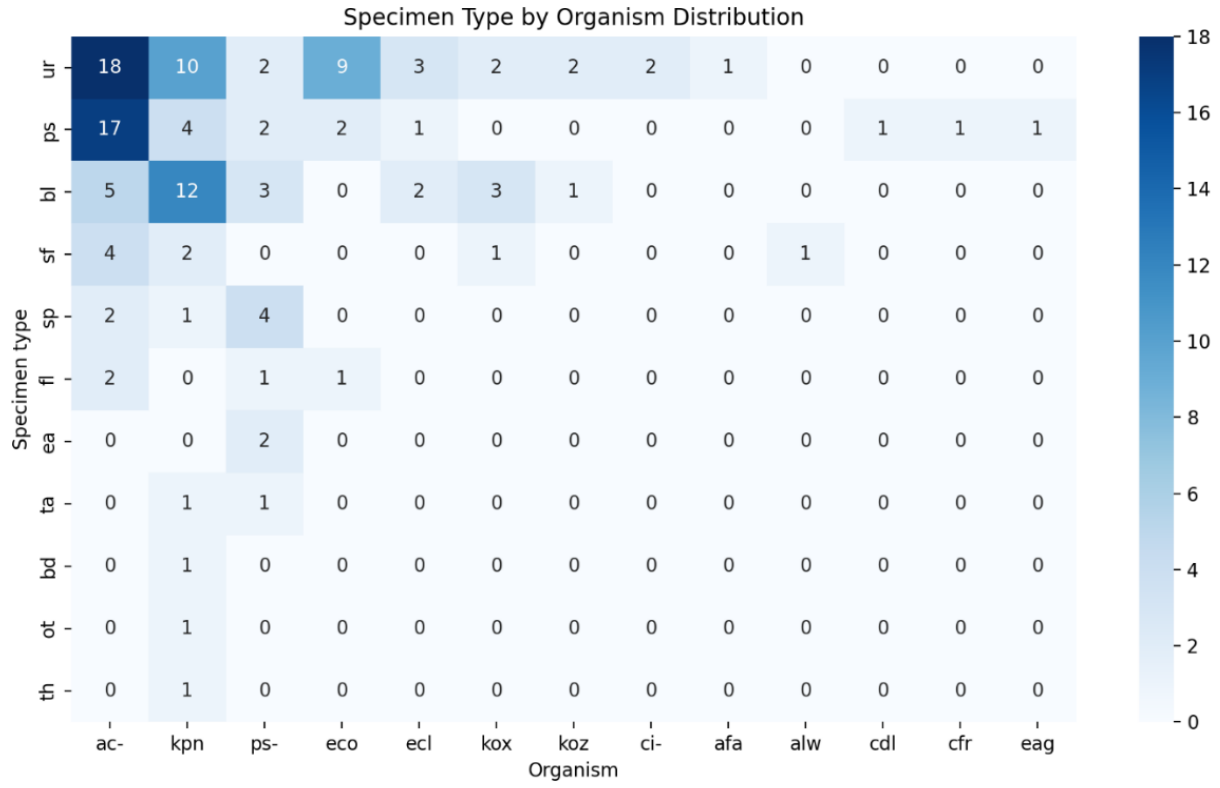


Figure 4: The heatmap shows the distribution of CRGNB across different specimen types. The heatmap demonstrates that urine (ur) and pus (ps) specimens had the highest number of isolates, particularly for *Acinetobacter spp.* and *Klebsiella pneumoniae*. The organism abbreviations shown on the x-axis include ac (*Acinetobacter spp.*), afa (*Alcaligenes/Aeromonas faecalis* group), cfr (*Citrobacter freundii*), kpn (*Klebsiella pneumoniae*), *Escherichia coli (eco)*, *Enterobacter cloacae (ecl)*, and *Pseudomonas spp. (ps)*. Samples, like blood (bl) and stool (st) specimens, also showed moderate distribution of resistant organisms. In contrast, other specimen types such as ear aspirate (ea), tracheal aspirate (ta), body fluid (bd), and other samples (ot) had relatively low frequencies. *Acinetobacter spp.* (ac) and *CRKP (kpn)*, suggesting a greater burden of CRGNB infections in this hospital.

5.3 Magnitude of carbapenemase enzyme and ESBL production

Phenotypic detection of carbapenemase production showed clear variation across species. Among CRKP, 14/33 (42.4%) isolates were positive by the modified carbapenem inactivation method, while 18/33 (54.5%) were negative. For beta-lactamase confirmation, 30/33 (90.9%) were negative and 2/33 (6.1%) were positive, with one isolate lacking a final recorded confirmation result. *Klebsiella oxytoca* showed a higher carbapenemase burden, with 5/6 (83.3%) mCIM positive and 1/6 (16.7%) negative, while all 6/6 (100.0%) were negative on the beta-lactamase confirmation test. In the small species groups, CR-*Klebsiella ozaenae* and *Citrobacter diversus* showed complete mCIM positivity and no confirmed beta-lactamase positivity in the available results. *Enterobacter cloacae* also showed frequent phenotypic carbapenemase detection. Among the six isolates with available confirmatory results, 4/6 (66.7%) were mCIM positive and 2/6 (33.3%) were negative, while all 6/6 (100.0%) were negative on beta-lactamase confirmation. These Enterobacterales findings reinforce the importance of carbapenemase-mediated resistance in this isolate group, especially in *Klebsiella* and *Enterobacter* species.

Contrastingly, CRAB showed a high rate of mCIM negativity, with 46/48 (95.8%) negative and only 1/48 (2.1%) positive. Beta-lactamase confirmation was also negative in nearly all Acinetobacter baumannii isolates, with 47/48 (97.9%) negative. Pseudomonas aeruginosa showed the opposite pattern, with 12/15 (80.0%) mCIM positive and 3/15 (20.0%) negative, while beta-lactamase confirmation was negative in 13/15 (86.7%) and positive in 2/15 (13.3%). Taken together, these findings highlight marked diversity in carbapenem resistance mechanisms among the major Gram-negative species in the study collection. The outer mCIM disks demonstrate a lack of a zone of inhibition (bacterial growth up to the disk edge), indicating active carbapenemase destruction of the meropenem disk. The mCIM and eCIM results are shown below in Figure 5.

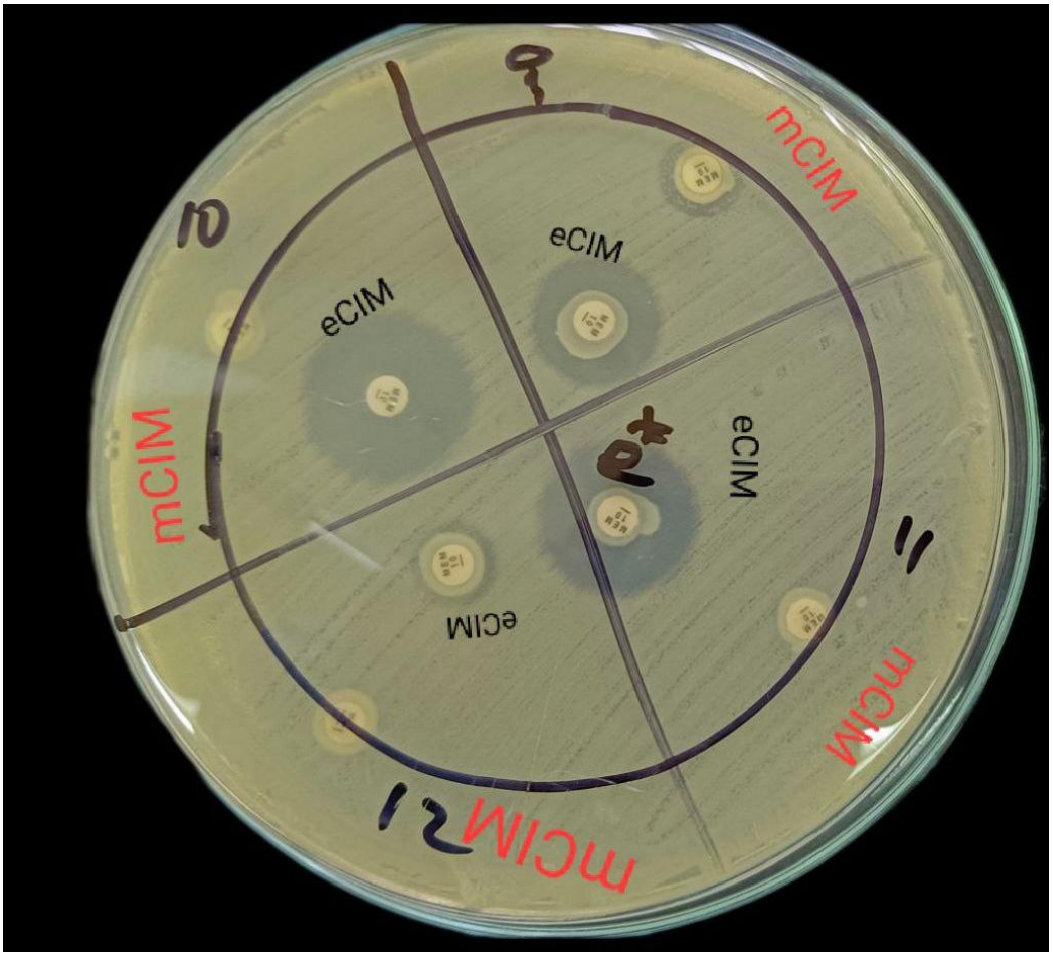


Figure 5: Photograph of Mueller-Hinton agar plates inoculated with a carbapenem-susceptible isolate (*E. coli* ATCC 25922) to which meropenem disks have been applied after disks were incubated with select isolates in the absence or presence of EDTA. Phenotypic evaluation of carbapenemase production using the modified carbapenem inactivation method (mCIM) and EDTA-modified carbapenem inactivation method (eCIM). The agar plate displays a comparative disk diffusion assay split into four distinct test sectors (labeled 9, 10, 11, and 12) using *Escherichia coli* ATCC 25922 as the indicator strain lawn. Each sector contains a paired setup consisting of an outer mCIM zone and a corresponding inner eCIM zone to differentiate between serine carbapenemases and metallo-beta-lactamases (MBLs). Sectors 9, 10, and 11 (Positive for Metallo-beta-Lactamases):

Ultimately, CRCE, 33.3% (7 isolates), was ESBL-positive, with a notable ESBL positivity rate of 38.89%. CRKP, 61.9% (13 isolates), was ESBL-positive, with a positivity rate of 28.26%, highlighting its significant role in resistance. Klebsiella oxytoca and Klebsiella ozaenae showed low ESBL positivity and limited MBL positivity, indicating varied resistance mechanisms. For CRPA, a low ESBL positivity rate of 6.25% (1 isolate) was observed. Rare isolates (Alcaligenes faecalis, Acinetobacter lwoffii, Citrobacter diversus, and Pantoea agglomerans) were consistently mCIM negative and lacked ESBL production. The Photograph of Mueller-Hinton agar plates ESBL results are shown below in Figure 6.

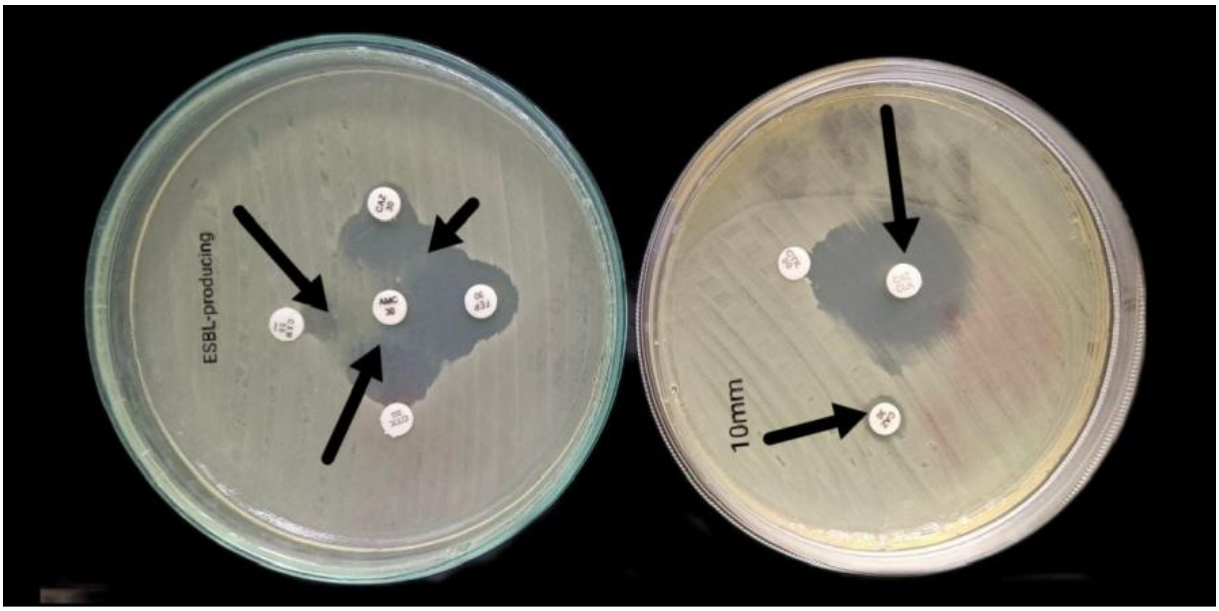


Figure 6: Photograph of Mueller-Hinton agar plates representative of phenotypic detection of ESBL-producing organisms by double-disc-based testing (left **side**) and combination (**right side**) disk diffusion. This image shows a phenotypic test used to detect Extended-Spectrum Beta-Lactamase (ESBL)-producing bacteria, which are resistant to many common antibiotics. The plate on the left demonstrates a double-disk synergy test, where antibiotic disks are placed around an amoxicillin-clavulanic acid disk to check for enhanced inhibition (synergy). The arrow on the left shows the "keyhole" effect, or expansion of the inhibition zone toward the center disk, indicating that the clavulanic acid is inhibiting the ESBL enzymes, confirming the presence of these resistance enzymes. **Right** confirmation was carried out using BD BBL™ Sensi-Disc™ combination disks: cefotaxime/clavulanic acid (CTX/CLA 30/10) and ceftazidime/clavulanic acid (CAZ/CLA 30/10). An isolate was considered ESBL positive when the inhibition zone around the cephalosporin-plus-clavulanic acid disk was at least 5 mm larger than that around the corresponding cephalosporin disk alone.

Overall, among the 128 isolates with complete phenotypic confirmatory results, 44, 34.4 percent, were mCIM positive; 40, 31.3 percent, were eCIM positive and therefore consistent with metallo-β-lactamase production; 3, 2.3 percent, showed a serine carbapenemase pattern, and 5, 3.9 percent, were ESBL confirmation positive. The organism-level distribution was clearly heterogeneous. High mCIM and eCIM positivity were seen in CR-*Klebsiella oxytoca*, CRPA, *Enterobacter cloacae*, and several low-count Enterobacterales species, whereas *Acinetobacter baumannii* showed only 1 mCIM-positive isolate out of 48 tested. CRKP, the largest *Enterobacterales* subgroup, showed mCIM positivity in 14 of 33 isolates and metallo-β-lactamase positivity in 13 of 33 isolates, supporting the view that carbapenemase-mediated resistance, particularly metallo-β-lactamase activity, contributed materially to resistance in this species. The detail finding has been shown in Table 3.

Table 3: Distribution of mCIM, eCIM, and serine ESBL beta-lactamase confirmation results among carbapenem-resistant gram-negative bacterial isolates by organism.

Organism	Total Isolates (N)	(mCIM Positive n/N (%)	(mCIM negative n/N (%)	eCIM Metallo-β-lactamase Positive n/N (%)	Serine Carbapenemase Positive n/N (%)	ESBL Confirmation Positive n/N (%)	ESBL Confirmation Negative n/N (%)
Klebsiella pneumoniae	33	14/33 (42.4%)	18/33 (54.5%)	13/33 (39.4%)	1/33 (3.0%)	3/33 (9.1%)	30/33 (90.9%)
Klebsiella oxytoca	6	5/6 (83.3%)	1/6 (16.7%)	5/6 (83.3%)	1/6 (16.7%)	0/6 (0.0%)	6/6 (100.0%)
Klebsiella pneumoniae ss. *ozaenae*	3	3/3 (100.0%)	0/3 (0.0%)	1/3 (33.3%)	0/3 (0.0%)	0/3 (0.0%)	3/3 (100.0%)
Enterobacter cloacae	6	4/6 (66.7%)	2/6 (33.3%)	4/6 (66.7%)	1/6 (16.7%)	0/6 (0.0%)	6/6 (100.0%)
Citrobacter diversus	2	2/2 (100.0%)	0/2 (0.0%)	1/2 (50.0%)	0/2 (0.0%)	0/2 (0.0%)	2/2 (100.0%)
Acinetobacter baumannii	48	1/48 (2.1%)	46/48 (95.8%)	1/48 (2.1%)	0/48 (0.0%)	0/48 (0.0%)	47/48 (97.9%)
P. aeruginosa	15	12/15 (80.0%)	3/15 (20.0%)	12/15 (80.0%)	0/15 (0.0%)	2/15 (13.3%)	13/15 (86.7%)
Alcaligenes faecalis (odorans)	1	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)	1/1 (100.0%)
Acinetobacter lwoffii	1	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)	1/1 (100.0%)
Pantoea agglomerans	1	1/1 (100.0%)	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)	1/1 (100.0%)
Escherichia coli	12	2/12 (16.7%)	10/12 (83.3%)	2/12 (16.7%)	0/12 (0.0%)	0/12 (0.0%)	12/12 (100.0%)
Total	**128**	**44/128 (34.4%)**	**82/128 (64.1%)**	**40/128 (31.3%)**	**3/128 (2.3%)**	**5/128 (3.9%)**	**123/128 (96.1%)**

The very low ESBL confirmation positivity suggests that classical ESBL phenotypes explained only a small part of resistance in this collection. Instead, the phenotypic profile points more strongly toward carbapenemase-mediated resistance, especially metallo-β-lactamase activity, in key Enterobacterales and in CRPA. This also helps explain why resistance to cephalosporins and several β-lactam/β-lactamase inhibitor combinations remained so high in the study isolates. At the same time, the near-universal mCIM negativity in CRAB should be interpreted cautiously, because carbapenem resistance in this organism is often driven by mechanisms that are not captured well by *Enterobacterales*-oriented phenotypic algorithms.

Overall, the results suggest that carbapenemase-mediated resistance was more evident among CRE and CRPA, whereas CRAB appeared to rely more often on resistance mechanisms not consistently captured by mCIM or the applied beta-lactamase confirmation approach. This distinction is important because it suggests that carbapenem resistance in the present collection does not arise from a single shared pathway across all species.

5.4 Susceptibility to Polymyxin B and Tigecycline

Overall, TGC retained better in vitro activity than PMB in the stored isolate collection. The categorical susceptibility summary indicated that PMB results were dominated by the intermediate category, whereas TGC remained active against most isolates with valid results. Because the raw categorical summaries and some of the underlying frequency tables were not fully concordant, the detailed minimum inhibitory concentrations (MIC) and zone diameter distributions are presented descriptively below. Susceptibility data for PMB and TGC were available for the stored CRGNB isolates. For PMB, all 130/130 (100.0%) isolates had valid E-test results. In the summary used for final interpretation, 119/130 (92.3%) were classified as intermediate and 10/130 (7.7%) as resistant, suggesting limited in vitro activity of PMB in this dataset. The species-level summary showed stronger in vitro activity for TGC than for PMB, as has been shown in Table 4 below. TGC was reported as susceptible in 109/117 (94.1%) isolates and resistant in 8/117 (5.9%) isolates, with no intermediate category reported. In the earlier dataset summary, 115/130 (88.5%) isolates had valid TGC results, and 15/130 (11.5%) were missing. See the summary detailed in Table 4.

Table 4. Susceptibility profile of carbapenem-resistant gram-negative bacterial isolates to polymyxin B and tigecycline.

Antibiotic	Susceptible	Intermediate	Resistant	Missing
Polymyxin B	NA	120 (92.3%)	10 (7.6%)	-
Tigecycline	109 (93.1%)	NA	8 (6.8%)	13 (10.0%)

*NA indicates not applicable or not reported in the species-level summary. * *EUCAST guidelines were followed to define breakpoints for tigecycline, % R (resistant), and CLSI-M100 for polymyxin B. There is NO "Susceptible" category in standard CLSI interpretation.*

5.4.1 Minimum inhibitory concentration values for Polymyxin B

The raw PMB minimum inhibitory concentrations (MIC) table for 130 isolates showed that most values clustered in the low to moderate range. The commonest MIC was 1.5 mg/L per mL, observed in 62 isolates, or 47.7%. This was followed by 1.0 milliliter per mL in 36 isolates, or 27.7%, and 0.5 mg/L per mL or lower in 21 isolates, or 16.2%. High MIC values were less frequent, with 1 isolate at 4.0 mg/L per mL, 6 isolates above 4.0 mg/L per mL, and 3 isolates at 6.0 mg/L per mL. Taken together, 119 of 130 isolates, or 91.5%, had MIC values of 1.5 mg/L or lower, whereas 11 isolates, or 8.5%, were in the high MIC range from 4.0 to 6.0 mg/L.

Species-specific inspection showed that CRAB had mainly low PMB MIC values, with 25 isolates at 1.5 mg/L per mL, 12 at 0.5 mg/L per mL or lower, and 8 at 1.0 mg/L per mL; no CRAB isolate was reported with a PMB MIC of 4.0 mg/L per mL or higher in the raw species sheet. CRPA also clustered largely at 1.0 to 1.5 mg/L per mL, with 7 isolates at 1.5 mg/L per mL, 6 at 1.0 mg/L per mL, 1 at 4.0 mg/L per mL, and 1 at 0.5 mg/L per mL or lower. In CRKP, the most frequent MIC was again 1.5 mg/L per mL, followed by 1.0 mg/L per mL, but a small resistant subset showed values above 4.0 mg/L per mL and at 6.0 mg/L per mL. CREC showed mostly moderate MIC values, with 8 isolates at 1.5 mg/L per mL, 3 at 1.0 mg/L per mL, and 1 above 4.0 mg/L per mL. These results show that most isolates had PMB MICs at or below 1.5 mg/L, but a small high MIC tail was present, especially among some *Enterobacterales*. The resistant range of 4.0 to 6.0 mg/L per mL is concerning because it suggests the presence of isolates with reduced last-line treatment options. See the detail that has been shown in Table 5 below.

Table 5. MIC range µg/mL distributions for isolates for Polymyxin B

Polymyxin B MIC range mg/L	Number of isolates	%	Interpretation
0.5 or lower	21	16.2	Intermediate
1.0	36	27.7	Intermediate
1.5	62	47.7	Intermediate
4.0	1	0.8	Resistant
Above 4.0	6	4.6	Resistant
6.0	3	2.3	Resistant
Total	130	100.0	—

** CLSI M100 guidelines were followed to define breakpoints for tigecycline (susceptible, intermediate, and resistant). According to current CLSI/EUCAST recommendations for polymyxins: ≤2 µg/mL = susceptible and >2 µg/mL = resistant.*

5.4.2 Tigecycline susceptibility and zone diameter distribution

For TGC, the categorical summary sheet recorded valid results for 115 isolates, of which 107, or 93.0%, were susceptible and 8, or 7.0%, were resistant. In the raw zone diameter sheet, measurements were available for 117 isolates, and 13 were missing. Most zone diameters clustered between 20 and 25 mm, supporting the overall pattern of retained tigecycline activity in this collection. At the species level, the raw species summary indicated that CRAB remained largely susceptible to TGC, with 44 susceptible and 4 resistant isolates among 48 tested. Klebsiella pneumoniae also showed substantial retained activity, with 28 susceptible and 4 resistant isolates in the available species-specific worksheet. CREC, CR *Klebsiella oxytoca*, and *Enterobacter cloacae* were reported as fully susceptible in the same raw summary, as has been shown in Table 6 below.

Minor species such as Alcaligenes faecalis, CRA *Alwoffii, Citrobacter diversus*, *Pantoea agglomerans*, and CRPA were also recorded as fully susceptible in the species-level table supplied. The zone diameter distribution showed that the most frequent values were 22 mm, 23 mm, 25 mm, and 20 mm. Only a small number of isolates fell at the lower end of the distribution. Overall, the tigecycline data indicate substantially better retained activity than the PMB categorical summary, even though resistant isolates were still present. See details in Table 6.

Table 6. Zone of inhibition distributions for isolates for tigecycline

Tigecycline zone diameter mm	Frequency	%	Interpretation
15#*	1	0.8	Resistant
18#*	7	5.4	Resistant
19	1	0.8	Susceptible
20	17	13.1	Susceptible
21	11	8.5	Susceptible
22	27	20.8	Susceptible
23	23	17.7	Susceptible
24	8	6.2	Susceptible
25	19	14.6	Susceptible
26	3	2.3	Susceptible
Total valid	117	90.0% of all isolates	—
Missing	**13	10.0 of all isolates	—
Total study isolates	130	100.0	Interpretation

For E.coli **EUCAST and '#' indicate clinical breakpoints. FDA, and USA guidelines were followed to define breakpoints for tigecycline disk diffusion (susceptible, intermediate, and resistant). **Intrinsic Resistance isolate*

5.5 Trends of resistance to Polymyxin B and tigecycline over five years

A total of 130 bacterial isolates were recovered between 2019 and 2023. CRAB was the most frequently isolated organism, accounting for 48 (36.9%) isolates, followed by CRKP 33 (25.4%), CRPA 15 (11.5%), and CREC 12 (9.2%). The highest number of isolates was recorded in 2019 (53, 40.8%), followed by 2021 (38, 29.2%). CRAB predominated in 2019, constituting 62.3% of isolates recovered during that year, whereas CRKP showed an increasing relative contribution over time, representing 42.9% of isolates in 2023. The predominance of Gram-negative organisms throughout the study period suggests a substantial burden of healthcare-associated infections and highlights the potential role of antimicrobial resistance among the circulating bacterial pathogens.

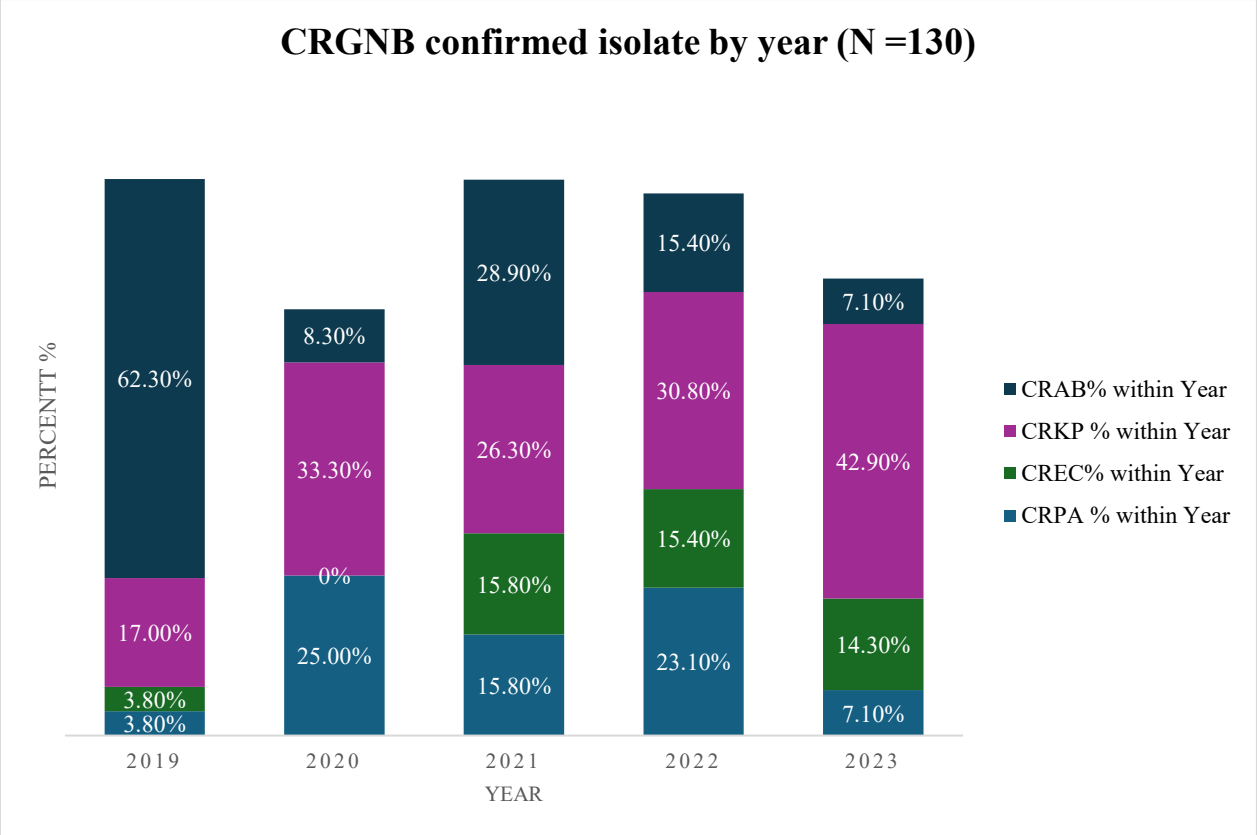


Figure 7. A key bacterial species isolated yearly distribution (percents %) dominated by Gram-negative pathogens.

The yearly isolate distribution showed a marked fluctuation across the study period from 2019 to 2023. The largest number of isolates was recorded in 2019, with 53 of 130 isolates, or 40.8%. This dropped sharply in 2020 to 12 isolates, or 9.2%, then rose again in 2021 to 38 isolates, or 29.2%. Lower numbers were recorded in 2022 and 2023, with 13 isolates, or 10.0%, and 14 isolates, or 10.8%, respectively. The sharp reduction in 2020 likely reflects disruption of routine clinical and laboratory activity during the coronavirus disease pandemic, with partial recovery in the following years. The isolate distribution, across from the onset of the COVID-19 pandemic in November 2019 through to October 2022, shows the distribution of specimen collection over this period. The majority of specimens, 53 in total (40.8%), were collected in 2019, likely representative pre-pandemic or early pandemic samples. In 2020, a notable decrease occurred, with only 12 specimens (9.2%) collected, which may reflect the disruptions in healthcare and laboratory testing services during the initial COVID-19 response. Collection increased again in 2021, with 38 specimens (29.2%), as healthcare systems began to stabilize and testing efforts resumed. (Figure 8).

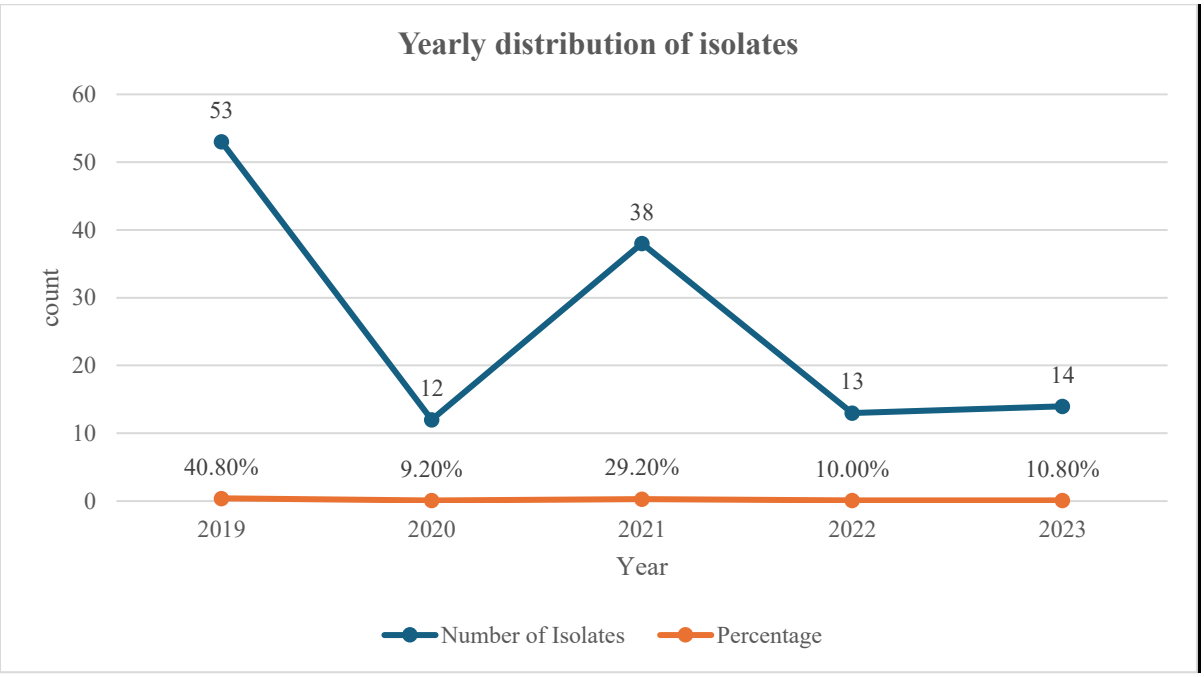


Figure 8. CRGNBs' trend frequencies over five years, 2019-2023

For Polymyxin B, the dominant pattern throughout the five-year period was intermediate susceptibility. In 2019, 47 of 53 isolates, or 88.7%, were intermediate, 5 isolates, or 9.4%, were resistant, and 1 isolate, or 1.9%, was susceptible. In 2020, 83.3 % of isolates were intermediate and 8.3 % were resistant. In 2021 and 2022, more than 92% of isolates remained intermediate, while resistance stayed below 8%. In 2023, no resistant isolate was recorded, with 13 of 14 isolates, or 92.9%, intermediate and 1 isolate, or 7.1%, susceptible. Overall, the PMB profile remained fairly stable across the study period, with resistance present at a low level and most isolates falling in the intermediate category (Figure 9).

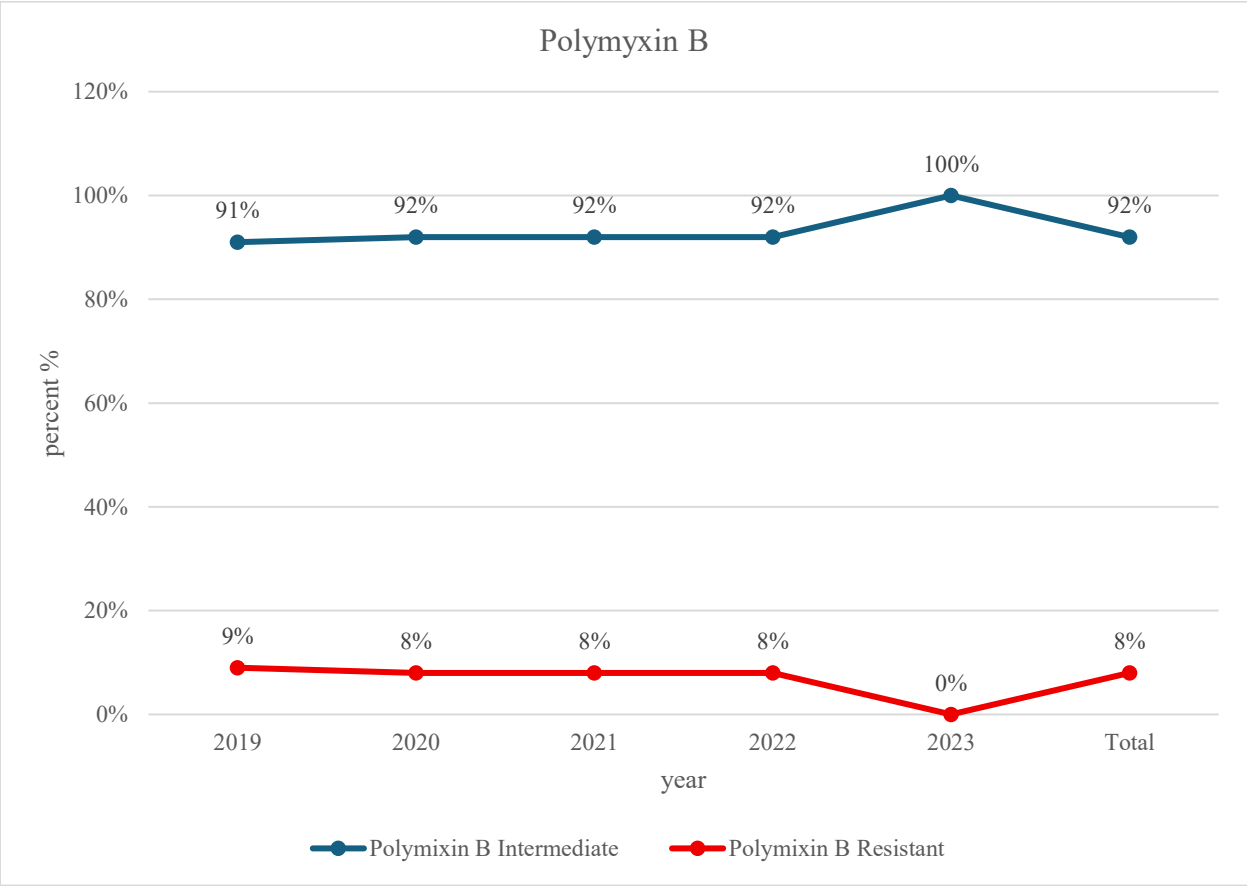


Figure 9. PMB showed a more favorable pattern over time from 2019 to 2023.

Using valid yearly results only, susceptibility was 92.0 % in 2019, 88.9 % in 2020, 6.0 % in 2021, 100.0 % in 2022, and 92.3 % in 2023. Resistance remained low in every year, ranging from 0.0% in 2022 to 11.1% in 2020. Across the full study period, TGC susceptibility remained high and resistance remained uncommon. These results indicate that TGC preserved better in vitro activity than polymyxin B, although both drugs require continued surveillance because resistant isolates were present. For TGC, among the 115 specimens collected, a high level of susceptibility was observed across the years. In 2019, 92% of the isolates were susceptible, with only 8% showing resistance. In 2020, susceptibility slightly declined to 88.9%, with a minor increase in resistance to 11.1%. However, susceptibility improved in the following years, reaching 100% in 2022. In 2023, susceptibility remained high at 92.3%, with only a single isolate (7.7%) showing resistance. Across the entire period, TGC susceptibility was maintained at 93%, and resistance was minimal (7%) (Figure 10).

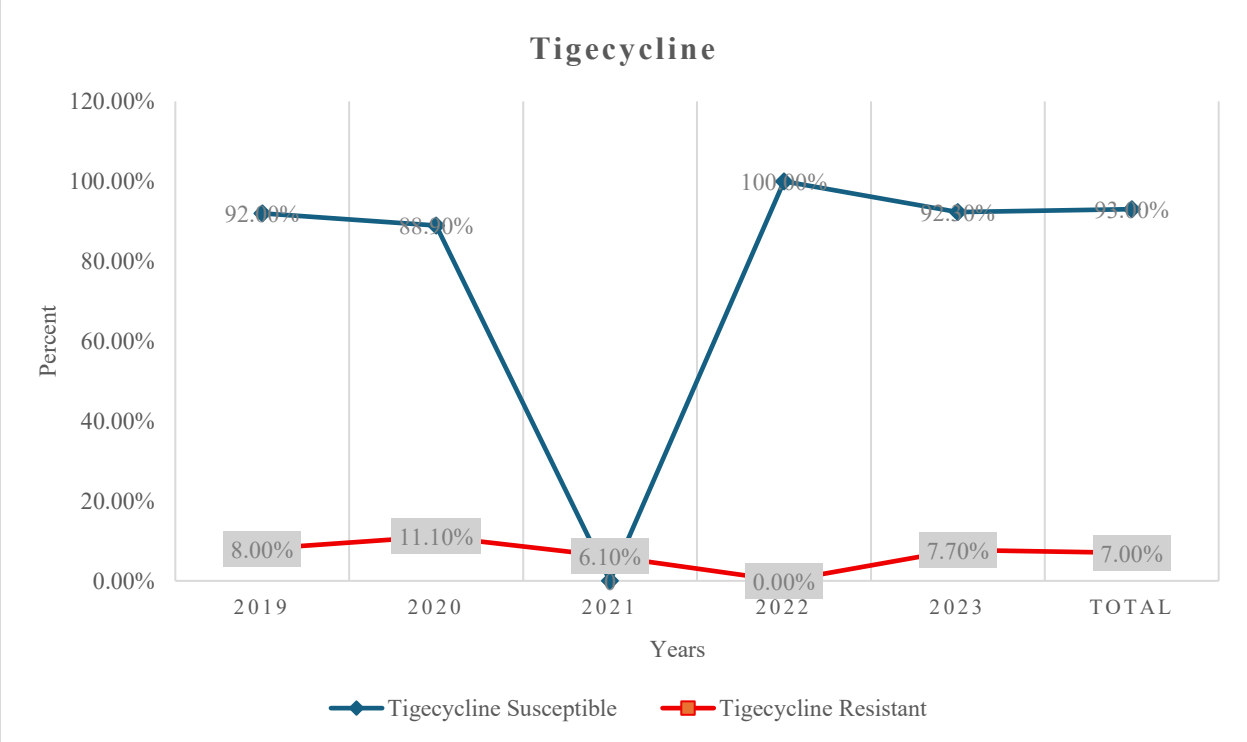


Figure 10. The figures illustrate the antimicrobial susceptibility trend of tigecycline from 2019 to 2023.

5.4.4 Susceptibility pattern of tigecycline and polymyxin B with phenotypic resistance markers by age group, sex, and health facility

Overall, tigecycline resistance remained uncommon across demographic subgroups, while polymyxin B resistance and mCIM positivity were more frequent than tigecycline resistance. Among age groups, tigecycline resistance was highest in patients older than 65 years (2/16, or 12.5%) and in those aged 15 to 24 years (2/18, or 11.1%), whereas polymyxin B resistance was highest in children aged 1 to 14 years (3/11, or 27.3%). mCIM positivity was highest in patients older than 65 years (4/16, 25.0%) and in those aged 25 to 44 years (11/45, 24.4%). By sex, tigecycline resistance was slightly higher in males (6/85, 7.1%) than in females (2/45, 4.4%), whereas polymyxin B resistance was more frequent in females (6/45, 13.3%) than in males (4/85, 4.7%).

Modified carbapenem inactivation positivity was observed in 13/45 (28.9%) female isolates and 16/85 (18.8%) male isolates. At the health facility level, AeBET Hospital contributed the highest absolute number of tigecycline-resistant isolates, 6/60 (10.0%), while mCIM positivity was proportionally highest in Tikur Anbessa Specialized Hospital (2/3, 66.7%), Ras Desta Referral Hospital (4/12, 33.3%), St. Paul Hospital (2/6, 33.3%), and Federal Police Referral Hospital (3/10, 30.0%). Metallo-beta-lactamase and ESBL positivity remained infrequent across most institutions. A detailed summary has been shown in Table 7 below.

Table 7: Summarizes tigecycline resistance, polymyxin B resistance, modified carbapenem inactivation positivity, metallo-beta-lactamase positivity, and ESBL positivity across age group, sex, and health facility.

Category	**Subgroup**	**Tigecycline-resistant**	**Polymyxin B-resistant**	**mCIM positive**	**MBL positive**	**ESBL positive**
Age group	Less than 1 year	0/17 (0.0%)	1/17 (5.9%)	3/17 (17.6%)	1/17 (5.9%)	1/17 (5.9%)
	1 to 14 years	1/11 (9.1%)	3/11 (27.3%)	2/11 (18.2%)	0/11 (0.0%)	0/11 (0.0%)
	15 to 24 years	2/18 (11.1%)	0/18 (0.0%)	4/18 (22.2%)	0/18 (0.0%)	0/18 (0.0%)
	25 to 44 years	3/45 (6.7%)	4/45 (8.9%)	11/45 (24.4%)	1/45 (2.2%)	1/45 (2.2%)
	45 to 64 years	0/23 (0.0%)	2/23 (8.7%)	5/23 (21.7%)	2/23 (8.7%)	2/23 (8.7%)
	More than 65 years	2/16 (12.5%)	0/16 (0.0%)	4/16 (25.0%)	1/16 (6.3%)	1/16 (6.3%)
Sex	Female	2/45 (4.4%)	6/45 (13.3%)	13/45 (28.9%)	4/45 (8.9%)	4/45 (8.9%)
	Male	6/85 (7.1%)	4/85 (4.7%)	16/85 (18.8%)	1/85 (1.2%)	1/85 (1.2%)
Health facility	AeBET Hospital	6/60 (10.0%)	4/60 (6.7%)	11/60 (18.3%)	1/60 (1.7%)	1/60 (1.7%)
	Bethezata Hospital	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)
	Minilik I Referral Hospital	0/17 (0.0%)	1/17 (5.9%)	5/17 (29.4%)	0/17 (0.0%)	0/17 (0.0%)
	Private clinic	1/4 (25.0%)	1/4 (25.0%)	0/4 (0.0%)	1/4 (25.0%)	1/4 (25.0%)
	Government health center	0/7 (0.0%)	0/7 (0.0%)	0/7 (0.0%)	0/7 (0.0%)	0/7 (0.0%)
	Federal Police Referral Hospital	0/10 (0.0%)	2/10 (20.0%)	3/10 (30.0%)	0/10 (0.0%)	1/10 (10.0%)
	Ras Desta Referral Hospital	0/12 (0.0%)	0/12 (0.0%)	4/12 (33.3%)	2/12 (16.7%)	2/12 (16.7%)
	St. Paul Hospital	0/6 (0.0%)	1/6 (16.7%)	2/6 (33.3%)	1/6 (16.7%)	1/6 (16.7%)
	St. Peter Hospital	0/7 (0.0%)	0/7 (0.0%)	1/7 (14.3%)	0/7 (0.0%)	0/7 (0.0%)
	Tikur Anbessa Specialized Hospital	0/3 (0.0%)	0/3 (0.0%)	2/3 (66.7%)	0/3 (0.0%)	0/3 (0.0%)
	Yekatit 12 Hospital	0/3 (0.0%)	0/3 (0.0%)	0/3 (0.0%)	0/3 (0.0%)	0/3 (0.0%)

5.4 Resistance trends among other classes of antibiotics in key carbapenem-resistant Gram-negative bacilli

5.5.1 *Carbapenem-resistant Enterobacterales*

Among the 130 CRGNB included in the study, *Enterobacterales* made up about half of the isolate set. The main *Enterobacterales* species were CRKP 33/130 (25.4%), CREC 12/130 (9.2%), *Enterobacter cloacae* 8/130 (6.2%), *Klebsiella oxytoca* 6/130 (4.6%), *Klebsiella ozaenae* 3/130 (2.3%), *Citrobacter diversus* 2/130 (1.5%), and *Pantoea agglomerans* 1/130 (0.8%). These organisms were recovered mainly from urine, blood, and pus, showing that carbapenem-resistant Enterobacterales in the present collection were concentrated in clinically important urinary, bloodstream, and wound-associated infections. Within this group, CRKP was the dominant species and the clearest *Enterobacterales* contributor to carbapenem-resistant infection. It was followed by CREC and *Enterobacter cloacae*, while the remaining species contributed smaller numbers. See Fig. 11 below.

5.5.2 Carbapenem-resistant *Escherichia coli, Klebsiella pneumoniae*, and *Enterobacter cloacae*

CREC demonstrated a slightly lower but still concerning resistance pattern. Complete resistance (100%) was observed for cefazolin and levofloxacin, while very high resistance (>90%) was recorded for most cephalosporins and fluoroquinolones. Aminoglycosides showed variable performance, with gentamicin resistance reaching 91.7% but amikacin showing comparatively lower resistance (25.0%), suggesting partial retention of activity. Resistance to amoxicillin/clavulanic acid (83.3%) and piperacillin/tazobactam (83.3%) indicates widespread β-lactamase production, consistent with ESBL-producing E. coli. Notably, fosfomycin (25.0%) and ceftazidime-avibactam (91.7%) resistance patterns suggest limited but not absent therapeutic options. See the detail that has been shown in Figure. 12 below.

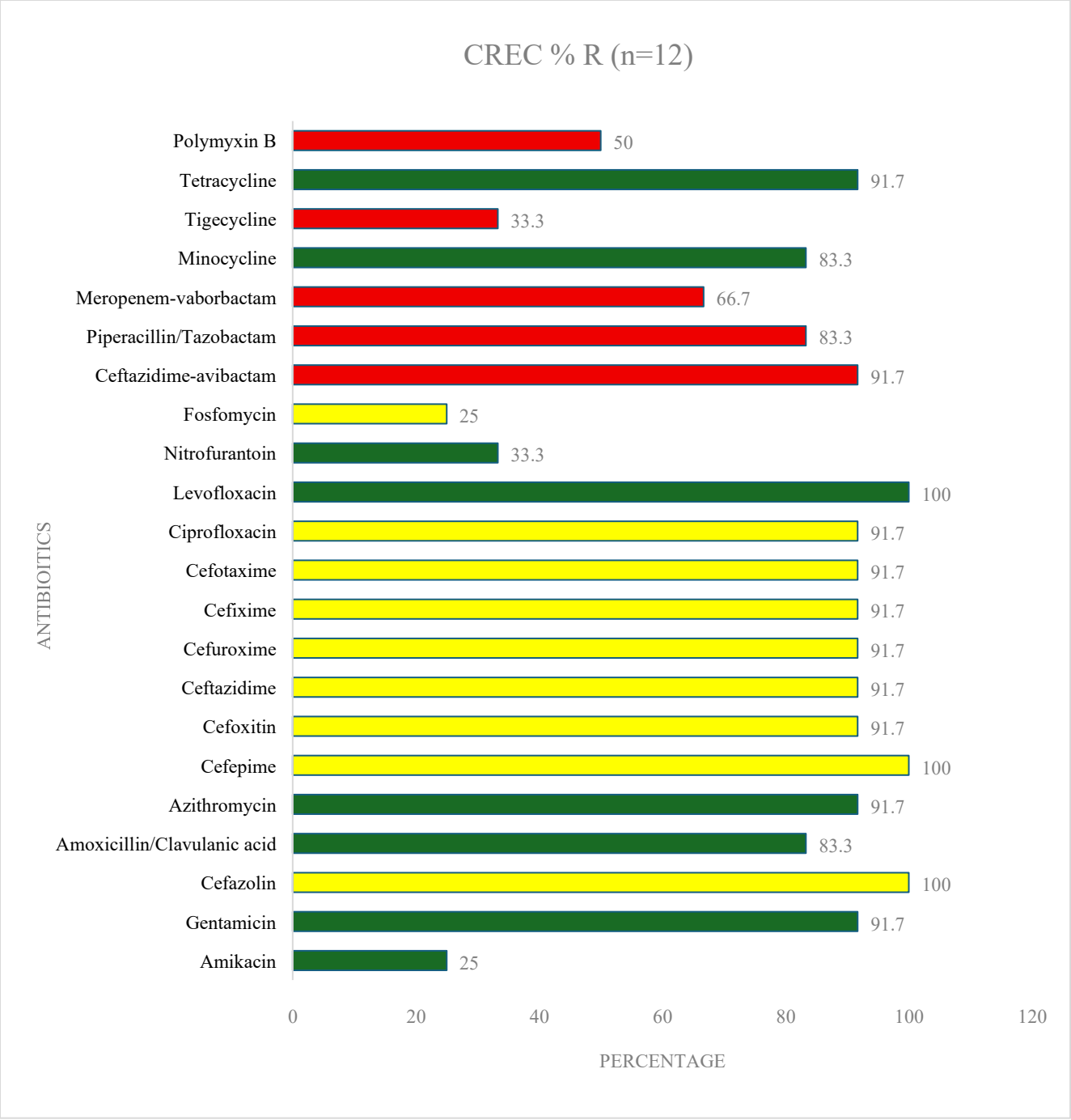


Figure 12. Percentage distribution of antibiotic resistance among carbapenem-resistant *Escherichia coli* (CREC) isolates. *CLSI M100 and * EUCAST guidelines were followed to define breakpoints for tigecycline. *color shows WHO & EML AWaRe classification AWaRe, green (Access), yellow (Watch), and red (Reserve) antibiotics include last-line agents for resistance.*

CRKP exhibited a highly resistant phenotype, with universal resistance (100%) to most cephalosporins, including cefazolin, cefepime, cefoxitin, and ceftazidime, indicating a strong presence of carbapenemase and ESBL-producing strains. High resistance was also observed for ciprofloxacin (93.9%), levofloxacin (93.9%), and tigecycline (97.0%), further limiting treatment options. Aminoglycoside resistance was also substantial, with amikacin (57.6%) and gentamicin (75.8%) showing poor activity. The relatively lower resistance to Polymyxin B (12.1%) and meropenem-vaborbactam (84.8%) suggests that only a few last-resort agents retain partial effectiveness, although even these are increasingly compromised. See the detail that has been shown in Figure. 13 below.

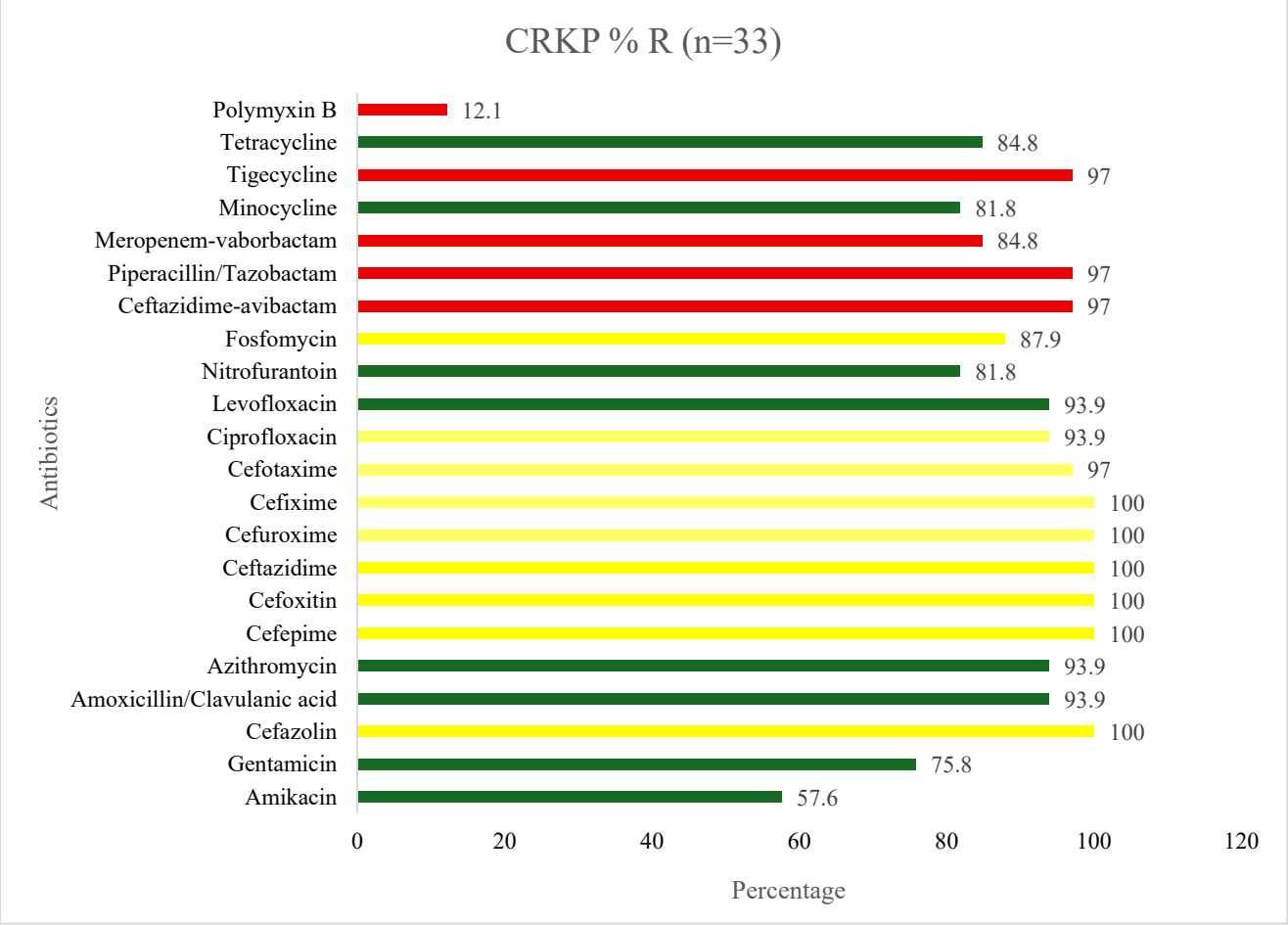
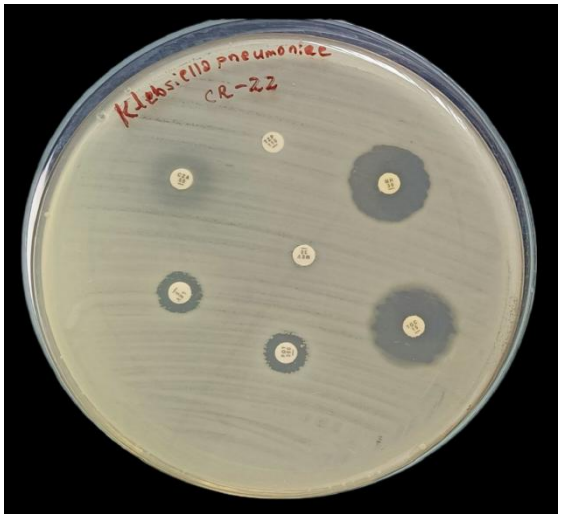


Figure 13: Percentage distribution of antibiotic resistance among CRKP isolates. * CLSI M100 * EUCAST guidelines were followed to define breakpoints for tigecycline). The WHO & EML AWaRe classification AWaRe includes green (Access), yellow (Watch), and red (Reserve) antibiotics, which are last-line agents for resistance.



(A) (B)

(C) 

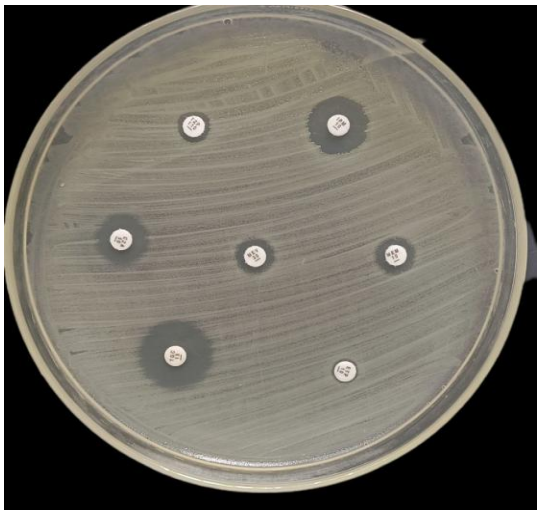
(D) 

Figure 11. The photographic plate shows antimicrobial susceptibility testing of CRKP and CREC, a significant multidrug-resistant pathogen. Key Takeaway: These findings confirm that the strain is not only resistant to standard treatments but also specifically produces enzymes that drive its "central role in the local burden of CRGNB (carbapenem-resistant Gram-negative bacteria)." * CLSI M100 and * EUCAST guidelines were followed to define breakpoints for tigecycline. The plates illustrate different laboratory methods used to identify resistance patterns: (A) CRKP & (B) CREC E-tests with MIC = 2 µg/ml. and 22 mm : These use strips with a concentration gradient of an antibiotic to determine the Minimum Inhibitory Concentration (MIC). The elliptical "zone of inhibition" shows where the bacteria can no longer grow with an intermediate MIC = 2 µg/ml. (C) & (D) Disk Diffusion: These plates use small paper disks impregnated with antibiotics. Plate (C): CRKP, Shows several disks with small surrounding clear zone, indicating the bacteria are highly resistant to those specific drugs.

5.5.3 *Carbapenem-resistant Acinetobacter baumannii*

Among the individual organisms, Acinetobacter baumannii showed the most extreme resistance profile overall, with resistance reaching 100% for most cephalosporins and β-lactam agents, including cefepime and cefotaxime. High resistance was also observed for amikacin (68.8%) and gentamicin (85.4%), reflecting reduced efficacy of aminoglycosides. Additionally, resistance to ciprofloxacin (91.7%) and levofloxacin (89.6%) further highlights its multidrug-resistant nature. Even last-line or advanced agents such as tigecycline (97.9% resistance) and piperacillin/tazobactam (97.9% resistance) showed poor performance, indicating a highly resistant hospital-associated strain population. See the detail that has been shown in Figure. 14 below.

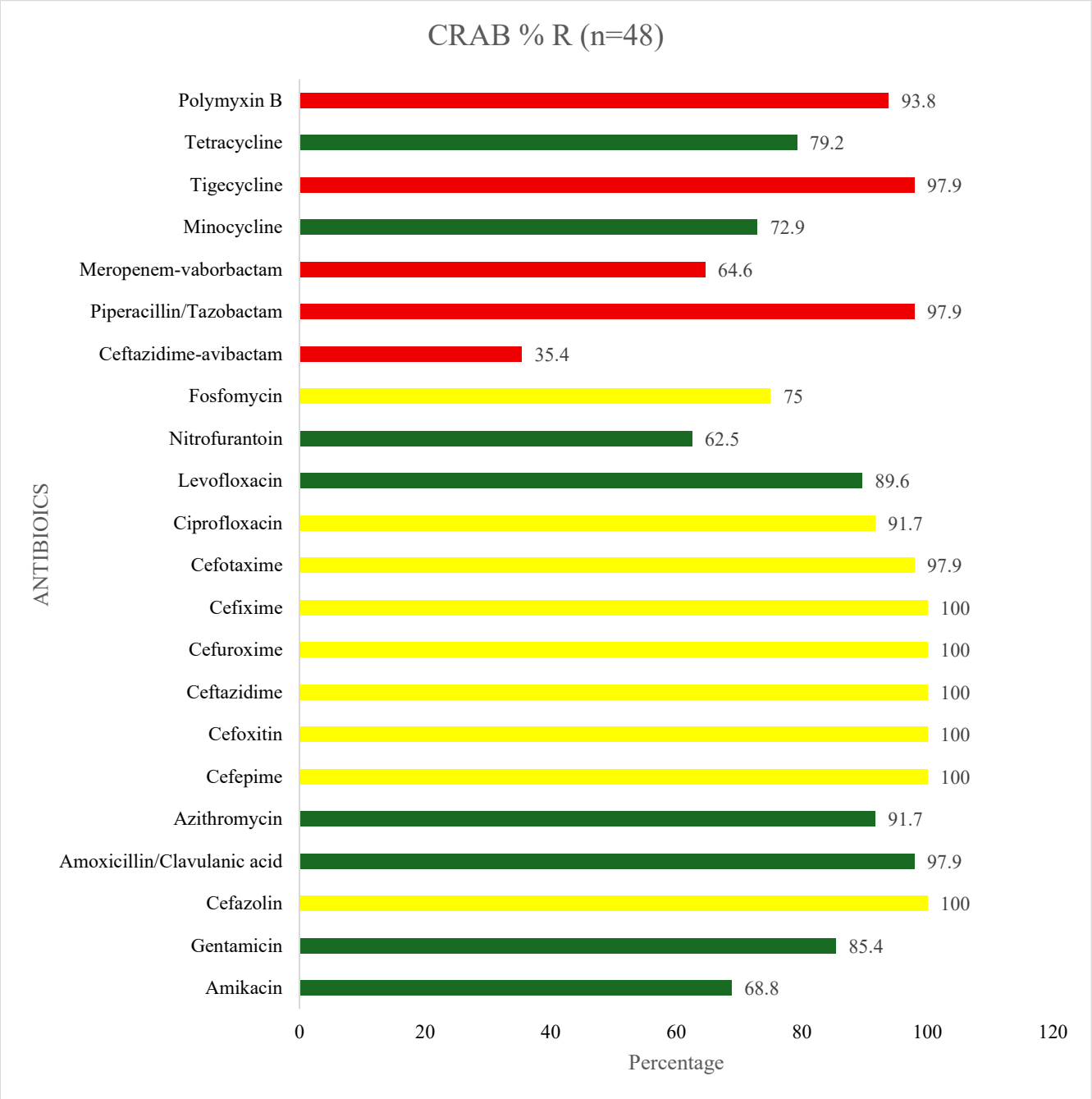


Figure 14: Percentage distribution of antibiotic resistance among carbapenem-resistant *A. baumannii* (CRAB) isolates. * EUCAST guidelines were followed to define breakpoints for tigecycline; % R (Resistant) color shows WHO & EML AWaRe classification. AWaRe, green (Access); yellow (Watch) and red (Reserve) antibiotics include last-line agents for resistance.

5.5.4 *Carbapenem-resistant Pseudomonas aeruginosa*

CRPA demonstrated a distinct but still highly resistant profile. Complete resistance (100%) was observed for cefazolin, amoxicillin/clavulanic acid, and most cephalosporins, as well as levofloxacin. High resistance was also seen for ciprofloxacin (86.7%) and fosfomycin (86.7%),. However, comparatively lower resistance was observed for gentamicin (46.7%) and meropenem-vaborbactam (53.3%), suggesting some retained susceptibility to selected aminoglycosides and carbapenem-based combinations. Importantly, Polymyxin B showed 0% resistance, confirming it as the most effective agent against this organism Figs. 15 below.

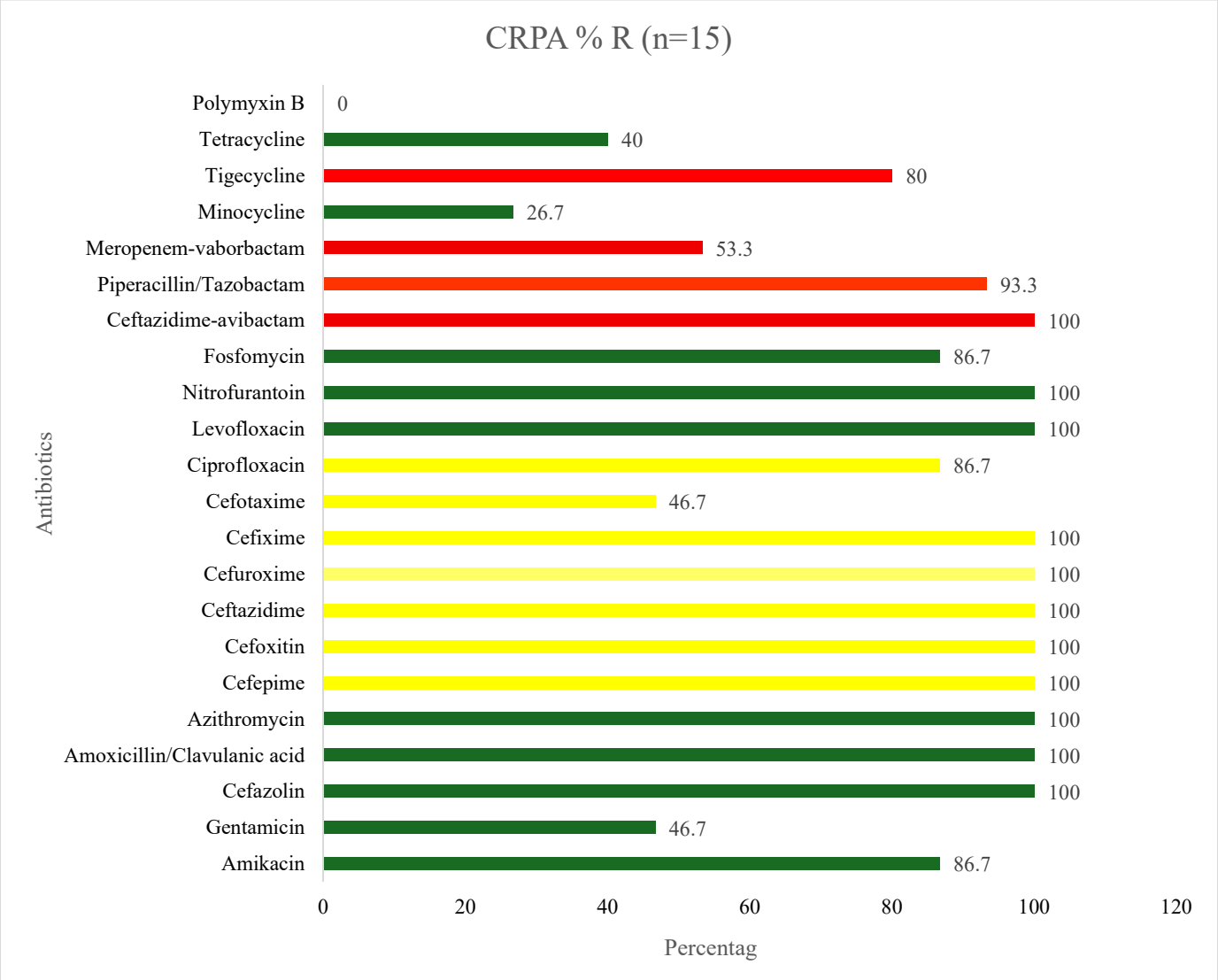
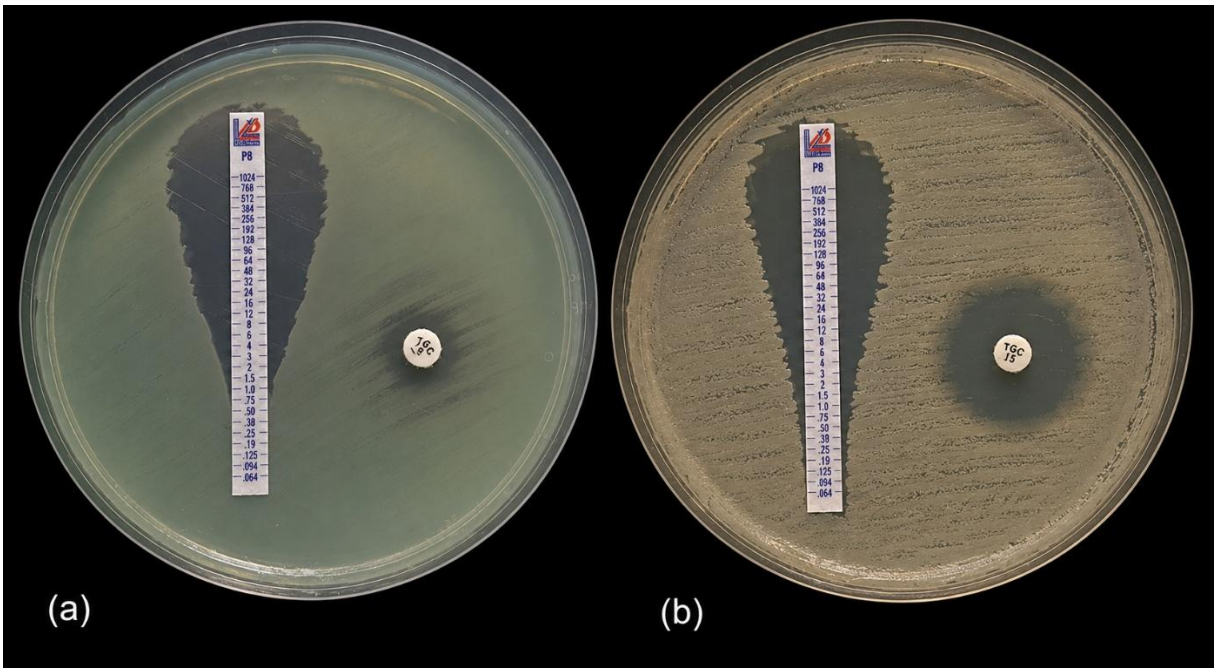


Figure 15: Percentage distribution of antibiotic resistance among carbapenem-resistant Pseudomonas aeruginosa (CRPA) isolates. * EUCAST guidelines were followed to define breakpoints for tigecycline, % R (Resistant). * CLSI

M100 guidelines were followed to define breakpoints for tigecycline; % R (Resistant) color shows WHO & EML AWaRe classification. AWaRe, green (Access); yellow (Watch) and red (Reserve) antibiotics include last-line agents for resistance.

PMB emerged as the most reliable agent overall, with 0% resistance in CRPA and very low resistance in CRKP (12.1%), although higher resistance was observed in CRAB (93.8%) and CREC (50.0%), indicating emerging resistance even to last-line therapy. The Photographic plate results are shown below in (Photogeraph image on Figure 16).



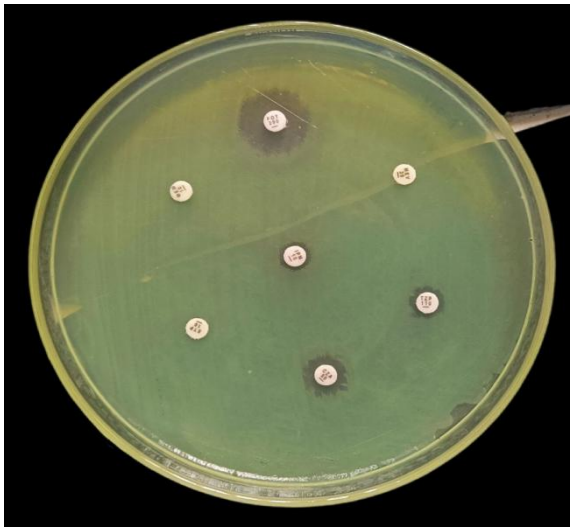
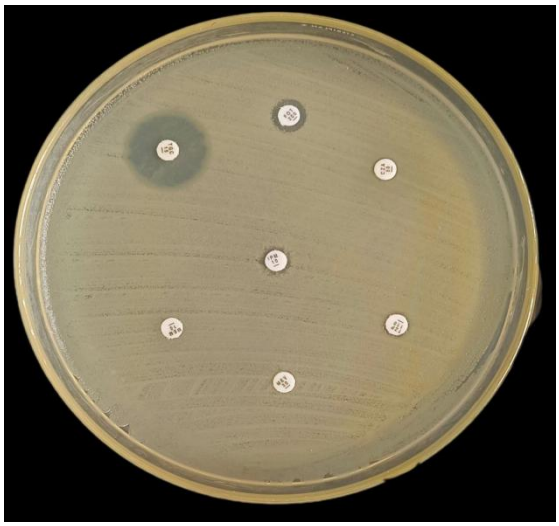
(C)  (D) 

Figure 16: Photographic plate showing antimicrobial susceptibility testing of carbapenem-resistant Pseudomonas aeruginosa (CRPA) and carbapenem-resistant Acinetobacter baumannii (CRAB), significant multidrug-resistant pathogens. The plates illustrate different laboratory methods used to identify resistance patterns: (A) CRPA – Disk Diffusion: The disk showed a zone diameter of 6 mm, consistent with CLSI and EUCAST guidelines, demonstrating intrinsic resistance to the 15 µg tigecycline (TGC) disk. (B) CRAB – Disk Diffusion: The isolate was susceptible to the 15 µg TGC disk. (A) E-test: Uses strips with a concentration gradient of an antibiotic to determine the Minimum Inhibitory Concentration (MIC). Bothe are SEnsetive to PMB with the elliptical zone (1.5 mm) of inhibition shows where bacterial growth is inhibited. The plates illustrate (C&D) Disk Diffusion: Shows multiple disks with no surrounding clear zone of inhibition (zone diameter of 6 mm), except for C, only folcomycin show sensitive for D only tigecycline is sensative, indicating the bacteria are highly resistant to those specific antimicrobial agents.

5.6 Susceptibility to other antimicrobial classes

Similarly, fluoroquinolones such as ciprofloxacin and levofloxacin showed very poor activity, with susceptibility rates generally below 10% across all pathogens. Likewise, β-lactam/β-lactamase inhibitor combinations such as amoxicillin/clavulanic acid and piperacillin/tazobactam demonstrated minimal effectiveness, reinforcing the limited utility of standard empirical therapies in this context.

CRAB exhibited the most alarming resistance profile, with very low susceptibility to all tested antibiotics. The highest susceptibility rates were observed with amikacin (29.2%), nitrofurantoin (31.3%), fosfomycin (22.9%), and minocycline (20.8%), while most other agents showed near-complete inactivity. Even newer combinations such as ceftazidime-avibactam (47.9% S, 16.7% I) and meropenem-vaborbactam (20.8% S, 14.6% I) demonstrated only partial effectiveness, reflecting the organism's well-known capacity to acquire multiple resistance determinants, including OXA-type carbapenemases and efflux-mediated resistance mechanisms.

CREC showed comparatively higher susceptibility than the other pathogens, particularly to amikacin (75.0%), fosfomycin (66.7%), tigecycline (66.7%), and nitrofurantoin (50.0%). Despite this, resistance to cephalosporins and fluoroquinolones remained extremely high, indicating a strong ESBL-producing population. The moderate susceptibility to nitrofurantoin and fosfomycin suggests their continued usefulness, especially in urinary tract infections where these drugs achieve high urinary concentrations and clinical efficacy despite in vitro resistance in other agents.

CRKP demonstrated a highly resistant phenotype with very limited therapeutic options. The highest susceptibility was observed with amikacin (30.3%), followed by minocycline (15.2%) and nitrofurantoin (12.1%), while most β-lactams and fluoroquinolones showed complete or near-complete resistance. The presence of some intermediate susceptibility to agents such as meropenem-vaborbactam (12.1% I) suggests emerging but insufficient activity of newer β-lactamase inhibitor combinations. Overall, this pattern is consistent with carbapenemase-producing *K. pneumoniae*, which represents a major global clinical concern.

CRPA showed a somewhat distinct susceptibility pattern compared to the other organisms. It retained relatively higher susceptibility to minocycline (73.3%), tetracycline (60.0%), and gentamicin (53.3%), while most cephalosporins and fluoroquinolones showed complete resistance. The moderate response to meropenem-vaborbactam (46.7% S) suggests partial retained activity of this combination against some isolates. However, the overall resistance profile still indicates limited treatment options and highlights the organism's intrinsic and acquired resistance mechanisms, including efflux pumps and reduced outer membrane permeability.

Polymyxin B demonstrated the highest overall activity against the tested organisms and remains the most reliable last-resort antibiotic. It showed particularly high susceptibility against CRKP (87.9% S) and *P. aeruginosa* (100% S), while CRCE showed only 50% susceptibility and *A. baumannii* showed very limited intermediate susceptibility (6.3%). Although polymyxins remain effective for many isolates, the reduced susceptibility in some species is concerning and may indicate the early emergence of resistance to last-line therapies. See Table 8 for more detail.

Table 8. Distribution of antimicrobial susceptibility patterns among CRGNB.

Antimicrobial Class	Antibiotic	CRAB (S%/I%)	CREC (S%/I%)	CRKP (S%/I%)	CRPA (S%/I%)
Aminoglycoside	Amikacin	29.2 / 2.1	75.0 / 0.0	30.3 / 12.1	13.3 / 0.0
Aminoglycoside	Gentamicin	14.6 / 0.0	8.3 / 0.0	21.2 / 3.0	53.3 / 0.0
Cephalosporin	Cefazolin	0.0 / 0.0	0.0 / 0.0	0.0 / 0.0	0.0 / 0.0
β-lactam/β-lactamase inhibitor	Amoxicillin/clavulanic acid	0.0 / 2.1	8.3 / 8.3	0.0 / 6.1	0.0 / 0.0
Macrolide	Azithromycin	6.3 / 2.1	0.0 / 8.3	3.0 / 3.0	0.0 / 0.0
Cephalosporin	Cefepime	0.0 / 0.0	0.0 / 0.0	0.0 / 0.0	0.0 / 0.0
Cephalosporin	Cefoxitin	0.0 / 0.0	8.3 / 0.0	0.0 / 0.0	0.0 / 0.0
Cephalosporin	Ceftazidime	0.0 / 0.0	8.3 / 0.0	0.0 / 0.0	0.0 / 0.0
Cephalosporin	Cefuroxime	0.0 / 0.0	8.3 / 0.0	0.0 / 0.0	0.0 / 0.0
Cephalosporin	Cefixime	0.0 / 0.0	8.3 / 0.0	0.0 / 0.0	0.0 / 0.0
Cephalosporin	Cefotaxime	0.0 / 2.1	8.3 / 0.0	0.0 / 3.0	53.3 / 0.0
Fluoroquinolone	Ciprofloxacin	6.3 / 2.1	8.3 / 0.0	3.0 / 3.0	13.3 / 0.0
Fluoroquinolone	Levofloxacin	8.3 / 2.1	0.0 / 0.0	3.0 / 3.0	0.0 / 0.0
Nitrofuran	Nitrofurantoin	31.3 / 6.3	50.0 / 16.7	12.1 / 6.1	0.0 / 0.0
Phosphonic acid derivative	Fosfomycin	22.9 / 2.1	66.7 / 8.3	9.1 / 3.0	13.3 / 0.0
Cephalosporin/β-lactamase inhibitor	Ceftazidime-avibactam	47.9 / 16.7	0.0 / 8.3	0.0 / 3.0	0.0 / 0.0
β-lactam/β-lactamase inhibitor	Piperacillin/Tazobactam	0.0 / 2.1	8.3 / 8.3	0.0 / 3.0	6.7 / 0.0
Carbapenem/β-lactamase inhibitor	Meropenem-vaborbactam	20.8 / 14.6	25.0 / 8.3	3.0 / 12.1	46.7 / 0.0
Tetracycline	Minocycline	20.8 / 6.3	16.7 / 0.0	15.2 / 3.0	73.3 / 0.0
Glycylcycline	Tigecycline	0.0 / 2.1	66.7 / 0.0	3.0 / 0.0	13.3 / 6.7
Tetracycline	Tetracycline	18.8 / 2.1	8.3 / 0.0	12.1 / 3.0	60.0 / 0.0
Polymyxin	Polymyxin B	0.0 / 6.3	0.0 / 50.0	0.0 / 87.9	0.0 / 100.0

Abbreviations: S = Susceptible; I = Intermediate; NA = Not Available. Antimicrobial agents are grouped according to their pharmacological class.

5.7. Interpretation of multidrug resistance category

The CR-GNB isolates from Ethiopia's National Reference Laboratory (January 2019–December 2023, N = 130) display a resistance profile that is overwhelmingly multidrug-resistant (MDR), with a large proportion likely meeting the criteria for extensively drug-resistant (XDR) and only a very small fraction, if any, being pan-drug-resistant (PDR). By the standard Magiorakos definitions (98), MDR is defined as non-susceptibility to at least one agent in at least three antimicrobial categories, XDR as non-susceptibility to at least one agent in all but two or fewer categories, and PDR as non-susceptibility to all agents in all tested categories.

In this study, all isolates are already carbapenem-resistant by inclusion criteria, and they show near-universal resistance to cephalosporins (100% resistance to cefazolin, ceftazidime, cefuroxime, cefixime, and cefotaxime; 99.2% to cefepime); very high resistance to fluoroquinolones (93.8% ciprofloxacin, 90.8% levofloxacin); piperacillin/tazobactam (96.2%); and aminoglycosides (gentamicin 86.9%, amikacin 64.6%), plus high resistance to newer β-lactam/β-lactamase inhibitor combinations such as ceftazidime/avibactam (98.9%) and meropenem/vaborbactam (75.3%). This pattern of non-susceptibility across at least five to six antimicrobial categories (carbapenems, cephalosporins, fluoroquinolones, penicillin/β-lactamase inhibitors, aminoglycosides, and often carbapenem combinations) means that virtually 100% of these CR-GNB isolates are MDR. Many isolates are likely XDR, as they remain susceptible mainly to only two categories: tigecycline (93% susceptible among 115 tested) and polymyxin B (92.3% susceptible among 130 tested), with fosfomycin and nitrofurantoin showing mixed susceptibility but tested only in subsets (53 and 36 isolates, respectively).

Consequently, these organisms are resistant in all but two categories, fitting the XDR definition. PDR is unlikely to be common, because most isolates retain susceptibility to tigecycline and/or polymyxin B; PDR would require resistance to all agents, including these last-line options, which appears to occur in only a very small minority, if any, of isolates. Clinically, this pattern indicates a high burden of XDR CR-GNB at the national reference level, with treatment options severely limited to tigecycline and polymyxin B as the most reliable agents, while newer combination β-lactams and traditional classes offer minimal coverage.

In the present study, viewed through the WHO AWaRe (Access, Watch, Reserve) antibiotic classification. Under AWaRe, green (Access) antibiotics include first-line agents such as amikacin, gentamicin, cefazolin, cefuroxime, cefixime, cefotaxime, piperacillin/tazobactam, and nitrofurantoin; yellow (Watch) antibiotics include higher-resistance-risk agents such as cefepime, ceftazidime, ciprofloxacin, levofloxacin, fosfomycin, minocycline, and tigecycline; and red (Reserve) antibiotics include last-line agents for resistant infections such as meropenem/vaborbactam, ceftazidime/avibactam, and polymyxin B. In this dataset, the WHO Access (green) category is almost entirely compromised: resistance is 100% for cefazolin, cefuroxime, cefixime, cefotaxime, and ceftazidime; 99.2% for cefepime; 86.9% for gentamicin; 64.6% for amikacin; 96.2% for piperacillin/tazobactam; and 47.2% for nitrofurantoin (tested in only 36 isolates). This means that virtually all CR-GNB isolates are resistant to multiple Access-class antibiotics, reinforcing their MDR status.

In the WHO Watch (yellow) category, resistance remains very high for most agents: 93.8% for ciprofloxacin, 90.8% for levofloxacin, 98.9% for ceftazidime/avibactam (though this is technically a Reserve agent in AWaRe, it is often grouped with Watch-like β-lactam/β-lactamase inhibitors), 75.4% for minocycline, and 49.1% for fosfomycin (tested in only 53 isolates). However, tigecycline, a key Watch agent, shows 93% susceptibility (107/115), standing out as a major remaining option within the Watch class. In the WHO Reserve (red) category, which includes the most critical last-line agents for resistant Gram-negatives, meropenem/vaborbactam shows only 16.9% susceptibility (75.3% resistance), and ceftazidime/avibactam shows only 1.1% susceptibility (98.9% resistance), but polymyxin B shows 92.3% susceptibility (only 7.7% resistance), making it the most effective Reserve-class agent in this dataset.

The fact that resistance spans nearly all Access (green) antibiotics, most Watch (yellow) antibiotics, and most Reserve (red) agents except polymyxin B means that these isolates are non-susceptible to at least three AWaRe categories, confirming MDR; they are resistant in all but one or two AWaRe categories (mainly tigecycline in Watch and polymyxin B in Reserve), which fits the XDR definition; and true PDR is unlikely because most isolates remain susceptible to at least tigecycline and/or polymyxin B. Clinically, this AWaRe-framed interpretation highlights that the WHO Access (green) pool is effectively unusable for these CR-GNB, the Watch (yellow) pool is largely compromised except for tigecycline, and the Reserve (red) pool is critically limited to polymyxin B as the primary reliable option. This pattern underscores a high burden of XDR CR-GNB in Ethiopia, with urgent needs for antimicrobial stewardship, strengthened infection prevention and control, and continued AMR surveillance with molecular characterization of carbapenemases to guide public health responses and preserve the remaining effectiveness of TGC and PMB.

Table 9: Summary of different antibiotic category susceptibility patterns among carbapenem-resistant gram-negative bacilli at the National Reference Laboratory, Addis Ababa, Ethiopia, January 2019 to December 2023.

Antimicrobial class category	**Antibiotic**	**2019 N=53**			**2020 N=12**			**2021 N=38**			**2022 N=13**			**2023 N=14**			**Total N=130**			
		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	*N*	*S (%)*	*I (%)*	*R (%)*
Aminoglycosides	**Amikacin**	16	3	34	4	1	7	11	0	27	6	1	6	4	0	10	130	41 (31.5)	5 (3.8)	84 (64.6)
	Gentamicin	7	0	45	1	0	8	5	0	31	1	0	11	1	1	11	122	15 (12.3)	1 (0.8)	106 (86.9)
Cephalosporins	**Cefazolin**	0	0	53	0	0	12	0	0	38	0	0	13	0	0	14	130	0 (0.0)	0 (0.0)	130 (100)
	Cefepime	0	0	53	0	0	12	1	0	37	0	0	13	0	0	14	130	1 (0.8)	0 (0.0)	129 (99.2)
	Ceftazidime	0	0	53	0	0	12	0	0	38	0	0	13	0	0	14	130	0 (0.0)	0 (0.0)	130 (100)
	Cefuroxime	0	0	53	0	0	12	0	0	38	0	0	13	0	0	14	130	0 (0.0)	0 (0.0)	130 (100)
	Cefixime	0	0	53	1	0	11	0	0	38	0	0	13	0	0	14	130	1 (0.8)	0 (0.0)	129 (99.2)
	Cefotaxime	0	0	51	0	0	9	0	0	34	0	0	11	0	0	13	118	0 (0.0)	0 (0.0)	118 (100)
Fluoroquinolones	**Ciprofloxacin**	1	1	51	0	0	12	5	0	33	1	0	12	0	0	14	130	7 (5.4)	1 (0.8)	122 (93.8)
	Levofloxacin	2	2	49	0	0	12	3	2	33	1	0	12	1	1	12	130	7 (5.4)	5 (3.8)	118 (90.8)
Nitrofurans	**Nitrofurantoin**	6	1	4	2	1	3	5	0	7	2	0	1	1	1	2	36	16 (44.4)	3 (8.3)	17 (47.2)
Polyphosphates	**Fosfomycin**	7	1	8	5	0	3	5	2	12	2	0	3	3	2	0	53	22 (41.5)	5 (9.4)	26 (49.1)
β-Lactam Combinations	**Piperacillin/Tazobactam**	0	1	52	0	0	12	2	0	36	0	1	12	1	0	13	130	3 (2.3)	2 (1.5)	125 (96.2)
	Ceftazidime/avibactam	1	0	22	0	0	12	0	0	28	0	0	12	0	0	14	89	1 (1.1)	0 (0.0)	88 (98.9)
Carbapenems	**Meropenem/vaborbactam**	8	4	16	0	2	7	5	0	24	1	1	8	1	0	12	89	15 (16.9)	7 (7.9)	67 (75.3)
Tetracyclines	**Minocycline**	11	2	38	3	0	6	6	1	27	1	0	10	3	2	8	118	24 (20.3)	5 (4.2)	89 (75.4)
Glycylcycline	**Tigecycline**	46	0	4	8	0	1	31	0	2	10	0	0	12	0	1	115	107 (93)	0 (0.0)	8 (7.0)
Antipseudomonal Penicillins	**Piperacillin-tazobactam**	0	1	52	0	0	12	2	0	36	0	1	12	1	0	13	130	3 (2.3)	2 (1.5)	125 (96.2)
Polymyxins	**Polymyxin B**	NA	48	5	NA	11	1	0	35	3	0	12	1	NA	14	0	130	NA	120(92.3)	10 (7.7)

N.B.: Susceptible (S), Intermediate (I), and Resistant (R) % in 100 NB. Not all antimicrobial agents were tested against all isolates because of intrinsic resistance patterns and sample limitations, like *Nitrofurantoin, tested only for urine in 36 isolates; similarly, Fosfomycin, only in 53; and except for CRPA, Tigecycline, only in 115.*

6. Discussion

The present study showed that carbapenem-resistant Gram-negative bacilli stored at the Ethiopian Public Health Institute (EPHI) from 2019 to 2023, Addis Ababa, the isolate distribution was characterized primarily by CRAB 48/130 (36.9%) and CRKP 33/130 (25.4%), with CRPA 15/130 (11.5%) and CREC (9.2%) contributing smaller but clinically important proportions. This pattern is broadly consistent with Ethiopian evidence showing that Acinetobacter species and CRKP are major contributors to carbapenem resistance in referral and reference settings (94, 99, 100). The prominence of CRAB in the current study is especially plausible in light of the earlier Ethiopian Public Health Institute study, where CRAB accounted for a smaller share of all Gram-negative isolates but made up a much larger share of extensively drug resistant isolates (72.7%) (100).

This suggests that CRAB becomes more dominant as resistance severity increases. However, the present findings differ from some international studies, particularly South African CRE bacteremia surveillance, where CRKP was overwhelmingly dominant 79.8% and CREC accounted for only 4.1% (101). They also differ from Ghanaian reports in which CREC (46.0%) and CRKP 17.0% were more prominent, while CRAB contributed only 2.2% (82). These differences indicate that species distribution is strongly shaped by local case mix, hospital ecology, and the relative contribution of non-fermenters and Enterobacterales. The same pattern is also consistent with regional evidence from East Africa, where these organisms repeatedly dominate carbapenem-resistant hospital infections.

The demographic findings of the present study also fit the broader hospital-based resistance literature. Male patients accounted for 65.4% of isolates, which is close to the male distribution reported in Addis Ababa CRKP isolates 62.9% (20) and Kenyan carbapenem non-susceptible isolates 69.0% (81), although it is higher than the earlier Ethiopian Public Health Institute phenotypic study 54.3% (3) and South African CRE bacteremia surveillance 54.2% (101). The largest age group in the present study was 25 to 44 years 34.6%, which broadly aligns with the Ghanaian study where the mean patient age was 41.1 years (7) and with South African surveillance where adults aged 20 to 59 years accounted for (50.1%) of cases (20). In contrast, one Addis Ababa CRKP study reported a strong pediatric concentration, with children younger than 5 years contributing 56.1% of isolates (2). This difference is likely due to institutional case mix rather than a fundamental contradiction in resistance epidemiology.

In terms of specimen distribution, urine was the leading source in the present study, 49/130 (37.7%), followed by pus 29/130 (22.3%) and blood 27/130 (20.8%). CRAB was particularly common in urine 18/48 (37.5%) and pus 17/48 (35.4%), while Klebsiella pneumoniae was common in blood 13/33 (39.4%) and urine 10/33 (30.3%). This pattern is similar to earlier Ethiopian findings. At the Ethiopian Public Health Institute, urine contributed 48.5% of XDR isolates, pus 24.2%, and blood 21.2% (100). In the Addis Ababa CRKP carbapenemase study, urine accounted for 35.7% and blood for 25.0% of carbapenemase-positive isolates (99). The present specimen profile therefore appears locally credible and supports the view that CRGNB at EPHI are involved in both invasive and non invasive infection. Compared with other African studies, the present study was more balanced across specimen types. Ghana showed a stronger urine concentration 63.0% (82), while the Kenyan carbapenem non susceptible series was

dominated by skin and soft tissue infection 64.0% (81). This makes the present dataset more representative of a broad referral laboratory burden rather than a single syndrome.

The carbapenemase findings of the present study suggest mechanistic heterogeneity across species. Modified carbapenem inactivation testing identified carbapenemase activity mainly among Enterobacterales and part of the CRPA subgroup, whereas most CRBA isolates were negative. The carbapenemase findings suggest marked biological differences between species groups. In the present study, modified carbapenem inactivation positivity was seen in 14/33 (42.4%) CRKP, 5/6 (83.3%) *Klebsiella oxytoca*, 4/6 (66.7%) *Enterobacter cloacae*, and 12/15 (80.0%) CRPA isolates, but in only 1/48 (2.1%) CRAB isolates. This pattern agrees with Ethiopian studies showing that carbapenemase positivity is substantial but species-dependent.

At the Ethiopian Public Health Institute, 15.4% of all Gram-negative isolates were carbapenem non-susceptible, and 34.8% of those were phenotypically confirmed carbapenemase producers (100). In Tikur Anbessa, carbapenemase positivity among CRKP reached 21.2% (102). In Addis Ababa bloodstream Gram negative isolates, carbapenemase prevalence was 25.1% overall (103). The molecular evidence is also important. In Addis Ababa, CRKP, blaNDM was detected in 92.9% of gene positive isolates, whereas blaKPC* was found in only 3.6% (102). In Jimma, blaNDM accounted for 35.0%, blaVIM for 24.0%, and blaKPC* for 13.0% of detected carbapenemase genes (104). These findings support the interpretation that carbapenem resistance in Ethiopian Enterobacterales is strongly NDM linked, while resistance in CRAB may depend more on mechanisms not fully captured by *Enterobacterales* oriented phenotypic tests.

The main therapeutic finding is the clear difference between tigecycline and PMB in the present dataset. TGC showed the best overall in vitro activity in the present study, with susceptibility in 109/117 (93.0%) of isolates with valid results and resistance in only 8/117 (7.0%). This high activity is consistent with international and regional studies reporting retained TGC susceptibility against many carbapenem-resistant isolates. In China, TGC susceptibility among CRE was (97.0%) (25). In the Africa and Middle East ATLAS surveillance study, TGC susceptibility among Enterobacterales was (96.0%) (105). Even though the South African CRE bacteraemia study reported a lower TGC susceptibility 80.2%, the drug still remained one of the more active agents in that setting (80). The present findings therefore support the view that TGC remains an important in vitro option in Ethiopia. However, this interpretation should remain cautious because TGC activity can vary by species and testing method, and clinical usefulness is more limited in bloodstream infection (106).

The PMB findings require more careful interpretation. In the present study, most MIC values were in the low to moderate range, with 91.5% of isolates at 1.5 mg/L per mL or lower, but 8.5% had elevated MIC values in the range of 4.0 to 6.0 mg/L per mL. Categorical results showed intermediate (92.3%) and resistant (10/130, or 7.7%) isolates. Species-level resistance patterns add further meaning to the reserve-drug results. CRAB contributed 4/10 (40.0%) of PMB resistant isolates and 4/8 (50.0%) of TGC resistant isolates, making it the single most important resistant species in the collection. This is consistent with the broader Ethiopian and African literature, where Acinetobacter baumannii is repeatedly the hardest carbapenem-resistant pathogen to treat because it combines hospital persistence, multidrug resistance, and broad capacity to acquire new resistance determinants (10, 94, 107).

CRKP also remains important because of its strong association with carbapenemase production and bloodstream infection in both the present study and prior Ethiopian work (20, 108). Compared with international data, this pattern appears less favorable than some Asian studies. In the Chinese CRE study, PMB susceptibility was 97.0%, with only 3.0% non susceptible isolates (25). In Singapore CRAB isolates, PMB susceptibility was 100.0%, with an MIC range of 0.5 to 2 mg per L (21). However, the present results are not worse than all African data. In South African CRE bacteraemia, colistin resistance was 18.6% (101). Across African reports, crude Polymyxin resistance proportions were 2.4% for CREC and 1.8% for Klebsiella species (79), although those continent wide figures are not directly comparable to a mixed CRGNB collection enriched with A. baumannii. Overall, the current findings suggest that Polymyxin activity is still partly preserved locally, but it is less dependable than TGC and should be interpreted cautiously.

The broader susceptibility antimicrobial profile confirms that the study isolates occupied a severely narrowed therapeutic space. Resistance to cephalosporins was nearly universal, with cefazolin 100.0%, ceftazidime 100.0%, cefuroxime 100.0%, cefotaxime 100.0%, and cefepime 99.2% showing very poor activity. Fluoroquinolone resistance was also extremely high, with ciprofloxacin resistance 93.8% and levofloxacin resistance 90.8%. Amikacin retained limited activity 31.5% susceptible, whereas gentamicin activity was much lower 12.3% susceptible. These findings are in line with Ethiopian reports showing that carbapenem resistant isolates are usually embedded within broader MDR and XDR phenotypes (88,100). In Addis Ababa carbapenemase producing CRKP, ciprofloxacin resistance was 89.3%, gentamicin resistance 85.7%, and amikacin resistance only 14.3%, again suggesting that amikacin may retain some residual activity even when most comparators fail (99). The present lower overall amikacin activity likely reflects the heavier non fermenter burden in the study population.

The poor performance of ceftazidime-avibactam, 1.1% susceptible, and meropenem-vaborbactam, 16.9% susceptible, in the present study is also notable. In the Africa and Middle East ATLAS study, ceftazidime avibactam (CAZ) susceptibility among all *Enterobacterales* was 96.6%, but in meropenem-nonsusceptible *Enterobacterales*, it fell to 45.7% (105). The much lower activity in the present study is therefore biologically plausible and probably reflects the influence of NDM producers and non-fermenters, since these newer combinations perform best in KPC dominant settings and are less reliable in NDM rich contexts (16,105).

The five-year trend in the present study showed marked fluctuation in isolate numbers, with the highest number in 2019 (40.8%) and a sharp decline in 2020 (9.2%), followed by partial recovery in 2021 (29.2%), 2022 (10.0%), and 2023 (10.8%). This likely reflects disruption of specimen flow and laboratory activity during the coronavirus disease period. Despite these fluctuations, TGC susceptibility remained high across years, ranging from 88.9% to 100.0%, whereas PMB remained dominated by the intermediate category in every year, 83.3% to 92.9%. This suggests that while isolate numbers changed, the relative therapeutic pattern remained stable. This interpretation is supported by the earlier EPHI non-fermenter study, where carbapenem resistance in Acinetobacter rose from 50.0% to 76.2% over time, while CRPA fluctuated without a clear trend (94).

Overall, the present study is consistent with Ethiopian referral and surveillance literature showing that CRGNB are a major hospital-based resistance problem dominated by CRAB and CRKP. The study adds important local value by directly measuring PMB and TGC activity in a mixed carbapenem-resistant isolate set. In percentage terms, TGC remained highly active at 93.0%, whereas PMB showed a more difficult pattern, with a high intermediate proportion of 90.0% and a smaller elevated MIC tail of 8.5%. These findings suggest that TGC remains the more reliable

in vitro option in the present setting, while PMB should be interpreted with caution and cannot be considered uniformly dependable. The results also confirm that most routinely used alternatives have already lost activity, which strengthens the need for continued surveillance, improved reference susceptibility testing, and strong antimicrobial stewardship in Addis Ababa and Ethiopia more broadly (18, 20, 94 , 100).

From a public health and laboratory standpoint, three points follow from the data. First, the Ethiopian Public Health Institute isolate bank captures a clinically meaningful burden of CRAB, CRKP, and CRPA, which supports the national relevance of the study. Second, tigecycline appears to retain substantial in vitro activity in this collection, whereas PMB results are much more equivocal. Third, reserve-drug surveillance in Ethiopia should move toward more standardized MIC-based methods, especially for polymyxins, because treatment decisions at this stage depend on a very small number of last-line agents (24,109).

7. Strengths and limitations

7.1. Strengths

A major strength of this study is that it provides local Ethiopian data over a five-year period on an important set of last-line antibiotics against World Health Organization-classified priority carbapenem-resistant isolates stored at a national public health institute. It also combines organism distribution, phenotypic resistance mechanism testing, PMB, MIC data, TGC susceptibility data, and other broader class-level susceptibility patterns in one dataset.

7.2. Limitations

The study also has limitations. It relied on stored isolates rather than a prospective clinical cohort, so the findings describe laboratory resistance patterns more than clinical outcomes. Some of the raw tables showed denominator inconsistencies, especially with intrinsically resistant organisms; TGC counts are on the border; they may need the MIC test and a few species-specific summaries, which limits precision in some subgroup comparisons. Also, PMB was interpreted from a testing framework known to be method-sensitive, and TGC interpretation is also affected by breakpoint choice.

8. Conclusion and recommendations

8.1 Conclusion

In this study, it was demonstrated that CRGNBs stored at the Ethiopian Public Health Institute were dominated by CRAB and CRKP and were recovered mainly from urine, pus, and blood specimens. The overall resistance pattern showed extensive resistance to many commonly used antibiotic classes, especially cephalosporins and fluoroquinolones. Carbapenemase activity was detected mainly among CRE and part of the CRPA subgroup, whereas most CRAB isolates were negative by the phenotypic confirmatory method used.

TGC retained the best overall in vitro activity among the study drugs, with high susceptibility among isolates with valid results. PMB showed a less favorable categorical profile because most isolates fell in the intermediate range, although the MIC distribution demonstrated that many isolates remained in the low to moderate range and only a smaller subset showed high MIC values consistent with resistance. The presence of resistant isolates to both TGC and PMB indicates that the effectiveness of these last-line agents cannot be assumed and requires continuous local verification. Overall, the findings confirm that CRGNB remains an important clinical and public health problem in Addis Ababa, Ethiopia. Continued surveillance, improved reference susceptibility testing, and stronger antimicrobial stewardship are necessary to preserve the usefulness of PMB, TGC, and other remaining treatment options. Without strengthened antimicrobial stewardship, standardized susceptibility testing, and expanded molecular surveillance capacity, therapeutic options for carbapenem-resistant Gram-negative infections in Ethiopia may become increasingly limited even for last-line antibiotics.

8. Recommendations

8.1 For clinical laboratories

- Both antimicrobials have different efficacy in various geographical locations; routine surveillance of CRGNB should be strengthened at EPHI and at major referral hospitals.
- Broth microdilution should be prioritized or used as a confirmatory method for polymyxin B and tigecycline whenever feasible.
- Tigecycline resistance results should be interpreted using clearly stated breakpoint criteria, and MIC-based methods should be adopted where resources allow.
- Phenotypic carbapenemase detection should be complemented by molecular methods whenever possible, especially for epidemiologically important isolates.

8.2 For clinicians and antimicrobial stewardship programs

- Tigecycline and polymyxin B should be used cautiously and only with laboratory susceptibility guidance, taking into account the infection site and the pharmacologic limitations of these agents.
- Reserve group antibiotics should be protected through antimicrobial stewardship programs in line with the AWaRe approach adopted in Ethiopia.
- Empirical and targeted treatment protocols for severe Gram-negative infections should be updated regularly using local surveillance data.

8.3 For policymakers and public health institutions

- National and regional AMR surveillance systems should continue to monitor, especially CRE, CRAB, and CRPA.
- Investment in reference laboratory capacity should be expanded to include molecular characterization of carbapenemase genes and improved susceptibility testing infrastructure.

- National treatment guidelines and essential medicines policy should continue to integrate stewardship principles and local susceptibility evidence.

8.4 For future researchers

- Prospective multicenter and method comparison studies should be conducted to confirm these findings in current clinical isolates.
- Future work should include molecular detection of carbapenemase genes, ESBL genes, and polymyxin or tigecycline resistance determinants.
- Clinical outcome studies are needed to relate laboratory susceptibility findings to treatment response and patient survival.

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10. Annexes

Appendix A: Preparation of MacConkey Agar

MacConkey agar was prepared from commercially available dehydrated medium containing peptone, lactose, bile salts, sodium chloride, neutral red, and agar. The medium was prepared at a concentration of 5.2 g per 100 mL of distilled water, or according to the manufacturer's instructions. The dehydrated powder was dissolved in distilled water and sterilized by autoclaving at 121°C for 15 minutes. After sterilization, the medium was allowed to cool to 50-55°C, mixed thoroughly, and dispensed aseptically into sterile Petri dishes. Each batch was dated and labeled appropriately. Prepared plates were stored at 2-8°C, preferably in sealed plastic bags to prevent moisture loss. The plates were used for up to 4 weeks, provided there was no evidence of contamination or change in appearance suggestive of pH alteration. At room temperature, the pH of the prepared medium was maintained within the range of 7.2-7.6.

Appendix B: Preparation of Mueller-Hinton Agar

Mueller-Hinton agar (MHA) was prepared from commercially available dehydrated medium containing beef extract, acid digest of casein, and starch. The required amount of powder was dissolved in distilled water and heated gently until fully dissolved. The medium was sterilized by autoclaving at 121°C for 20 minutes. The pH of the final medium was maintained at 7.2-7.4. After sterilization, the molten agar was poured into sterile 100 mm or 150 mm Petri dishes to a uniform depth of 4 mm, corresponding to approximately 25 mL per 100 mm plate. Plates were poured on a level surface to ensure even depth throughout. Care was taken to avoid variation in agar depth, since excessively thin agar may produce falsely large inhibition zones, whereas overly thick agar may produce falsely small zones.

Appendix C. Preparation of Tryptic Soy Broth with 20% Glycerol

Tryptic Soy Broth (TSB) was prepared from commercially available dehydrated medium containing pancreatic digest of casein, papaic digest of soybean meal, sodium chloride, dipotassium phosphate, and dextrose.

Thirty grams of dehydrated powder were suspended in 1 L of distilled water. The pH was adjusted to 7.3 ± 0.2, and the medium was warmed gently until fully dissolved. The broth was then dispensed and sterilized by autoclaving at 121°C for 15 minutes.

To prepare TSB containing 20% glycerol, 800 mL of sterile TSB was mixed with 200 mL of sterile glycerol. This medium was used for long-term preservation of bacterial isolates.

Appendix D. Antimicrobial Susceptibility Testing by the Kirby-Bauer Disk Diffusion Method

Antimicrobial susceptibility testing was performed by the Kirby-Bauer disk diffusion method on Mueller-Hinton agar in accordance with standard laboratory procedures and CLSI recommendations.

Preparation of inoculum

Three to five well-isolated colonies of the same morphological type were selected from a pure overnight culture. The colonies were suspended in 4-5 mL of sterile normal saline or Tryptic Soy Broth and mixed. The turbidity of the suspension was adjusted to match a 0.5 McFarland standard.

Inoculation of plates

A sterile cotton swab was dipped into the standardized suspension, rotated firmly against the inner wall of the tube to remove excess fluid, and used to inoculate the entire surface of a Mueller-

Hinton agar plate. The plate was streaked evenly in three directions, rotating the plate approximately 60° between streakings, to obtain a uniform lawn of growth. The surface was allowed to dry for a few minutes before application of antibiotic disks.

Application of antibiotic disks

Antibiotic disks were brought to room temperature before use and placed aseptically on the inoculated agar surface using sterile forceps or a disk dispenser. The disks were distributed evenly and pressed gently to ensure complete contact with the agar surface.

Incubation

The plates were incubated at 35 ± 2°C for 18-24 hours under the atmospheric conditions appropriate for the test organism.

Reading and interpretation

After incubation, only plates showing confluent lawn growth were interpreted. If individual colonies were visible, the inoculum was considered too light, and the test was repeated. The diameters of inhibition zones were measured in millimeters using a ruler or caliper. Results were interpreted as susceptible, intermediate, or resistant according to current CLSI interpretive criteria for the antimicrobial agents tested.

Recording and reporting

For each isolate, the organism name, antimicrobial agents tested, measured zone diameters, and final susceptibility interpretation were recorded.

Tigecycline susceptibility profile of selected aerobic Gram-negative bacteria

The expected natural susceptibility profile of tigecycline for selected Gram-negative bacteria is summarized below.

Pathogen organism	Natural state or susceptibility to tigecycline
Commonly susceptible gram-negative species	
Citrobacter freundii	Susceptible
Citrobacter koseri	Susceptible
Escherichia coli	Susceptible
Species for which acquired resistance may be a problem	
Acinetobacter baumannii spp.	Acquired resistance
Burkholderia cepacia.	Acquired resistance
Klebsiella spp.	Acquired resistance
Enterobacter cloca	Acquired resistance
Stenotrophomus maltopilia	Intrinsically resistance
Pseudomonas aeruginosa	Intrinsically resistant
Morganella morganii	Intrinsically resistant
Serratia marcescens	Intrinsically resistant
Providencia spp.	Intrinsically resistant
Proteus spp.	Intrinsically resistant

Appendix E. Minimum Inhibitory Concentration Determination by Epsilometer Test (E-test)

Minimum inhibitory concentrations (MICs) of polymyxin B, colistin, and tigecycline were determined using E-test strips on Mueller-Hinton agar.

Principle

The E-test is a quantitative agar diffusion method that uses a plastic strip impregnated with a predefined gradient of antimicrobial concentration. After incubation, an elliptical zone of inhibition forms around the strip. The MIC is read at the point where the edge of the inhibition ellipse intersects the scale on the strip.

Materials

The following materials were used:

- Mueller-Hinton agar plates
- E-test strips for polymyxin B, colistin, and tigecycline
- Sterile normal saline (0.85% NaCl)
- Sterile cotton swabs
- Sterile test tubes
- Forceps
- 0.5 McFarland standard or densitometer
- Incubator set at 35 ± 2°C
- Quality control strains

Storage and handling of strips

E-test strips were stored at -20°C or -70°C until use. Before opening, unopened packages were allowed to equilibrate to room temperature for approximately 30 minutes, and any surface moisture was permitted to evaporate completely. Packages were inspected for damage before use.

Preparation of inoculum

Well-isolated colonies from an overnight culture were suspended in sterile saline and adjusted to a turbidity equivalent to a 0.5 McFarland standard. A correct inoculum density was expected to produce a confluent or nearly confluent lawn of growth after incubation.

Inoculation of agar plates

A sterile swab was dipped into the standardized inoculum and rotated against the wall of the tube to remove excess liquid. The entire agar surface was inoculated evenly in three directions, rotating the plate approximately 60° between streakings. The plate surface was allowed to dry for 10-15 minutes before application of the strips.

Application of E-test strips

The strips were applied to the inoculated agar surface using sterile forceps, with the MIC scale facing upward and the antimicrobial gradient in full contact with the agar surface. The coded end of the strip was placed toward the outside of the plate. Once applied, the strip was not moved.

Incubation

The plates were incubated in an inverted position at 35 ± 2°C for approximately 16-24 hours. Slow-growing organisms were incubated longer when necessary, according to standard laboratory guidance.

Reading of MIC results

After incubation, the MIC was read at the point where the inhibition ellipse intersected the scale on the strip. MIC values falling between two standard dilutions were rounded up to the next higher value. If the ellipse intersected the strip at different points on either side, the higher reading was recorded. Faint growth at the edge of the strip was ignored, and the MIC was read at the point of complete inhibition.

If growth extended along the entire strip without an inhibition ellipse, the MIC was recorded as greater than or equal to the highest value on the strip. If the ellipse intersected below the lower limit of the scale, the MIC was recorded as less than the lowest value on the strip.

Interpretation

MIC values were interpreted as susceptible, intermediate, or resistant using current CLSI breakpoints or manufacturer-approved interpretive criteria where applicable. Reported reference MIC readings included:

- Polymyxin B: 1.0 µg/mL
- Colistin: 1.5 µg/mL

Precautions

Care was taken to ensure that:

1. The strip was firmly applied and not moved after placement.
2. Agar surfaces were completely dry before inoculation.
3. Unused strips were returned promptly to appropriate storage conditions.
4. Quality control strains were tested routinely, and any out-of-range result prompted repeat testing.

Note on method limitations

The E-test method is convenient for MIC determination; however, it may underestimate Polymyxin resistance in some strains and may overestimate susceptibility in others. Results should therefore be interpreted cautiously and, where required, confirmed using reference methods.

Pathogen organism	**Natural state or susceptibility to polymyxins B & E**
Commonly susceptible gram-negative species	
Escherichia coli	Susceptible
Klebsiella spp.	Susceptible
Enterobacter spp.	Susceptible
Pseudomonas aeruginosa	Susceptible
A. baumannii	Susceptible
Salmonella spp.	Susceptible
Shigella spp.	Susceptible
Pasteurella spp.	Susceptible
Haemophilus spp.	Susceptible
Commonly intrinsically resistant aerobic gram-negative species	
Proteus spp.	Intrinsically resistant
Providencia spp.	Intrinsically resistant
Morganella morganii	Intrinsically resistant
Serratia spp.	Intrinsically resistant
Brucella spp.	Intrinsically resistant
Neisseria spp.	Intrinsically resistant

Chromobacterium spp.	Intrinsically resistant
Burkholderia spp.	Intrinsically resistant

Appendix F. Detection of Extended-Spectrum Beta-Lactamase (ESBL) Production

ESBL production was detected by the double-disk synergy method and confirmatory disk testing using clavulanic acid-based combinations, in accordance with standard laboratory methods and CLSI guidance.

Principle

Extended-spectrum beta-lactamases are inhibited by clavulanic acid. An increase in the inhibition zone of an extended-spectrum cephalosporin tested in combination with clavulanic acid, compared with the cephalosporin alone, indicates ESBL production.

Test organisms

This method was applied primarily to *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Proteus mirabilis*.

Materials

The following materials were used:

- Mueller-Hinton agar plates
- Amoxicillin-clavulanate disk (20/10 µg)
- Ceftazidime disk (30 µg)

- Cefotaxime or ceftriaxone disk (30 µg)
- Aztreonam disk (30 µg)
- Cefpodoxime disk (optional)
- Cefoxitin disk
- Sterile saline
- Sterile swabs
- 0.5 McFarland standard
- Quality control strains, including *Klebsiella pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922

Preparation of inoculum

A bacterial suspension equivalent to a 0.5 McFarland standard was prepared from a pure culture.

Inoculation of plates

The suspension was used to inoculate Mueller-Hinton agar uniformly to produce a confluent lawn of growth.

Placement of disks

The amoxicillin-clavulanate disk was placed near the center of the plate. Ceftazidime, cefotaxime or ceftriaxone, aztreonam, and optionally cefpodoxime were placed around it so that the inner edge of each disk was approximately 15 mm from the clavulanate disk. A cefoxitin disk was placed in the remaining available space.

For confirmatory testing with ESBL disks, cefotaxime and ceftazidime were tested alone and in combination with clavulanic acid on the same inoculated plate.

Incubation

The plates were incubated at 35-37°C for 16-20 hours in ambient air or according to standard conditions for the organism under study.

Interpretation

Zone diameters were measured in millimeters. ESBL production was considered present when there was clear potentiation of the inhibition zone of ceftazidime, cefotaxime, ceftriaxone, aztreonam, or cefpodoxime toward the amoxicillin-clavulanate disk or when confirmatory testing showed an increase of at least 5 mm in the inhibition zone for cefotaxime or ceftazidime tested in combination with clavulanic acid compared with the antimicrobial alone.

Interpretive patterns

The following interpretive patterns were used:

- **Class A ESBL is present.**
    - Potentiation with clavulanic acid present
    - Cefoxitin susceptible
    - Variable susceptibility to ceftazidime, ceftriaxone, or aztreonam
- **Class A and Class C ESBL are present**
    - Potentiation with clavulanic acid present
    - Cefoxitin-resistant or intermediate
    - Variable susceptibility to ceftazidime, ceftriaxone, or aztreonam
- **Class C ESBL present**
    - No potentiation with clavulanic acid
    - Cefoxitin-resistant or intermediate

- Resistance to one or more of ceftazidime, ceftriaxone, or aztreonam

- **ESBL is not Class A or Class C**
    - No potentiation with clavulanic acid
    - Cefoxitin susceptible
    - Resistance to one or more of ceftazidime, ceftriaxone, or aztreonam
- **ESBL absent**
    - No potentiation with clavulanic acid
    - Variable cefoxitin susceptibility
    - Susceptibility to ceftazidime, ceftriaxone, and aztreonam

Quality control

Quality control strains were included routinely. *Klebsiella pneumoniae* ATCC 700603 was used as a supplemental ESBL-positive control strain, and *Escherichia coli* ATCC 25922 was used as a negative control strain. If quality control results were outside the acceptable range, testing was repeated.

Reporting

For confirmed ESBL-producing isolates, susceptibility results were interpreted and reported according to current CLSI criteria. Where required by local policy, agents such as moxalactam, cefonicid, cefamandole, and cefoperazone were reported as resistant for ESBL-positive isolates.

Appendix G. Standard CLSI SOP for Modified Carbapenem Inactivation Method (mCIM) for Suspected Carbapenemase Production in *Enterobacterales* and *Pseudomonas aeruginosa (page 168).*

Introduction

The modified carbapenem inactivation method (mCIM) is a phenotypic test recommended by CLSI for the detection of carbapenemase-producing organisms among *Enterobacterales* and *Pseudomonas aeruginosa.* The method uses routine laboratory reagents and may be performed to support treatment decisions, infection prevention measures, and epidemiological investigations.

Materials Required

The following materials were used:

- Tryptic Soy Broth (TSB), 2 mL aliquots
- Meropenem disks, 10 µg
- 1 µL and 10 µL inoculating loops
- Sterile nutrient broth or normal saline, 3.0-5.0 mL aliquots
- Mueller-Hinton agar (MHA) plates, 100 mm or 150 mm
- Meropenem-susceptible indicator strain: *Escherichia coli* ATCC 25922
- 0.5 M EDTA (for eCIM when required)
- Sterile forceps
- The incubator is set at 35-37°C.

Procedure

1. Using a 10 µL loop, a full loopful of the test organism was taken from an overnight blood agar plate culture and emulsified in 2 mL of TSB.
2. The suspension was vortexed for 10-15 seconds to ensure adequate mixing.
3. One 10 µg meropenem disk was added aseptically to each tube using sterile forceps, ensuring that the disk was fully immersed in the suspension.
4. The tubes were incubated at 35-37°C in ambient air for 4 hours ± 15 minutes.
5. Just before or immediately after completion of the disk suspension incubation, a 0.5 McFarland suspension of *CREC* ATCC 25922 was prepared in sterile saline or nutrient broth using the direct colony suspension method.
6. A Mueller-Hinton agar plate was inoculated with the *CREC* ATCC 25922 suspension for routine disk diffusion testing. Preparation of the inoculum and inoculation of the plate were completed within 15 minutes. The plate was allowed to dry for 3-10 minutes before placement of the meropenem disks.
7. Each meropenem disk was removed from the TSB suspension using a 10 µL loop. The flat side of the loop was placed against the edge of the disk, and the disk was carefully lifted from the broth. Excess liquid was removed by dragging the loop along the inside wall of the tube. The disk was then placed onto the previously inoculated MHA plate containing the *CREC* ATCC 25922 indicator lawn.
    - Up to 4 disks were placed on a 100 mm MHA plate.
    - Up to 8 disks were placed on a 150 mm MHA plate.
8. The plates were inverted and incubated at 35-37°C in ambient air for 18-24 hours.

9. After incubation, the zone of inhibition around each meropenem disk was measured in millimeters as in routine disk diffusion testing.

Interpretation

Carbapenemase-positive

- Zone diameter of 6-15 mm, or
- Presence of pinpoint colonies within a 16-18 mm zone

A positive result indicates that the test isolate produced carbapenemase, which hydrolyzed the meropenem in the disk, resulting in reduced inhibition of the *CREC*ATCC 25922 indicator strain.

Carbapenemase negative

- Zone diameter of ≥19 mm with a clear inhibition zone

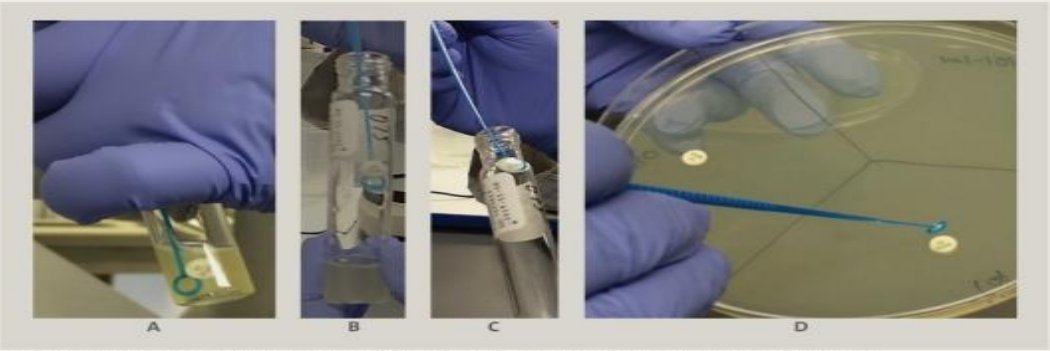
A negative result indicates that the test isolate did not produce carbapenemase, and the meropenem remained active against the *CREC*ATCC 25922 indicator strain.

Carbapenemase indeterminate

- Zone diameter of 16-18 mm, or
- Zone diameter of ≥19 mm with pinpoint colonies present within the zone

An indeterminate result indicates that the presence or absence of carbapenemase production cannot be confirmed.

Table 3C. (Continued)



Abbreviations: ATCC®, American Type Culture Collection; mCIM, modified carbapenem inactivation method; MHA, Mueller-Hinton agar.

Figure 1. Procedure for Placing Meropenem Disks for the mCIM.

Remove the meropenem disk with a 10-µL loop (A) and drag the loop against the inside edge of the tube to expel any excess liquid (B). Use the same loop to remove the disk from the tube (C) and place it on the MHA plate (D) previously inoculated with the meropenem-susceptible *E. coli* (ATCC® 25922) indicator strain.

Table 3C. (Continued)



Abbreviations: ATCC®, American Type Culture Collection; mCIM, modified carbapenem inactivation method; QC, quality control; TSB, trypticase soy broth.

Figure 2A. mCIM Results for QC Strains: Negative Control *K. pneumoniae* ATCC® BAA-1706™ (A) and Positive Control *K. pneumoniae* ATCC® BAA-1705™ (B).

NOTE: A narrow ring of growth around the meropenem disk as seen with the negative control (A) results from carryover of the test organism in the TSB and should be ignored.

Table 3C
Modified Carbapenem Inactivation Methods

Table 3C. (Continued)



Abbreviations: mCIM, modified carbapenem inactivation method; TSB, trypticase soy broth.

Figure 2B. mCIM Test Interpretation.

- Result: positive mCIM
- Report: carbapenemase detected

NOTE: A narrow ring of growth around the meropenem disk results from carryover of the test organism in the TSB and should be ignored.

Table 3C. (Continued)



Abbreviations: eCIM, EDTA-modified carbapenem inactivation method; mCIM, modified carbapenem inactivation method.

Figure 3A. mCIM and eCIM Test Interpretation: Negative mCIM.

"A" shows an mCIM negative result (zone diameter = 20 mm) and "B" shows an eCIM invalid result. Do not interpret the eCIM result when the mCIM is negative as the isolate is negative for carbapenemase production.

- Result: negative for carbapenemase production
- Report: carbapenemase not detected

Table 3C
Modified Carbapenem Inactivation Methods

Table 3C. (Continued)



Abbreviations: eCIM, EDTA-modified carbapenem inactivation method; EDTA, ethylenediaminetetraacetic acid; mCIM, modified carbapenem inactivation method.

Figure 3B. mCIM and eCIM Test Interpretation: Positive mCIM and eCIM.

"A" shows an mCIM positive result (zone diameter of 6 mm) and "B" shows an eCIM positive result (zone diameter = 15 mm with pinpoint colonies throughout the zone of inhibition). **NOTE:** The pinpoint colonies throughout the zone of inhibition are ignored when measuring the zone for the eCIM test. A ≥ 5-mm increase in zone diameter for eCIM vs zone diameter for mCIM (15 mm − 6 mm = 9 mm) demonstrates the inhibition of the metallo-β-lactamase in the presence of EDTA.

- Result: positive mCIM and eCIM
- Report: metallo-β-lactamase detected

Table 3C. (Continued)



Abbreviations: eCIM, EDTA-modified carbapenem inactivation method; EDTA, ethylenediaminetetraacetic acid; mCIM, modified carbapenem inactivation method.

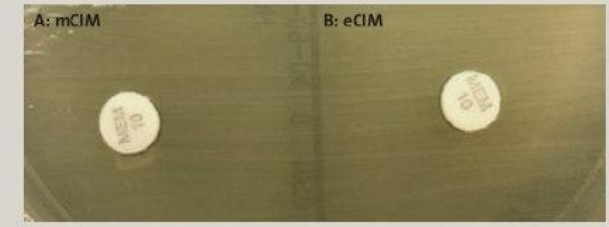
Figure 3C. mCIM and eCIM Test Interpretation: Positive mCIM and eCIM.

"A" shows an mCIM positive result (zone diameter = 6 mm) and "B" shows an eCIM positive result (zone diameter = 19 mm). A ≥ 5-mm increase in zone diameter for eCIM vs diameter for mCIM zone (19 mm − 6 mm = 13 mm) demonstrates the inhibition of the metallo-β-lactamase in the presence of EDTA.

- Result: positive mCIM and eCIM
- Report: metallo-β-lactamase detected

Table 3C
Modified Carbapenem Inactivation Methods

Table 3C. (Continued)



Abbreviations: eCIM, EDTA-modified carbapenem inactivation method; EDTA, ethylenediaminetetraacetic acid; mCIM, modified carbapenem inactivation method

Figure 3D. mCIM and eCIM Test Interpretation: Positive mCIM and Negative eCIM.

"A" shows an mCIM positive result (zone diameter = 6 mm) and "B" shows an eCIM negative result (zone diameter = 6 mm). Serine carbapenemases are not inhibited by EDTA and demonstrate a ≤ 4-mm increase in zone diameter for eCIM vs zone diameter for mCIM.

- Result: positive mCIM and negative eCIM
- Report: serine carbapenemase detected

Source: CLSI M-100, 2026, Vol. 35, Page 168

Appendix H: Recommended Antimicrobial Disks and E-Test Zone Sizes: Result of AST Pattern of the Isolates According to 2024 Clinical Laboratory Standards Institute (CLSI-M100) and EUCAST Breaking Point.

Table 5: CLSI-M100 standard spectrum of activity of colistin against selected pathogenic aerobic gram-negative bacteria Organism's natural state (resistant or susceptible to two polymyxins, B & E).

No	Antimicrobial Agent	Disk content /ug	Enterobacterial spss			*Pseudomonas aeruginosa*			*Acintobacter baumannii*			Comments	page 1/2
			≤ S	**I**	**≥R**	**≤ S**	**I**	**≥ R**	**≤ S**	**I**	**≥ R**		
1.	Ampicillin	30	17	14-16	13	-	-	-	_	_	_		
2.													
3.	Cefazolin	30	23	20-22	19	_	_	_	_	_	_		
4.													
5.	Gentamicin	30	18	15-17	14				15	13-14	12		
6.													
7.	Cefepime	30	25	19-24	18	18	15-17	14	18	15-17	14		
8.													
9.	Amikacin	30	20	17-19	16	17	15-16	14	17	15-16	14		
10.													
11.	Amoxicillin+clavulanic acid	30	18	14-17	13	_	_	_	_	_	_		
12.													
13.	Aztreonam	30	21	18-20	17	22	16-21	15	_	_	_		
14.													
15.	Cefuroxime	30	18	15-17	14	_	_	_	_	_	_		

16.													
17.	Cefotaxime	30	18	15-17	14	_	_	_	_	_	_		
18.													
19.	Ceftriaxone	30	23	20-22	19	_	_	_	23	15-22	14		
20.													
21.	Cefoxitin	30	18	15-17	14	_	_	_	_	_	_		
22.													
23.	Ceftazidime		21	18-20	17	18	15-17	14	18	15-17	14		
24.													
25.	Ceftazidime-avibactam	50	21	-	20	21	-	20	_	_	_		
26.													
27.	Imipenem	10	23	20-22	19	19	16-18	15	12	19-21	18		
28.													
29.	Ertapenem	10	22	19-21	18	-	-	-	-	-	-		
30.													
31.	Meropenem	10	23	20-22	19	19	16-18	15	18	15-17	14		
32.													
33.	Ciprofloxacin	30	26	22-25	21								
34.													
35.	Trimethoprim + Sulfamethoxazole	30	16	11-15	10		_	_	16	11-15	10		
36.													
37.	Piperacillin-Tazobactam	30	25	21-24	20	22	18-21	17	21	18-20	17		
38.													
39.	Nitrofurantoin	300	17	15-16	14	_	_	_	_	_	_	*Urine	
40.													

41.	Tetracycline	30	15	12-14	11				15	12-14	11		
42.													
43.	Levofloxacin	30	_	_	_	_	_	_	21	16-20	15	*Urine	
44.													
45.	Fosfomycin	200	16	13-15	12								
46.													
47.	Levofloxacin	30	21	17-20	16	_	_	_	_	_	_		
48.													
49.	Meropenem-vaborbactam	30	18	15-17	14	_	_	_	_	_	_		
50.													
51.	Tigecycline	15	18*	_-	18*	_	_	_	_	_	_	*EUCAST	
			19**	15-18**	14**							**FDA	
52.	Minocycline	50	16	13-15	12	_	_	_	16	13-15	12		
53.													
54.	Colistin or Polymyxin B	0.06-1024 mg/L	-	2	4	-	2	4	-	2	4	*MIC by CLSI-M100	
55.													

*In this table: S = sensitive; R = resistant; I = Intermediate** disk zone of inhibition braking point (mg/dl) established by the European Committee on Antimicrobial Subtility Testing (EUCAST)* CLSI-M100 = Clinical and Laboratory Standards Institute; MIC = minimum inhibitory concentration. Tigecycline was evaluated according to the FDA guidelines

Key Notes:

- The dash ("—") indicates missing data or non-applicable breakpoints.
- MIC values given represent the concentrations that define susceptibility or resistance:
    - For Colistin, organisms with MIC ≤ 2 μg/mL are considered susceptible (S) under EUCAST guidelines.
    - MICs > 2 μg/mL indicate resistance (R) for both Colistin and Polymyxin B for some pathogens.
- For Polymyxin B and Colistin:

- Enterobacterales data is not specified for CLSI-M100 criteria.
- Pseudomonas spp. and CRAB spp. have defined MIC breakpoints for susceptibility with these drugs under both CLSI-M100 and EUCAST guideline

Appendix I: Gram-Negative Bacilli Isolate Collection and Strain Refreshment Log Sheet

No	No Laboratory	Referring Site	Hospital Id Number	Age	Sex	Date Stored	Specimen Type	Name Of Organism	carbapenem drug	Refrigerator #No	Rack #No	Box #No	Cell #No	Remark
1.														
2.														
3.														
4.														
5.														
6.														
7.														
8.														
9.														
10.														
11.														
12.														
13.														
14.														
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17.														
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21.														
22.														
23.														
24.														
25.														
26.														
27.														
28.														
29.														
30.														

Appendix J, CLSI-M100 standard spectrum of Activity of resistant or susceptible to tigecycline against selected pathogenic aerobic gram-negative bacteria Organism Natural state.

Intrinsic Resistance

A. Enterobacterial

Antibiotic Agent	*Ampicillin*	*Amoxilin clavulanic acid*	*Amp-sulbactam*	*Ticarcilin*	*Cephalosporins I*	*Cephamycin*	*Cephalosporin II*	*Imipenem*	*Tetracycline*	*Tigecycline*	*Nitrofurantoin*	*Polymyxin B Colistin*	*Aminoglycosides*
Organism													
Citrobacter freundii	*R*	*R*	*R*		*R*	*R*	*R*						
Citrobacter koseri *Citrobacter amalonaticus*	*R*			*R*									
Enterobacter claocoae complex	*R*	*R*	*R*		*R*	*R*							
Eshercia coli -there is no intrinsic resistance to beta lactams in this organism													
Klebsiella spp	*R*			*R*									
Morganella morgani	*R*	*R*			*R*		*R*			*R*	*R*	*R*	
P.mirabilis	*There is no intrinsic resistance to pencillin and cephalosporin in this organism*								*R*	*R*	*R*	*R*	
P.vulgaris	*R*				*R*		*R*		*R*	*R*	*R*	*R*	
P.penerri	*R*				*R*		*R*		*R*	*R*	*R*	*R*	
Providencia stewarti	*R*	*R*			*R*				*R*	*R*	*R*	*R*	
Providencia retgeri	*R*	*R*			*R*				*R*	*R*	*R*	*R*	
Serratia marcences	*R*	*R*	*R*		*R*	*R*	*R*				*R*	*R*	

N.B In this table:

- "R" denotes resistance to the specific antibiotic agent.
- The explanations are included for organisms like *CREC*and *Proteus mirabilis*, which have specified resistance profiles.

B. *non-Enterobacterals*

Antibiotic Agent	***Ampicillin/ Amoxicillin***	***Amoxilin clavulanic acid***	***Amp-sulbactam***	***Ticarcillin***	***Piperacillin***	***Piperacillin-tazobactam***	***Cefotaxime***	***Ceftriaxone***	***Ceftazidime***	***Cefepime***	***Imipenem***	***Meropenem***	***Ertapenem***	***Aztreonam***	***Polymyxin B, Colistin***	***Aminoglycosides***	***Tetracycline/ Tigecycline***	***Trimethoprim***	***SXT***	***Chloramphenicol***	***Fosfomycin***
Organism																					
Acinitobacter baumani	*R*	*R*											*R*	*R*				*R*		*R*	*R*
Burkholderia cepacia	*R*	*R*	*R*	*R*	*R*								*R*		*R*						*R*
Pseudomonas aureginosa	*R*	*R*	*R*				*R*	*R*					*R*				*R*	*R*	*R*	*R*	
Stenotrephomonas maltophilia	*R*	*R*	*R*	*R*	*R*	*R*	*R*	*R*			*R*	*R*	*R*	*R*		*R*		*R*			*R*

NB. In this table:

- "R" denotes resistance to the specific antibiotic agent.

The explanations are included for organisms like *CREC*and *Proteus mirabilis*, which have specified resistance

Appendix K. Result recording format

Sno	Lab ID	Age	Sex	Types of Specimens	Isolate Organism	CTX (S/R)	CAZ (S/R)	FEP (S/R)	FOX (S/R)	MEM (S/R)	SXT (S/R)	CRO (S/R)	GNT (S/R)	AMC (S/R)	TGI (S/R)	mm	PMB (S/R)	mg/dl	CPO		eCIM	ESBL
																			Pos	Neg		
1																						
2																						
3																						
4																						
5																						
6																						
7																						
8																						
9																						
10																						
11																						
12																						
13																						
14																						

N.B *The antimicrobial susceptibility testing panel included Cefotaxime (CTX), Ceftazidime (CAZ), Cefepime (FEP), Cefoxitin (FOX), Meropenem (MEM), Trimethoprim-Sulfamethoxazole (SXT), Ceftriaxone (CRO), Gentamicin (GNT), Amoxicillin-Clavulanic Acid (AMC), Tigecycline (TGI), and Polymyxin B (PMB). Zone diameters were measured in millimeters (mm), while concentrations were expressed in mg/dl where applicable. Carbapenemase-producing organisms (CPO) were identified based on standard laboratory testing methods.*

Appendix L: WHO Current adopted AWaRe classification of antibiotics in the EEML-2020.

Access	Watch	Reserve
1. Amoxicillin	1. Ampicillin + Sulbactam	1. Piperacillin + tazobactam
2. Amoxicillin + Clavulanic Acid	2. Cefuroxime	2. Meropenem
3. Ampicillin	3. Cefixime	3. Meropenem + Vaborbactam
4. Penicillin G, Benzathine	4. Cefpodoxime Cefotaxime Sodium	4. Ceftazidime + Avibactam
5. Penicillin G, Sodium Crystalline	5. Ceftriaxone	5. Colistin
6. Cloxacillin	6. Ceftazidime	6. Polymyxin B
7. Cephalexin	7. Cefepime	Vancmycin
8. Cefazolin	8. Ceftriaxone + sulbactam	
9. Azithromycin	9. Ciprofloxacin	
10.Clarithromycin	10. Clindamycin	
11.Sulphamethoxazole + Trimethoprim		
12.Nitrofurantoin		
13.Norfloxacin		
14.Gentamicin		
15.Metronidazole		
16.Doxycycline		

Sorce, EEML 2020

Table 1: AWaRe classification of antibiotics in the EEML2024

Access	Watch	Reserve
1. Amoxicillin	1. Amikacin	1. Ceftazidime + Avibactam
2. Amoxicillin + Clavulanic acid	2. Ampicillin + Sulbactam	2. Colistin
3. Ampicillin	3. Azithromycin	3. Linezolid
4. Benzathine benzylpenicillin (Penicillin G, Benzathine)	4. Cefepime	4. Meropenem
5. Benzylpenicillin (Penicillin G)	5. Cefixime	5. Meropenem + aborbactam
6. Cefalexin	6. Cefotaxime sodium	6. Neomycin
7. Cefazolin	7. Ceftazidime	7. Polymyxin B
8. Cloxacillin	8. Ceftriaxone	
9. Doxycycline	9. Cefuroxime	
10. Gentamicin	10. Ciprofloxacin	
11. Metronidazole	11. Clarithromycin	
12. Nitrofurantoin	12. Clindamycin	
13. Procaine-benzylpenicillin	13. Norfloxacin	
14. Sulphamethoxazole + Trimethoprim	14. Piperacillin + Tazobactam	
	15. Vancomycin	

Sorce, EEML Seventh Edition October 2024

10. Declaration

I, the undersigned agree to accept responsibility for the scientific ethical, and technical conduct of the research project and for the provision of required progress reports as per the terms and conditions of the research publications office.

M.Sc. Candidate: Bruke Gezhagne (B.Sc.)

Signature: _____

Date of submission: _____

This proposal has been submitted with our approval as advisors.

Advisor: Professor. Mistire Wolde

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Dr. Biruk zerfu

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia

External advisor: - Dr. Abebe Aseffa

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.