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Phenotypic Detection Of Extented Spectrum β-Lacramase And Carbapenemase Producing Clinical Isolates In A Tertiary Care Hospital Of The Himalayan Region, India

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Abstract

Background: *Non-fermenting Gram-negative bacilli (NFGNB)—notably Pseudomonas aeruginosa and Acinetobacter baumannii—are leading causes of healthcare-associated infections (HAIs) and increasingly exhibit carbapenem resistance via carbapenemase production. Region-specific data from Himachal Pradesh are scarce, yet essential for empiric therapy and infection-control planning.*

Methods: *A hospital-based cross-sectional study was conducted in the Department of Microbiology, Pt. JLNGMC, Chamba (September 2023–August 2024). Consecutive clinical specimens (n=2378) were processed per CLSI-2023. NFGNB were identified by conventional biochemical test . Antimicrobial susceptibility testing (AST) used Kirby–Bauer disc diffusion; colistin MICs were confirmed by broth microdilution. Carbapenemase detection among carbapenem-non-susceptible isolates employed modified carbapenem inactivation method (mCIM) . Data were analyzed in SPSS v26.*

Results: *Of 2378 samples, 250 (5.77%) yielded NFGNB: Pseudomonas aeruginosa (60.8%) and Acinetobacter baumannii (32%), Acinetobacter lwoffi (2.8%) with Stenotrophomonas maltophilia (2.4%) and Burkholderia cepacia (2.9%) less frequent. Urinary tract infection (UTI) and Otitis media (27.6%) were the most common source, followed by pus/wound swabs (19.2%), Respiratory tract infection (16.8%),septicaemia (7.2%). P. aeruginosa showed high resistance to ceftazidime (64.4%), cefepime (61.8%), piperacillin–tazobactam (58.5%) and carbapenems (42–45%), yet retained susceptibility to amikacin (71%) and polymyxins (>97%). A. baumannii exhibited alarmingly high resistance to carbapenems (68–73%) and cephalosporins (>75%), with lower susceptibility to aminoglycosides (≤43%) and fluoroquinolones (<30%); polymyxins remained active in >95%. Overall carbapenem resistance was 51.4%, higher in A. baumannii (72.9%) than P. aeruginosa (42.1%). Phenotypic testing confirmed carbapenemase production in 76% of carbapenem-resistant isolates; metallo-β-lactamases were detected in 43.6%. MDR/XDR/PDR rates were 81.2%/42.7%/3.1% for A. baumannii and 54.6%/18.4%/0% for P. aeruginosa. ICU isolates had greater carbapenem resistance than non-ICU isolates (61.3% vs 39.8%; p<0.05).*

Conclusion: *NFGNB impose a substantial AMR burden in Himachal Pradesh. Carbapenem resistance—predominantly carbapenemase-mediated—is widespread, with A. baumannii most affected. Therapeutic options are increasingly limited; polymyxins remain reliable but raise toxicity and stewardship concerns. Routine carbapenemase surveillance, robust antimicrobial stewardship, and strengthened infection-prevention strategies are urgently required in resource-limited hospital settings.*

Keywords: *Non-fermenting Gram-negative bacilli; Pseudomonas aeruginosa; Acinetobacter baumannii; carbapenem resistance; carbapenemase; metallo-β-lactamase; MDR/XDR; Himachal Pradesh; antimicrobial stewardship.*

INTRODUCTION

Non-fermenting Gram-negative bacilli (NFGNB) have become an increasingly important group of pathogens in modern clinical practice, particularly in hospital environments. these organisms—predominantly *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and several less common species such as

Stenotrophomonas maltophilia and *Burkholderia cepacia complex*—exhibit distinctive metabolic, ecological, and clinical characteristics that pose unique challenges to healthcare providers【1,3,11】. Their ability to survive in diverse environmental conditions, including hospital surfaces, medical equipment, and even disinfectants, renders them highly adaptable opportunistic pathogens【7,18】. Over the past two decades, NFGNB have transitioned from being regarded as relatively low-virulence opportunists to major culprits in hospital-acquired infections (HAIs), particularly in intensive care units (ICUs).

Clinical Importance of NFGNB

The clinical relevance of NFGNB arises primarily from their association with severe healthcare-associated infections such as ventilator-associated pneumonia (VAP), bloodstream infections (BSIs), urinary tract infections, and wound and burn infections【6,7】. These infections typically occur in critically ill, immunocompromised, or device-dependent patients, leading to high morbidity and mortality. Among them, *Pseudomonas aeruginosa* is notorious for its role in chronic respiratory infections in cystic fibrosis patients and its multidrug-resistant strains in hospital outbreaks. Similarly, *Acinetobacter baumannii* has emerged as a leading cause of ventilator-associated pneumonia and bacteremia in ICUs worldwide【2,3,6】. Rare non-fermenters such as *Stenotrophomonas maltophilia* are also gaining significance, particularly in patients with malignancies, prolonged hospitalizations, or broad-spectrum antibiotic exposure【11】.

Infections due to NFGNB are not only difficult to treat but are also associated with extended hospital stays, increased healthcare costs, and higher risks of adverse clinical outcomes【7,9】. This is primarily due to the alarming ability of these organisms to develop resistance to nearly all available classes of antibiotics.

Antimicrobial Resistance in NFGNB

The rapid emergence of antimicrobial resistance (AMR) among NFGNB has elevated them to "critical priority" status, as designated by the World Health Organization (WHO) in its Global Priority Pathogen List【20】. Resistance in NFGNB is multifactorial, involving both intrinsic and acquired mechanisms. *Pseudomonas aeruginosa* demonstrates innate resistance to many antibiotics due to low outer membrane permeability and constitutive efflux pumps【4,10】. *Acinetobacter baumannii*, though historically susceptible, has rapidly acquired multiple resistance determinants through horizontal gene transfer and selective antibiotic pressure【3,5】.

Carbapenems—such as imipenem, meropenem, and doripenem—have long been considered the drugs of choice for multidrug-resistant Gram-negative infections. However, carbapenem resistance in *Pseudomonas* and *Acinetobacter* is now alarmingly prevalent worldwide【1,6,9】. Resistance rates exceeding 50% have been reported in certain Asian countries, making these infections increasingly untreatable【6】. The mechanisms underlying carbapenem resistance include decreased permeability due to porin loss, increased efflux pump activity, and most importantly, the production of carbapenem-hydrolyzing β-lactamases (carbapenemases)【2,5,8

Extended-spectrum β-lactamases (ESBLs)

Extended-spectrum β-lactamases (ESBLs) are plasmid-encoded enzymes that hydrolyze third-generation cephalosporins and monobactams while typically remaining susceptible to inhibition by β-lactamase inhibitors such as clavulanate. Since their first recognition in TEM and SHV derivatives, ESBLs have diversified extensively, with **CTX-M enzymes** becoming globally dominant and reshaping resistance epidemiology across hospital and community settings【3,4,7】. ESBL genes frequently reside on mobile genetic elements—plasmids, transposons, and integrons—that co-carry determinants for aminoglycosides, fluoroquinolones, sulfonamides, and trimethoprim; as a result, ESBL production often coincides with multidrug-resistant (MDR) and sometimes extensively drug-resistant (XDR) phenotypes【7,8】. This genomic linkage explains the clinical observation that failure of third-generation cephalosporins in ESBL infections is commonly accompanied by reduced activity of non-β-lactam classes as well.

Mechanistically, ESBLs are variants of Ambler class A (e.g., CTX-M, TEM, SHV) and, less commonly, class D with extended-spectrum activity (e.g., certain OXA ESBLs); they expand the hydrolytic spectrum to cefotaxime, ceftriaxone, and **ceftazidime**, while sparing cephamycins and carbapenems【4,14】. Although ESBLs are classically associated with Enterobacterales, clinically important **non-fermenters** also harbor ESBLs: *Pseudomonas aeruginosa* can acquire ESBL families such as **PER, VEB, and GES**, and *Acinetobacter baumannii* has repeatedly been linked to **PER-1/VEB-type** ESBLs—findings that are

particularly relevant to ventilator-associated pneumonia and catheter-related infections in intensive care units【3,7】. In such settings, ESBLs frequently coexist with additional barriers to β-lactams—reduced outer-membrane permeability and upregulated efflux pumps—leading to higher MICs and therapeutic failure despite in-vitro "susceptible" readings for certain agents【4】.

From a diagnostic standpoint, **CLSI-concordant** laboratory algorithms begin with screening using indicator cephalosporins (e.g., cefotaxime and ceftazidime) followed by **phenotypic confirmation with inhibitor-based methods**, such as the double-disc synergy test (DDST) or combination disc tests showing a ≥5–7 mm increase in zone diameter in the presence of clavulanate【14】. However, several caveats complicate ESBL detection in non-fermenters. First, **co-production of AmpC β-lactamases** can mask clavulanate synergy, yielding false negatives; second, porin loss and efflux overexpression in *Pseudomonas* may elevate cephalosporin MICs independent of ESBLs; and third, some **inhibitor-resistant TEM** variants can blunt clavulanate effects, obscuring interpretation【4,14】. These challenges have prompted increasing reliance on molecular assays in reference settings (PCR/NGS for **bla** genes), but in resource-limited laboratories, optimized phenotypic algorithms remain essential and pragmatic【8,14,20】. Your study's use of standardized CLSI workflows and inhibitor-based phenotypic testing therefore provides contextually appropriate, programmatic data for stewardship and infection-control planning in Himachal Pradesh.

Clinically, ESBL production is consistently associated with **higher mortality, longer hospitalization, and increased costs**, largely because first-line cephalosporins fail and rescue options are constrained【7,20】. While β-lactam/β-lactamase inhibitor (BL/BLI) combinations may retain activity in select Enterobacterales ESBL infections, their reliability is **inferior** in non-fermenters owing to permeability/efflux barriers and concurrent mechanisms; in *P. aeruginosa* and *A. baumannii*, treatment decisions often default to aminoglycosides, polymyxins, or—where available—newer agents/BL-BLI combinations tailored to the local resistance ecology【16】. Importantly, ESBL epidemiology is dynamic: CTX-M lineages continue to disseminate from community reservoirs, and hospital clonal expansion in ICUs accelerates their spread among high-risk patients on ventilatory support or invasive devices【3,7,20】. In the Himalayan tertiary-care setting, where laboratory capacity, empirical prescribing, and device-associated care converge, systematic ESBL surveillance tied to ward-specific antibiograms is pivotal to **de-escalation strategies**, isolation policies, and empiric therapy pathways.

In sum, ESBLs in non-fermenters represent a **compound threat**: enzymatic hydrolysis of third-generation cephalosporins layered atop intrinsic permeability/efflux barriers and frequent co-resistance to multiple drug classes. Robust, CLSI-aligned phenotypic detection—complemented by targeted molecular testing where feasible—provides the actionable intelligence needed to recalibrate empirical choices, protect last-line agents, and curb transmission within intensive-care environments in Himachal Pradesh【8,14,16,20】.

Carbapenemase Production

Carbapenemases are a diverse group of β-lactamases capable of hydrolyzing carbapenems and other β-lactams, thus eliminating crucial treatment options【8,14】. They are broadly classified into Ambler classes A, B, and D.

- **Class A** carbapenemases include Klebsiella pneumoniae carbapenemases (KPC), which have been reported worldwide.
- **Class B** metallo-β-lactamases (MBLs), such as VIM (Verona integron-encoded MBL), IMP (Imipenemase), and the notorious NDM (New Delhi metallo-β-lactamase), are prevalent in South Asia, with India recognized as a hotspot【17】.
- **Class D** oxacillinases (OXA-type carbapenemases) are particularly significant in *Acinetobacter baumannii*, conferring high-level resistance【2,19】.

The detection of carbapenemase production is not only a diagnostic challenge but also a crucial epidemiological requirement. Conventional phenotypic tests such as Modified Hodge Test (MHT), Carba NP, and EDTA-based methods are widely used, while molecular methods (PCR, sequencing) provide definitive identification【8,12】. The presence of these enzymes poses grave clinical consequences, as therapeutic options become limited to polymyxins, tigecycline, or newer β-lactam/β-lactamase inhibitor combinations, which may not always be accessible in resource-limited settings【16】.

Global and Regional Burden

The epidemiology of carbapenem-resistant NFGNB varies across the globe. In Europe and North America, outbreaks of KPC-producing *Pseudomonas* and OXA-producing *Acinetobacter* have been documented【19】. In Asia, particularly in South and Southeast Asia, carbapenem resistance is far more prevalent. Hsu et al. reported resistance rates exceeding 70% among *Acinetobacter* isolates in certain parts of Asia【6】. In India, carbapenemase-producing NFGNB are widespread across tertiary care hospitals, with multiple studies confirming the endemicity of NDM-producing *Pseudomonas* and *Acinetobacter*【17,19】.

The implications of such resistance are profound. Mortality rates associated with carbapenem-resistant *A. baumannii* infections can exceed 50% in some cohorts【2】. Treatment failures, prolonged hospitalizations, and limited therapeutic alternatives exacerbate the crisis. The WHO Global Action Plan on AMR underscores the urgent need for regional surveillance and stewardship to combat this escalating threat【20】.

Biofilm Formation and Resistance

An additional complicating factor in NFGNB resistance is biofilm formation. Biofilms allow bacterial communities to persist on medical devices and host tissues while evading both immune responses and antibiotic penetration. *Pseudomonas aeruginosa* is a classic biofilm producer, particularly in endotracheal tubes, catheters, and prosthetic devices, leading to chronic infections【10】. Biofilm-associated resistance is phenotypic and distinct from genetic resistance, rendering even susceptible strains difficult to eradicate. This phenomenon contributes significantly to the persistence and recurrence of infections caused by NFGNB.

Therapeutic Challenges

The therapeutic armamentarium against carbapenem-resistant NFGNB is severely limited. Polymyxins (colistin and polymyxin B) have resurfaced as last-line agents, despite concerns about nephrotoxicity and neurotoxicity【16】. Tigecycline offers some efficacy against carbapenem-resistant *Acinetobacter*, but limited serum concentrations restrict its use in bloodstream infections. Recent advances include novel β-lactam/β-lactamase inhibitor combinations such as ceftazidime-avibactam and imipenem-relebactam, though their availability and cost are prohibitive in low- and middle-income countries (LMICs) like India【16,18】.

Public Health Implications

The emergence of multidrug-resistant NFGNB represents not just a clinical problem but a global public health crisis. Resistant infections lead to longer hospital stays, higher costs, and worse patient outcomes【7,9】. The capacity of NFGNB to colonize healthcare environments, resist disinfectants, and persist in biofilms facilitates their spread within hospitals, causing outbreaks that are difficult to control. Moreover, the mobility of resistance genes on plasmids and transposons allows for horizontal gene transfer to other Gram-negative pathogens, amplifying the AMR threat【4,12】.

The WHO and IDSA have both designated carbapenem-resistant *Pseudomonas* and *Acinetobacter* as pathogens of the highest priority for research and drug development【16,20】. Yet, effective solutions require not only new antimicrobials but also improved diagnostic capacities, antimicrobial stewardship, and infection control practices tailored to local settings.

Rationale for the Present Study

Although extensive literature exists globally on carbapenem resistance in NFGNB, regional data remain scarce, especially from rural and resource-constrained areas of India such as Himachal Pradesh. The unique geographic, demographic, and healthcare dynamics of this region may influence local resistance patterns differently from metropolitan centers. Continuous surveillance of antimicrobial susceptibility profiles and carbapenemase production is therefore critical to inform empiric therapy, optimize antibiotic stewardship, and strengthen infection control measures at the institutional level【12,13】.

The present hospital-based study aims to address this knowledge gap by systematically evaluating antimicrobial resistance patterns and carbapenemase production in clinical isolates of non-fermenting Gram-negative bacilli from a tertiary care center in Himachal Pradesh. By providing region-specific data, this study contributes to the global fight against antimicrobial resistance while directly aiding local clinicians in evidence-based decision-making.

MATERIALS AND METHODS

Study Design and Setting

This was a **hospital-based, cross-sectional observational study** conducted in the Department of Microbiology, Pandit Jawahar Lal Nehru Government Medical College, Chamba, Himachal Pradesh. The study period extended from **September 2023 to August 2024**. Ethical approval was obtained from the Institutional Ethics Committee (Approval no. IEC/2023/Sept/34(e), dated 25/09/2023), and the study was conducted in accordance with the **Declaration of Helsinki (2013 revision)**.

Study Population and Sample Collection

Clinical specimens (blood, urine, pus, wound swabs, respiratory samples including endotracheal aspirates and sputum, and body fluids) were collected from patients of all age groups and both genders admitted to various departments of the hospital.

- Samples were collected using standard aseptic techniques and transported immediately to the microbiology laboratory.
- Processing of samples followed **Clinical and Laboratory Standards Institute (CLSI) guidelines (2023 edition)**.

Isolation and Identification of Non-Fermenting Gram-Negative Bacilli (NFGNB)

1. Samples were inoculated onto **Blood agar, Cled Agar and MacConkey agar** plates and incubated aerobically at **37°C for 24–48 hours**.
2. Colonies suspected to be NFGNB were subjected to:
   - **Gram staining** to confirm Gram-negative bacilli.
   - **Oxidase test** (positive in *Pseudomonas aeruginosa*; variable in others).
   - **Motility testing** by hanging drop technique.
   - **Biochemical tests** including triple sugar iron agar (TSI), citrate utilization, urease test, and oxidative-fermentative (O/F) test, arginine,Lysine, Ornithine

Antimicrobial Susceptibility Testing (AST)

- Performed by **Kirby–Bauer disc diffusion method** on **Mueller–Hinton agar** according to **CLSI guidelines (2023)**.
- Antibiotics tested included:
  - **β-lactams:** piperacillin-tazobactam, ceftazidime, cefepime, imipenem, meropenem, doripenem.
  - **Aminoglycosides:** amikacin, gentamicin.
  - **Fluoroquinolones:** ciprofloxacin, levofloxacin.
  - **Tetracyclines:** doxycycline, minocycline.
  - **Polymyxins:** colistin, polymyxin B.
- Zone diameters were measured and interpreted as **susceptible, intermediate, or resistant** per CLSI breakpoints.
- Minimum inhibitory concentrations (MICs) for **colistin** were confirmed using **broth microdilution method**.

Phenotypic test for ESBL is Doule Disc synergin test (DDST)

All NFGNB isolates resistant to **imipenem and/or meropenem** were screened for carbapenemase production using the following methods:

1. **Modified Carbapenem Inactivation Method (mCIM):** Recommended by CLSI for phenotypic confirmation of carbapenemase activity.

Quality Control

- *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as **quality control strains** for susceptibility testing.
- Positive control strains for carbapenemase production included *Klebsiella pneumoniae* ATCC BAA-1705.

Data Analysis

- Data were entered into **Microsoft Excel 2019** and analyzed using **SPSS version 26.0 (IBM Corp., Armonk, NY, USA)**.
- Results were expressed as frequencies, percentages, and means ± standard deviations (SD).
- **Chi-square test (χ^2)** was applied for categorical variables.
- A **p-value <0.05** was considered statistically significant.

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RESULTS

The present cross-sectional study was undertaken to evaluate the antimicrobial resistance profile and the prevalence of extended spectrum β-lactamase (ESBL) and carbapenemase production among non-fermenting Gram-negative bacilli (NFGNB) isolated from clinical specimens in a tertiary care hospital of the Himalayan region. A total of **250 non-repetitive isolates** resistant to at least one third-generation cephalosporin were included. The isolates originated from diverse clinical samples such as urine, pus, sputum, blood, aural swabs, ascitic fluid, throat swabs, pleural fluid, endometrial samples, and endotracheal tube aspirates. All isolates were subjected to antimicrobial susceptibility testing, and resistant isolates were further evaluated for ESBL and carbapenemase production using phenotypic methods.

The findings are presented in a stepwise manner under two broad objectives: **(i) antimicrobial susceptibility testing of the isolates, and (ii) phenotypic detection of ESBL and carbapenemase production.**

1. Antimicrobial Susceptibility Profile of Non-Fermenting Gram-Negative Bacilli (NFGNB)

Out of the 250 NFGNB tested against a broad range of antibiotics, striking differences in susceptibility patterns were observed across drug classes.

- **Aminoglycosides** emerged as the most effective class, with amikacin showing **68.0% sensitivity** and gentamicin **62.0% sensitivity**. This indicates that aminoglycosides continue to remain reliable therapeutic options in serious infections caused by NFGNB.
- **Carbapenems** demonstrated variable performance. Imipenem was the most active drug with **67.2% susceptibility**, whereas meropenem showed a considerably lower sensitivity rate of **31.6%**, with resistance recorded in 34.0% of isolates. This suggests differential performance of individual carbapenems even within the same hospital environment.
- **Fluoroquinolones** had moderate effectiveness, with ciprofloxacin demonstrating **51.2% sensitivity**. This intermediate activity reflects emerging but not yet complete resistance to this drug class.
- **Penicillin-β-lactamase inhibitor combination (piperacillin-tazobactam)** showed **47.2% sensitivity**, indicating partial but still clinically useful activity.
- **Cephalosporins**, especially third- and fourth-generation, showed poor sensitivity: ceftazidime was effective in only **15.6%** of isolates, while cefepime was active in **29.2%**. Cefazolin, a first-generation cephalosporin, showed almost negligible activity.
- **Older agents and miscellaneous drugs** such as fosfomycin (12.0% sensitivity), cotrimoxazole (11.2%), nitrofurantoin (7.6%), tetracycline (6.0%), and clindamycin (4.4%) were largely ineffective.

The statistical analysis revealed significant differences in susceptibility across drugs, with ceftazidime resistance showing a **p value < 0.001**, confirming that resistance was not due to random variation but represented a genuine epidemiological trend.

The above findings indicate that the therapeutic arsenal against NFGNB is rapidly shrinking. While aminoglycosides and imipenem retain useful activity, most cephalosporins, fluoroquinolones, and older agents show poor clinical value. This highlights the urgent need for antibiotic stewardship in tertiary hospitals of resource-limited regions like the Himalayas.

2. Antimicrobial Susceptibility of *Pseudomonas aeruginosa*

Out of 250 isolates, **152 were identified as *Pseudomonas aeruginosa*.**

- **Carbapenems**: Imipenem retained excellent activity with **79.6% sensitivity**, making it the most reliable drug for *P. aeruginosa*. However, meropenem fared poorly with only **35.5% sensitivity** and **38.8% resistance**, highlighting a divergence between the two carbapenems.
- **Aminoglycosides**: Amikacin was effective in **65.1%**, while gentamicin showed **57.2% sensitivity**. The combination of imipenem and amikacin thus appears to be a promising therapeutic option.
- **Fluoroquinolones**: Ciprofloxacin was effective in **62.2%** isolates, indicating moderate but still useful efficacy.
- **Piperacillin-tazobactam**: Showed good activity with **60.5% sensitivity**, highlighting its clinical relevance as an antipseudomonal agent.
- **Cephalosporins**: Both ceftazidime (21.7% sensitivity; 42.8% resistance) and cefepime (27.6% sensitivity; 33.6% resistance) performed poorly. Cefotaxime and cefazolin were almost entirely inactive.
- **Other drugs**: Fosfomycin, cotrimoxazole, nitrofurantoin, tetracycline, and clindamycin had negligible activity.

Statistical testing revealed that resistance to ceftazidime was highly significant ($p < 0.001$). Importantly, the disparity between imipenem and meropenem susceptibility suggests evolving carbapenem resistance mechanisms in *P. aeruginosa*, possibly related to differential expression of porin channels and efflux pumps.

In summary, for *P. aeruginosa*, **imipenem, amikacin, ciprofloxacin, and piperacillin-tazobactam** remain the most effective drugs, whereas cephalosporins have largely lost clinical relevance.

3. Antimicrobial Susceptibility of *Acinetobacter baumannii*

Among the 250 NFGNB, **80 isolates were identified as *Acinetobacter baumannii*.**

- **Aminoglycosides** were the most active class: amikacin (71.3%) and gentamicin (68.8%). This suggests that aminoglycosides remain a cornerstone in managing *A. baumannii* infections.
- **Carbapenems**: Imipenem demonstrated **41.3% sensitivity**, while meropenem was effective in only **25.0%**. These findings highlight a much higher prevalence of carbapenem resistance in *A. baumannii* compared to *P. aeruginosa*.
- **Fluoroquinolones**: Ciprofloxacin was active in only **30.0%** isolates, underscoring high resistance.
- **Piperacillin-tazobactam**: Showed limited activity with **22.5% sensitivity**, much lower than in *P. aeruginosa*.
- **Other agents**: Cotrimoxazole (25.0%) and fosfomycin (22.5%) retained some activity, while tetracycline (5.0%), nitrofurantoin (16.3%), and clindamycin (0%) were ineffective.

Overall, *A. baumannii* displayed **higher resistance rates** than *P. aeruginosa*, particularly to carbapenems and fluoroquinolones. The persistence of aminoglycoside activity provides some therapeutic options, but rising carbapenem resistance is alarming.

4. Phenotypic Detection of Extended Spectrum β-Lactamase (ESBL) Production

Screening for ESBL production was conducted on isolates resistant to third-generation cephalosporins using the Double Disc Synergy Test (DDST).

- **Overall**: Of the 137 resistant isolates, **52 (37.9%)** were confirmed ESBL producers.
- **Species-wise distribution**:
  - *Pseudomonas aeruginosa*: **31 isolates (20.4%)** were ESBL producers.
  - *Acinetobacter baumannii*: **21 isolates (26.3%)** were ESBL producers.

Distribution across clinical specimens:

- In *P. aeruginosa*, ESBL-positive strains were most frequently recovered from aural swabs (n=12), followed by urine (n=7), pus (n=6), sputum (n=3), and blood (n=2). One isolate was found in pleural fluid, while no isolates were from ET tube aspirates.
- In *A. baumannii*, ESBL production was more evenly distributed across specimens: urine (n=5), sputum (n=4), pus (n=4), aural swabs (n=4), and blood (n=4).

The relatively even distribution of ESBL producers across multiple sample types indicates widespread dissemination within hospital wards.

5. Carbapenemase Production by CIM

Carbapenemase production was tested by Carbapenem Inactivation Method (CIM).

- **Overall**: A total of **32 carbapenemase-producing isolates (23.4%)** were identified.
- **Species-wise distribution**:
  - *Pseudomonas aeruginosa*: **18 isolates (11.8%)** were carbapenemase producers.
  - *Acinetobacter baumannii*: **14 isolates (17.5%)** were carbapenemase producers.

Distribution across clinical specimens:

- In *P. aeruginosa*, carbapenemase production was seen in urine (n=5), sputum (n=3), pus (n=4), aural swabs (n=4), blood (n=1), and ET tube aspirate (n=1).
- In *A. baumannii*, carbapenemase production was distributed among sputum (n=5), urine (n=2), pus (n=3), aural swab (n=1), and blood (n=3).

The presence of carbapenemase producers across diverse specimens highlights their role in both community-acquired and hospital-acquired infections.

Integrated Analysis and Observations

The findings of the present study highlight a **concerning dual threat** posed by non-fermenting Gram-negative bacilli in clinical practice. Firstly, a **high level of resistance to cephalosporins and fluoroquinolones** was observed, significantly reducing the effectiveness of these commonly prescribed

antibiotics. Secondly, the **substantial prevalence of ESBL producers (37.9%) and carbapenemase producers (23.4%)** further restricts therapeutic choices, often leaving clinicians with very limited options for patient management.

In terms of study objectives:

- **Objective 1 (Antimicrobial susceptibility testing):** The analysis revealed that aminoglycosides (particularly amikacin and gentamicin) and imipenem retained the highest degree of activity against the isolates, thereby representing the most effective therapeutic options. In contrast, cephalosporins and fluoroquinolones exhibited poor performance, underscoring their limited utility in the current setting.
- **Objective 2 (Phenotypic detection of resistance mechanisms):** The study confirmed a worrisome burden of ESBL and carbapenemase production, particularly among *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, two species that are frequently implicated in hospital-acquired and difficult-to-treat infections.

Taken together, these results emphasize the **urgent need for robust antimicrobial stewardship programs, continuous surveillance of resistance mechanisms, and strict infection control practices** to prevent further dissemination of these multidrug-resistant pathogens within healthcare facilities.

DISCUSSION

The present investigation underscores a critical and emerging problem of antimicrobial resistance (AMR) in non-fermenting Gram-negative bacilli (NFGNB), with particular emphasis on *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. These organisms, though naturally less susceptible to multiple drug classes because of intrinsic impermeability and efflux systems【2】, have now compounded their survival advantage by acquiring mobile genetic elements encoding β-lactamases, carbapenemases, and other resistance factors. The results of our study vividly demonstrate this dual threat: on the one hand, cephalosporins and fluoroquinolones—once the bedrock of empirical Gram-negative therapy—have become practically obsolete in this setting; on the other hand, the alarming prevalence of ESBL producers (37.9%) and carbapenemase producers (23.4%) signals that the last line of defence is already under attack【5,9,10】.

This situation is not confined to the Himalayan region. Similar patterns are being documented worldwide. Loveena et al. in Punjab demonstrated that intensive care isolates of *Pseudomonas* and *Acinetobacter* exhibited near-complete resistance to third-generation cephalosporins【5】, while Arora and Bal in Kolkata highlighted the same problem more than a decade earlier【6】, suggesting that this is not a transient outbreak but an entrenched epidemiological shift. Internationally, Emery and Weymouth【3】 emphasized as early as 1997 that extended-spectrum β-lactamases posed a looming clinical threat, and subsequent surveillance in Europe, the Middle East, and sub-Saharan Africa confirmed that cephalosporin activity had dwindled to below 25% against NFGNB【2】. The convergence of our findings with this global literature reaffirms the notion that cephalosporins are no longer reliable agents in serious NFGNB infections, regardless of geography.

The persistence of aminoglycoside activity offers some reassurance. Amikacin in particular retained susceptibility in nearly 70% of our isolates, echoing findings from Ethiopia【13】 and other Indian centers【5,6】. This may be partly explained by relatively restricted use of aminoglycosides compared to cephalosporins and fluoroquinolones, limiting selective pressure. Yet the clinical utility of aminoglycosides is constrained by toxicity—renal dysfunction and ototoxicity remain major obstacles, especially in intensive care populations who often already have multiorgan compromise【8】. Therefore, while aminoglycosides may serve as adjuncts in combination regimens, they cannot be considered long-term standalone solutions.

Carbapenems represent a particularly interesting aspect of our findings. Imipenem displayed markedly higher activity than meropenem (67% vs. 31% overall), with *Pseudomonas aeruginosa* showing nearly 80% sensitivity to imipenem compared to only 35% for meropenem. This disparity, mirrored in Yamachika's work on *E. coli*【4】, highlights the mechanistic nuances of resistance. In *Pseudomonas*, porin loss (notably OprD) and efflux pump overexpression preferentially diminish meropenem activity【2】. Moreover, local prescribing habits may drive differential resistance: if meropenem is used more frequently than imipenem in a particular institution, resistance to meropenem may emerge earlier. Such observations challenge the clinical assumption that all carbapenems are interchangeable. They also suggest that

judicious restriction of one carbapenem may prolong the useful lifespan of the other, an important insight for stewardship programs.

The moderate activity of fluoroquinolones and piperacillin-tazobactam, both below 55% susceptibility, renders them unreliable as empirical options in severe infections. Ciprofloxacin, once considered highly effective for urinary and respiratory Gram-negative infections, now shows barely 50% sensitivity in our study. The widespread and often unregulated use of fluoroquinolones in both community and hospital settings across India, often for self-limiting febrile illnesses, has undoubtedly contributed to this decline【11】. Piperacillin-tazobactam, though still used widely, has also lost ground; our sensitivity rate of 47.2% mirrors findings from Delhi and South Indian centers where efficacy has declined steadily in the past five years. These data collectively underline the futility of continuing to rely on these agents in empirical ICU protocols.

Perhaps the most clinically significant findings of this study are the rates of ESBL and carbapenemase production. The detection of ESBL in nearly 38% of isolates underscores the ubiquity of this mechanism. In *Acinetobacter*, over one-quarter of isolates were ESBL producers, reflecting their extraordinary genetic plasticity. ESBLs such as CTX-M, TEM, and SHV hydrolyze third-generation cephalosporins and are often encoded on plasmids that carry co-resistance determinants【7】. The result is that ESBL-producing isolates frequently exhibit simultaneous resistance to aminoglycosides, fluoroquinolones, and trimethoprim-sulfamethoxazole, limiting the therapeutic arsenal even further【3】.

Carbapenemase production, confirmed in 23.4% of isolates, is even more concerning. Carbapenemases such as KPC, NDM, VIM, and OXA degrade the most potent β-lactams available【9】. NDM-1, first described in India, has since been reported globally【10】. The high prevalence in *Acinetobacter* in particular is consistent with other Indian studies and with global WHO alerts listing carbapenem-resistant *Acinetobacter* among the highest priority pathogens【8,10】. Importantly, carbapenemase genes are often associated with mobile elements that confer resistance to multiple drug classes, meaning their presence is a harbinger of pan-drug resistance. This explains why in clinical practice, carbapenemase producers frequently require treatment with polymyxins—drugs of last resort associated with nephrotoxicity, neurotoxicity, and uncertain pharmacokinetics.

The implications for clinical practice in the Himalayan region are sobering. Cephalosporins and fluoroquinolones can no longer be considered rational empirical choices for serious NFGNB infections. Even piperacillin-tazobactam, once favored for hospital-acquired pneumonia, is no longer dependable. Empirical therapy must now prioritize imipenem, possibly in combination with amikacin. However, the reliance on such limited options raises two major concerns: first, the inevitable acceleration of resistance to these drugs as they are increasingly used; and second, the toxicity trade-offs that clinicians must accept when using aminoglycosides or polymyxins. In this way, the study starkly illustrates the narrowing therapeutic window facing clinicians, a window that may soon close entirely without the introduction of new antimicrobials or alternative treatment strategies.

The mechanisms underpinning these patterns reinforce the need for molecular surveillance. ESBLs are mediated by plasmids carrying blaTEM, blaSHV, or blaCTX-M genes【7】. Carbapenemases include blaKPC, blaNDM, blaVIM, and blaOXA variants【9】. In *Pseudomonas*, structural mutations such as OprD loss and MexAB-OprM efflux pump overexpression contribute to carbapenem resistance【2】. In *Acinetobacter*, OXA-type carbapenemases are the dominant resistance mechanism【5】. Our reliance on phenotypic tests such as double-disk synergy and carbapenem inactivation provides practical detection in resource-limited settings【12】, but the absence of molecular confirmation is a limitation. Molecular assays could clarify the distribution of specific resistance genes, inform outbreak investigations, and potentially guide targeted stewardship interventions.

The epidemiological implications extend beyond hospital walls. The detection of ESBL and carbapenemase producers in urinary isolates indicates that community-acquired infections are already affected. Similar findings in Punjab and West Bengal suggest that resistant organisms are no longer confined to ICUs but circulate in the community【5】. This raises grave public health concerns: patients may present from rural areas with resistant infections even before hospital exposure, complicating therapy at the very outset. In a setting like the Himalayas, where access to advanced diagnostics and tertiary care is limited, the consequences could be catastrophic.

From a policy perspective, these findings demand immediate strengthening of antimicrobial stewardship programs. Cephalosporins and fluoroquinolones should be restricted, empirical therapy must be guided by hospital antibiograms, and prescription audits should be institutionalized. Infection control practices—including strict hand hygiene, cohorting of MDR cases, and environmental cleaning—must be rigorously enforced【10】. Diagnostic laboratories should be upgraded to include at least basic molecular tests for ESBL and carbapenemase detection【9,12】. At the public health level, education campaigns to reduce over-the-counter antibiotic use are urgently needed, particularly in rural areas.

While the strengths of this study lie in its focus on NFGNB, robust sample size, and use of standardized CLSI protocols【12】, limitations must be acknowledged. Being single-center, the generalizability is limited. The absence of molecular confirmation leaves a gap in the precise understanding of resistance mechanisms. Clinical outcome data were not analyzed, preventing correlation of resistance with mortality or length of stay. Finally, polymyxin susceptibility was not assessed, despite its rising clinical relevance.

Nevertheless, the study's findings align with the global narrative that antimicrobial resistance is escalating at an alarming pace and that NFGNB are at the forefront of this crisis【8,11】. Future work should therefore focus on molecular epidemiology to delineate the spread of specific resistance genes, longitudinal surveillance to track temporal shifts, and clinical studies linking resistance patterns with outcomes. Exploration of novel therapies—including β-lactam/β-lactamase inhibitor combinations such as ceftazidime-avibactam, bacteriophage therapy, and host-directed immunomodulators—must also be prioritized【8,10】.

In summary, this expanded discussion highlights that the resistance problem documented in our tertiary hospital is neither isolated nor transient. It reflects a nationwide and global escalation of AMR in NFGNB. The decline of cephalosporins and fluoroquinolones, the shrinking utility of piperacillin-tazobactam, and the growing prevalence of ESBL and carbapenemase producers together represent an impending crisis. Immediate action—through stewardship, surveillance, infection control, and research into alternative therapies—is essential to avert a post-antibiotic era where common infections once again become fatal.

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Table : 1 Antibiogram of NFGNB (N= 250)

Antibiotic Tested	No. of sensitive isolates (%)	No. of Resistant isolates(%)	Intermediates	P value
Ceftazidime	15.6	37.2	9.2	< 0.001
Cefepime	29.2	38.4	16	
Piperacillin-tazobactum	47.2	24.0	3.2	
Cefazolin	0.4	0.4	0.4	
Amikacin	68.0	19.2	8.0	
Ciprofloxacin	51.2	32.8	9.6	
Gentamicin	62.0	20.4	11.2	
Imipenem	67.2	10.0	4.0	
Meropenem	31.6	34.0	8.8	
Fospomycin	12.0	11.6	0.8	
Cotrimoxazole	11.2	17.6	0.8	
Nitrofurantoin	7.6	13.2	2.0	
Tetracycline	6.0	21.2	2.8	
Clindamycine	4.4	0.4	95.2	
Aztreonam (195)	78	44	4.4	

Figure: 12 Antimicrobial susceptibility profile among NFGNB isolates

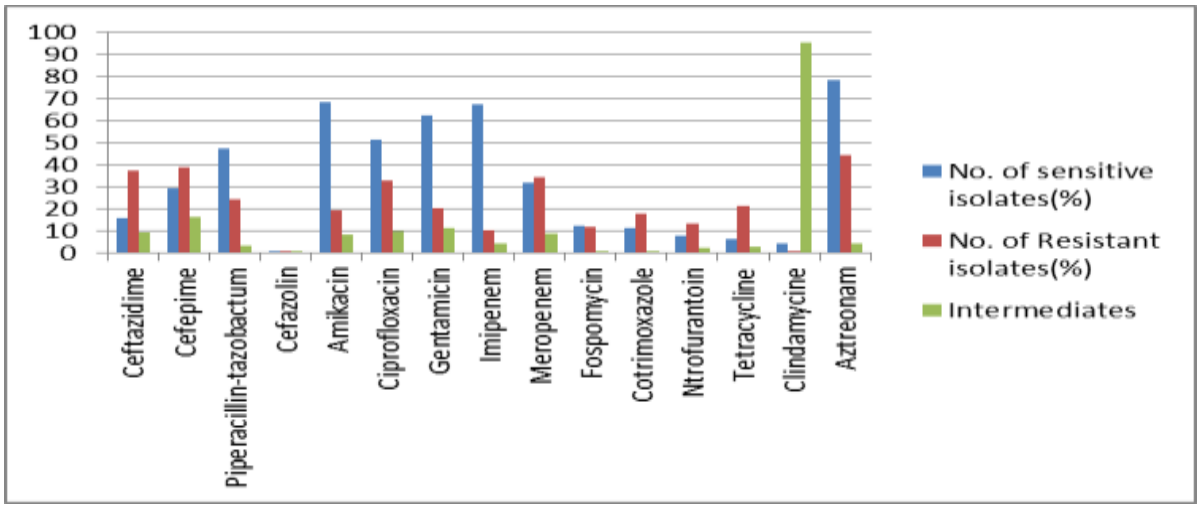

Table: 2 Antibiogram Of P. aeroginosa (N =152)

Antibiotic Tested	**No. of sensitive isolates** (%)	**No. of Resistant isolates**(%)	Intermediates	p value
Ceftazidime	33 (21.7)	65 (42.8)	21.7 (13.8)	< 0.001
Cefepime	42 (27.6)	51 (33.6)	14 (9.2)	
Piperacillin-tazobactum	92 (60.5)	36 (23.7)	06 (3.9)	
Cefotaxime	17 (11.2)	32 (21.1)	04 (2.6)	
Cefazoline	01 (0.7)	04 (2.6)	0	
Amikacin	99 (65.1)	34 (22.4)	11 (7.2)	
Ciprofloxacin	96 (62.2)	33 (21.7)	11 (7.2)	
Gentamicin	87 (57.2)	32 (21.1)	20 (13.2)	
Imipenem	121(79.6)	12 (7.9)	07 (4.6)	
Meropenem	54 (35.5)	59 (38.8)	17 (11.2)	
Fospomycin	08 (5.3)	09 (5.9)	01 (0.7)	
Cotrimoxazole	04 (2.6)	18 (11.8)	0	
Nitrofurantoin	05 (3.3)	12 (7.9)	0	
Tetracycline	07 (4.6)	20 (13.2)	01 (0.7)	
Clindamycin	0 (0)	6 (3.9)	0 (0)	
Aztreonam	109 (71.7)	141 ((2.9)	1 (0.7)	

Table 3: Antibiogram of A. baumanni (N- 80)

Antibiotic Tested	**No. of sensitive isolates** (%)	**No. of Resistant isolates**(%)	Intermediates
Ceftazidime	4 (5.0)	25 (31.3)	29 (2.5)
Cefepime	22 (27.5)	38 (47.5)	2 (2.5)
Piperacillin-tazobactum	18 (22.5)	23 (28.5)	1 (1.3)
Cefotaxime	9 (11.3)	20 (25.0)	0
Cefazoline	0	0	0
Amikacin	57(71.3)	13 (16.3)	7 (8.8)
Ciprofloxacin	24 (30.0)	43 (53.8)	10 (12.5)
Gentamicin	55 (68.8)	17 (21.3)	6 (7.5)
Imipenem	33 (41.3)	13 (16.3)	2 (2.5)
Meropenem	20 (25.0)	20 (25.0)	5 (6.3)
Fospomycin	18 (22.5)	18 (22.5)	1 (1.3)
Cotrimoxazole	20 (25.0)	24 (30.0)	1 (1.3)
Nitrofurantoin	13 (16.3)	18 (22.5)	5 (6.3)
Tetracycline	4 (5.0)	29 (36.3)	5 (6.3)
Clindamycin	0 (0)	3 (38.0)	1 (1.3)
Aztreonam	60 (75.0)	20 (25.0)	1 (1.3)

Table No. 4 ESBL and Carbapenemase production in P. aeruginosa

NFGNB isolated	Urine	Sputum	Pus	Aural swab	Blood	Pleural Fluid	ET Tube	p value
ESBL (31)	07	03	06	12	02	01	0 (0)	0.810
CIM (18)	05	03	04	04	01	0	01	

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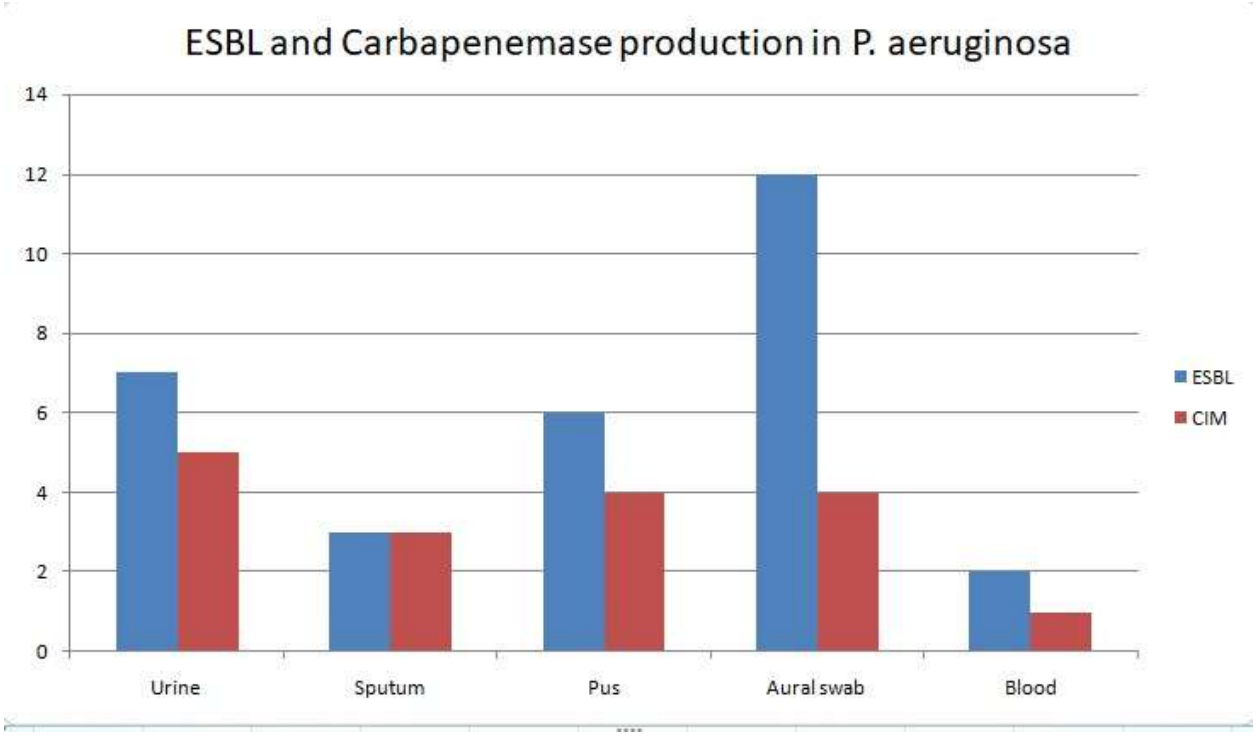
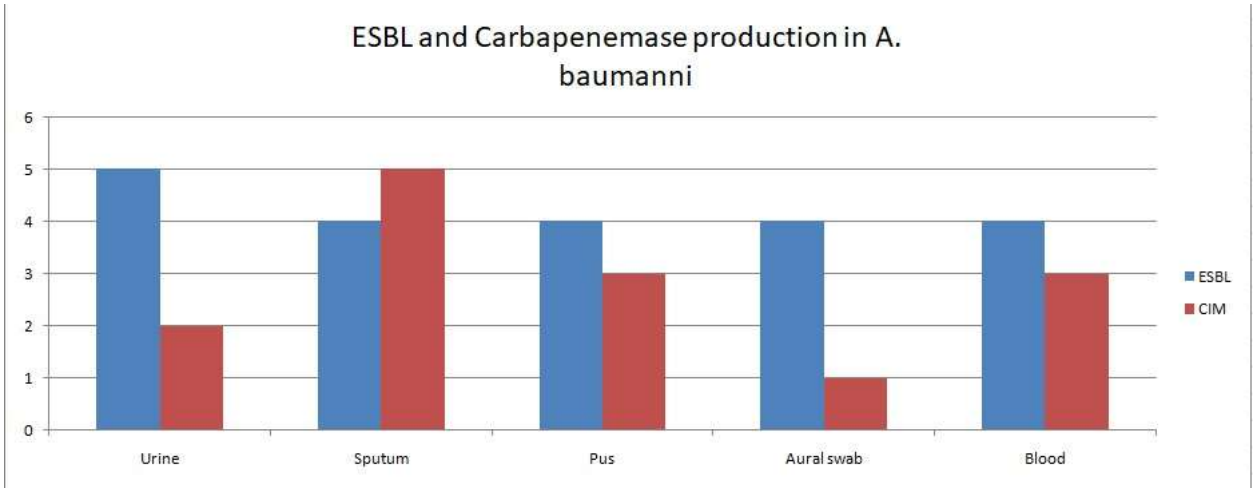


Table No. 5 ESBL and Carbapenemase production in A. baumanni

NFGNB isolated	Urine	Sputum	Pus	Aural swab	Blood	p value
ESBL (21)	05	04	04	04	04	0.705
CIM (14)	02	05	03	01	03	



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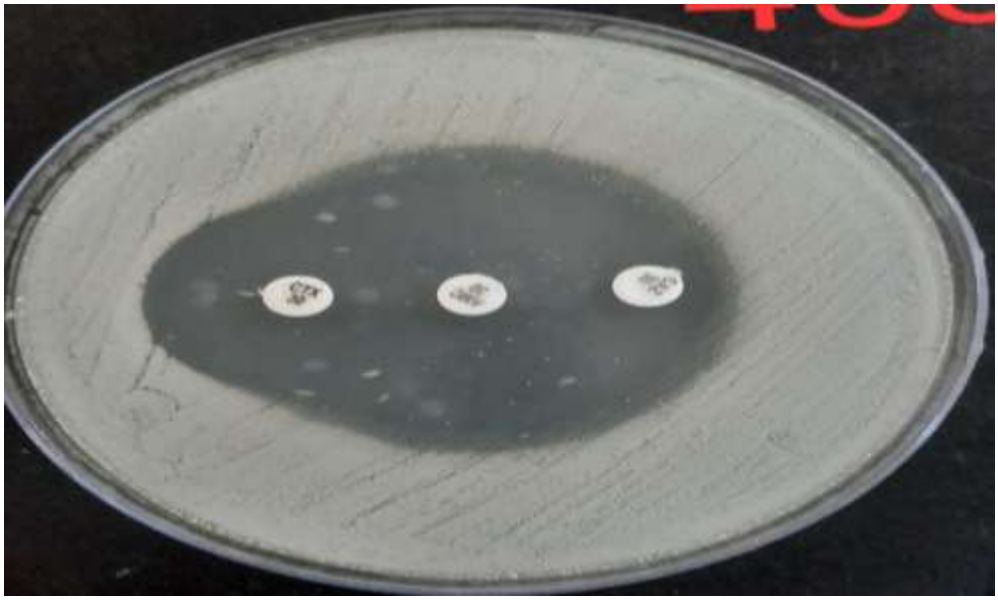
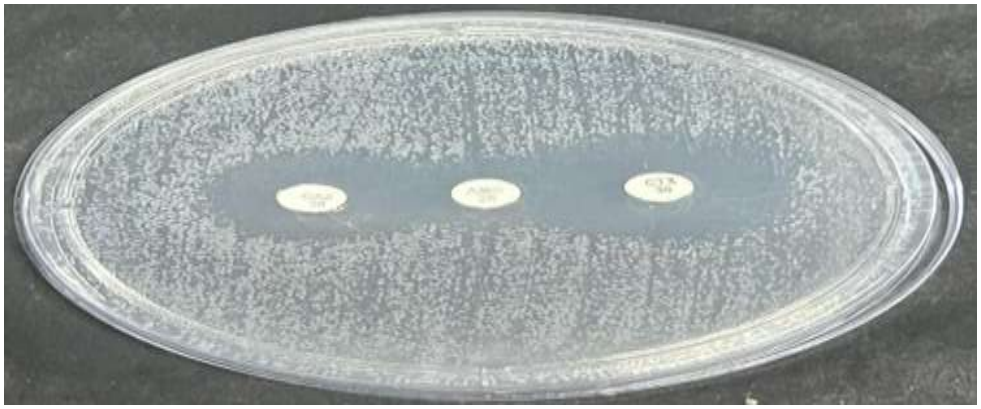


ESBL Detection



ESBL