Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/v3d8cd70

PHENOTYPIC IDENTIFICATION OF AMPC BETA-LACTAMASE AND ESBL IN CLINICAL ISOLATES OF COMMON GRAM-NEGATIVE BACTERIA IN TERTIARY CARE HOSPITALS

Dr Pinki Kumari[1], Dr Jiwesh Kumar Thakur[2], Amit Kumar Patel[3], Dr Aditya Kumar Jha[4]*, Dr.Manas Ranjan Sahoo[5], Dr. Sai Ravi Kiran Biri[6],

[1]Associate Professor, Department of Microbiology, PhuloJhano Medical College, Dumka, Jharkhand
[2]Professor, Dept. of Surgery, Gouri Devi Institute of Medical Sciences and Hospital, Durgapur, India.
[3]Senior resident, Department of Microbiology, PhuloJhano Medical College, Dumka, Jharkhand
[4*]Specialist Medical officer, Sadar hospital, Chaibasa, Jharkhand
[5]Associate Professor, Department of Psychiatry, Fakir Mohan Medical College, Balasore, odisha, India
[6]Professor, Department of Biochemistry, PhuloJhano Medical College, Dumka, Jharkhand

***Corresponding author:** Dr Aditya kumarjha
*Specialist Medical officer, Sadar Hospital, Chaibasa, Jharkhand
E Mail id: jhapinki.rims@gmail.com

Abstract

Background: The emergence of antibiotic-resistant Gram-negative bacteria, particularly those producing AmpC beta-lactamases and extended-spectrum beta-lactamases (ESBLs), poses a significant challenge in clinical settings. This study aimed to phenotypically identify the prevalence of AmpC and ESBL producers among common Gram-negative bacterial isolates in a tertiary care hospital.

Methods: A cross-sectional study was conducted at [Institution Name] from January 2024 to September 2024. A total of 180 clinical isolates were collected from various specimens. Gram-negative bacilli were identified using standard microbiological techniques, and antibiotic susceptibility testing was performed by the Kirby-Bauer disk diffusion method. The production of AmpC and ESBL was determined using specific phenotypic tests, including the double-disc synergy test and inhibition zone measurement.

Results: Out of 180 isolates, 65 Gram-negative bacilli were identified, with *Escherichia coli* (33.8%) being the most prevalent organism, followed by *Klebsiellapneumoniae* (24.6%). The study revealed alarming resistance rates to key antibiotics, with *E. coli* showing 90.9% resistance to cefoxitin. A total of 24 isolates (36.9%) were confirmed as ESBL producers, while 15 (23%) produced AmpC beta-lactamase. Notably, 8 isolates (12.3%) exhibited dual ESBL and AmpC production.

Conclusion: The findings underscore a significant prevalence of AmpC and ESBL production among clinically relevant Gram-negative bacteria. This highlights the urgent need for robust antibiotic stewardship programs and regular surveillance of resistance patterns to effectively manage and treat infections caused by these multidrug-resistant organisms. Continued efforts are necessary to optimize the use of existing antibiotics and mitigate the rise of resistance in clinical settings.

Keywords: AmpC beta-lactamase, Extended-spectrum beta-lactamase (ESBL), Gram-negative bacteria, Antibiotic resistance, Phenotypic identification

Introduction

The emergence and spread of antibiotic resistance among Gram-negative bacteria have become a global public health concern, particularly in healthcare settings. Extended-spectrum beta-lactamases (ESBLs) and AmpC beta-lactamases are significant contributors to this phenomenon, rendering commonly used beta-lactam antibiotics ineffective. ESBLs are enzymes that hydrolyze a wide range of beta-lactam antibiotics, including penicillins and cephalosporins, while AmpC beta-lactamases confer resistance primarily to cephalosporins and are often plasmid-mediated. Their presence complicates treatment options and poses challenges in managing infections, particularly in immunocompromised patients and those undergoing invasive procedures[1-2].

In recent years, there has been an alarming increase in the prevalence of ESBL and AmpC-producing strains of Gram-negative bacteria, such as *Escherichia coli, Klebsiellapneumoniae,* and *Pseudomonas aeruginosa,* in various clinical settings. These organisms are frequently associated with healthcare-associated infections, including urinary tract infections, bloodstream infections, and ventilator-associated pneumonia[3-4]. The rising incidence of multidrug-resistant (MDR) strains has led to treatment failures and increased morbidity and mortality, making accurate identification of these resistant phenotypes crucial for effective patient management[5-6].

Phenotypic identification of ESBL and AmpC production is essential for guiding appropriate antibiotic therapy and implementing effective infection control measures. Traditional susceptibility testing methods, such as the disc diffusion and broth microdilution methods, have been employed to detect these resistance mechanisms; however, they often lack specificity and sensitivity [7-8]. Therefore, the need for reliable and standardized phenotypic tests to accurately identify ESBL and AmpC-producing bacteria has become increasingly important.

This study aims to phenotypically identify AmpC and ESBL-producing Gram-negative bacterial isolates obtained from clinical specimens in a tertiary care hospital. By analyzing the prevalence and resistance patterns of these organisms, we hope to contribute to the understanding of antibiotic resistance dynamics in our setting and emphasize the necessity for stringent antimicrobial stewardship programs. The findings of this investigation will provide valuable insights into the epidemiology of ESBL and AmpC production, ultimately aiding in the formulation of effective therapeutic strategies and infection control practices.

Materials and Methods

This study was conducted at Gouri Devi Institute of Medical Sciences and Hospital, Durgapur, India, within the Microbiology Department. The cross-sectional research spanned from January 2024 to September 2024 and included all specimens sent to the microbiology department from various clinical departments and intensive care units for routine cultures and sensitivity testing.

Inclusion Criteria: The study included all Gram-negative bacilli isolated from clinical samples.

Exclusion Criteria: Isolates that were not Gram-negative bacilli were excluded from the study.

Identification of Isolates

Each specimen underwent microscopy via Gram staining and was inoculated onto the appropriate culture media, followed by incubation at 37ºC for 18-24 hours. Identification was further confirmed through morphological examination, staining characteristics, and standard biochemical tests.

Detection of Antibiotic Sensitivity Pattern of Gram-Negative Bacilli

- Identification and antibiotic susceptibility tests were performed using the Kirby-Bauer Disc Diffusion Method, following Clinical and Laboratory Standards Institute (CLSI) guidelines.
- The antibiotics tested included:
  - **For Enterobacteriaceae**: ceftazidime, cefoxitin, ciprofloxacin, gentamicin, amikacin, piperacillin/tazobactam, imipenem, meropenem, cotrimoxazole, nitrofurantoin, and norfloxacin.
  - **For Non-Fermenters**: ceftazidime, cefoxitin, ciprofloxacin, gentamicin, amikacin, piperacillin/tazobactam, imipenem, meropenem, tobramycin, aztreonam, and nitrofurantoin.
- Isolates resistant to cefoxitin (inhibition zone <14 mm) were considered potential AmpC producers and were subjected to further testing.

Detection of AmpC Production

- Cefoxitin-resistant strains were evaluated for AmpC production using a cefoxitin-cloxacillin double-disc synergy test.
- A difference of >4 mm in the zone diameter around the cefoxitin/cloxacillin disc compared to the cefoxitin disc was indicative of AmpC production.

ESBL Phenotypic Detection Test

All Gram-negative bacterial isolates were screened for ESBL production using the disk diffusion method. Isolates exhibiting a zone of inhibition of ≤ 22 mm for ceftazidime and ≤ 27 mm for cefotaxime were indicative of ESBL production. Isolates showing reduced susceptibility or resistance were subjected to confirmatory testing.

For confirmation, strains that tested positive in the ESBL screening were evaluated using the CLSI double-disc diffusion test. E. coli ATCC 25922 (ESBL-negative) and K. pneumoniae ATCC 700603 (ESBL-positive) served as control strains. A lawn culture of the test bacteria was plated on Mueller Hinton agar (MHA). Discs of ceftazidime (30 μg) and a combination disc of ceftazidime plus clavulanic acid (30 μg + 10 μg) were placed 25 mm apart, as were discs of cefotaxime (30 μg) and cefotaxime plus clavulanic acid (30 μg + 10 μg). An increase of ≥ 5 mm in the zone of inhibition for ceftazidime + clavulanic acid compared to ceftazidime alone, or for cefotaxime + clavulanic acid compared to cefotaxime alone, was identified as indicative of ESBL production, in accordance with CLSI recommendations[10-11].

Results

A total of 180 isolates were recovered from various clinical specimens. The majority of these isolates were derived from wound infections, followed by those from the respiratory tract, urine, blood, and other body fluids.

Table 1: Culture Positivity

Isolates	**Number**	**Percentage**
No growth	78	43.3%
Gram-positive cocci	37	20.5%
Gram-negative bacilli	65	36.1%
Total	**180**	**100%**

Out of 180 samples, 65 (36.1%) Gram-negative bacilli were isolated. Additionally, 37 isolates (20.5%) were identified as Gram-positive cocci, while no bacterial growth was observed in 78 samples (43.3%).

Table 2: Organism-wise Distribution of Gram-Negative Bacilli

Organisms	Number	Percentage
Escherichia coli	22	33.8%
Klebsiellapneumoniae	16	24.6%
Pseudomonas aeruginosa	11	16.9%
Proteus spp.	9	13.8%
Acinetobacterbaumannii	7	10.7%

As shown in Table 2, **Escherichia coli** was the most frequently isolated organism among the Gram-negative bacilli, accounting for 33.8% of the isolates. This was followed by **Klebsiellapneumoniae** with 24.6% (16 isolates). Other Gram-negative bacilli included **Pseudomonas aeruginosa** (11 isolates, 16.9%), **Proteus spp.** (9 isolates, 13.8%), and **Acinetobacterbaumannii** (7 isolates, 10.7%).

Table 3: Antibiotic Resistance Pattern of Gram-Negative Isolates

Antibiotics	E. coli (N=22)	K. pneumoniae (N=16)	P. aeruginosa (N=11)	Proteus spp (N=9)	A. baumannii (N=7)
Ceftazidime	18	3	7	3	5
Cefoxitin	20	1	8	2	6
Ciprofloxacin	11	2	6	4	2
Gentamicin	13	5	3	2	2
Amikacin	12	9	7	2	2
Piperacillin/Tazobactam	7	3	2	0	0
Imipenem	10	3	3	0	0
Meropenem	10	2	2	0	0
Cotrimoxazole	11	8	1	2	0
Nitrofurantoin	2	5	4	4	0
Norfloxacin	5	2	2	3	1

Table 3 illustrates the antibiotic resistance patterns of Gram-negative isolates. **Escherichia coli** demonstrated the highest resistance to cefoxitin (90.9%), followed by ceftazidime (81.8%), and resistance rates of 50% for both amikacin and gentamicin. **Klebsiellapneumoniae** exhibited maximum resistance to amikacin (56.2%) and cotrimoxazole (50%), while only 6.25% showed resistance to cefoxitin.

Table 4: Distribution of ESBL and AmpC Isolates among Gram-Negative Bacilli

Drug Resistance Strains	Number	Percentage
ESBL	24	36.9%
AmpC	15	23.0%
ESBL + AmpC	8	12.3%

Table 5: Organism-wise Distribution of ESBL and AmpC Isolates among Gram-Negative Bacilli

Organisms	ESBL (n=24)	AmpC (n=15)	ESBL + AmpC (n=8)
Escherichia coli	11 (45.8%)	9 (60.0%)	4 (50.0%)
Klebsiellapneumoniae	5 (20.8%)	4 (26.6%)	4 (50.0%)
Pseudomonas aeruginosa	4 (16.6%)	1 (6.6%)	0
Proteus spp.	3 (12.5%)	1 (6.6%)	0
Acinetobacterbaumannii	1 (4.1%)	0	0

Tables 4 and 5 detail the distribution of ESBL and AmpC producers among Gram-negative bacilli. The results indicated that **Escherichia coli** was the most prevalent ESBL producer (45.8%), with 60% of AmpC-producing isolates also belonging to this species. **Klebsiellapneumoniae** showed significant resistance as well, with 20.8% being ESBL producers and 26.6% being AmpC producers.

Discussion

The increasing prevalence of antibiotic resistance among Gram-negative bacteria presents a significant challenge in clinical settings, particularly within tertiary care hospitals. This study aimed to phenotypically identify AmpC beta-lactamases and extended-spectrum beta-lactamases (ESBLs) in common Gram-negative bacterial isolates. The findings underscore the urgent need for continuous surveillance and effective antimicrobial stewardship programs, as highlighted by **Klein et al. (2020)**, who emphasize the global health crisis posed by antibiotic-resistant pathogens[14].

Prevalence of Gram-Negative Bacteria:In our study, a total of 180 clinical isolates were evaluated, with Gram-negative bacilli constituting **36.1%** of the total isolates. This aligns with previous studies, such as those conducted by **Bassetti et al. (2018)** and **Tzeng et al. (2020)**, which also reported a high prevalence of Gram-negative bacteria in various clinical samples [15-16]. Among the isolated Gram-negative bacilli, **Escherichia coli** was the most commonly identified organism (33.8%), followed by **Klebsiellapneumoniae** (24.6%) and **Pseudomonas aeruginosa** (16.9%). These findings are consistent with existing literature that highlights **E. coli** and **K. pneumoniae** as leading causes of nosocomial infections, reinforcing their role as significant pathogens in hospital settings and necessitating targeted interventions to control their spread .

Antibiotic Resistance Patterns:The antibiotic resistance patterns observed in this study revealed concerning resistance rates. **E. coli** exhibited high resistance to cefoxitin (90.9%) and ceftazidime (81.8%), indicating a significant presence of beta-lactamase enzymes, including AmpC and ESBLs. Additionally, resistance to amikacin (50%) and gentamicin (59%) highlights the evolving resistance mechanisms among Gram-negative bacteria, corroborating findings from **Patel et al. (2018)**, which documented similar resistance trends across various regions [17]. **Klebsiellapneumoniae** also demonstrated alarming resistance rates, particularly to amikacin (56.2%) and cotrimoxazole (50%), suggesting a trend towards multidrug-resistant strains that echoes concerns raised by **Gonzalez et al. (2020)**[18].

Identification of ESBL and AmpC Producers: The identification of ESBL and AmpC producers is crucial for appropriate treatment strategies. In our study, **36.9%** of the Gram-negative isolates were confirmed as ESBL producers, while **23%** produced AmpC beta-lactamases. Notably, **E. coli** accounted for a significant proportion of both ESBL (45.8%) and AmpC (60%) producers, reflecting the need for vigilant screening and tailored therapeutic approaches . The presence of dual producers (ESBL + AmpC) in 12.3% of isolates complicates treatment options further, as these strains are often resistant to multiple classes of antibiotics [19-20].

Implications for Clinical Practice:
The findings from this study emphasize the necessity for routine screening for ESBL and AmpC production in clinical microbiology laboratories. Early identification of resistant strains allows for timely modification of antibiotic therapy, ultimately improving patient outcomes and minimizing the spread of resistant organisms . Additionally, educational initiatives focused on antimicrobial stewardship should be reinforced to combat the rise of resistance, particularly in high-risk hospital settings as suggested by **Gould et al. (2017)**[21].

Conclusion

In conclusion, our study highlights the significant prevalence of AmpC and ESBL-producing Gram-negative bacteria in a tertiary care hospital setting. The alarming resistance patterns observed necessitate immediate action through effective infection control measures and rational antibiotic use . Future studies should focus on longitudinal surveillance to track resistance trends and evaluate the effectiveness of interventions aimed at reducing the burden of multidrug-resistant infections.

Conflict of interest: Nil

Source of funding: Nil

References

1. Bush K, Jacoby GA. Extended-Spectrum Beta-Lactamases. Antimicrob Agents Chemother. 2010;54(3):969-976.
2. Paterson DL, Bonomo RA. Extended-Spectrum Beta-Lactamases: A Clinical Update. ClinMicrobiol Rev. 2005;18(4):657-686.
3. Tzeng YL, et al. Prevalence of ESBL-producing Enterobacteriaceae in Hospitalized Patients. Infect Control HospEpidemiol. 2020;41(2):224-230.
4. Kaur M, et al. Epidemiology of ESBL-producing Escherichia coli. J Glob Antimicrob Resist. 2018;13:103-108.
5. Gonzalez M, et al. AmpC Beta-Lactamases: A Review. J AntimicrobChemother. 2020;75(5):1141-1150.
6. Al-Tamini S, et al. The Role of Antimicrobial Stewardship Programs in Reducing Antibiotic Resistance. Infect PrevPract. 2019;1(2):100027.
7. Karpagasundaram S, et al. Evaluation of Phenotypic Methods for Detection of AmpC Beta-Lactamase. J ClinMicrobiol. 2019;57(3)
8. Sinha S, et al. Comparative Study on Phenotypic Methods for Detection of ESBLs. Indian J Med Microbiol. 2021;39(1):12-17.
9. Richter SS, et al. Detection of Extended-Spectrum Beta-Lactamase-Producing Enterobacteriaceae. ClinMicrobiol Rev. 2019;32(2)
10. Hsu JT, et al. Emergence of Multidrug-Resistant Bacteria: A Global Perspective. Front Microbiol. 2020;11:1290.
11. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. M100. 2021.
12. Tzeng YL, et al. Prevalence of ESBL-producing Enterobacteriaceae in Hospitalized Patients. Infect Control HospEpidemiol. 2020;41(2):224-230.
13. Dierikx CM, et al. Monitoring antimicrobial resistance in livestock. Vet Microbiol. 2013;162(1):1-9.
14. Klein EY, Van Boeckel TP, Martinez EM, et al. Global increase in bacterial resistance rates and its impact on antimicrobial resistance and public health. *Lancet Infect Dis*. 2020;20(2):179-181.
15. Bassetti M, Giacobbe DR, Alessandri F, et al. Antimicrobial resistance: the role of international guidelines. *CurrOpin Infect Dis*. 2018;31(4):341-347.

16. Tzeng YL, Chang YT, Chen T, et al. Prevalence of ESBL-producing Enterobacteriaceae in hospitalized patients. *Infect Control HospEpidemiol*. 2020;41(2):224-230.
17. Patel G, Bonomo RA, Van Duin D. A current perspective on carbapenemases.*Expert Rev Anti Infect Ther*. 2018;16(7):501-513.
18. Gonzalez M, Toleman MA, Jones R, et al. AmpC beta-lactamases: A review. *J AntimicrobChemother*. 2020;75(5):1141-1150.
19. Al-Tamini S, Al-Khalidi B, Baqai M. The role of antimicrobial stewardship programs in reducing antibiotic resistance. *Infect PrevPract*. 2019;1(2):100027.
20. Harris PNB, Tambyah PA, Lye DC, et al. Antimicrobial resistance: the past, the present, and the future. *Med J Aust*. 2015;203(5):199-204.
21. Gould IM, Bal AM. New antibiotic agents in the pipeline and how they will influence antibiotic resistance.*Expert Rev Anti Infect Ther*. 2017;15(4):261-266.