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Original Research Article

Multidrug-Resistance Profile of Non-Fermenting Gram Negative Bacilli Isolated from Various Clinical Samples Received in a Tertiary Care Hospital, Ballari - Cross Sectional Study

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ABSTRACT

Background: Non-fermenting Gram-negative bacilli (NF-GNB) are important causes of healthcare-associated infections and are increasingly associated with multidrug resistance, posing serious therapeutic challenges.
Objectives: To determine the prevalence of NF-GNB among culture-positive clinical samples and to assess their antimicrobial susceptibility patterns in a tertiary care hospital.
Materials and Methods: A cross-sectional study was conducted over six months (July–December 2025) in a tertiary care hospital laboratory. A total of 388 clinical specimens were processed using standard microbiological techniques. Identification of NF-GNB was performed by conventional biochemical methods. Antimicrobial susceptibility testing was carried out using the Kirby–Bauer disc diffusion method, and results were interpreted according to CLSI guidelines. Multidrug resistance was defined as resistance to three or more antibiotic classes.
Results: Of 223 culture-positive samples, 50 NF-GNB were isolated, of which 25 (50%) were multidrug resistant. *Acinetobacter* species predominated (76%), followed by *Pseudomonas aeruginosa* (16%). Blood samples were the major source (44%). High resistance was observed to gentamicin (76%), ciprofloxacin (80%), and imipenem (60%), while moderate susceptibility was noted for meropenem (40%), tigecycline (32%), and cotrimoxazole (32%).
Conclusion: The study demonstrates a high burden of MDR NF-GNB, emphasizing the need for regular surveillance, antimicrobial stewardship, and strict infection-control measures.

Keywords: Non-fermenting Gram-negative bacilli; Multidrug resistance; Acinetobacter; Pseudomonas aeruginosa; Antimicrobial susceptibility; Healthcare-associated infections; Kirby–Bauer disc diffusion.

INTRODUCTION

Antimicrobial resistance is a growing global health concern, contributing to increased morbidity, mortality, and healthcare burden, particularly due to multidrug-resistant (MDR) Gram-negative bacteria causing various hospital-acquired infections [1] A consistent rise in MDR organisms has been observed over time, especially in tertiary care settings, with higher prevalence in intensive care units and association with increased antibiotic use [2] These organisms have become endemic in hospitals and exhibit multiple resistance mechanisms, making treatment difficult and necessitating awareness of local resistance patterns [3]

These organisms are widely distributed in nature, especially in soil and water, and within hospital settings they can be recovered from medical equipment such as ventilators, humidifiers, mattresses, other devices, as well as from the skin of healthcare workers [4]. Once considered contaminants, they are now recognized as significant nosocomial pathogens with intrinsic and acquired resistance mechanisms [5]

Their clinical importance is further reinforced by their environmental ubiquity and ability to persist under adverse conditions, contributing to their increasing prevalence in hospital settings and necessitating regular surveillance of antimicrobial susceptibility patterns [4]. The rising incidence of infections is compounded by the rapid emergence of multidrug resistance, significantly limiting therapeutic options and highlighting the need for strict antimicrobial stewardship [6]. Advances in diagnostic techniques have enabled earlier and more accurate identification of these pathogens, thereby improving clinical outcomes [7].

In recent years, the situation has worsened due to the rising emergence of antimicrobial resistance among non-fermenting organisms, which are commonly targeted by widely used antibiotics. This has led to their recognition as significant healthcare-associated pathogens. The global increase in multidrug-resistant strains in hospital settings has greatly reduced available treatment options for clinicians and has negatively impacted patient outcomes. The rapid development of resistance has created serious challenges in both disease management and therapy worldwide. Therefore, early identification of the causative organisms and timely initiation of appropriate treatment, guided by antimicrobial susceptibility testing, have become essential to ensure effective management and prevent treatment failure. [8]. In addition, inappropriate antibiotic usage and limited diagnostic resources continue to drive the burden of resistance, emphasizing the importance of effective infection control strategies and rational antimicrobial use [9].

In this context, continuous monitoring of the antimicrobial resistance patterns of NFGNB is essential. Understanding their resistance profiles across various clinical samples can aid in optimizing antimicrobial stewardship strategies and improving patient outcomes. Therefore, the present cross-sectional study was undertaken to assess the MDR profile, distribution, and antimicrobial susceptibility patterns of non-fermenting Gram-negative bacilli isolated from diverse clinical specimens in a tertiary care hospital.

Aims & Objectives

- To determine the prevalence of NF-GNB in culture-positive clinical samples.
- To evaluate the antibiotic susceptibility patterns of these isolates.
- To provide data that supports improved infection control and antibiotic stewardship practices.

MATERIALS & METHODS

A cross-sectional study was conducted over a period of six months 1st July 2025 to 31st December 2025 in the central laboratory of a tertiary care hospital after obtaining approval from the Institutional Ethics Committee (approval number: 129/2025). The samples comprised urine, blood, pus, sputum, cerebrospinal fluid (CSF), pleural fluid, peritoneal fluid, ascitic fluid, endotracheal secretions, tracheal aspirates, ear swabs, and cervical/vaginal swabs. A total of 388 samples were processed using conventional microbiological techniques including Gram staining, culture on blood and MacConkey agar, and biochemical testing. Non-lactose-fermenting colonies isolated on MacConkey agar were subjected to Gram staining. All isolates identified as Gram-negative bacilli, cocci, or coccobacilli were further processed for identification using standard microbiological procedures.

The isolates were characterized based on motility (by the hanging drop method) and a battery of biochemical tests, including catalase and oxidase tests; indole, methyl red, Voges–Proskauer, citrate utilization, urease, and triple sugar iron (TSI) tests. Additional identification was carried out using the oxidative–fermentative (O/F) test, nitrate reduction test, and carbohydrate utilization tests with glucose, lactose, sucrose, mannose, maltose, mannitol, arabinose, and xylose. Enzyme activity was assessed using lysine and ornithine decarboxylase tests and the arginine dihydrolase test. Bacterial identification was confirmed using VITEK automated system.

Antimicrobial susceptibility testing of the identified isolates was performed using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar, and the results were interpreted in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. Following Antibiotic discs and the disc potencies used were gentamicin 10 µg, amikacin 30 µg, ampicillin 10 µg, piperacillin–tazobactam 100/10 µg, amoxicillin–clavulanate 20/10 µg, cefepime 30 µg, cefotaxime 30 µg, ceftazidime 30 µg, ceftriaxone 30 µg, meropenem 10 µg, imipenem 10 µg, levofloxacin 5 µg, ciprofloxacin 5 µg, tigecycline 15 µg, tetracycline 30 µg, minocycline 30 µg, doxycycline 30 µg, nitrofurantoin 300 µg, and cotrimoxazole (trimethoprim/ sulfamethoxazole) 1.25/23.75 µg.

The inoculum was adjusted to match 0.5 McFarland turbidity and inoculated onto Mueller–Hinton agar plates using the lawn culture method. The plates were incubated at 37 °C for 18–24 hours, and the results were interpreted by measuring zone diameters in accordance with CLSI guidelines *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were used as quality control strains. Culture media and antibiotic discs were procured from Hi Media Laboratories.

Statistical Analysis

Descriptive statistics were expressed as percentages and proportions.

RESULT

Out of 388 clinical samples processed, growth was obtained in 223 cases, among these, 50 isolates were identified as non-fermenting Gram-negative bacilli, of which 25 isolates (50%) were found to be multidrug-resistant (MDR).

Within the MDR group, Acinetobacter species were the most common (19 isolates), followed by Pseudomonas aeruginosa (4 isolates) other Pseudomonas species (2 isolates).

Table 1 : Distribution of multidrug-resistant non-fermenting Gram-negative bacilli among all isolates

No table of figures entries found.	No of Isolates
Pseudomonas Aeruginosa	4
Pseudomonas	2
Acinetobacter SPP	19
TOTAL	25

Specimen-wise, the majority of MDR isolates were from blood samples (44%), followed by urine (32%), sputum (12%), and fluid, pus, and ear swabs—each contributing 4%

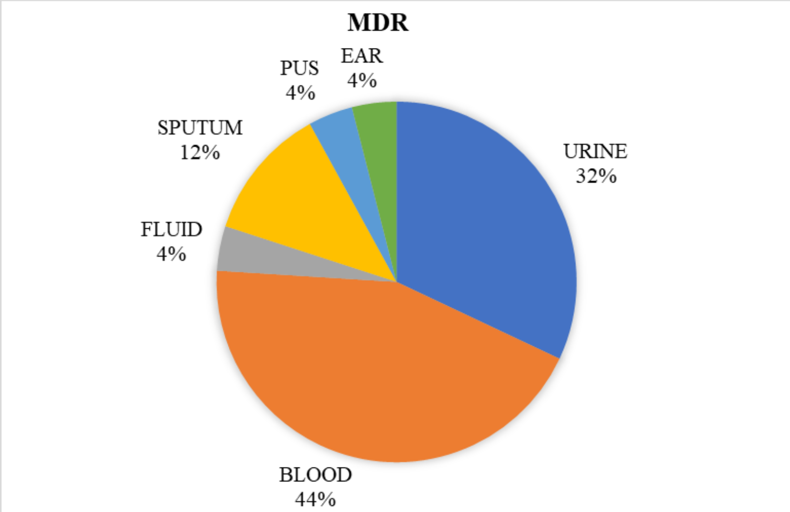

Figure 1 : Specimen-wise distribution of multidrug-resistant non-fermenting Gram-negative bacilli

A slight male predominance was observed, with 52% of isolates from males and 48% from females.

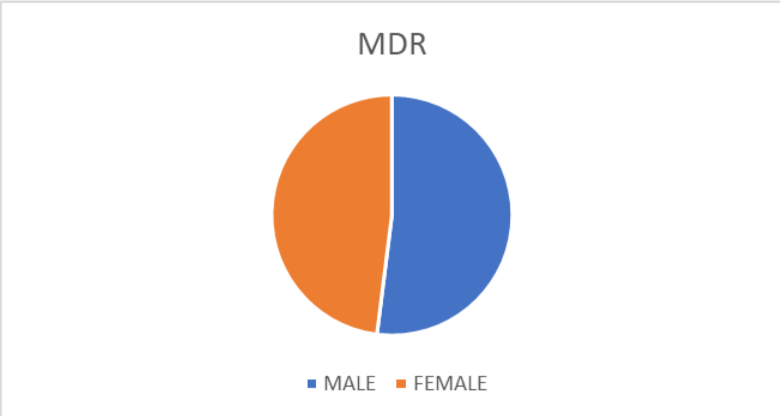

Figure 2 : Gender-wise distribution of multidrug-resistant non-fermenting Gram-negative bacilli isolates

Regarding age distribution, the highest prevalence was noted among adults and newborns—each accounting for 36% of MDR isolates—followed by paediatric cases (16%) and geriatric patients (12%).

Table 2 : Age-wise distribution of multidrug-resistant non-fermenting Gram-negative bacilli isolates

ADULTs	9	36
PAEDIATRICS	4	16
GERATRIC	3	12
NEWBORN	9	36
TOTAL	**25**	

On departmental analysis, the Paediatrics Department recorded the highest number of MDR isolates (48%), followed by Medicine (24%), Obstetrics and Gynaecology (16%), and smaller proportions from Surgery, ENT, and Emergency departments (each 4%).

Table 3 : Department-wise distribution of multidrug-resistant non-fermenting Gram-negative bacilli isolates

DEPARTMENT	MDR	%
MEDICINE	6	24
SURGERY	1	4
OBG	4	16
ENT	1	4
PEADS	12	48
EMERGENCY	1	4
TOTAL	25	

In terms of antibiotic susceptibility, isolates showed high resistance to Gentamicin (76%), Ciprofloxacin (80%), and Imipenem

(60%), indicating limited efficacy of these commonly used agents.

However, moderate sensitivity was observed with Meropenem (40%), Tigecycline (32%), and Cotrimoxazole (32%), suggesting these drugs may still retain some therapeutic value against MDR non-fermenters.

Table 4 :Antimicrobial susceptibility pattern of multidrug-resistant non-fermenting Gram-negative bacilli isolates

Drugs	Sensitive	%Sensitive	Resistant	%Resistant
Gentamycin	2	8	19	76
Amikacin	7	28	18	72
Ampicillin	0	0	11	44
Piptaz	5	20	11	44
Moxclav	0	0	7	28
Cefepime	5	20	7	28
Cefotaxime	0	0	5	20
Ceftazidime	0	0	5	20
Ceftriaxone	5	20	4	16
Meropenem	10	40	9	36
Imipenem	4	16	15	60
Levofloxacin	2	8	4	16
Ciprofloxacin	4	16	20	80
Tigecycline	8	32	2	8
Tetracycline	3	12	5	20
Minocycline	2	8	3	12
Doxycycline	0	0	1	4
Nitrofurantoin	0	0	6	24
Cotrimoxazole	8	32	8	32
Total	25		25	

DISCUSSION

In the present study, 388 clinical specimens were processed, of which 223 yielded positive cultures. Among these, 50 isolates were identified as non-fermenting Gram-negative bacilli (NF-GNB), and 25 isolates (50%) exhibited multidrug resistance. This substantial proportion indicates that one in every two NF-GNB isolates is resistant to multiple antibiotic classes, underscoring the clinical difficulty in selecting effective empirical therapy.

A study by Benachinmardi et al. reported an NF-GNB isolation rate of 3.58%, which was lower than that observed in the present study, indicating a comparatively higher prevalence of NF-GNB isolates in our setting.[10]

In contrast, Pal et al. [11] reported a lower culture positivity rate of 22.3% among suspected septicemia cases, with the majority of isolates being bacterial (88.9%). This contrast may be attributed to differences in study design, as Pal et al. [11] focused exclusively on bloodstream infections, whereas the present study included a wider spectrum of clinical specimens, potentially leading to a higher yield of culture positivity. Iincreased MDR prevalence has also been demonstrated by trend analysis of ICU isolates reported in Eastern India, where 61.76% of NF-GNB strains were multidrug resistant [12]. The slightly lower proportion observed in the present study compared to ICU- based reports may reflect differences in patient population and intensity of antibiotic exposure

This high proportion of MDR NF-GNB in the present study reflects the growing antimicrobial resistance burden reported in tertiary care centers across India. Such elevated resistance rates may be attributed to prolonged hospital stay, increased use of broad-spectrum antibiotics, and selective pressure within hospital environments. A similar rise in multidrug-resistant non-fermenters was documented in the antibiotic susceptibility study from Patiala, where MDR Pseudomonas aeruginosa and Acinetobacter baumannii strains were noted as significant healthcare concerns [13].

Acinetobacter species accounted for the majority of MDR isolates in the present study (76%), followed by *Pseudomonas aeruginosa* and other *Pseudomonas* species. This predominance highlights the remarkable ability of Acinetobacter spp. to survive in hospital environments and acquire resistance determinants rapidly. The predominance of Acinetobacter correlates with findings from the ICU-based trend analysis, where Acinetobacter spp. represented the most frequent NF-GNB isolates [12]. In contrast, Grewal et al. reported Pseudomonas aeruginosa as the predominant NF-GNB isolate (87.96%), followed by Acinetobacter baumannii (7.87%) [13]. A similar distribution pattern was also observed in the study from a hospital in Tirupati where Pseudomonas spp. and Acinetobacter spp. were commonly isolated from clinical specimens [14]. The consistent predominance of these organisms across studies emphasizes their role as key nosocomial pathogens requiring targeted infection control strategies.

In the current work, the highest proportion of MDR isolates originated from blood cultures (44%), followed by urine and sputum. This suggests a strong association of MDR NF-GNB with invasive infections and possible progression to sepsis, particularly in hospitalized patients. This distribution aligns with the Ethiopian bloodstream infection study, which highlighted MDR Gram-negative organisms, including non-fermenters, as major contributors to septicemia [9]. The Patiala study similarly reported blood among the key clinical sources for NF-GNB recovery [13]. The predominance of bloodstream isolates further indicates the need for early detection and prompt antimicrobial intervention to reduce morbidity and mortality.

A slight male predominance in MDR infections was observed in the present study (52%). Although the difference is marginal, it may reflect increased exposure of males to hospital settings or underlying risk factors such as comorbidities and occupational hazards. This trend is comparable to the male-to-female ratios documented in the Patiala and Tirupati studies, both of which reported higher male susceptibility to NF-GNB infections [13,14].

Adults and newborns represented the leading age groups affected by MDR isolates in our findings, each contributing 36%. The involvement of neonates is particularly concerning, as it indicates vulnerability due to immature immunity and frequent exposure to invasive procedures in neonatal care units. A similar predominance of adult cases, along with a significant burden among neonates, has also been reported by Pal et al. [11], where adults and neonates constituted a major proportion of septicemia cases. Comparable vulnerability among paediatric and neonatal patients was noted in the bloodstream infection prevalence report of Ethiopian bloodstream infection study, which described high proportions of MDR isolates in neonates and immunocompromised children [9]. The predominance of isolates from paediatric departments in the present study similarly reflects the patterns observed in the ICU surveillance study, which reported significant MDR NF-GNB isolation from critically ill patient units [12]. This highlights the importance of stringent infection control measures in neonatal and pediatric intensive care settings.

Antimicrobial susceptibility analysis in this study recorded high resistance to gentamicin (76%), ciprofloxacin (80%) and imipenem (60%). These findings indicate a marked decline in the effectiveness of commonly used first-line and reserve antibiotics, limiting therapeutic options. These patterns correspond closely with resistance profiles presented in the Patiala and Tirupati studies, where cephalosporins, fluoroquinolones and carbapenems exhibited declining susceptibility against NF-GNB isolates [13,14]. The observed carbapenem resistance is particularly alarming, as it suggests the possible presence of carbapenemase-producing strains.

Moderate susceptibility to meropenem (40%), tigecycline (32%), and cotrimoxazole (32%) remained in our isolates. Although partial susceptibility persists, these agents cannot be relied upon as uniformly effective therapies and should ideally be guided by susceptibility testing. Comparable patterns were documented in the Tirupati study, which reported meropenem as one of the most active therapeutic options among tested antibiotics [14]. This partially correlates with findings by Grewal et al. [13], who reported higher susceptibility to drugs like polymyxin B and imipenem, though resistance trends were still evident. The ICU resistance trend study further noted decreasing carbapenem resistance over time for some isolates, suggesting retained therapeutic potential for these agents despite emerging resistance challenges [12]. This indicates that antimicrobial stewardship interventions may help in preserving the efficacy of these drugs.

Taken together, the present findings demonstrate that MDR NF-GNB infection remains a critical challenge in tertiary healthcare settings, with Acinetobacter spp. predominating and bloodstream infections representing a major clinical concern.

The resistance profiles highlight diminishing efficacy of first-line and second-line antibiotics, emphasizing the need for rational antimicrobial use, periodic antibiogram surveillance, and strengthened infection-prevention practices to limit further spread of MDR NF-GNB in vulnerable populations, particularly paediatric and critical-care patients.

Limitations

- Molecular mechanisms of resistance were not investigated.
- Clinical outcomes and comorbidities of patients were not assessed.

Recommendations

Regular surveillance of antimicrobial susceptibility patterns of non-fermenting Gram-negative bacilli should be carried out to guide appropriate empirical therapy and detect emerging resistance trends. Strict implementation of antimicrobial stewardship programs is essential to promote rational antibiotic use and reduce the development of multidrug resistance. Adherence to standard infection control practices, particularly in high-risk areas such as paediatric units and intensive care settings, is crucial to limit the spread of these pathogens. Early detection and prompt reporting of multidrug-resistant isolates should be ensured to facilitate timely clinical management. Additionally, periodic preparation of institutional antibiograms is recommended to support evidence-based antibiotic selection. Further studies incorporating molecular methods are needed to better understand the mechanisms of resistance in these organisms.

CONCLUSION

The present study demonstrates a significant burden of multidrug-resistant non-fermenting Gram-negative bacilli, with 50% of isolated non-fermenters exhibiting multidrug resistance. Acinetobacter species predominated among MDR isolates, followed by *Pseudomonas aeruginosa*. Blood was the major specimen source, and paediatric departments accounted for nearly half of the MDR isolates, indicating increased vulnerability among neonates and children. High resistance rates to gentamicin, ciprofloxacin, and imipenem highlight reduced efficacy of commonly used antimicrobial agents, while moderate susceptibility to meropenem, tigecycline, and cotrimoxazole suggests that therapeutic options remain limited.

These findings reinforce the need for continuous antimicrobial resistance surveillance, strict adherence to antimicrobial stewardship practices, and implementation of effective infection-control strategies. Early detection of MDR strains and judicious antibiotic use are essential to prevent further escalation of resistance and to safeguard available treatment options in tertiary healthcare settings.

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