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CLINICO-MICROBIOLOGICAL PROFILE OF NON-FERMENTING GRAM-NEGATIVE BACILLI IN BLOODSTREAM INFECTIONS: A RETROSPECTIVE STUDY FROM A TERTIARY CARE CENTER"

Dr Ankita Porwal[1], Dr P Sravanasandya Md[2], Abdullah[3], Dr Srinivas[4]*, Dr Mithilesh Krushnarap Raut[5]

[1]Consultant Microbiologist, Department of Microbiology, Medicover Superspecialty Hospitals, Hyderabad, Telangana
[2]Assistant Professor, Department of Pharmacology, ICARE Institute of Medical Sciences and Research, Haldia
[3]Clinical Pharmacist, Department of Clinical Pharmacy, Medicover Superspecialty Hospitals, Hyderabad, Telangana
[4]Consultant Neuropsychiatrist, Department of Psychiatry, Medicover Superspecialty Hospitals, Hyderabad, Telangana
[5]Consultant Intensivist, Department of Critical Care, Medicover Superspecialty Hospitals, Hyderabad, Telangana

***Corresponding Author:** (email - dr.ks.osm@gmail.com)

ABSTRACT

Introduction: Non-fermenting Gram-negative bacilli (NFGNB) have emerged as important opportunistic pathogens causing bloodstream infections, particularly in hospitalised and critically ill patients. Their intrinsic resistance and increasing multidrug resistance pose significant therapeutic challenges. This study was conducted to characterise NFGNB isolated from blood samples and to determine their antimicrobial susceptibility patterns in a tertiary care hospital.

Materials and Methods: This was a hospital-based, retrospective observational study conducted in the Department of Microbiology at a tertiary care hospital in Hyderabad. The study was carried out over a period of one year. A total of 1800 blood samples received for culture and sensitivity were included. Standard microbiological techniques were used for isolation and identification of NFGNB. Antimicrobial susceptibility testing was performed as per CLSI guidelines.

Results: Out of 1800 blood samples, 412 (22.9%) were culture positive, of which 96 (23.3%) isolates were identified as NFGNB. Pseudomonas aeruginosa (50.0%) was the most common isolate, followed by Acinetobacter spp. (35.4%). A higher proportion of isolates were obtained from ICU patients (60.4%). High resistance was observed to cephalosporins (62.5% to ceftazidime) and fluoroquinolones (54.2% to ciprofloxacin). Carbapenems showed moderate sensitivity (imipenem 63.5%), while aminoglycosides, particularly amikacin (68.8%), were more effective. Colistin demonstrated the highest sensitivity (95.8%). Multidrug resistance was observed in 51.0% of isolates.

Conclusion: NFGNB are significant pathogens in bloodstream infections with high antimicrobial resistance rates. The predominance of Pseudomonas and Acinetobacter spp. and rising multidrug resistance emphasize the need for continuous surveillance and rational antibiotic use.

Keywords: Non-fermenting Gram-negative bacilli, bloodstream infections, antimicrobial resistance, Pseudomonas aeruginosa, Acinetobacter, multidrug resistance, colistin

INTRODUCTION

Non-fermenting Gram-negative bacilli (NFGNB) are a heterogeneous group of aerobic, non-spore-forming organisms that do not ferment carbohydrates for energy production but utilize oxidative metabolic pathways [1]. These organisms are widely distributed in nature, particularly in soil and water, and are commonly found in hospital environments including medical equipment, humidifiers, and surfaces, contributing to their role in healthcare-associated infections [2].Over the past few decades, NFGNB have emerged as important opportunistic pathogens, particularly in hospitalized and immunocompromised patients [3,4]. They are increasingly implicated in a wide spectrum of infections such as bacteremia, pneumonia, urinary tract infections, wound infections, and meningitis, with bloodstream infections being associated with significant morbidity and mortality [5,6]. In particular, species such as Pseudomonas aeruginosa, Acinetobacter baumannii, and Stenotrophomonas maltophilia are among the most clinically relevant NFGNB and are frequently isolated from blood cultures in septic patients [7-9].NFGNB are responsible for approximately 10–15% of all Gram-negative infections in hospital settings and are often associated with nosocomial outbreaks [10]. Their ability to survive in adverse environmental conditions, colonize hospital surfaces, and persist on the hands of healthcare workers contributes to their transmission and infection potential [11]. Additionally, risk factors such as prolonged hospitalization, invasive procedures, mechanical ventilation, and underlying comorbidities further predispose patients to NFGNB infections [11].

A major concern associated with NFGNB is their intrinsic and acquired resistance to multiple classes of antimicrobial agents [12]. These organisms possess diverse resistance mechanisms including production of β-lactamases, efflux pumps, reduced membrane permeability, and biofilm formation, which significantly limit therapeutic options [13]. The increasing prevalence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains has made the management of infections caused by NFGNB challenging and has been recognized as a global public health threat [14].In bloodstream infections, NFGNB are particularly important due to their association with severe sepsis, prolonged hospital stay, increased healthcare costs, and higher mortality rates [15]. Early identification and appropriate antimicrobial therapy based on susceptibility patterns are crucial for improving patient outcomes. However, the antimicrobial susceptibility profiles of NFGNB vary significantly across geographical regions and healthcare settings, necessitating continuous local surveillance [16].Given the rising incidence of NFGNB infections and the alarming trend of antimicrobial resistance, it is essential to characterize these organisms and monitor their susceptibility patterns, especially in tertiary care hospitals where critically ill patients are managed. Therefore, the present study was undertaken to characterize non-fermenting Gram-negative bacilli isolated from clinical blood samples and to determine their antimicrobial susceptibility patterns in a tertiary care hospital setting.

MATERIALS AND METHODS

This was a hospital-based, retrospective observational study conducted in the Department of Microbiology at a tertiary care hospital in Hyderabad. The study was carried out over a period of one year with the objective of characterizing non-fermenting Gram-negative bacilli (NFGNB) isolated from blood samples and determining their antimicrobial susceptibility patterns.A total of 1800 blood samples received in the microbiology laboratory during the study period were included. These samples were collected from patients attending outpatient departments as well as those admitted to various wards and intensive care units (ICUs) with clinical suspicion of bloodstream infections.Only non-repetitive blood culture samples from individual patients were considered for analysis. The samples were processed and incubated using the Bac-T Alert system, which is typically used for detecting bacterial growth. Positive samples were then identified by the Vitek-2 compact system, which automates bacterial identification and susceptibility testing.

In addition to the Vitek-2 compact system, the modified Kirby-Bauer method was used for antibiotic susceptibility testing in cases where it was necessary. This method involves the use of antibiotic-impregnated discs placed on an agar plate inoculated with the bacteria, and the zone of inhibition is measured to determine bacterial resistance or susceptibility.The results from both testing methods were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines, which provide standardized procedures and criteria for antimicrobial susceptibility testing to ensure consistent and reliable results

Inclusion Criteria

- All blood samples received for culture and sensitivity during the study period.
- Blood cultures yielding non-fermenting Gram-negative bacilli.

Exclusion Criteria

- Repeat blood culture isolates from the same patient.
- Blood cultures showing polymicrobial growth or contamination.
- Isolates identified as fermenting Gram-negative bacilli or Gram-positive organisms.

Sample Collection and Processing

Blood samples were collected under strict aseptic precautions and inoculated in automated blood culture bottles containing appropriate culture media. The inoculated bottles were incubated in automated blood culture system (BacT/ALERT; bioMeriux, Inc. Durham, North California, USA) and monitored for growth.Positive blood cultures were subcultured onto blood agar and MacConkey agar plates and incubated at 37°C for 18–24 hours. Colony morphology and Gram staining were performed for preliminary identification. Identification of NFGNB and antimicrobial susceptibility pattern was performed by Vitek 2 Compact system.

Data Collection and Statistical Analysis

Patient-related variables such as age, sex, and hospital location (ward/ICU) were recorded.Data were entered into Microsoft Excel and analyzed using SPSS version 11. Descriptive statistics such as frequencies and percentages were used to summarize the distribution of isolates and their antimicrobial susceptibility patterns.

RESULTS

A total of 1800 blood samples were processed during the study period, as shown in Table 1. Out of these, 412 (22.9%) samples yielded positive growth, while the majority, 1388 (77.1%), were culture negative. The clinical characteristics of patients with bloodstream infections caused by NFGNB were diverse, with a significant number of isolates originating from critically ill patients admitted to the Intensive Care Unit (ICU). The demographic and clinical distribution of these patients revealed a predominance of patients aged between 30 to 60 years, with a higher proportion of male patients (64.5%) compared to females (35.5%).

The majority of patients presented with symptoms indicative of systemic infection, including fever (80%), chills (72%), and septic shock (40%). A significant proportion of cases were associated with underlying comorbidities, particularly diabetes mellitus (35%), chronic obstructive pulmonary disease (COPD) (28%), and chronic kidney disease (CKD) (18%). The use of invasive devices such as central venous catheters (CVC) and mechanical ventilation was common among these patients, with 60% of ICU-admitted patients having a history of invasive interventions. Additionally, a notable proportion of NFGNB isolates were associated with multi-organ involvement, including pneumonia (55%), urinary tract infections (18%), and wound infections (12%). Mortality rates associated with bloodstream infections in this cohort were high, with a 30-day mortality rate of 22%, predominantly

in patients with Acinetobacter baumannii and Pseudomonas aeruginosa infections.Among the culture-positive samples, 96 isolates were identified as non-fermenting Gram-negative bacilli (NFGNB), accounting for 5.3% of all blood samples and 23.3% of the total culture-positive isolates. This finding highlights the significant contribution of NFGNB to bloodstream infections in the study population.The species-wise distribution of NFGNB isolates is presented in Table 2. Among the 96 isolates, Pseudomonas aeruginosa was the most frequently isolated organism, accounting for 48 (50.0%) cases. This was followed by Acinetobacter spp., which constituted 34 (35.4%) isolates. Less commonly isolated organisms included Stenotrophomonas maltophilia, which accounted for 8 (8.3%) cases, and Burkholderia spp., comprising 6 (6.3%) isolates.This distribution clearly demonstrates the predominance of Pseudomonas aeruginosa as the leading cause of NFGNB bloodstream infections, followed by Acinetobacter spp., both of which are well-known opportunistic pathogens associated with hospital-acquired infections.

The antimicrobial susceptibility pattern of the NFGNB isolates is summarized in Table 3. Among the β-lactam antibiotics, piperacillin-tazobactam showed moderate effectiveness, with 54 (56.3%) isolates being sensitive, whereas higher resistance rates were observed for ceftazidime and cefepime, with 60 (62.5%) and 56 (58.3%) isolates resistant, respectively.Carbapenems demonstrated comparatively better activity against NFGNB isolates. Imipenem showed sensitivity in 61 (63.5%) isolates, while meropenem was effective in 58 (60.4%) isolates. However, a considerable proportion of isolates exhibited resistance to carbapenems, indicating an emerging concern of carbapenem resistance among NFGNB.Among aminoglycosides, amikacin showed higher sensitivity, with 66 (68.8%) isolates susceptible, compared to gentamicin, which showed sensitivity in 50 (52.1%) isolates. This suggests that amikacin may be a more effective option for the treatment of infections caused by NFGNB.Fluoroquinolones demonstrated relatively lower sensitivity, with ciprofloxacin and levofloxacin showing sensitivity rates of 45.8% and 48.9%, respectively, indicating that more than half of the isolates were resistant to these agents.Colistin was found to be the most effective antimicrobial agent, with 92 (95.8%) isolates showing sensitivity and only 4 (4.2%) isolates demonstrating resistance. This indicates that colistin remains a highly effective therapeutic option against multidrug-resistant NFGNB.

Table 1. Culture Positivity and Isolation of NFGNB (n = 1800)

Parameter	Number (n)	Percentage (%)
Total blood samples processed	1800	100
Culture positive samples	412	22.9
Culture negative samples	1388	77.1
NFGNB isolates (out of total samples)	96	5.3
NFGNB among culture positive	96/412	23.3

Table 2. Distribution of NFGNB Isolates (n = 96)

Organism	Number (n)	Percentage (%)
Pseudomonas aeruginosa	48	50.0
Acinetobacter spp.	34	35.4
Stenotrophomonas maltophilia	8	8.3
Burkholderia spp.	6	6.3
Total	96	100

Table 3. Antimicrobial Susceptibility Pattern of NFGNB Isolates (n = 96)

Antibiotic	Sensitive (n, %)	Resistant (n, %)
Piperacillin-tazobactam	54 (56.3%)	42 (43.7%)
Ceftazidime	36 (37.5%)	60 (62.5%)
Cefepime	40 (41.7%)	56 (58.3%)

Imipenem	61 (63.5%)	35 (36.5%)
Meropenem	58 (60.4%)	38 (39.6%)
Amikacin	66 (68.8%)	30 (31.2%)
Gentamicin	50 (52.1%)	46 (47.9%)
Ciprofloxacin	44 (45.8%)	52 (54.2%)
Levofloxacin	47 (48.9%)	49 (51.1%)
Colistin	92 (95.8%)	4 (4.2%)

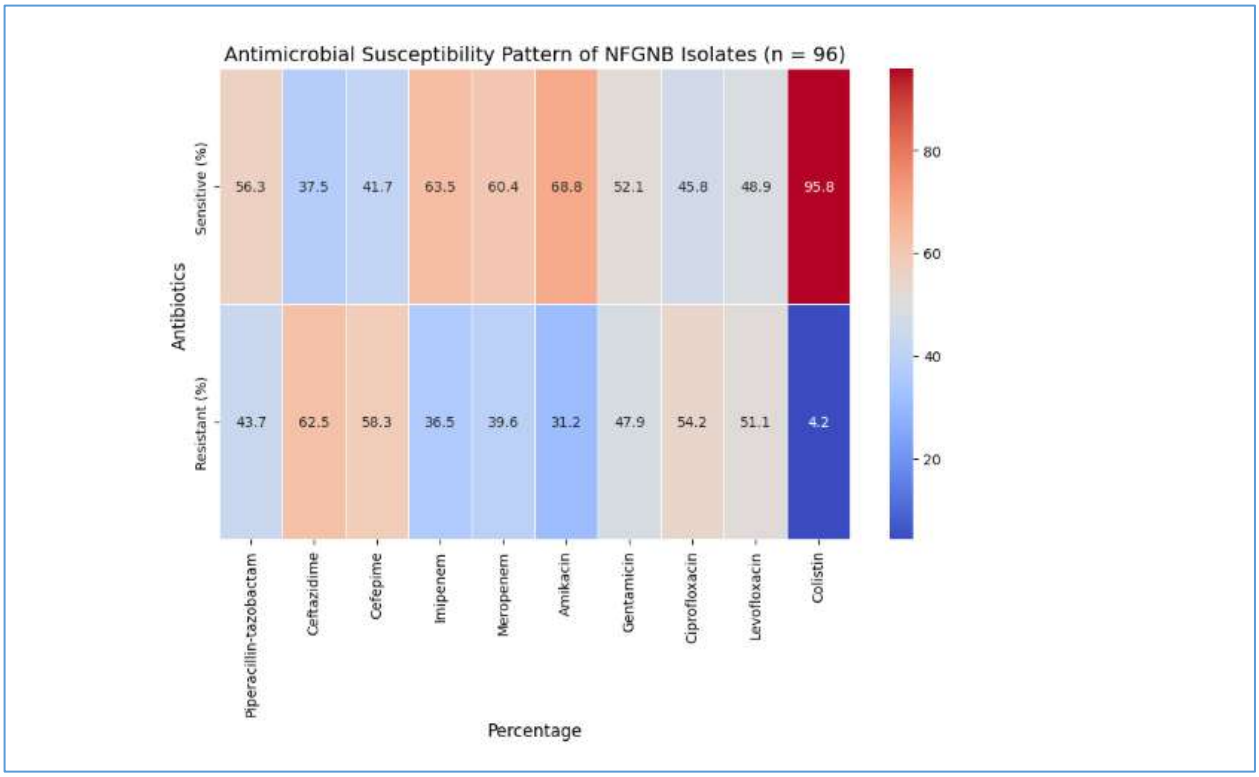


Figure 1: Heat map of Antimicrobial Susceptibility Pattern of NFGNB Isolates

DISCUSSION

In the present study, out of 1800 blood samples processed, 412 (22.9%) were culture positive, and 96 (5.3%) isolates were identified as non-fermenting Gram-negative bacilli (NFGNB), accounting for 23.3% of all culture-positive isolates. This indicates that NFGNB form a substantial proportion of bloodstream infections in our setting.Comparable findings were reported by Previous study [16], who analyzed 24,179 nosocomial bloodstream infection cases and observed that Gram-negative organisms accounted for approximately 30–40% of all bloodstream infections, with non-fermenters forming a significant subset. Their study highlighted that Pseudomonas aeruginosa alone contributed to about 8–10% of bloodstream infections, emphasizing the importance of NFGNB in hospital settings. A previous study [17] reported that non-fermenting Gram-negative bacilli accounted for 7.8% of all bacteremia cases in their study. In comparison, our study showed a slightly lower overall prevalence (5.3% of total samples) but a higher proportion among culture-positive isolates (23.3%). This difference may be attributed to variations in patient population, ICU burden, and regional microbial patterns.In the present study, Pseudomonas aeruginosa was the most common isolate, accounting for 50.0% (48/96), followed by Acinetobacter spp. (35.4%, 34/96), Stenotrophomonas maltophilia (8.3%), and Burkholderia spp. (6.3%). Previous study [18] reported that Pseudomonas aeruginosa is one of the most predominant pathogens in ICU settings, contributing to approximately 30–40% of non-fermenting Gram-negative infections, particularly in critically ill patients. This is slightly lower than the 50.0% observed in our study but still supports the predominance of Pseudomonas species. In contrast, another study [17] reported Acinetobacter baumannii as the most common isolate,

accounting for 32.7%, followed by Pseudomonas aeruginosa at 27.8%. In our study, although Acinetobacter spp. constituted a comparable proportion (35.4%), Pseudomonas aeruginosa was significantly higher (50.0%), indicating regional variation in pathogen distribution.The relative proportions of Stenotrophomonas maltophilia (8.3%) and Burkholderia spp. (6.3%) in our study were also similar to those reported in previous studies, where these organisms typically account for less than 10% of NFGNB isolates.Thus, while the predominance between Pseudomonas and Acinetobacter may vary across studies, both remain the principal pathogens in bloodstream infections.In the present study, high resistance rates were observed to cephalosporins, with 62.5% resistance to ceftazidime and 58.3% resistance to cefepime. Fluoroquinolone resistance was also high, with ciprofloxacin and levofloxacin resistance rates of 54.2% and 51.1%, respectively. Previous study [18] reported that Pseudomonas aeruginosa exhibited resistance rates of approximately 60–70% to ceftazidime and 50–60% to fluoroquinolones, which closely parallels our findings (62.5% and ~52–54%, respectively). This similarity indicates a consistent global trend of increasing resistance to commonly used antibiotics.Carbapenems in our study showed moderate sensitivity, with imipenem (63.5%) and meropenem (60.4%). Previous study [17] reported carbapenem susceptibility rates of approximately 65–75% for NFGNB, which is slightly higher but comparable to our findings, suggesting a gradual decline in carbapenem effectiveness.Among aminoglycosides, amikacin showed 68.8% sensitivity compared to 52.1% for gentamicin in our study. Similar findings have been reported in previous studies, where amikacin sensitivity ranged between 65–75%, indicating its relatively better efficacy compared to other aminoglycosides.Colistin demonstrated the highest sensitivity in our study (95.8%), with only 4.2% resistance. This is consistent with previous reports, where colistin sensitivity ranged from 90–100% in NFGNB isolates. Studies summarized by Suryasa IW et al. [19] and Thaib PKP et al. [20] also emphasized that colistin remains one of the most effective last-resort antibiotics against multidrug-resistant organisms.Additionally, the prevalence of multidrug resistance (MDR) in our study was 51.0%, with higher MDR rates in Acinetobacter spp. (70.6%) compared to Pseudomonas aeruginosa (37.5%). Similar high MDR rates ranging from 40–70% have been reported in previous studies, indicating the growing challenge of antimicrobial resistance among NFGNB.

CONCLUSION

Non-fermenting Gram-negative bacilli were identified as significant contributors to bloodstream infections, with Pseudomonas aeruginosa and Acinetobacter spp. being the predominant isolates. A high level of antimicrobial resistance, particularly to cephalosporins and fluoroquinolones, was observed. Carbapenems and aminoglycosides showed moderate efficacy, while colistin remained the most effective agent. These findings highlight the need for continuous surveillance and strict antimicrobial stewardship to manage emerging resistance.

LIMITATIONS OF THE STUDY

This was a single-centre study limited to blood culture isolates, which may restrict the generalizability of the findings to other settings and specimen types. Molecular characterisation, and MIC testing (especially for colistin) was not performed.

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