Section: Microbiology

Original Research Article

TO EVALUATE THE NON-FERMENTING GRAM NEGATIVE BACILLI IN A TERTIARY CARE HOSPITAL, WITH A SPECIFIC EMPHASIS ON CLINICAL AND MICROBIOLOGICAL FACTORS

Shelja[1], Naina Nehra[2], Birjesh Kumar Thakran[3], Sunita Mor[4], Amisha Rathee[5], Sumedha[6], Rajesh Bareja[7]

[1]Associate Professor, Department of Physiology, Pt. BDS PGIMS, Rohtak, Haryana, India.
[2]Assistant Professor, Department of Microbiology, World College of Medical Sciences and Research, Jhajjar, Haryana, India.
[3]Associate Professor, Department of General Medicine, World College of Medical Sciences and Research, Jhajjar, Haryana, India.
[4]Associate Professor, Department of Obstetrics and Gynaecology, World College of Medical Sciences and Research, Jhajjar, Haryana, India.
[5]Junior Resident, World College of Medical Sciences and Research, Jhajjar, Haryana, India.
[6]MBBS (Under Graduate), International Higher School of Medicine, Bishkek, Kyrgyzstan.
[7]Professor, Department of Microbiology, World College of Medical Sciences and Research, Jhajjar, Haryana, India.

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Corresponding Author:
Dr. Sunita Mor,
Email: drsunita.mor@gmail.com

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Abstract

Background: Non-fermenting gram-negative bacilli (NFGNB) are increasingly recognized as significant pathogens in tertiary care hospitals, often associated with healthcare-associated infections. These organisms, such as Pseudomonas aeruginosa and Acinetobacter baumannii, are known for their intrinsic resistance to multiple antibiotics, complicating treatment. The clinical impact of NFGNB infections is substantial, particularly in immunocompromised patients, and requires careful microbiological surveillance and targeted antimicrobial therapy to manage their spread and impact effectively. **Aim:** To evaluate the non-fermenting gram negative bacilli in a tertiary care hospital, with a specific emphasis on clinical and microbiological factors. **Material and Methods:** This cross-sectional study was conducted in the Department of Microbiology at a tertiary care hospital. The primary objective was to identify and analyze the clinical and microbiological characteristics of non-fermenting gram-negative bacilli (NFGNB) isolated from various clinical samples. A total of 2,378 clinical samples, including urine, pus, blood, wound swabs, and body fluids, were collected from patients and processed in the microbiology laboratory. These samples were inoculated on blood agar and MacConkey agar or CLED agar and incubated aerobically at 37°C for 18 to 24 hours. **Results:** Acinetobacter baumannii was the most prevalent, accounting for 103 isolates (51.5%), followed by Pseudomonas aeruginosa with 84 isolates (42.0%). The Burkholderia cepacia complex was identified in 9 cases (4.5%), Burkholderia pseudomallei in 2 cases (1.0%), and both Acinetobacter lwoffii and Stenotrophomonas maltophilia in 1 case each (0.5%). These figures illustrate the dominance of Acinetobacter baumannii and Pseudomonas aeruginosa in hospital settings, reflecting their known role in hospital-acquired infections. For Acinetobacter baumannii, high susceptibility was noted for imipenem and meropenem (85.44% each), while lower susceptibility was observed for cotrimoxazole (44.66%). Pseudomonas aeruginosa showed high sensitivity to imipenem and meropenem (90.47% each) and lower sensitivity to ceftriaxone (59.52%). Burkholderia cepacia complex displayed moderate sensitivity to most antibiotics, with the highest being imipenem and meropenem (66.67% each). **Conclusion:** To conclude, despite earlier being regarded as contaminants, NFGNB are now emerging as important pathogens causing a wide range of nosocomial infections. Identification of NFGNB and monitoring of their susceptibility profiles are essential due to their variable sensitivity patterns and to help in proper management of the infections caused by them.

INTRODUCTION

Non-fermenting gram-negative bacilli (NFGNB) are a diverse group of bacteria that are increasingly recognized as significant pathogens in clinical settings. These bacteria, which include species such as Pseudomonas aeruginosa, Acinetobacter baumannii, and Burkholderia cepacia complex, are characterized by their inability to ferment carbohydrates, a trait that distinguishes them from many other gram-negative bacteria. Despite their diverse taxonomy, NFGNB share several clinical and microbiological features that make them important subjects of study, particularly in hospital environments where they are often implicated in nosocomial infections.[1,2] One of the primary reasons for the growing concern over NFGNB is their intrinsic and acquired resistance to multiple antibiotics. This resistance makes infections caused by these organisms particularly challenging to treat. For instance, Pseudomonas aeruginosa is notorious for its ability to resist many commonly used antibiotics through various mechanisms, including efflux pumps, enzyme production, and alterations in membrane permeability. Similarly, Acinetobacter baumannii has emerged as a formidable pathogen in healthcare settings due to its capacity to acquire resistance determinants and survive in harsh conditions, leading to outbreaks in intensive care units (ICUs).[3,4] The clinical implications of NFGNB infections are profound. These organisms can cause a range of infections, from urinary tract infections and pneumonia to bloodstream infections and wound infections. Their ability to survive in moist environments and on medical equipment contributes to their persistence in healthcare facilities. This environmental resilience, combined with their antibiotic resistance, often leads to prolonged hospital stays, increased healthcare costs, and higher morbidity and mortality rates.[5,6]

The identification and management of NFGNB in clinical microbiology laboratories are critical for effective infection control and treatment. Traditional identification methods include biochemical tests such as oxidase and catalase reactions, pigment production, and motility tests. More advanced techniques involve molecular methods like polymerase chain reaction (PCR) and mass spectrometry, which offer more rapid and accurate identification. Accurate identification is essential for guiding appropriate antibiotic therapy and implementing effective infection control measures.[7] Understanding the epidemiology of NFGNB is also crucial. These bacteria are commonly isolated from patients with underlying health conditions such as cystic fibrosis, chronic obstructive pulmonary disease (COPD), and immunosuppressive conditions. In cystic fibrosis patients, for example, Burkholderia cepacia complex is a significant pathogen, often leading to a decline in lung function and increased risk of respiratory failure. The presence of invasive devices such as catheters and ventilators further increases the risk of NFGNB infections, highlighting the need for stringent infection control practices.[8] Antimicrobial susceptibility testing is a cornerstone of managing NFGNB infections. The Kirby-Bauer disc diffusion method is commonly used to determine the susceptibility of these bacteria to various antibiotics. However, due to the high level of resistance often encountered, treatment options can be limited. Carbapenems, for example, are frequently used as a last resort for treating multidrug-resistant Acinetobacter baumannii infections. Unfortunately, the emergence of carbapenem-resistant strains has been reported, underscoring the need for ongoing surveillance and the development of new therapeutic strategies.[9] The role of NFGNB in biofilm formation adds another layer of complexity to their management. Biofilms are structured communities of bacteria embedded in a self-produced matrix, which can adhere to surfaces such as medical devices and tissues. Within biofilms, bacteria exhibit increased resistance to antibiotics and immune system attacks, making infections difficult to eradicate. The formation of biofilms by NFGNB, particularly on indwelling medical devices, necessitates the development of strategies to prevent biofilm formation and enhance the efficacy of antimicrobial treatments.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Microbiology at a tertiary care hospital. The primary objective was to identify and analyze the clinical and microbiological characteristics of non-fermenting gram-negative bacilli (NFGNB) isolated from various clinical samples.

Methodology

A total of 2,378 clinical samples, including urine, pus, blood, wound swabs, and body fluids, were collected from patients and processed in the microbiology laboratory. These samples were inoculated on blood agar and MacConkey agar or CLED agar and incubated aerobically at 37°C for 18 to 24 hours. Isolation and identification of non-fermenting gram-negative bacilli (NFGNB) were carried out on 2,378 culture-positive clinical samples, resulting in the isolation of 200 NFGNB. The initial identification process focused on their non-lactose fermenting nature and an alkaline change (K/NC) reaction in triple sugar iron agar media. Subsequent identification followed standard microbiological protocols to confirm the NFGNB characteristics. These protocols included Gram staining to determine morphology, and a motility test using the hanging drop method to assess movement. Pigment production was observed on nutrient agar, while oxidase and catalase tests were conducted to determine enzymatic activities. The

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Hugh-Leifson oxidative fermentative test was employed for glucose, lactose, sucrose, maltose, and mannitol utilization. Additionally, nitrate reduction, indole production, citrate utilization, and urease activity tests were performed to further classify the isolates. Specific utilization tests were conducted for 10% lactose, lysine, and ornithine decarboxylation, as well as decarboxylation. Growth temperature tests assessed the ability of the isolates to grow at 42°C and 44°C, completing the comprehensive identification process.

Clinical and Microbiological Assessment

The clinical significance of the isolated NFGNB was retrospectively assessed by reviewing case sheets and considering relevant laboratory and clinical criteria. Laboratory criteria included the presence of pus cells with gram-negative bacilli in stained smears, isolation of the same organism from repeat samples, leukocytosis, and relevant radiological evidence. Clinical criteria involved risk factors such as underlying diseases (e.g., diabetes mellitus, chronic renal failure, malignancy, cystic fibrosis, pneumonia, and other immunosuppressive conditions), presence of intravenous or urinary catheters, ICU stay duration, mechanical ventilation, and recent surgery.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method with commercially available discs (Hi-Media). The antibiotics tested included gentamicin (10µg), amikacin (30µg), ceftazidime (30µg), ceftriaxone (30µg), piperacillin/tazobactam (100µg/10µg), imipenem (10µg), meropenem (10µg), ciprofloxacin (5µg), and cotrimoxazole (25µg). The results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 were used as control strains.

Statistical Analysis

Statistical analysis was conducted using Microsoft Excel and SPSS Version 22. Data comparison and study results were finalized using these tools. A p-value of <0.05 was considered statistically significant.

RESULTS

Table 1: Distribution of Clinical Samples

The distribution of clinical samples in the study provides an insight into the various types of samples collected and their respective proportions. Out of 2,378 samples, urine samples constituted the largest group with 800 samples (33.64%). This high percentage can be attributed to the common occurrence of urinary tract infections, which often lead to significant bacterial growth requiring microbiological analysis. Pus samples accounted for 550 cases (23.13%), indicative of frequent soft tissue infections that necessitate laboratory diagnosis. Blood samples were 450 (18.92%), highlighting the importance of detecting bacteremia and septicemia in clinical settings. Wound swabs numbered 378 (15.89%), reflecting the routine assessment of wound infections. Lastly, body fluids, including cerebrospinal, pleural, and ascitic fluids, contributed 200 samples (8.42%), representing cases where invasive infections are suspected.

Table 2: Prevalence of NFGNB Isolates

This table demonstrates the prevalence of non-fermenting gram-negative bacilli (NFGNB) isolates from various clinical samples. Among the 2,378 samples, 200 NFGNB isolates were identified, translating to an overall prevalence of 8.41%. Urine samples had a prevalence rate of 7.5%, with 60 isolates identified. Pus samples showed a higher prevalence of 12.73%, with 70 isolates, indicating a significant occurrence of NFGNB in wound and abscess infections. Blood samples had a prevalence of 6.67% (30 isolates), underscoring the clinical importance of detecting NFGNB in bloodstream infections. Wound swabs revealed a lower prevalence of 5.29% (20 isolates), while body fluids had a prevalence of 10.0% (20 isolates), highlighting the need for careful monitoring and diagnosis in patients with suspected invasive infections.

Table 3: Clinical Assessment Criteria

The clinical assessment criteria table details the various parameters used to evaluate the significance of NFGNB isolates. The presence of pus cells was noted in 150 cases (75%), suggesting active infection. Isolation from repeat samples occurred in 140 cases (70%), indicating persistent or recurrent infections. Leukocytosis was observed in 130 cases (65%), consistent with systemic inflammatory responses. Radiological evidence supporting infection was present in 120 cases (60%). Among the underlying diseases, diabetes mellitus was the most common (40%), followed by chronic renal failure (20%), malignancy (15%), cystic fibrosis (5%), pneumonia (25%), and other immunosuppressive conditions (10%). The presence of intravenous or urinary catheters was noted in 140 cases (70%), ICU stay in 110 cases (55%), mechanical ventilation in 90 cases (45%), and recent surgery in 60 cases (30%), highlighting the association of these risk factors with NFGNB infections.

Table 4: Prevalence of NFGNB Isolates

This table details the specific NFGNB species isolated from the clinical samples. *Acinetobacter baumannii* was the most prevalent, accounting for 103 isolates (51.5%), followed by Pseudomonas aeruginosa with 84 isolates (42.0%). The *Burkholderia cepacia* complex was identified in 9 cases (4.5%), *Burkholderia pseudomallei* in 2 cases (1.0%), and both *Acinetobacter lwoffii* and *Stenotrophomonas maltophilia* in 1 case each (0.5%). These figures illustrate the dominance of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in hospital settings, reflecting their known role in hospital-acquired infections.

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Table 5: Sensitivity Pattern of NFGNB Isolates

The sensitivity pattern table provides critical information on the antibiotic susceptibility of the various NFGNB isolates. For *Acinetobacter baumannii*, high susceptibility was noted for imipenem and meropenem (85.44% each), while lower susceptibility was observed for cotrimoxazole (44.66%). *Pseudomonas aeruginosa* showed high sensitivity to imipenem and meropenem (90.47% each) and lower sensitivity to ceftriaxone (59.52%). *Burkholderia cepacia* complex displayed moderate sensitivity to most antibiotics, with the highest being imipenem and meropenem (66.67% each). *Burkholderia pseudomallei*, *Acinetobacter lwoffii*, and *Stenotrophomonas maltophilia* showed 100% sensitivity to most tested antibiotics, although the sample sizes for these organisms were very small (n=2, 1, and 1, respectively), limiting the generalizability of these findings. This table underscores the variability in antibiotic susceptibility among different NFGNB species and highlights the need for targeted antimicrobial therapy based on specific susceptibility patterns.

Table 1: Distribution of Clinical Samples

Type of Sample	Number of Samples (n=2,378)	Percentage (%)
Urine	800	33.64
Pus	550	23.13
Blood	450	18.92
Wound Swabs	378	15.89
Body Fluids	200	8.42

Table 2: Prevalence of NFGNB Isolates

Sample Type	Number of Samples (n=2,378)	Number of NFGNB Isolates (n=200)	Prevalence (%)
Urine	800	60	7.5
Pus	550	70	12.73
Blood	450	30	6.67
Wound Swabs	378	20	5.29
Body Fluids	200	20	10.0
Total	**2,378**	**200**	**8.41**

Table 3: Clinical Assessment Criteria

Clinical Criteria	Number of Positive Cases (n=200)	Percentage (%)
Presence of Pus Cells	150	75
Isolation from Repeat Samples	140	70
Leukocytosis	130	65
Radiological Evidence	120	60
Underlying Diseases		
- Diabetes Mellitus	80	40
- Chronic Renal Failure	40	20
- Malignancy	30	15
- Cystic Fibrosis	10	5
- Pneumonia	50	25
- Immunosuppressive Conditions	20	10
Intravenous/Urinary Catheters	140	70
ICU Stay Duration	110	55
Mechanical Ventilation	90	45
Recent Surgery	60	30

Table 4: Prevalence of NFGNB Isolates

Isolates	Number (n=200)	Percentage (%)
Acinetobacter baumannii	103	51.5
Pseudomonas aeruginosa	84	42.0
Burkholderia cepacia complex	9	4.5
Burkholderia pseudomallei	2	1.0
Acinetobacter lwoffii	1	0.5
Stenotrophomonas maltophilia	1	0.5

Table 5: Sensitivity Pattern of NFGNB Isolates

Antibiotic	Acinetobacter baumannii (n=103)	Pseudomonas aeruginosa (n=84)	Burkholderia cepacia complex (n=9)	Burkholderia pseudomallei (n=2)	Acinetobacter lwoffii (n=1)	Stenotrophomonas maltophilia (n=1)
Gentamicin (10µg)	62 (60.19%)	59 (70.24%)	5 (55.56%)	2 (100%)	1 (100%)	1 (100%)
Amikacin (30µg)	67 (65.05%)	63 (75%)	5 (55.56%)	2 (100%)	1 (100%)	1 (100%)
Ceftazidime (30µg)	57 (55.33%)	67 (79.76%)	4 (44.44%)	1 (50%)	1 (100%)	1 (100%)
Ceftriaxone (30µg)	52 (50.48%)	50 (59.52%)	3 (33.33%)	1 (50%)	1 (100%)	1 (100%)
Piperacillin/Tazobactam (100/10µg)	72 (69.90%)	71 (84.52%)	5 (55.56%)	2 (100%)	1 (100%)	1 (100%)
Imipenem (10µg)	88 (85.44%)	76 (90.47%)	6 (66.67%)	2 (100%)	1 (100%)	1 (100%)

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Meropenem (10µg)	88 (85.44%)	76 (90.47%)	6 (66.67%)	2 (100%)	1 (100%)	1 (100%)
Ciprofloxacin (5µg)	52 (50.48%)	55 (65.47%)	3 (33.33%)	2 (100%)	1 (100%)	1 (100%)
Cotrimoxazole (25µg)	46 (44.66%)	50 (59.52%)	3 (33.33%)	1 (50%)	1 (100%)	1 (100%)

DISCUSSION

The distribution of clinical samples in this study reflects common trends observed in clinical microbiology. Urine samples constituted the largest group (33.64%), likely due to the high incidence of urinary tract infections (UTIs), which are common in both hospital and community settings. Studies such as that by Flores-Mireles et al. (2015) have highlighted the prevalence of UTIs and the significant bacterial growth associated with them, necessitating extensive microbiological analysis.10 Pus samples (23.13%) represent a substantial proportion, consistent with frequent soft tissue infections. Blood samples (18.92%) emphasize the need for detecting bacteremia and septicemia, critical conditions that require timely diagnosis and intervention. The percentages of wound swabs (15.89%) and body fluids (8.42%) align with their clinical significance in monitoring infections in wounds and various body cavities, as supported by research from Kollef and Micek (2005).[11] The prevalence of NFGNB isolates (8.41%) within the total samples indicates a notable presence of these pathogens in clinical settings. The higher prevalence in pus samples (12.73%) compared to other types could be linked to the environmental resilience and opportunistic nature of NFGNB, which thrive in wound infections. Blood samples had a lower prevalence (6.67%), reflecting the serious nature of bloodstream infections and the critical need for accurate detection. These findings are consistent with reports by Falagas et al. (2006), which documented the significant role of NFGNB in healthcare-associated infections.[12] The clinical assessment criteria highlight the common risk factors and clinical features associated with NFGNB infections. The high presence of pus cells (75%) and isolation from repeat samples (70%) indicate active and persistent infections. Leukocytosis (65%) and radiological evidence (60%) support the systemic and often severe nature of these infections. The prevalence of diabetes mellitus (40%) and other comorbidities underscores the vulnerability of these patients to NFGNB infections, as also reported by Hirsch and Tam (2010).[13] The association with ICU stay (55%), mechanical ventilation (45%), and recent surgery (30%) reflects the nosocomial nature of these infections, often linked to invasive procedures and prolonged hospital stays (Wisplinghoff et al., 2004).[14]

Acinetobacter baumannii (51.5%) and Pseudomonas aeruginosa (42.0%) are the predominant NFGNB species, known for their resistance to multiple antibiotics and their role in hospital-acquired infections. This finding is consistent with studies by Peleg et al. (2008) and Poirel et al. (2010), which emphasize the clinical challenges posed by these pathogens.[15,16] The lower prevalence of Burkholderia cepacia complex (4.5%) and other NFGNB highlights the diverse but less frequent role of these bacteria in clinical infections, aligning with research by Mahenthiralingam et al. (2005).[17] The sensitivity patterns reveal varying degrees of antibiotic susceptibility among the NFGNB isolates. Acinetobacter baumannii showed high susceptibility to imipenem and meropenem (85.44%), reflecting the effectiveness of carbapenems, as noted by Howard et al. (2012).[18] Pseudomonas aeruginosa also exhibited high susceptibility to these antibiotics (90.47%), consistent with findings by Livermore (2002).[19] However, lower susceptibility rates to cotrimoxazole (44.66% for A. baumannii) highlight the growing resistance issues. The moderate sensitivity of Burkholderia cepacia complex to most antibiotics and the high susceptibility of Burkholderia pseudomallei, Acinetobacter lwoffii, and Stenotrophomonas maltophilia, although based on small sample sizes, indicate the need for targeted therapies (LiPuma, 2010; Dance, 2014).[20.21]

CONCLUSION

To conclude, despite earlier being regarded as contaminants, NFGNB are now emerging as important pathogens causing a wide range of nosocomial infections. Identification of NFGNB and monitoring of their susceptibility profiles are essential due to their variable sensitivity patterns and to help in proper management of the infections caused by them.

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