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Emergence of Multidrug-Resistant and Extensively Drug-Resistant Non-Fermenting Gram-Negative Bacilli in a Tertiary Hospital in India

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ABSTRACT

Introduction: Non-fermenting Gram-negative bacilli (NFGNB), particularly the *Acinetobacter baumannii* complex (ABC) and *Pseudomonas aeruginosa*, are common causes of infections in both hospitalized patients and outpatients, posing significant clinical and therapeutic challenges. The primary objective of this study was to conduct a comprehensive analysis of NFGNB, specifically ABC and *P. aeruginosa*, isolated from pus specimens obtained from both hospitalized patients and outpatients**. Methods**: This study investigated the antibiotic resistance patterns of NFGNB, focusing on ABC and *P. aeruginosa*, isolated from pus samples collected from both hospitalized patients and outpatients. The isolates were tested for multidrug resistance (MDR) and extensive drug resistance (XDR) using standardized microbiological protocols. The data were analyzed using descriptive statistics to summarize the findings. **Results:** Out of 1234 pus samples received, 117 (9.5%) NFGNB were isolated, accounting for 30% of the total Gram-negative bacilli (GNB) isolates. The majority of NFGNB (82.9%, n = 97/117) were isolated from inpatients, with surgical site infections being the most common clinical condition (33.3%, n = 39/117). Among the NFGNB isolates, *P. aeruginosa* was the predominant species (76.9%, n = 90/117), followed by A. *baumannii* (22.2%, n = 26/117). Antimicrobial susceptibility testing revealed that 37.7% (n = 34/90) of *P. aeruginosa* isolates were MDR and 13% (n = 12/90) were XDR, while 65% (n = 17/26) of *A. baumannii* isolates were MDR and 26.9% (n = 7/26) were XDR. **Conclusion:** This study highlights the emergence of NFGNB as significant nosocomial pathogens, exhibiting a high degree of resistance to commonly used antibiotics. The findings underscore the urgent need to enhance and strictly implement effective antibiotic stewardship policies, including the development of new antibiotic regimens and antimicrobial resistance surveillance programs**,** to combat the growing resistance of nosocomial pathogens and ultimately improve patient outcomes.

INTRODUCTION

Non-fermenting Gram-negative bacilli (NFGNB) are a significant cause of both nosocomial and community-acquired infections, with *Acinetobacter baumannii* complex (ABC), *P. aeruginosa*, and *Burkholderia cepacia* complex being prominent examples [1]. These bacteria are renowned for their intrinsic resistance to various antibiotics, including beta-lactams and aminoglycosides. Notably, *B. cepacia* complex has been reported to exhibit innate resistance to polymyxin B and colistin, which are often the last-resort therapeutic options for severe Gram-negative infections [1, 2]. The escalating spread of resistance mechanisms, including the

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production of carbapenemases such as Ambler classes A, B, and D enzymes, has become a critical concern, compromising treatment options for affected patients. The inadequate availability of effective therapeutic strategies for multidrug-resistant pathogens has further exacerbated this issue, highlighting the urgent need for novel antimicrobial agents and innovative treatment approaches [3].

Therefore, further studies are warranted to elucidate the epidemiology of MDR and XDR pathogens in India, which would enable a comprehensive comparison with existing literature and inform strategies for antimicrobial stewardship. The emergence of MDR organisms poses significant diagnostic and therapeutic challenges for patients, and the looming threat of untreatable infections in the near future is a pressing concern that warrants immediate attention and action [4, 5]. The selection of appropriate antibiotics for patients with resistant bacterial infections is a daunting task, and accurate and up-to-date local microbiological data play a crucial role in guiding treatment decisions. One of the key challenges in managing these infections is determining the most effective antibiotics, which can be informed by local microbiological epidemiological data. These data remain essential for defining the baseline risk of MDR-GNB infections and guiding targeted empirical treatment choices [6].

NFGNB account for approximately 15% of all bacterial isolates, a phenomenon attributed to the escalating medical support needs of patients, the prevalence of chronically ill patients colonized with multidrug-resistant bacteria, and the judicious but often widespread use of broad-spectrum antibiotics [1, 7].

The objective of this study was to investigate the presence and antimicrobial resistance patterns of NFGNB, specifically ABC and *P. aeruginosa*, in pus samples collected from both hospitalized patients and outpatients. Our hypothesis was that these organisms, commonly found in the hospital environment, would be frequently isolated in pus samples, potentially leading to colonization and infection.

MATERIAL AND METHODS

This hospital-based cross-sectional population based study was conducted in the Microbiology Department of the tertiary care University hospital of Maharishi Markandeshwar (Deemed to be University) located in a rural setting near the city of Ambala in the State of Haryana, in North India catering to rural patients and patients from neighbouring state of Uttar Pradesh, with a comprehensive range of medical facilities, including all departments as per the National Medical Commission guidelines. The study was conducted from March 2019 to March 2020. A total of 1234 clinical pus samples were collected from hospitalized patients and outpatients after obtaining approval from the Institutional Ethics Committee (IEC No. 1411) and written informed consent from the patients.

Criteria for pus sample collection. Given that pus and lower respiratory tract specimens are commonly associated with NFGNB isolation, we specifically selected pus samples for this study. We employed a prospective, consecutive sampling strategy to collect the specimens, ensuring that all eligible samples were included and minimizing potential biases.

Criteria for selection of patients. Patients presenting with signs and symptoms of clinically suspected surgical site infection (SSI), burn wound infections, trauma, abscesses (local, breast, and liver), otitis media, conjunctivitis, and urethritis were included in the study, and their pus samples were collected for microbiological analysis.

Inclusion criteria. All pus samples collected under aseptic conditions using sterile syringe aspiration or sterile swabs from both inpatients and outpatients with suspected bacterial infections were included in the study.

Exclusion criteria. Samples with mixed or polymicrobial growth (≥3 different microorganisms) were excluded due to potential contamination or the difficulty in attributing the infection to a single pathogen.

Inoculum preparation. The bacterial strains were screened for MDR and XDR according to the updated Centers for Disease Control and Prevention (CDC) guidelines. The defining criteria are as follows:

We defined a MDR isolate as a bacterium resistant to at least three classes of antimicrobial agents, including at least one of the following: fluoroquinolones, aminoglycosides, or carbapenems (including combinations with inhibitors like clavulanic acid, sulbactam, or tazobactam). We defined multidrug-resistant *P. aeruginosa* (MDRPA) as isolates exhibiting intermediate or resistant phenotypes to at least three antimicrobial classes, including beta-lactams, carbapenems, aminoglycosides, and fluoroquinolones [8]. Furthermore, we defined multidrug-resistant *A. baumannii* (MDRAB) as isolates exhibiting resistance to at least two specific representatives from at least two of the following antimicrobial classes: aminoglycosides, antipseudomonal penicillins, carbapenems, third- or fourth-generation cephalosporins, and fluoroquinolones [9].

We defined an XDR isolate as a bacterium that exhibited MDR and additional resistance to all available carbapenems and at least one agent in all but two or fewer antimicrobial categories (Figure 1).

All samples were processed in accordance with the latest standard microbiological guidelines, as recommended by the Clinical and Laboratory Standards Institute (CLSI, Wayne, PA, USA). Samples were aseptically streaked onto 5% sheep blood agar and MacConkey agar plates under strict aseptic conditions. The plates were then incubated aerobically at 37°C for 18-

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24 h. The sample was considered to have significant bacterial growth when it yielded ≥ two distinct colony-forming units (CFU) by qualitative culture method.

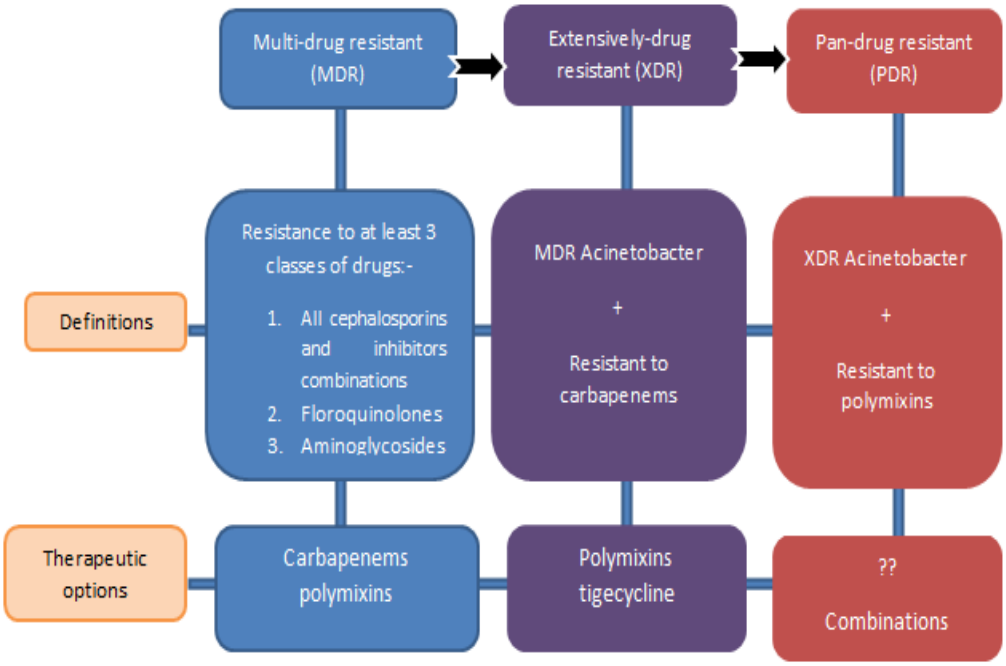


Fig. 1. Definitions and criteria for MDR, XDR, and pandrug-resistant (PDR) isolates

The isolated organisms were identified using a combination of standard biochemical tests [10] and automated identification systems. All organisms that exhibited growth on Triple Sugar Iron agar and produced an alkaline reaction were preliminarily identified as NFGNB. Further identification was performed using a standard protocol [1], which considers the following criteria: morphological appearance of colonies, Gram staining, motility, pigment production, and oxidase production. The following tests were performed: Oxidative-Fermentative (Hugh-Leifson's medium) for glucose, lactose, sucrose, maltose, mannitol, and xylose; MacConkey agar supplemented with 10% lactose; lysine decarboxylase test; and gelatin liquefaction test (Figure 2). Further identification and antimicrobial susceptibility testing were performed using the Vitek 2 compact system (bioMérieux, software version VT2-9.01) [11], which was calibrated and validated according to the manufacturer's instructions and CLSI guidelines. This system was selected as the standard method for accurate antibiotic susceptibility testing, enabling determination of the minimum inhibitory concentration (MIC) of antibiotics, with results interpreted according to the CLSI breakpoints. It was the latest automated method available in the institute during the study period, regularly updated and maintained to ensure compliance with current CLSI guidelines.

Statistical analysis. The descriptive analyses were performed using SAS statistical software (Version 9.3 for Windows; SAS Institute, Inc., Cary, NC, USA) to analyze the distribution of observations in variables. The results are presented as percentages, figures and tables.

RESULTS

Of the 1234 pus samples analyzed, 740 (59.9%) exhibited significant bacterial growth, defined as culture positivity with the growth of at least two distinct colony-forming units (CFU) by qualitative culture method.

NFGNB accounted for 117 isolates (15.8% of culture-positive samples), which represented 30% of the total 390 Gram-negative bacteria isolated. *P. aeruginosa* was the most prevalent NFGNB isolated in this study, accounting for 76.9% of all NFGNB isolates, followed by *Acinetobacter baumannii* (23.1%) (Figure 3).

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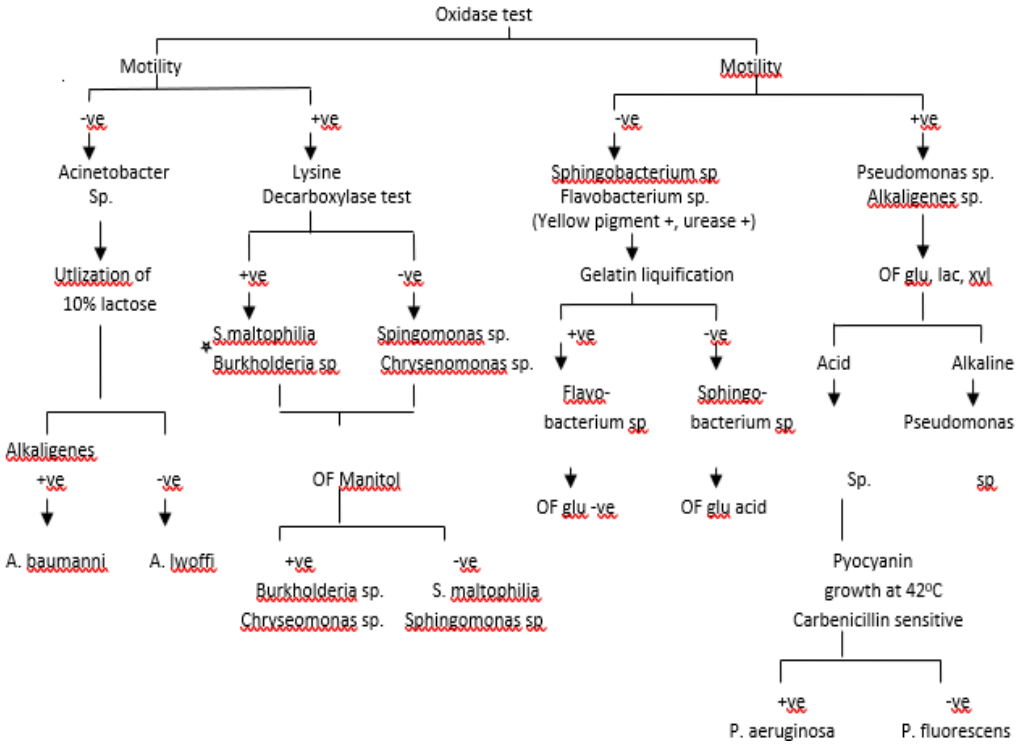


Fig. 2. Schematic representation of the identification protocol used in this study for non-fermenting Gram-negative bacilli [1]

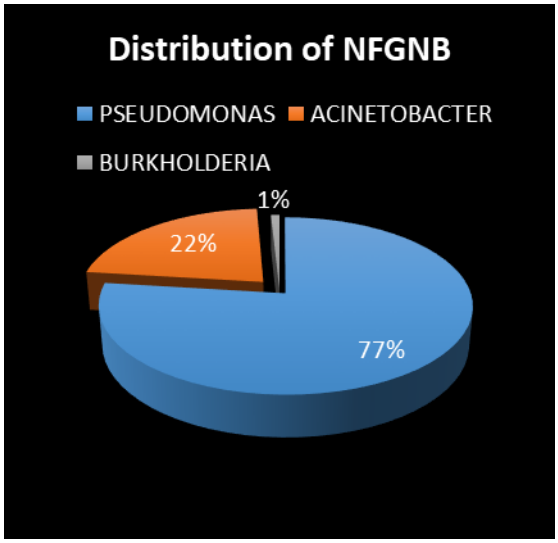


Fig. 3. Distribution of NFGNB among the total Gram-negative bacterial isolates from pus samples

Among the 117 NFGNB isolates, 74 (63.2%) were from male patients and 43 (36.8%) were from female patients. The highest proportion of NFGNB isolates (37.6%) was observed in the age group of ≥60 years, while the lowest proportion (11.9%) was observed in the age group of 0-20 years.

According to clinical status, 97 (82.9%) of NFGNB isolates were obtained from hospitalized patients. Among the isolates from hospitalized patients, 69 (71.1%) were acquired from various inpatient wards, including surgery, gynecology, dermatology, and others. Additionally, 23 isolates (23.7%) were obtained from patients in the intensive care unit (ICU), while 5 (5.2%) were isolated from the outpatient department (OPD). The distribution of NFGNB isolates by clinical source revealed that surgical site infections (SSIs) accounted for the highest prevalence (33.3%), followed by Local Abscess (14.5%), Liver Abscess and Otitis (each accounting for 11.9%), Burn

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Wound (10.2%), Eye Discharge (7.6%), Accidental Wounds (5.9%), Breast Abscess (3.4%), and Urethritis (0.85%) (Figure 4).

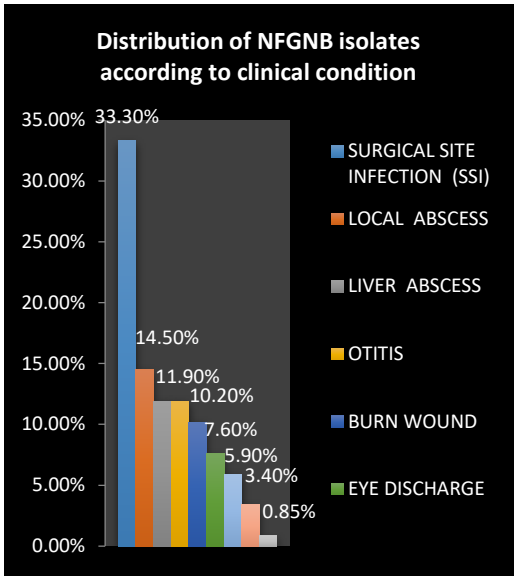


Fig. 4. Distribution of NFGNB isolates from pus samples by clinical source or diagnosis.

Table 1. Antibiotic susceptibility profile of commonly isolated NFGNB in present study.

Antibiotics	***Pseudomonas* (N=90)**		***Acinetobacter* (N=26)**		***Burkholderia* (N=1)**	
β-Lactams	**% (S)**	**% (R)**	**% (S)**	**% (R)**	**% (S)**	**% (R)**
Ceftriaxone (CTR)	48.34	51.64	30.76	69.23	-	100
Ceftazidime (CAZ)	52.2	47.77	23.07	76.92	-	100
Cefotaxime (CTX)	38.88	61.11	23.07	76.92	-	-
Cefipime (CPM)	40.00	60.00	26.92	73.07	-	-
Piperacillin (PI)	-	-	7.69	92.30	-	-
Mezlocillin (MZ)	42.2	57.77	30.76	69.23	-	-
Piperacillin/Tazobactum (PIT)	84.44	15.55	46.15	53.84	-	-
Ticarcillin/clavulanic acid (TCC)	-	-	15.38	84.61	-	100
Floroquinolones						
Ciprofloxacin (CIP)	53.31	46.66	23.07	76.92	-	100
Levofloxacin (LE)	44.42	55.55	34.6	65.38	-	100
Amnioglycosides						
Amikacin (AK)	70.00	30.00	38.46	61.53	-	
Gentamicin (GEN)	60.00	40.00	26.92	73.07	-	
Tobramycin (TOB)	65.51	34.44	38.46	61.53	-	100
Carbapenems						
Imipenem (IMP)	84.5	16.66	26.92	73.07		
Meropenem (MRP)	82.41	17.58	26.92	73.07	-	100
Tetracycline						
Minocycline (MI)	-	-	53.84	46.15	100	-
Tetracycline (TE)	-	-	57.69	42.30	-	-
Doxycycline (DO)	-	-	23.07	76.92	-	-
Sulphonamides						
Cotrimoxazole (COT)	21.11	78.88	11.53	88.4S6	-	
POLYMYXIN-B	100	-	100	-	-	100
Colistin (CL)	100	-	100	-	-	-
Glycylcycline						
Tigecycline (TGC)	47.77	52.22	80.76	19.23	100	-

The antibiotic resistance tests revealed that piperacillin-tazobactam was the most effective β-lactam antibiotic against *P. aeruginosa*, exhibiting a sensitivity of 84.44%, and *A.* baumannii, exhibiting a sensitivity of 46.15%. The

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highest resistance rates were observed for Piperacillin (92.3%) followed by ticarcillin-clavulanic acid (84.61%). Notably, *Burkholderia* spp. exhibited complete resistance to all tested β-Lactams, fluoroquinolones, polymyxins, and glycyclines. Colistin demonstrated exceptional efficacy against the majority of NFGNB isolates, with a 100% susceptibility rate (Table 1), indicating its potential as an effective treatment option.

A significant proportion of NFGNB isolates, particularly *P. aeruginosa*, exhibited MDR characteristics, with 37.7% classified as MDR and 13.3% as XDR. Similarly, a substantial proportion of *A. baumannii* isolates (65.3%) were classified as MDR and 26.9% as XDR (Table 2).

Table 2. Distribution of MDR and XDR NFGNB isolates among *A. baumannii* and *P. aeruginosa*

No. of antibiotics classes	*P. aeruginosa* (N=90)	*A. baumannii* (N=26)
MDR	34 (37.7%)	17 (65.3%)
XDR	12 (13.3%)	7 (26.9%)

DISCUSSION

Despite enhanced awareness among healthcare professionals and advancements in hospital care, nosocomial infections persist as a significant challenge, with hospitalized patients remaining at risk of developing infections [9]. NFGNB, previously considered mere contaminants or colonizers, have emerged as significant healthcare-associated pathogens, posing a substantial threat to patient safety and public health [12, 13].

In our study, 15.8% of culture-positive samples were NFGNB, a finding consistent with the prevalence reported by Rahbar *et al*. (2010) [13]. However, Bhargava *et al*. (2015) [14] in Nepal (29.6%) and Sharma *et al*. (2014) [15] in India (25.6%) documented higher prevalence rates. These variations in NFGNB prevalence across different healthcare facilities may be attributed to differences in antimicrobial stewardship programs, infection control practices, and the circulation of these bacterial pathogens in respective hospitals, highlighting the need for tailored interventions to address local epidemiology.

In this study, the highest prevalence of NFGNB was found in the age group ≥60 years (37.6%), which is consistent with the findings of Nivitha *et al.* (25.6%) [16]. However, Al-Zaidi *et al*. (2016) [17] reported the highest number of isolates in the age group 5–25 years (38.9%) [17]. The disparity in these findings may be attributed to differences in the study populations and demographics.

A total of 97 NFGNB isolates (82.9% of the total NFGNB isolates) were obtained from inpatient settings. Among these isolates, 71.1% were from ward patients, 23.7% from ICU patients, and 5.2% from outpatients. This finding is consistent with the study by Chaudhary *et al*. (2021), which reported 74.95% IPD and 25% OPD isolates [18]. In contrast, Gupta *et al*. (2017) found a higher proportion of isolates from outpatients (76%) compared to inpatients (24%) in their study, which focused on skin infections and had a larger proportion of outpatients [19].

The prevalence of major NFGNB in our study was led by *P. aeruginosa* (76.9%), followed by *A. baumannii* (23.1%) and a single isolate of *Burkholderia* spp. This distribution pattern is consistent with the findings of previous studies, which reported similar proportions. For instance, Somily *et al*. (2021) isolated *P. aeruginosa* in 73.6% of cases, followed by *A. baumannii* in 21.0% [20]. Similarly, studies by Namita *et al*. (2017) [21], Grewal *et al*. (2017) and Soni *et al* (2023) reported a higher prevalence of non-fermenting *Pseudomonas* species compared to Acinetobacter species [22, 23]. The ubiquitous nature and ability to tolerate harsh environments of both *P. aeruginosa* and *A. baumannii* enable them to colonize the skin of hospitalized patients more commonly. The emergence of antibiotic resistance in these bacteria is likely attributed to a multifaceted array of factors, including hospital overcrowding, the increasing number of immunocompromised patients, the growing elderly population, increased international travel, the widespread use of broad-spectrum antibiotics, over-the-counter availability of antibiotics, inappropriate antibiotic use (including self-treatment and non-compliance), inadequate resources for in-service training of health workers, insufficient resources for infection control, and decreased funding for public health surveillance.

In the present study, the distribution of NFGNB isolates among various clinical specimens revealed a predominance in surgical site infections (33.3%), followed by local abscesses (14.5%), liver abscesses (11.9%), otitis media (11.9%), burn wound infections (10.2%), eye discharge (7.6%), accidental wounds (5.9%), breast abscesses (3.4%), and urethritis (0.85%). This finding is consistent with that of Rao *et al*. (2014) [24], which reported a similar distribution of MDR NFGNB in various clinical specimens.

Among all β-lactam drugs, piperacillin-tazobactam exhibited the highest susceptibility, with 84.44% for *P. aeruginosa* and 46.15% for *A. baumannii*. In contrast, *Burkholderia* spp. showed resistance to all β-lactam drugs. This finding is consistent with that of Kishor *et al*. (2021) [25], which reported piperacillin-tazobactam as the most effective β-lactam drug (63.4% overall susceptibility), with a susceptibility of 50% in *P. aeruginosa* and 46.15% in *A. baumannii*. Similarly,

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studies conducted by Maniyan *et al*. (2016) [26] and Sambyal *et al*. (2017) [27] reported comparable results. Notably, a high degree of resistance (69-73%) was observed for both 3rd- and 4th-generation cephalosporins, which may be attributed to their widespread use in hospital settings. This finding aligns with recent reports from other studies, highlighting the increasing resistance of NFGNB to cephalosporins.

In the present study, among the fluoroquinolone drugs, levofloxacin exhibited resistance in 55.55% of *Pseudomonas* isolates, while ciprofloxacin showed resistance in 46.66% of isolates. The high resistance to fluoroquinolones may be attributed to their overuse, potentially driven by their previous over-the-counter availability in some settings. Notably, all *A. baumannii* isolates resistant to Ciprofloxacin harbored mutations in *gyrA* and *parC* genes. However, other mechanisms, such as alterations in efflux systems, may also contribute to this resistance. Our findings align with those of *Buzilă* et al. (2021) [7], who reported resistance to Levofloxacin in 67% of isolates and to Ciprofloxacin in 66.7% of isolates. These findings underscore the need for monitoring the sale of fluoroquinolones and implementing policies to restrict their use to prescription-only to curb the emergence of MDR NFGNB.

Carbapenems are the primary antibiotic class preferred for treating *Acinetobacter* and *Pseudomonas* infections. Our study revealed 82.41% sensitivity to imipenem and 84.5% sensitivity to meropenem among *Pseudomonas* isolates, while both imipenem and meropenem showed 26.92% sensitivity against *Acinetobacter* isolates. These findings are consistent with the study by Kaur *et al*. (2014) [28], which reported 71.4% imipenem and 64.5% meropenem sensitivity against *Pseudomonas*, and 39.6% sensitivity against *Acinetobacter*. However, Kakati et al. (2022) [29] reported higher resistance rates, with 92.2% of *Acinetobacter* isolates resistant to imipenem and 92.6% resistant to meropenem, and 53.4% of Pseudomonas isolates resistant to imipenem and 48.8% resistant to meropenem [29]. Carbapenems have been the drug of choice for treating infections caused by penicillin- or cephalosporin-resistant Gram-negative bacilli, particularly Extended Spectrum Beta Lactamase (ESBL) Gram-negative infections. Resistance to carbapenems is attributed to various mechanisms, including decreased outer membrane permeability, increased efflux systems, altered penicillin-binding proteins, carbapenemase enzymes, and increased Amp-C β-lactamase production. *A. baumannii* exhibits diverse resistance mechanisms [5], with carbapenem resistance being a significant concern, observed in 73.1% of our isolates. The limited treatment options for carbapenem-resistant *A. baumannii* (CRAB) may result in high mortality rates [6]. Currently, no superior agent or combination regimen has been proven in randomized clinical trials, although ampicillin-sulbactam appears to be a viable initial treatment option due to its ability to saturate penicillin-binding proteins 1 and 3 at high doses [30].

Combination therapy is likely the most effective treatment approach and should include high-dose ampicillin-sulbactam in combination with another active agent, such as high-dose tigecycline, polymyxins, or newer agents like cefiderocol or eravacycline. However, the emergence of resistant infections is already limiting available options, making it challenging for clinicians to select effective drugs in the management of critically ill patients. The judicious use of combination therapy may help to mitigate the risk of further resistance development and improve treatment outcomes [30, 31].

The primary factors contributing to this issue are the widespread availability and inadvertent use of broad-spectrum antibiotics, leading to the rapid evolution of resistance mechanisms in these organisms. NFGNB, such as *Pseudomonas* and *Acinetobacter* spp., exhibit intrinsic resistance to various antibiotics, including ampicillin, amoxicillin, amoxicillin-clavulanate, cefotaxime, ceftriaxone, cefazolin, ertapenem, tetracyclines, trimethoprim, and chloramphenicol [31, 32]. Consequently, carbapenems (imipenem, meropenem), aminoglycosides, β-lactam/β-lactamase inhibitors, quinolones, polymyxins, and monobactams have become the drugs of choice for treating infections caused by these bacteria [33, 34]. However, the indiscriminate use of these antibiotics has led to the emergence of highly resistant strains, which have developed various resistance mechanisms, including carbapenemase production, decreased permeability due to porin channel loss, overexpression of efflux pumps, and alterations in penicillin-binding proteins, over time [4].

In Metallo-Beta-Lactamase-expressing *P. aeruginosa* and *Acinetobacter* spp., aztreonam's potency is impacted by efflux resistance mechanisms, and avibactam fails to effectively reverse aztreonam's activity. Notably, only a single *Burkholderia* isolate showed sensitivity to Tigecycline and Minocycline, while resisting all other antibiotics. Unfortunately, the limited data for this isolate precludes an extensive discussion of susceptibility [35, 36]. Our study also investigated tetracycline antibiotics for *Acinetobacter*-related infections, revealing that tetracycline was effective against 57.69% of isolates, followed by minocycline (53.84%), and doxycycline (23.07%). These findings are in partial agreement with Batarseh *et al*. (2016) [37], who reported sensitivities of 42.7% for tetracycline and 87.5% for minocycline. Similarly, Yeruva *et al*. reported a Minocycline sensitivity of 40% [38]. The rising resistance rate of tetracyclines is attributed to their extensive use in human and animal treatment, leading to strong selective pressure and the emergence of resistant organisms. Polymyxin-B showed sensitivity to *Pseudomonas* and *Acinetobacter* isolates, but resistance to one *Burkholderia* isolate, which is intrinsically resistant to polymyxin [30]. Colistin exhibited 100% sensitivity to all organisms, consistent

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with findings from Kakati *et al*. (2022) [29], where colistin showed 100% sensitivity to all NFGNB. However, a study by Ko *et al*. (2007) [39] in Korea reported a high colistin resistance rate (30.6%), attributed to its extensive use in that country. Fortunately, colistin resistance in India is relatively low, making it a viable drug of choice for multidrug-resistant strains [40].

The sensitivity of tigecycline against *P. aeruginosa* was 47.77%, which is consistent with the findings reported by Premanadham *et al*. (2018) [41], who also observed a similar sensitivity profile. This is in contrast to our findings for *Acinetobacter* isolates, where tigecycline demonstrated a sensitivity of 80.76%, which is comparable to the 90% sensitivity reported by Yeruva *et al*. (2018) [38]. However, in contrast to both of these findings, a study by Jaggi *et al*. (2012) [42] reported a high rate of resistance to tigecycline (74.8%).

In this study, the isolates were categorized as MDR and XDR based on the definitions outlined in the methodology section. Among the *Pseudomonas* isolates, 37.7% exhibited MDR and 12.3% showed XDR. In contrast, *Acinetobacter* isolates demonstrated higher resistance rates, with 65.3% classified as MDR and 26.9% as XDR. These findings are consistent with a study by Gill *et al*. (2011) [43], which reported 22.7% MDR, 11% XDR, and 4.3% pandrug resistance (PDR) among *Pseudomonas* isolates. Furthermore, a study conducted by Yadav *et al*. (2020) [44] reported a high MDR rate of 82% among *Acinetobacter* isolates, which aligns with our findings. The variations in MDR and XDR rates observed across this study and others may be attributed to the fact that these patterns are influenced by environmental factors and the specific antimicrobial susceptibility patterns employed. The indiscriminate use of antibiotics in healthcare settings is a significant contributor to the emergence of MDR, while prolonged hospital stays, particularly in ICUs, serve as an additional risk factor for MDR development. The multidrug resistance in NFGNB is typically attributed to the presence of antimicrobial-inactivating enzymes, overexpression of multidrug efflux pumps, and alterations in penicillin-binding proteins (PBPs) resulting from genetic mutations [34].

One limitation of this study was our inability to comprehensively assess all risk factors and outcomes associated with NFGNB infections in patients, due to the constraints of a shorter follow-up period. Additionally, molecular analysis of the resistant phenotypes and genetic mechanisms of drug resistance could not be performed, as the Microbiology Department lacked a functional molecular laboratory during the study period. Despite these limitations, this study provides valuable insights into the resistance patterns of NFGNB and can serve as a foundation for further research in this area**.** These findings underscore the need for effective antibiotic stewardship policies aimed at reducing resistance and its subsequent impact on patient morbidity and mortality.

In conclusion, this study revealed a notable prevalence of multidrug resistance among NFGNB, with 37.7% of *Pseudomonas* isolates exhibiting MDR and 12.3% displaying XDR. Moreover, *Acinetobacter* isolates demonstrated even higher resistance rates, with 65.3% classified as MDR and 26.9% as XDR. This finding raises concerns about the rapid emergence of antibiotic resistance in NFGNB, which have demonstrated resistance to a broad spectrum of commonly used antibiotics, including β-lactams, sulfonamides, fluoroquinolones, and aminoglycosides. The emergence of MDR and XDR NFGNB in hospitalized patients is a significant public health concern, warranting enhanced antimicrobial stewardship and infection control measures. Our study also found that SSIs were the most common clinical condition associated with MDR and XDR NFGNB, highlighting the need for enhanced infection control measures, particularly in inpatient wards and ICUs where these bacteria are more prevalent. Therefore, we recommend implementing proper screening protocols for NFGNB in healthcare settings, regularly monitoring their antibiotic susceptibility profiles, and promoting judicious antibiotic use to effectively manage infections caused by these pathogens and mitigate the emergence of multidrug resistance. Additionally, our study underscores the gravity of carbapenem-resistant NFGNB, which pose a significant threat to infection control efforts in healthcare facilities, further emphasizing the need for enhanced surveillance and preventative measures.

Although this study was conducted in a single tertiary care hospital, the data obtained provide valuable insights into the emergence of drug-resistant NFGNB, contributing to the existing body of knowledge essential for managing infections caused by these pathogens. Strict adherence to proper infection control practices, including hand hygiene, and thorough disinfection of environmental surfaces and patient care items, is crucial to reduce colonization rates. Moreover, implementing effective antibiotic stewardship programs, such as hospital-based initiatives featuring case-based education through prospective audit and feedback, preauthorization, and personalized in-person interventions (*e.g*., 'handshake stewardship'), is essential to mitigate resistance patterns.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interests associated with this manuscript.

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