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Identification, Antibiogram, and Phenotypic Detection of β-Lactamase-Mediated Resistance in Non-Fermenting Gram-Negative Bacilli (NFGNB) from a Tertiary Care Hospital in Mysore

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ABSTRACT

Introduction: Non-fermenting Gram-negative bacilli (NFGNB) are emerging healthcare-associated pathogens whose rapidly escalating antimicrobial resistance poses substantial therapeutic challenges. This study aimed to determine the prevalence and antimicrobial resistance patterns of NFGNB and to detect β-lactamase-mediated resistance mechanisms (extended-spectrum β-lactamase [ESBL], metallo-β-lactamase [MBL], and AmpC) in a tertiary care hospital in Mysore, Karnataka, India. **Methods:** In this prospective two-month study, NFGNB were isolated from various clinical specimens (blood, pus, urine, synovial fluid, suction catheter tips, and endotracheal aspirates). Blood specimens were inoculated into brain–heart infusion broth and subcultured onto blood agar and MacConkey agar; all other specimens were plated directly and incubated aerobically at 37 °C for 16–18 h. Bacterial identification and antimicrobial susceptibility testing were performed using the BD Phoenix M50 system. Phenotypic detection of ESBL, MBL, and AmpC was carried out using combined disc-based methods, as the Clinical and Laboratory Standards Institute (CLSI) does not provide standardized confirmatory tests for these mechanisms in non-fermenters. **Results:** Of 3,000 clinical specimens, 50 yielded NFGNB (prevalence, 1.7%), with 64% originating from ICUs (95% CI: 49.2–77.1%). The predominant species were *Pseudomonas putida* (18%), *Pseudomonas aeruginosa* (16%), and *Acinetobacter baumannii* (16%). *P. putida* exhibited 100% resistance to ceftazidime, cefepime, ciprofloxacin, levofloxacin, piperacillin–tazobactam, tetracycline, and cefotaxime, while amikacin and gentamicin retained activity against 77.8% and 88.9% of isolates, respectively. *P. aeruginosa* showed high resistance to ceftazidime and cefepime (87.5% each) and to piperacillin–tazobactam and levofloxacin (75% each). *A. baumannii* was uniformly susceptible to colistin but resistant to most other agents. Phenotypic analysis revealed ESBL production in 28%, MBL production in 34%, and presumptive AmpC production in 92% of isolates, of which 60.9% were confirmed. Multiple β-lactamase types co-occurred in several isolates. **Conclusion:** The high prevalence of multidrug-resistant NFGNB, particularly extensively drug-resistant *P. putida* and carbapenem-resistant *Acinetobacter* species, underscores the urgent need for strengthened local antimicrobial resistance surveillance, timely identification, and continuous monitoring of resistance trends to guide targeted therapy and improve patient outcomes.

INTRODUCTION

Non-fermenting Gram-negative bacilli (NFGNB) comprise a diverse group of aerobic, non-spore-forming bacilli that cannot ferment glucose and instead utilize it oxidatively or not at all [1]. A study from the Kalinga Institute of Medical Sciences reported that the prevalence of NFGNB was 5.10% among all bacterial isolates, and 61.76% of them were identified as multidrug-resistant (MDR) [2]. Infections caused by NFGNB are typically opportunistic and include ventilator-associated pneumonia, bloodstream infections, and surgical site infections,

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particularly in patients with predisposing factors such as prolonged antimicrobial therapy, immunosuppression, and burn injuries. Additional risk factors include invasive medical procedures such as catheter use, surgery, tracheostomy, dialysis, and the presence of implanted devices [3].

Antimicrobial resistance among NFGNB has escalated significantly over recent years. Multiple molecular and enzymatic mechanisms contribute to antibiotic resistance in NFGNB, including mutations in porin genes, which reduce antibiotic entry, as well as the overexpression of efflux pumps, which actively expel antibiotics from the bacterial cell. Furthermore, resistance can arise from alterations in penicillin-binding proteins, which are the primary targets of β-lactam antibiotics. The production of chromosomal β-lactamases, enzymes that inactivate β-lactam antibiotics, also plays a significant role in antimicrobial resistance [4–6]. These mechanisms, including enzymatic inactivation of antibiotics, reduced outer membrane permeability, active efflux, and target-site modifications, contribute to a multifactorial resistance phenotype that limits treatment options for these organisms.

The increasing prevalence of MDR NFGNB in hospital-acquired infections presents a significant clinical challenge. NFGNB frequently exhibit high levels of antibiotic resistance, complicating clinical management. However, limited data exist on the species distribution and antimicrobial resistance profiles of NFGNB from tertiary care hospitals in Mysore, Karnataka. Therefore, the present study aimed to determine the prevalence and antimicrobial resistance patterns of NFGNB and to detect β-lactamase-mediated resistance mechanisms in Mysore, Karnataka.

MATERIAL AND METHODS

Study design and setting. A prospective study was conducted in the Department of Microbiology, Mysore Medical College and Research Institute, Mysore, Karnataka, over a 2-month period from 1 September 2024 to 31 October 2024. The study aimed to isolate, identify, and determine the antimicrobial susceptibility profiles and resistance mechanisms of NFGNB from clinical specimens.

Study population and sampling. The study population comprised inpatients admitted to general wards and intensive care units (ICUs). Of 3,000 clinical samples processed during the study period, all consecutive non-duplicate NFGNB isolates recovered from these samples were included in the study.

Specimen types and eligibility. The study included patients from whom the following clinical specimens were obtained: pus, blood, urine, synovial fluid, suction catheter tips, and endotracheal aspirates. Patients of all ages and both sexes admitted to ICUs and general wards were eligible. Sputum samples were excluded due to the high likelihood of oropharyngeal contamination. Endotracheal aspirates were included but interpreted using quantitative culture thresholds ($\geq 10^5$ CFU/mL) to increase specificity for true infection.

Sample collection. Clinical samples, including blood, pus, urine, synovial fluid, suction catheter tips, and endotracheal aspirates, were collected in sterile containers. Following aseptic technique, blood samples were inoculated into brain–heart infusion (BHI) broth bottles at blood-to-broth ratios of approximately 1:5 for neonates and 1:10 for adults. Clean-catch midstream urine samples were collected in sterile containers after cleansing of the perineal area. For catheterized patients, urine was aseptically aspirated from the catheter sampling port. Synovial fluid was obtained by percutaneous aspiration and transferred into a sterile screw-capped container. Endotracheal aspirates were collected aseptically by suctioning tracheal secretions through the endotracheal tube using a sterile suction trap. For suction catheter tip cultures, the distal 5-cm segment of the aseptically removed suction catheter was excised and placed in a sterile container for transport to the laboratory. For closed wounds, pus was aspirated from the wound cavity using a sterile syringe after skin antisepsis. For open wounds, surface debris was removed by irrigation with sterile saline or by debridement, and a tissue specimen was collected from the wound bas.

Sample processing and identification. Upon receipt, clinical specimens were inoculated onto blood agar and MacConkey agar. Blood culture broths were subcultured onto blood agar and MacConkey agar upon detection of growth. Urine samples were cultured by the semi-quantitative standard loop method, and a colony count of $\geq 10^5$ CFU/mL was considered significant. Endotracheal aspirates were processed by quantitative culture, with bacterial growth of $\geq 10^5$ CFU/mL interpreted as indicative of significant infection. All inoculated culture plates were incubated aerobically under ambient atmospheric conditions at 37 °C for 16–18 h. Bacterial identification and antimicrobial susceptibility testing were performed using the BD Phoenix M50 Automated Microbiology System.

Detection of resistance mechanisms. Phenotypic tests for β-lactamase production (Extended-spectrum β-lactamase (ESBL), Metallo-β-lactamase (MBL), and AmpC) were performed using combined disc-based methods, as the Clinical and Laboratory Standards Institute (CLSI) M100 does not provide standardized confirmatory tests for these mechanisms in non-fermenting Gram-negative bacilli [7].

A. ESBL production: ESBL production was detected using the combined-disc test. A ≥5 mm increase in the zone of inhibition for the ceftazidime–clavulanate disc compared to the ceftazidime disc alone was considered positive.

B. MBL production: MBL production was detected using the imipenem–EDTA combined disc test. A ≥7 mm increase in the zone of inhibition for the imipenem–EDTA disc compared to the imipenem disc alone was considered positive.

C. AmpC production: Isolates were screened for AmpC β-lactamase production using a cefoxitin (30 µg) disc; a zone of inhibition ≤18 mm was considered a positive screen. This screening cutoff captures both intrinsic chromosomal AmpC (common in non-fermenters) and acquired AmpC. All

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isolates that screened positive were subjected to a confirmatory cloxacillin-based combined-disc test using cefoxitin (30 µg) and cefoxitin/cloxacillin (30/200 µg) discs. A ≥4 mm increase in the zone of inhibition around the combination disc was considered indicative of AmpC production.

Quality control. Quality control for antimicrobial susceptibility testing using the BD Phoenix M50 system was performed with each new batch of media and identification/susceptibility panels using *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853, in accordance with CLSI guidelines. All quality-control results fell within the acceptable CLSI-defined ranges before patient results were reported.

Data analysis. Data were entered into Microsoft Excel and analyzed using IBM SPSS Statistics (Version 22; IBM Corp., Armonk, NY, USA). Categorical variables were expressed as frequencies (n) and proportions (%). The chi-square test was applied to compare resistance rates between ICU and ward isolates, and among the predominant bacterial species identified. A two-tailed *P*-value ≤ 0.05 was considered statistically significant.

RESULTS

Isolate distribution and antimicrobial susceptibility. A total of 50 NFGNB were isolated, with the majority (64%; 95% CI: 49.2–77.1%) isolated from ICU patients (P = 0.046) (Table 1). The most frequently identified species were *P. putida* (18%), *P. aeruginosa* (16%), and *A. baumannii* (16%). Antimicrobial susceptibility testing demonstrated widespread resistance, particularly to cephalosporins and fluoroquinolones. A high degree of multidrug resistance was observed across most species; notably, all *A. baumannii* isolates were susceptible to colistin. Phenotypic screening for resistance mechanisms indicated that of the 50 isolates, 14 (28%) were ESBL producers, 17 (34%) were MBL producers, and 46 (92%) screened positive by the cefoxitin disc test for presumptive AmpC production.

Table 1. Distribution of NFGNB isolates from ICUs and general wards

Ward/Unit	Isolates, n	Percentage (%)
Neonatal intensive care unit (NICU)	4	8
Medical intensive care unit (MICU)	16	32
Respiratory intensive care unit (RICU)	7	14
Surgical intensive care unit (SICU)	4	8
Paediatric intensive care unit (PICU)	1	2
General wards	18	36
Total	50	100

A significantly higher proportion of isolates originated from ICUs (64%, 32/50) than from general wards (36%, 18/50) (χ^2 = 3.92, df = 1, P = 0.048). Among the ICUs, the medical intensive care unit (MICU) was the most frequent source of NFGNB, accounting for 32% of all isolates. Among the 50 NFGNB isolates, 24 (48%) were recovered from blood samples. Suction catheter tips and urine accounted for 8 (16%) isolates each, followed by pus (7, 14%), endotracheal aspirates (2, 4%), and synovial fluid (1, 2%) (Table 2).

Notably, *P. putida* was isolated predominantly from blood and urine, whereas *S. maltophilia* was most frequently recovered from suction catheter tips.

Table 2. Distribution of NFGNB isolates from clinical samples

Organism	Blood	Pus	Suction catheter tip	Urine	Synovial fluid	Endotracheal aspirate	Total
Acinetobacter baumannii	4	1	1	1	0	1	8
Acinetobacter lwoffii/haemolyticus	5	0	1	0	0	0	6
Pseudomonas aeruginosa	0	4	1	3	0	0	8
Pseudomonas putida	4	1	1	2	1	0	9
Pseudomonas species	2	0	0	1	0	1	4
Pseudomonas stutzeri	1	0	1	0	0	0	2
Stenotrophomonas maltophilia	3	1	3	0	0	0	7
Sphingomonas paucimobilis	3	0	0	0	0	0	3
Moraxella catarrhalis	1	0	0	0	0	0	1
Achromobacter species	1	0	0	0	0	0	1
Burkholderia cepacia complex	0	0	0	1	0	0	1
Total	24	7	8	8	1	2	50

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Table 3. Antimicrobial susceptibility profile of *P. aeruginosa* isolates (n=8)

Antibiotic	Resistant, n (%)	Intermediate, n (%)	Susceptible, n (%)
Ceftazidime	7 (87.5)	0 (0)	1 (12.5)
Cefepime	7 (87.5)	0 (0)	1 (12.5)
Ciprofloxacin	2 (25.0)	0 (0)	6 (75.0)
Levofloxacin	6 (75.0)	0 (0)	2 (25.0)
Imipenem	4 (50.0)	0 (0)	4 (50.0)
Meropenem	4 (50.0)	0 (0)	4 (50.0)
Amikacin	5 (62.5)	0 (0)	3 (37.5)
Piperacillin-tazobactam	6 (75.0)	0 (0)	2 (25.0)
Aztreonam	4 (50.0)	0 (0)	4 (50.0)

P. aeruginosa isolates exhibited marked resistance to major anti-pseudomonal agents (Table 3). Notably, high rates of resistance were observed to third- and fourth-generation cephalosporins, with 87.5% resistance to both ceftazidime and cefepime, and 75% resistance to piperacillin–tazobactam. Additionally, 50% of the isolates were resistant to carbapenems, representing a considerable therapeutic concern.

Table 4. Antibiotic susceptibility patterns of *P. putida* and *P. stutzeri*

Antibiotic	*P. putida* (n = 9), n (%)			*P. stutzeri* (n=2), n (%)		
	*R, n (%)	**S, n (%)	***I, n (%)	R, n (%)	S, n (%)	I, n (%)
Ceftazidime	9 (100)	0 (0)	0 (0)	1 (50.0)	1 (50.0)	0 (0)
Cefepime	9 (100)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)
Ciprofloxacin	9 (100)	0 (0)	0 (0)	1 (50.0)	1 (50.0)	0 (0)
Levofloxacin	9 (100)	0 (0)	0 (0)	1 (50.0)	1 (50.0)	0 (0)
Imipenem	3 (33.3)	4 (44.4)	2 (22.2)	0 (0)	0 (0)	2 (100)
Meropenem	4 (44.4)	3 (33.3)	2 (22.2)	0 (0)	0 (0)	2 (100)
Amikacin	2 (22.2)	7 (77.8)	0 (0)	1 (50.0)	1 (50.0)	0 (0)
Piperacillin–tazobactam	9 (100)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)
Aztreonam	7 (77.8)	1 (11.1)	1 (11.1)	2 (100)	0 (0)	0 (0)
Gentamicin	0 (0)	8 (88.9)	1 (11.1)	1 (50.0)	1 (50.0)	0 (0)
Tetracycline	9 (100)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)
Cefotaxime	9 (100)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)

*R = Resistant; **S = Susceptible; ***I = Intermediate

P. putida isolates exhibited 100% resistance to ciprofloxacin, levofloxacin, piperacillin–tazobactam, ceftazidime, cefepime, tetracycline, and cefotaxime (Table 4), while resistance to imipenem and meropenem was observed in 33.3% and 44.4% of isolates, respectively. Notably, amikacin and gentamicin retained activity against 77.8% and 88.9% of isolates, respectively. The two *P. stutzeri* isolates showed 100% resistance to cefepime, piperacillin–tazobactam, aztreonam, tetracycline, and cefotaxime, and 50% resistance to ceftazidime, ciprofloxacin, levofloxacin, amikacin, and gentamicin; both isolates exhibited intermediate susceptibility to imipenem and meropenem.

Among the organisms isolated eight times each, *A. baumannii* and *P. aeruginosa* were the most common (n = 8 each), followed by *P. putida* **(**n = 9**)** as the single most frequent species. *A. baumannii* showed complete susceptibility (100%) to colistin, with imipenem and ciprofloxacin susceptibility of 50% and 37.5%, respectively. Resistance rates were highest for ceftazidime and cefotaxime (100% each), followed by ceftriaxone (87.5%), and cefepime, levofloxacin, gentamicin, meropenem, amikacin, piperacillin–tazobactam, and cotrimoxazole (75% each) (Table 5). Both *Acinetobacter* species remained fully susceptible to colistin. The *A. lwoffii*/*haemolyticus* group (n = 6) showed complete resistance (100%) to ceftazidime, cefepime, imipenem, meropenem, ceftriaxone, and cefotaxime.

Phenotypic characterization of β-lactamase-mediated resistance mechanisms among the NFGNB isolates (Table 6) revealed that of the 50 isolates tested, 14 (28%; 95% CI: 17.2–41.3 **%**) showed ESBL production, and 17 (34%; 95% CI: 22.1–47.4%) showed MBL production. In addition, 46 (92%; 95% CI: 80.8–97.8%) screened positive for presumptive AmpC production, of which 28 (60.9%; 95% CI: 45.4–74.9%) were confirmed by the cloxacillin combined-disc test.

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Table 5. Antimicrobial susceptibility profile of *A. baumannii* and *A. lwoffii/haemolyticus* group

Antibiotics	*A. baumannii* (n=8)			*A. lwoffi/hemolyticus* (n=6)		
	*R, n (%)	**S, n (%)	***I, n (%)	R, n (%)	S, n (%)	I, n (%)
Ceftazidime	8 (100)	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)
Cefepime	6 (75)	2 (25)	0 (0)	6 (100)	0 (0)	0 (0)
Ciprofloxacin	5 (62.5)	3 (37.5)	0 (0)	4 (66.7)	1 (16.7)	1 (16.7)
Levofloxacin	6 (75)	2 (25)	0 (0)	4 (66.7)	2 (33.3)	0 (0)
Gentamicin	6 (75)	1 (12.5)	1 (12.5)	3 (50)	2 (33.3)	1 (16.7)
Imipenem	4 (50)	4 (50)	0 (0)	6 (100)	0 (0)	0 (0)
Meropenem	6 (75)	2 (25)	0 (0)	6 (100)	0 (0)	0 (0)
Amikacin	6 (75)	2 (25)	0 (0)	3 (50)	1 (16.7)	2 (33.3)
Piperacillin–tazobactam	6 (75)	2 (25)	0 (0)	4 (66.7)	0 (0)	2 (33.3)
Cotrimoxazole	6 (75)	2 (25)	0 (0)	4 (66.7)	1 (16.7)	1 (16.7)
Ceftriaxone	7 (87.5)	1 (12.5)	0 (0)	6 (100)	0 (0)	0 (0)
Colistin	0 (0)	8 (100)	0 (0)	0 (0)	6 (100)	0 (0)
Cefotaxime	8 (100)	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)

*R = Resistant; **S = Susceptible; ***I = Intermediate

Table 6. Phenotypic detection of β-lactamase production among NFGNB isolates (n = 50)

Resistance mechanism/test	Positive, n (%)	Negative, n (%)	Total
ESBL production	14 (28.0)	36 (72.0)	50
MBL production	17 (34.0)	33 (66.0)	50
AmpC screening (cefoxitin disc)	46 (92.0)	4 (8.0)	50
AmpC confirmation (cloxacillin synergy test)	28 (60.9)	18 (39.1)	46*

*Denominator is 46; only isolates with a positive AmpC screening test underwent confirmatory testing.

Statistical analysis using the chi-square test revealed significant differences in susceptibility for several antibiotics between ICU and ward isolates. Significant differences were observed for ciprofloxacin (P = 0.040), gentamicin (P = 0.020), tetracycline (P = 0.005), imipenem (P = 0.002), meropenem (P = 0.003), and amikacin (P = 0.046); no significant differences were observed for the remaining antibiotics tested.

DISCUSSION

NFGNB are increasingly recognized as important opportunistic pathogens in hospital settings. Their frequent isolation from critically ill patients reflects both their environmental resilience and their ability to cause infection in individuals with compromised immunity or those undergoing invasive procedures [3]. The predominance of isolates from ICUs in this study underscores the significance of NFGNB as healthcare-associated pathogens and reinforces the need for stringent infection prevention measures in high-risk clinical settings.

Among the isolates, *P. putida* and *A. baumannii* emerged as key species, consistent with other regional reports [8-10]. Their high resistance rates to fluoroquinolones and cephalosporins emphasize the growing burden of multidrug-resistant NFGNB in clinical practice. These resistance patterns pose a significant clinical challenge owing to the limited therapeutic options available for such infections.

The phenotypic detection of ESBL, MBL, and AmpC β-lactamases in the current study indicates the presence of multiple resistance mechanisms within the NFGNB population, some of which coexisted within a single isolate. Such combined enzyme production severely limits treatment choices and has been associated with hospital outbreaks and poor patient outcomes [11].

These findings underscore the urgent need for sustained infection control practices, including hand hygiene, contact precautions, and environmental decontamination, combined with strict antimicrobial stewardship to prevent further dissemination of resistant NFGNB strains.

The ICU predominance observed here (64%) is comparable to the 56.9% ICU isolation rate reported by Adan *et al*. [12]. This high prevalence may be attributable to weakened immune systems, prolonged hospital stays, and the frequent use of invasive devices in critically ill patients.

Blood was the most common specimen type (48%), a finding that contrasts with the considerably higher proportion (91.4%) reported by Ndzabandzaba *et al*. [13]. The ability of NFGNB to colonize medical equipment, environmental surfaces, and healthcare workers increases the likelihood of their introduction into the bloodstream during invasive procedures or through breaches in skin integrity. Furthermore, their multidrug resistance facilitates survival and proliferation in the bloodstream in the presence

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of antibiotic therapy, potentially leading to persistent bloodstream infections [3].

Regarding species distribution, among the 50 NFGNB isolates, *P. putida* (n = 9) was the most frequently isolated, followed by *P. aeruginosa* (n = 8), *A. baumannii* (n = 8), and *S. maltophilia* (n = 7). Less commonly isolated organisms included *Achromobacter* spp., *M. catarrhalis*, and *Burkholderia cepacia* complex (n = 1 each). Although *P. putida* has traditionally been regarded as less pathogenic than other species within the genus *Pseudomonas*, it was the most common NFGNB in the present study. This finding is consistent with Usha Rani and Vijayalakshmi [8], who reported that 33.3% of their NFGNB isolates were *P. putida*. This higher prevalence may be associated with the ability of *P. putida* to survive under harsh conditions on hospital surfaces [14].

In the present study, *P. putida* isolates exhibited higher resistance rates to levofloxacin and ciprofloxacin (100% each) and 33.3% resistance to imipenem than those reported by Sader *et al*., who reported resistance rates of 20.2% for levofloxacin and 21.7% for ciprofloxacin [15]. This discrepancy may be attributable to selective antibiotic pressure contributing to localized resistance emergence [5, 6]. Furthermore, the *P. putida* isolates in this study demonstrated increased resistance to commonly used antimicrobials, including carbapenems. Although *P. putida* was historically considered an organism of low pathogenicity and high antimicrobial susceptibility [16], its isolation rate from clinical specimens has increased in recent years, paralleling reports of multidrug-resistant strains [17, 18]. This may be attributed to the ability of *P. putida* to survive for extended periods on environmental surfaces, overexpression of efflux pumps, and production of β-lactamases [16–18].

In the present study, among the *P. aeruginosa* isolates, 87.5% resistance to ceftazidime and cefepime was observed, followed by levofloxacin (75%). These findings are broadly comparable to those of Gondha *et al*., who reported resistance rates of 60.61% to ceftazidime, 61.66% to cefepime, and 69.93% to levofloxacin [9]. The high resistance rates to various antimicrobial classes may be attributed to multiple intrinsic and acquired mechanisms, such as active efflux, reduced membrane permeability, and enzymatic inactivation of antibiotics [9].

Among *Acinetobacter* species, *A. baumannii* (*n* = 8) showed susceptibility rates of 100% to colistin, 50% to imipenem, and 37.5% to ciprofloxacin. In comparison, Parandekar and Peerapur [19] reported 73.56% susceptibility to imipenem and 87.5% susceptibility to ciprofloxacin. The *A. baumannii* isolates in the present study showed 100% resistance to ceftazidime, 87.5% to ceftriaxone, and 75% to cefepime, levofloxacin, gentamicin, meropenem, amikacin, piperacillin–tazobactam, and cotrimoxazole. These findings align with those of Norris *et al.* [20], who reported resistance rates of 77.6% to ceftazidime, 82.3% to cefepime, 78.5% to gentamicin, 59.8% to amikacin, and 69.2% to cotrimoxazole. The *A. lwoffii/haemolyticus* group showed 100% susceptibility to colistin but only 33.3% susceptibility to tetracycline (Table 5). Given the high carbapenem resistance observed in *Acinetobacter* species, colistin remains one of the few reliably active agents, underscoring the limited therapeutic options for these infections [9]. This rapid emergence of multidrug-resistant and pandrug-resistant strains of *Acinetobacter* species reflects the organisms' ability to adapt to environmental pressures through enhancement of intrinsic resistance mechanisms and acquisition of exogenous resistance genes [19].

In the present study, 46 of 50 isolates (92%) screened positive for presumptive AmpC production by the cefoxitin disc test, a proportion notably higher than the 57% (80/140) reported by Inamdar and Anuradha [21]. This high screening positivity likely reflects the intrinsic chromosomal AmpC present in most non-fermenters, rather than acquired AmpC alone. Among these 46 screen-positive isolates, 28 (60.9%) were confirmed as AmpC producers by the cloxacillin double-combined-disc test. This finding is consistent with Wassef *et al.* [22], who reported that 56.5% of AmpC screen-positive isolates were confirmed by the cloxacillin combined-disc test. This may be attributable to mutations in the *ampD* gene, which can lead to derepression of AmpC production and constitutive hyperproduction in clinical isolates [23].

In settings with a high prevalence of AmpC-producing organisms, as observed in the present study, strict hand hygiene, contact precautions, patient isolation or cohorting, and thorough environmental cleaning are essential to prevent transmission [24]. Antimicrobial stewardship programs, active surveillance cultures, and staff education are critical to limiting the emergence and dissemination of AmpC-producing organisms [7, 24].

In the present study, 14 (28%) of the 50 isolates were ESBL producers and 17 (34%) were MBL producers, findings higher than those of Gupta *et al.* [25], who reported that 21.4% of their isolates were ESBL producers and 21.4% were MBL producers. The production of multiple β-lactamases has been associated with treatment failure, and poses a significant clinical challenge [20]. Reports of MBL-producing NFGNB are increasing globally, driven by the widespread use of carbapenems and the associated antibiotic selection pressure [11, 25]. Consequently, early detection of isolates producing these enzymes is crucial to prevent treatment failure and healthcare-associated outbreaks.

The high resistance rates to cephalosporins and fluoroquinolones observed in *P. aeruginosa* and *A. baumannii* isolates in this study align with reports from tertiary centers in Gujarat [9], Punjab [26], and Saudi Arabia [27]. *P. putida* was the predominant species in the present study, consistent with findings from Central China, where *P. putida* infections demonstrated high resistance to β-lactams and fluoroquinolones among hospitalized patients [10]. Comparable trends have been reported in Indian tertiary care centers, where *P. putida* and *A. baumannii* were frequently isolated from critically ill patients and exhibited high rates of multidrug resistance [9, 28].

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Consistent with the present findings, studies from Gujarat and Punjab identified *Pseudomonas* and *Acinetobacter* species as the most common NFGNB, with these organisms exhibiting high rates of resistance to cephalosporins and carbapenems [9, 26]. These reports underscore the widespread dissemination of resistant NFGNB across India and reinforce the urgent need for strengthened infection prevention measures and antimicrobial stewardship programs.

Recent reviews have highlighted the evolving microbiology and pathogenesis of NFGNB, noting that these organisms possess multiple intrinsic and acquired resistance mechanisms that promote their persistence in healthcare settings [5]. The diversity of these resistance mechanisms underscores the importance of combining phenotypic testing, as performed in the present study, with molecular surveillance to fully characterize the resistance profile of clinical isolates [5].

Studies from tertiary care hospitals in Karnataka and Kolkata also reported high resistance rates across multiple drug classes, with colistin remaining the only consistently active agent [29, 30]. These findings corroborate the results of the present study and underscore the therapeutic challenge posed by NFGNB infections, particularly in intensive care units.

Large-scale surveillance data from Saudi Arabia [27], together with reports from other regions [5, 9, 26], highlight the persistent and growing global challenge of antimicrobial resistance among NFGNB. Similar resistance trends have also been reported in Indian tertiary care settings [26, 28-30]. The resistance patterns observed in the present study are consistent with these findings, further emphasizing the increasing burden of multidrug-resistant NFGNB in healthcare settings.

The primary limitations of this study were the small sample size (n = 50), constrained by both the two-month study duration and the high cost of identification panels, and the single-center design, which limits generalizability. Molecular confirmation of resistance genes was not performed, which could have enabled characterization of the specific genetic determinants underlying the observed resistance phenotypes. Additionally, sputum samples were excluded owing to the difficulty in distinguishing true infection from colonization; this may have led to an underestimation of the role of NFGNB in respiratory infections, a major category of healthcare-associated infections. Moreover, the cefoxitin disc screen and cloxacillin-based confirmatory test for AmpC, as well as the combined-disc test for ESBL, are primarily validated for Enterobacterales; their application to NFGNB in this study should be interpreted with caution, as CLSI has not formally endorsed these methods for non-fermenters. Finally, the short study duration is unlikely to capture seasonal variations in NFGNB prevalence and resistance patterns.

In conclusion, this study underscores the clinical significance of NFGNB as multidrug-resistant pathogens, revealing a high prevalence of resistant strains in a tertiary care hospital in Mysore. The predominant species were highly multidrug-resistant *P. putida* and carbapenem-resistant *Acinetobacter* species, while *A. baumannii* and the *A. lwoffii/haemolyticus* group remained uniformly susceptible to colistin. β-Lactamase production was widespread: 28% (14/50) were ESBL producers, 34% (17/50) were MBL producers, and 92% (46/50) screened positive for presumptive AmpC production, of which 60.9% (28/46) were confirmed. These findings highlight the urgent need for sustained antimicrobial stewardship, robust infection control measures, and ongoing local surveillance to limit the dissemination of resistant NFGNB strains. Strengthening laboratory infrastructure and expanding diagnostic capacity, including the integration of molecular methods alongside phenotypic testing, are essential to enhance antimicrobial resistance monitoring and inform evidence-based policies. Timely identification, continuous susceptibility monitoring, and rigorous infection control practices remain critical for mitigating the clinical impact of NFGNB and improving patient outcomes.

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CONFLICTS OF INTEREST

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

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DATA AVAILABILITY

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

AI DISCLOSURE

Artificial intelligence (AI)-assisted language editing tools were used solely to improve the grammar and readability of the manuscript. All scientific content, data analysis, interpretation, and conclusions were conceived, verified, and approved by the authors.

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AUTHORS' CONTRIBUTIONS

SM & ShM: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Visualization. AKB: Investigation, Data curation, Formal analysis, Writing – review & editing. PR: Data curation, Validation, Writing – review & editing. BA and SM: Supervision, Project administration, Resources, and critical review of the manuscript.

ETHICS STATEMENT

This study was approved by the Institutional Ethics Committee, Mysore Medical College and Research Institute (Approval No.: ECR/Inst/KA/2013/RR-19).

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