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Original Article **Open Access**

Antibiotic resistance profile of Non-Fermenting Gram-negative bacteria recovered from clinical specimens in a tertiary care teaching hospital, Hyderabad, India

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Abstract

Background: Non-Fermenting Gram-negative bacilli (NFGNB), including *Pseudomonas aeruginosa*, *Acinetobacter* species, and *Burkholderia* species, are emerging as significant opportunistic pathogens, especially in immunocompromised and hospitalized patients. These organisms pose a major therapeutic challenge due to their multidrug-resistance (MDR) and intrinsic resistance mechanisms. The goal of this study is to determine antibiotic susceptibility pattern of NFGNB isolated from clinical specimens of patients in a tertiary care teaching hospital in Hyderabad, India.
Methodology: A descriptive cross-sectional observational study of patients with clinical infections was conducted between December 2024 and April 2025, to identify NFGNB from their clinical specimens. Different clinical samples (blood, sputum, urine, pus and others) were collected from patients of both sexes and all age groups for bacterial isolation using automated Bact/Alert for blood culture and conventional method for other samples. Identification of NFGNB isolates and antimicrobial susceptibility testing (AST) of the isolates against selected antibiotics were performed using the VITEK 2 Compact system, and AST interpretations were made in line with the Clinical and Laboratory Standards Institute (CLSI) guidelines.
Results: During the study period, clinical samples of 1575 patients were processed, 359 were positive for bacterial isolates on cultures, and 32 NFGNB were identified from clinical samples of 32 patients with clinical infections. Among the 32 isolates, *Pseudomonas* accounted for 78.0% (n=25) followed by *Acinetobacter* (13.0%, n=4) and *Burkholderia* (9.0%, n=3). NFGNB infections were more frequent in males (78.0%) and patients aged 51-75 years. Majority (78.0%) of isolates were from inpatients. *Pseudomonas* showed high resistance to fluoroquinolones (36.0%) but sensitive to piperacillin/tazobactam (84.0%) and β-lactam combinations (76.0%). *Acinetobacter* isolates exhibited 75.0% resistance to multiple antibiotics but 50.0% were sensitive to carbapenems and β-lactam/β-lactamase inhibitor combinations. *Burkholderia* isolates showed complete resistance to cefepime, cotrimoxazole and amikacin, but were 100.0% sensitive to piperacillin/tazobactam.
Conclusion: The study underscores the alarming levels of antimicrobial resistance among NFGNB, particularly in hospitalized and elderly patients. The findings highlight the necessity of continuous antimicrobial resistance surveillance, stringent infection control, and robust antibiotic stewardship to combat the growing threat of multidrug-resistant organisms.

Keywords: Non-Fermenting Gram-negative bacilli; Multi-drug resistance; Clinical specimens

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Profil de résistance aux antibiotiques des bactéries Gram-négatives Non-Fermentantes issues d'échantillons cliniques d'un hôpital universitaire de soins tertiaires, Hyderabad, Inde

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Résumé

Contexte: Les bacilles Gram-négatif Non-Fermentants (BGNF), notamment *Pseudomonas aeruginosa*, *Acinetobacter* et *Burkholderia*, apparaissent comme des pathogènes opportunistes importants, notamment chez

les patients immunodéprimés et hospitalisés. Ces organismes représentent un défi thérapeutique majeur en raison de leurs mécanismes de multirésistance aux antibiotiques (MDR) et de résistance intrinsèque. L'objectif de cette étude est de déterminer le profil de sensibilité aux antibiotiques du NFGNB isolé à partir d'échantillons cliniques de patients d'un hôpital universitaire de soins tertiaires à Hyderabad, en Inde.
Méthodologie: Une étude observationnelle descriptive transversale a été menée auprès de patients atteints d'infections cliniques entre décembre 2024 et avril 2025 afin d'identifier le NFGNB à partir de leurs échantillons cliniques. Différents échantillons cliniques (sang, expectorations, urine, pus et autres) ont été prélevés chez des patients des deux sexes et de tous âges afin d'être isolés bactériennement à l'aide du système automatisé Bact/Alert pour les hémocultures et de la méthode conventionnelle pour les autres échantillons. L'identification des isolats de NFGNB et les tests de sensibilité aux antimicrobiens (AST) des isolats contre certains antibiotiques ont été réalisés à l'aide du système VITEK 2 Compact. Les interprétations des AST ont été réalisées conformément aux recommandations du Institut des Normes Cliniques et de Laboratoire (CLSI).
Résultats: Au cours de la période d'étude, des échantillons cliniques de 1575 patients ont été traités, 359 étaient positifs pour les isolats bactériens sur les cultures et 32 NFGNB ont été identifiés à partir d'échantillons cliniques de 32 patients atteints d'infections cliniques. Parmi les 32 isolats, *Pseudomonas* représentait 78,0% (n=25), suivi par *Acinetobacter* (13,0%, n=4) et *Burkholderia* (9,0%, n=3). Les infections à NFGNB étaient plus fréquentes chez les hommes (78,0%) et les patients âgés de 51 à 75 ans. La majorité (78,0%) des isolats provenaient de patients hospitalisés. *Pseudomonas* a montré une résistance élevée aux fluoroquinolones (36,0%) mais une sensibilité aux combinaisons pipéracilline/tazobactam (84,0%) et β-lactamines (76,0%). Les isolats d'*Acinetobacter* présentaient une résistance de 75,0 % à de multiples antibiotiques, mais 50,0% étaient sensibles aux carbapénèmes et aux associations β-lactamines/inhibiteurs de β-lactamases. Les isolats de Burkholderia présentaient une résistance complète au céfépime, au cotrimoxazole et à l'amikacine, mais une sensibilité de 100,0% à la pipéracilline/tazobactam.
Conclusion: Cette étude souligne les niveaux alarmants de résistance aux antimicrobiens chez les patients atteints de NFGNB, en particulier chez les patients hospitalisés et les personnes âgées. Ces résultats soulignent la nécessité d'une surveillance continue de la résistance aux antimicrobiens, d'un contrôle rigoureux des infections et d'une gestion rigoureuse des antibiotiques pour lutter contre la menace croissante des organismes multirésistants.

Mots-clés: Bacilles à Gram-négatif Non-Fermentants; Multirésistance aux antibiotiques; Échantillons cliniques

Introduction:

Non-Fermenting Gram-negative bacilli (NFGNB) represent a varied group of bacteria that either utilize oxidative pathways to utilize carbohydrates for energy or are incapable of metabolizing them. These organisms have become causes of infections linked to healthcare, especially in immunocompromised patients (1). The two most frequently encountered non-fermenters that are pathogenic to humans are *Pseudomonas* and *Acinetobacter*. Other NFGNB species such as *Elizabethkingia meningoseptica*, *Burkholderia cepacia*, and *Stenotrophomonas maltophilia* are less frequent but are becoming increasingly reported, especially in immunocompromised patients, and those who have been hospitalised for an extended period (2).

These organisms are primarily saprophytic and were once regarded as mere contaminants or harmless commensals with minimal clinical importance (3). In the recent times however, NFGNB are responsible for several infections, including urinary tract infection (UTI), meningitis, wound infections, osteomyelitis, septicemia, and pneumonia (4). These infections are challenging to treat because of their multidrug-resistance (MDR) and rapid onset of high level MDR to different antibiotic classes such as fluoroquinolones, aminoglycosides and beta-lactam drugs (5). These organisms are often resistant to several antibiotics due to their capacity to produce enzymes such as metallo-beta-lactamase (MBL) and extended-spectrum beta lactamase (ESBL) which inactivate many beta-lactam drugs (5).

The increasing antibiotic resistance among NFGNB is a significant global concern, potentially leading to adverse clinical outcomes such as therapeutic failure, progression to bacteremia, the need for intravenous treatment, hospital admission, and prolonged hospital stays. A wide array of causes, such as improper antibiotic usage in humans and animals, environmental contamination, poor sanitation, and innate characteristics of bacteria, contribute to the complex, constantly evolving issue of antimicrobial resistance (6). Understanding the various antibiotic susceptibility pattern among NFGNB isolated from various clinical specimens was the goal of this study.

Materials and method:

Study setting, design and specimen collection:

This study was carried out in the Department of Microbiology of Apollo Institute of Medical Sciences and Research, Hyderabad, India. The study was a descriptive cross-sectional observational design carried out between December 2024 and April 2025 on patients who had Non-Fermenting Gram-negative bacilli (NFGNB) isolated from their clinical samples.

Specimens such as sputum, wound swabs, blood, urine, and others were collected from patients of both sexes and all ages, presenting with suspected infections in both

inpatient and outpatient departments. The samples were promptly transported to the microbiology laboratory for microbiological analysis.

Bacterial isolation:

To perform blood culture, 2-3 ml of paediatric blood and 5-10 ml of adult blood were aseptically collected into Bact/Alert PF Plus culture bottles for children and Bact/Alert FA Plus culture bottles for adults. The bottles were incubated in Bact/Alert 3D-60 automated system (BioMérieux SA) and monitored for a maximum of five days. An aliquot from every culture positive bottle was subcultured onto standard media (Chocolate, Blood and MacConkey agar plates) for bacteria isolation. Conversely, specimens such as urine, pus, sputum were directly seeded onto these media and subsequently incubated at 37℃ for 18 to 24 hours.

Bacterial identification and antibiotic susceptibility testing:

Non-Fermenting Gram-negative bacilli were identified based on conventional microbiological procedures such as colony morphology, Gram staining, and motility. Using the Vitek® 2 Compact system (BioMérieux, SA), Gram-negative identification card (ID-GN) was used for bacterial species identification, and AST-N406 susceptibility card was used for antibiotic susceptibility test (AST) in accordance with the manufacturer's standard protocol against piperacillin/tazobactam, levofloxacin, ciprofloxacin, ceftriaxone, ceftazidime, cefepime, cefoperazone/sulbactam, imipenem, meropenem, amikacin, minocycline, and trimethoprim-sulphamethoxazole (cotrimoxazole).

The turbidity of the inoculum prepared from bacterial colonies was adjusted to 0.5 MacFarland standard using DensiCHEK device (BioMérieux SA), after which the standardized inoculum was suspended in 3 ml sterile 0.45% sodium chloride solution. The results of AST were interpreted using the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results:

Distribution of Non-Fermenting Gram-negative bacilli (NFGNB) isolates:

Over the period of the study, clinical samples of 1575 patients were processed in the microbiology laboratory, and 359 were positive for bacterial isolates on cultures. Of these culture-positive samples, 32 Non-Fermenting Gram-negative bacilli (NFGNB) were identified from samples of 32 patients. Fig 1 shows that among the 32 NFGNB isolates, *Pseudomonas* accounted for 25 (78.0%), followed by *Acinetobacter* species with 4 isolates (13.0%), and *Burkholderia* species with 3 isolates (9.0%).

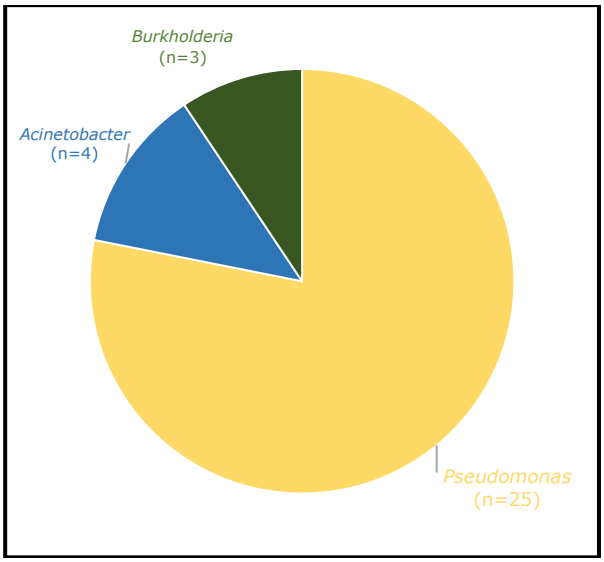

Fig 1: Frequency distribution of Non-Fermenting Gram-negative bacilli isolates from clinical samples of 32 patients

A higher frequency of NFGNB (n=25, 78.0%) were isolated on cultures from male patients compared to 7 (22.0%) from female patients (Table 1). The most frequently affected age group was between 51-75 years (n= 15, 47.0%) followed by 26-50 years group (n=14, 44.0%) and 1-25 years group (n=3, 9.0%). Twenty-five isolates (78.0%) were recovered from in-patient setting, while 7 (22%) were isolated from out-patient setting.

Table 1: Distribution of Non-Fermentative Gram-negative bacilli isolates with respect to sex, age group and location of patients

Variable	Frequency	Percentage
Age group (years)		
1-25	3	9.0
26-50	14	44.0
51-75	15	47.0
Sex		
Male	25	78.0
Female	7	22.0
Patient location		
In-patient	25	78.0
Out-patient	7	22.0

Antibiotic susceptibility test results of NFGNB isolates:

Antibiotic susceptibility results of the 25 *Pseudomonas* isolates revealed highest resistance to levofloxacin and ciprofloxacin (36.0%, n=9 each), followed by ceftazidime and imipenem (28.0%, n=7 each). In contrast, the isolates showed high sensitivity to piperacillin/tazobactam (84.0%, n=21), followed by cefepime, cefoperazone/sulbactam, meropenem and amikacin (76.0%, n=19 each) (Fig 2).

Fig 3 shows that among the 4 *Acinetobacter* isolates, resistance was most frequently observed against amikacin, cefepime,

ceftriaxone, ciprofloxacin, and cotrimoxazole (75.0%, n=3 each). However, 50.0% of the isolates (n=2) were sensitive to each of piperacillin/tazobactam, imipenem, meropenem, and cefoperazone/sulbactam.

For the 3 *Burkholderia* isolates, they were completely resistant (100.0%, n=3) to cefepime, cotrimoxazole and amikacin, but completely sensitive to piperacillin/tazobactam (100.0%, n=3), and 66.7% (n=2) sensitive to meropenem, ceftazidime and cefoperazone/sulbactam as shown in Fig 4.

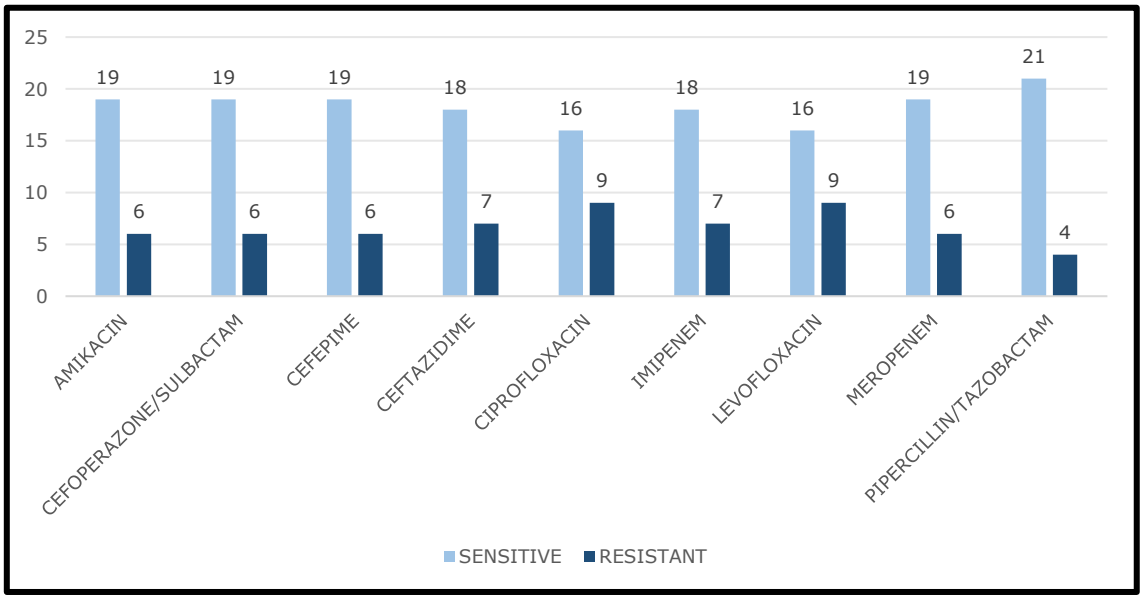

Fig 2: Antibiotic susceptibility of *Pseudomonas* isolates

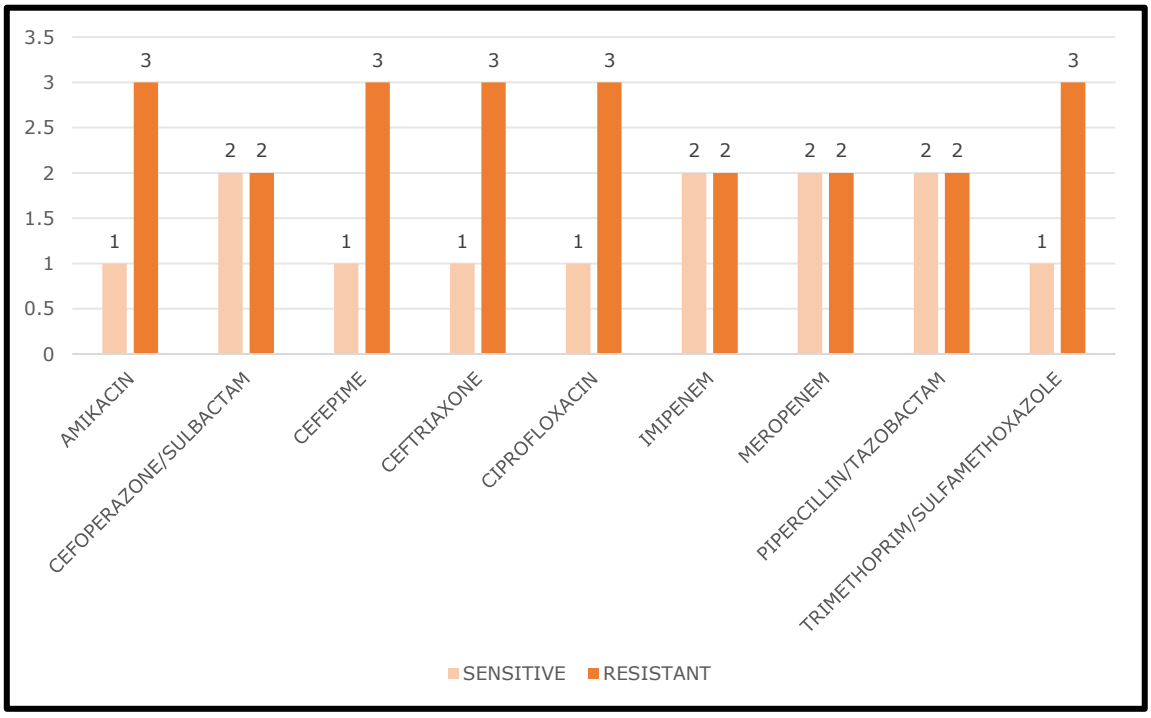

Fig 3: Antibiotic susceptibility of *Acinetobacter* isolates

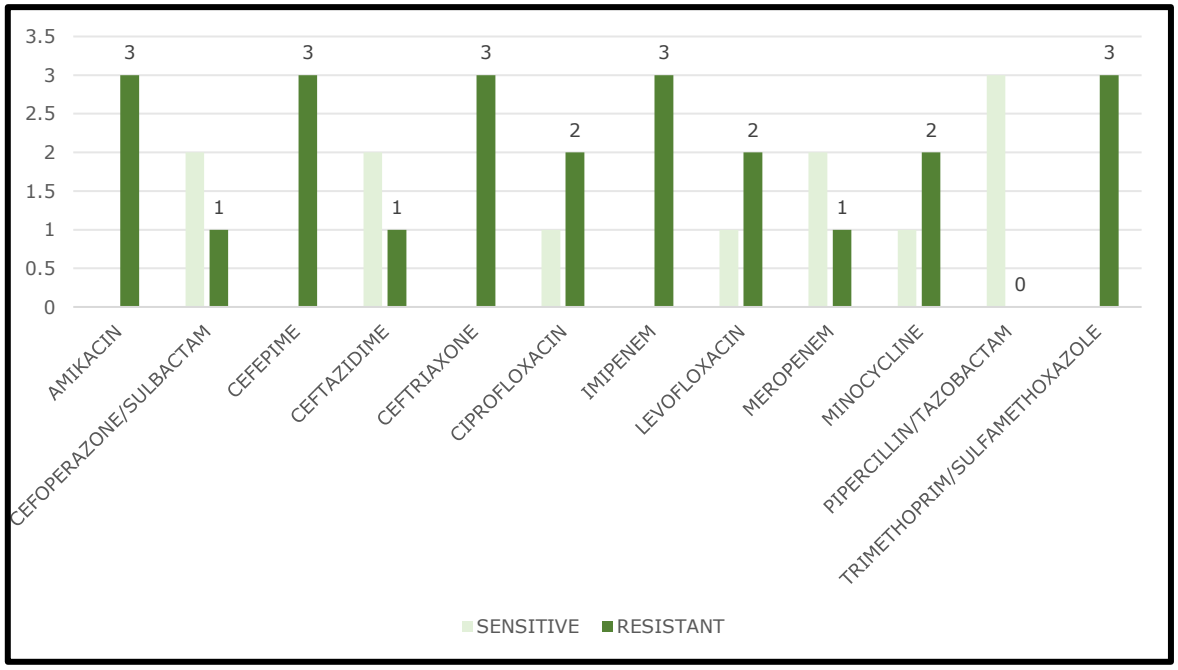

Fig 4: Antibiotic susceptibility of *Burkholderia* isolates

Multi-drug resistance among NFGNB isolates:

Multi-drug resistance (MDR), defined as resistance to at least three antibiotics in different classes of antimicrobial agents, is seen 10 of 25 (40.0%) *Pseudomonas* isolates, 3 of 4 (75.0%) *Acinetobacter* isolates, and all 3 (100.0%) *Burkholderia* isolates.

Discussion:

In our study, a higher proportion of NFGNB were isolated from males (78.0%) compared to females (22.0%). This male dominance is consistent with results from the findings of the study by Ghanshani et al., (7) who reported that 68% of their patients were men. Gender-based differences such as genetic and hormonal factors may account for this disparity (8). The present study showed that the most affected age group was 51-75 years (47.0%), followed by 26-50% (44.0%). This trend is consistent with findings by Taj et al., (9) where most of the patients with infections were in the 51-60 years group. Age-related decrease in immunity and increased risk of co-morbidities may explain the higher prevalence in older adults.

In terms of patient settings, 22.0% of isolates were from the outpatient and 78.0% came from inpatient. This distribution illustrates how hospitalized patients, are more likely to have infections caused by NFGNB as a result of factors such as invasive surgeries and prolonged hospital stay (10). *Pseudomonas aeruginosa* isolates in this study exhibited highest resistance to levofloxacin and ciprofloxacin (36%), which is comparable to the findings of a study by Tiwari et al., (12) of 43% and Kanthakumar et al., (11) of 33.2%. Resistance to imipenem and ceftazidime of 28.0% also aligned with other regional data, such as imipenem resistance rates of 26.2% and 16.2% reported by Kanthakumar et al., (11) and Bindu et al., (13) respectively. Piperacillin/tazobactam retained good efficacy (84.0% sensitivity), comparable to 86.5% reported by Kanthakumar et al., (11) and 74.0% by Tiwari (12). Sensitivity to cefepime, cefoperazone/sulbactam, meropenem and amikacin of 76.0% each aligns closely with the data reported by Bindu et al., (13) where sensitivity to cefepime and amikacin were 75.7% and 78.4% respectively.

In our study, *Acinetobacter baumannii* showed 75.0% resistance each to ciprofloxacin, cotrimoxazole, cefepime, amikacin and ceftriaxone. A study by Verma et al., (14) reported resistance rates of 72.16% to amikacin and 86.08% to ciprofloxacin, while high resistance was also reported by Gaur et al., (15), with over 80.0% of isolates resistant to cephalosporins and 81.0% to ciprofloxacin. The sensitivity of *Acinetobacter* isolates was 50.0% to each of cefoperazone/sulbactam, imipenem, meropenem and piperacillin/tazobactam, which is comparable to the findings of the studies by Gaur et al., (15) and Sivaranjani et al., (16), which reported some variations in susceptibility, with imipenem resistance ranging from 9.1% to 26.2%.

All three isolates of *Burkholderia* in this study showed 100.0% resistance to cefepime, cotrimoxazole and amikacin. A study conducted by Ibrahim et al., (17) reported 100.0% resistance to cefepime and 91.8% to amikacin, but 100.0% sensitivity to piperaci-

llin/tazobactam, followed by 67.0% sensitivity to meropenem, ceftazidime, and cefoperazone/sulbactam. This aligns with a study by Sethi (18) where *Burkholderia* isolates showed sensitivity rates of 89.0% to cotrimoxazole, 65.0% to ceftazidime and 43.0% to meropenem. These results mirror resistance trends that underscores the critical need for ongoing antimicrobial surveillance and judicious antibiotic use to maintain the effectiveness of current treatment options.

The resistance observed may be due to multiple factors, such as β-lactamase production, active efflux mechanisms, and genetic mutations at antibiotic target sites, all of which enhance the organism's resilience against several drug classes. These observations also point to variations in resistance patterns among NFGNB, reinforcing the necessity for region-specific monitoring and targeted antimicrobial stewardship.

Conclusion:

The high prevalence and resistance to antibiotics of clinical isolates of NFGNB, which includes *Pseudomonas*, *Acinetobacter* and *Burkholderia* species, are highlighted in this study. The predominance of infections in older males and in hospitalized patients emphasizes these group's vulnerability. High levels of resistance, especially to commonly used antibiotics such as fluoroquinolones, cephalosporins and aminoglycosides were observed, with some sensitivity retained to carbapenems and β-lactam/β-lactamase inhibitor combinations.

The concerning patterns of resistance, remarkably complete resistance observed in *Burkholderia* isolates, underscore the pressing need for strong infection control practices, antimicrobial resistance surveillance, and establishment of effective, community-based antibiotic stewardship initiatives to stop the spread of multidrug-resistant infections and guide rational therapeutic choices.

Contributions of authors:

Both authors contributed significantly to this study. KA and PG designed the study, performed the procedures and collected data, analyzed the data and drafted the manuscript. Both authors reviewed, revised, and approved the final version of the manuscript.

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Conflict of interest:

Authors declare no conflict of interest.

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