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Microbiological Profile and Antimicrobial Susceptibility Patterns of Non-fermenting Gram-Negative Bacilli Isolated From Clinical Specimens in a Tertiary Care Hospital: A Prospective Study

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Abstract

Background

Non-fermenting Gram-negative bacilli (NFGNB), including *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Burkholderia cepacia* complex, and *Stenotrophomonas maltophilia*, are important healthcare-associated pathogens with variable antimicrobial susceptibility patterns. This study assessed the microbiological profile and antimicrobial susceptibility patterns of NFGNB isolated from clinical specimens at a tertiary-care hospital in Gujarat, India.

Methods

This prospective, observational, laboratory-based study was conducted at the Central Microbiology Laboratory of Dhiraj Hospital, Vadodara, from April 1 to September 30, 2023. Clinical specimens from inpatients and outpatients were processed, and organism identification and antimicrobial susceptibility testing (AST) were performed using the VITEK 2 system (bioMérieux, Inc., Marcy-l'Étoile, France). The study documentation indicated interpretation according to the Clinical and Laboratory Standards Institute (CLSI) M100, 33rd edition (2023); however, the historical breakpoint implementation could not be independently verified.

Results

Of 278 initially identified laboratory records, 56 (20.14%) were excluded, leaving 222 clinical specimens for analysis. These specimens yielded 227 target NFGNB isolates, including five polymicrobial specimens containing both *P. aeruginosa* and *A. baumannii*. Among the 222 specimens, 109 (49.10%) yielded monomicrobial *P. aeruginosa*, 73 (32.88%) yielded monomicrobial *A. baumannii*, 20 (9.01%) yielded *B. cepacia* complex, and 15 (6.76%) yielded *S. maltophilia*. Including the co-isolates, the species-specific denominators for antimicrobial susceptibility analysis were 114 for *P. aeruginosa* and 78 for *A. baumannii*. Pus or wound swabs were the most common specimen type (37.39%). Among specimens with documented departmental information, intensive care units accounted for 77.85%. *P. aeruginosa* showed the highest recorded susceptibility to amikacin (50.00%), cefepime (46.49%), and cefoperazone-sulbactam and meropenem (45.61% each). *A. baumannii* showed the highest recorded susceptibility to minocycline (35.90%) and trimethoprim-sulfamethoxazole (24.36%), while susceptibility to imipenem and meropenem was 10.26% for each. *B. cepacia* complex showed the highest recorded susceptibility to meropenem (75.00%), whereas *S. maltophilia* showed the highest recorded susceptibility to minocycline (86.67%).

Conclusion

P. aeruginosa and *A. baumannii* were the predominant NFGNB in this study, with low recorded susceptibility to several tested antimicrobial agents. These findings provide institution-specific surveillance data that may inform local antimicrobial stewardship and infection-control initiatives. The results should be interpreted cautiously because historical breakpoint implementation could not be independently verified and should not be used alone to guide empirical treatment.

Categories: Pathology, Public Health, Infectious Disease
Keywords: antibiotic sensitivity testing, health care associated infections, multidrug resistance, non-fermenting gram-negative bacilli, pseudomonas aeruginosa

Introduction

Non-fermenting Gram-negative bacilli (NFGNB) are a heterogeneous group of aerobic, non-spore-forming Gram-negative bacteria characterized by their inability to ferment glucose under standard testing conditions, instead utilizing oxidative pathways or remaining non-saccharolytic [1]. The group includes several clinically important organisms, most notably *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia*, and *Burkholderia cepacia* complex [1]. Although historically regarded predominantly as environmental organisms of relatively low virulence, NFGNB are now recognized as important opportunistic pathogens, particularly among critically ill, immunocompromised, and hospitalized patients [1,2].

The clinical significance of these organisms is related to their ability to survive and persist in healthcare environments and to colonize susceptible hosts. *P. aeruginosa*, *A. baumannii*, *S. maltophilia*, and *B. cepacia* complex can cause a broad spectrum of healthcare-associated infections, including bloodstream infections, pneumonia, ventilator-associated pneumonia, urinary tract infections, and wound or surgical-site infections, particularly in patients with prolonged hospitalization, invasive devices, mechanical ventilation, or impaired host defenses [1,2]. While *P. aeruginosa* and *A. baumannii* remain the predominant clinically isolated NFGNB worldwide, less common species such as *B. cepacia* complex and *S. maltophilia* are increasingly recognized as causes of severe nosocomial infections [2].

A major concern associated with NFGNB is their ability to exhibit and accumulate multiple antimicrobial-resistance mechanisms. These organisms display intrinsic resistance as well as acquired resistance mediated through reduced outer-membrane permeability, active efflux pumps, modification of antimicrobial targets, and production of antimicrobial-inactivating enzymes [3]. Biofilm formation further facilitates persistence on medical devices, reduces antimicrobial penetration, and contributes to treatment failure [1]. Consequently, infections caused by resistant NFGNB substantially restrict therapeutic options and complicate clinical management [1,3].

The broader global burden of antimicrobial resistance underscores the severity of this challenge. The Global Burden of Disease 2021 Antimicrobial Resistance Collaborators estimated that bacterial antimicrobial resistance was associated with approximately 4.71 million deaths globally in 2021, including approximately 1.14 million deaths directly attributable to resistance [4]. Within the World Health Organization's 2024 Bacterial Priority Pathogens List, carbapenem-resistant *A. baumannii* is classified as a critical-priority pathogen, while carbapenem-resistant *P. aeruginosa* is classified as a high-priority pathogen, reflecting their substantial public-health burden and limited therapeutic options [5].

In India, substantial variations in the prevalence and antimicrobial susceptibility of NFGNB have been reported across different tertiary-care settings. A study from a tertiary-care teaching hospital in central India analyzed 2,106 NFGNB isolates and documented widespread resistance across multiple antimicrobial classes [6]. Similarly, a tertiary-care hospital study from Patiala identified NFGNB in 216 of 1,854 culture-positive samples (11.6%), with *P. aeruginosa* accounting for 190 (87.96%) isolates and *A. baumannii* accounting for 17 (7.87%) isolates [7]. Such inter-institutional differences in species distribution and susceptibility profiles are influenced by local patient case mix, critical-care utilization, antimicrobial prescribing practices, and infection-control measures [2,6,7].

Because susceptibility data generated in one institution cannot be reliably extrapolated to another, periodic local surveillance is essential for monitoring species distribution and resistance profiles. However, recent automated surveillance data characterizing the microbiological profile and phenotypic susceptibility of NFGNB from large tertiary-care teaching hospitals in Gujarat remain limited. Therefore, this study aimed to determine the distribution of NFGNB isolated from clinical specimens (across species, specimen sources, and hospital departments) and to characterize their antimicrobial susceptibility patterns during the study period at a tertiary-care teaching hospital in Vadodara, Gujarat, to provide institution-specific surveillance data supporting local antimicrobial stewardship.

Materials And Methods

Study design and setting

This prospective, observational, laboratory-based study was conducted from April 1 to September 30, 2023, at Dhiraj Hospital, a tertiary-care teaching hospital in Vadodara, Gujarat, India. Clinical specimens from inpatient, intensive care, and outpatient departments were processed in the hospital's NABL Medical Entry Level-accredited Central Microbiology Laboratory.

Sample size and sampling procedure

As this was a descriptive, laboratory-based prospective surveillance study conducted over a predefined six-month period, all consecutive, eligible, non-duplicate NFGNB isolates identified during the study period were included; therefore, no formal a priori sample-size calculation was performed. A non-duplicate isolate was defined as the first isolate of a given NFGNB species recovered from a patient during the study period, irrespective of anatomical site or specimen source. Subsequent isolates of the same species from the same patient were excluded. When a single specimen yielded two distinct target NFGNB species, both isolates

were retained and analyzed independently in their respective species-specific antimicrobial susceptibility analyses. Of the 278 initially identified laboratory records, 56 were excluded, leaving 222 eligible clinical specimens for the final analysis. These specimens yielded 227 target NFGNB isolates, including five polymicrobial specimens in which both *P. aeruginosa* and *A. baumannii* were recovered.

Clinical specimens

Clinical specimens submitted to the Central Microbiology Laboratory by treating physicians for suspected clinical infection during the study period were evaluated for the isolation of NFGNB. Specimens included pus and wound swabs, blood, sputum, endotracheal secretions, urine, catheter and device tips (such as endotracheal tube tips and central venous catheter tips), cerebrospinal fluid, pleural fluid, ascitic fluid, and other clinically indicated samples. Samples were collected from patients attending or admitted under various clinical departments according to standard clinical and laboratory procedures and were transported promptly to the microbiology laboratory for processing.

Inclusion criteria

Clinical specimens received in the Central Microbiology Laboratory from patients admitted to the hospital or attending various outpatient departments between April 1, 2023, and September 30, 2023, were included when they yielded NFGNB on microbiological examination and the isolate was reliably identified to the species level using the VITEK 2 (bioMérieux, Inc., Marcy-l'Étoile, France) automated system with complete antimicrobial susceptibility records. Clinically relevant isolates of target NFGNB species, including *P. aeruginosa*, *A. baumannii*, *B. cepacia* complex, and *S. maltophilia,* were included for analysis. Because bedside clinical correlation was not performed, all consecutive laboratory isolates from diagnostic clinical specimens submitted for suspected infection were included, acknowledging that true clinical infection versus colonization could not be definitively distinguished.

Exclusion criteria

Repeat specimens yielding the same bacterial species from the same patient after the initial isolation were considered duplicate isolates and excluded from the analysis. Isolates identified only to the genus level (e.g., *Pseudomonas* spp. or *Acinetobacter* spp.; n = 38), for which species-specific interpretive criteria could not be reliably applied, were excluded. Isolates belonging to NFGNB species outside the four predefined target groups (n = 12) and those with incomplete identification or uninterpretable antimicrobial susceptibility testing (AST) records (n = 6) were also excluded.

The screening and selection of eligible laboratory records, reasons for exclusion, distribution of monomicrobial and polymicrobial specimens, and final organism-specific isolate counts are summarized in Figure *1*.

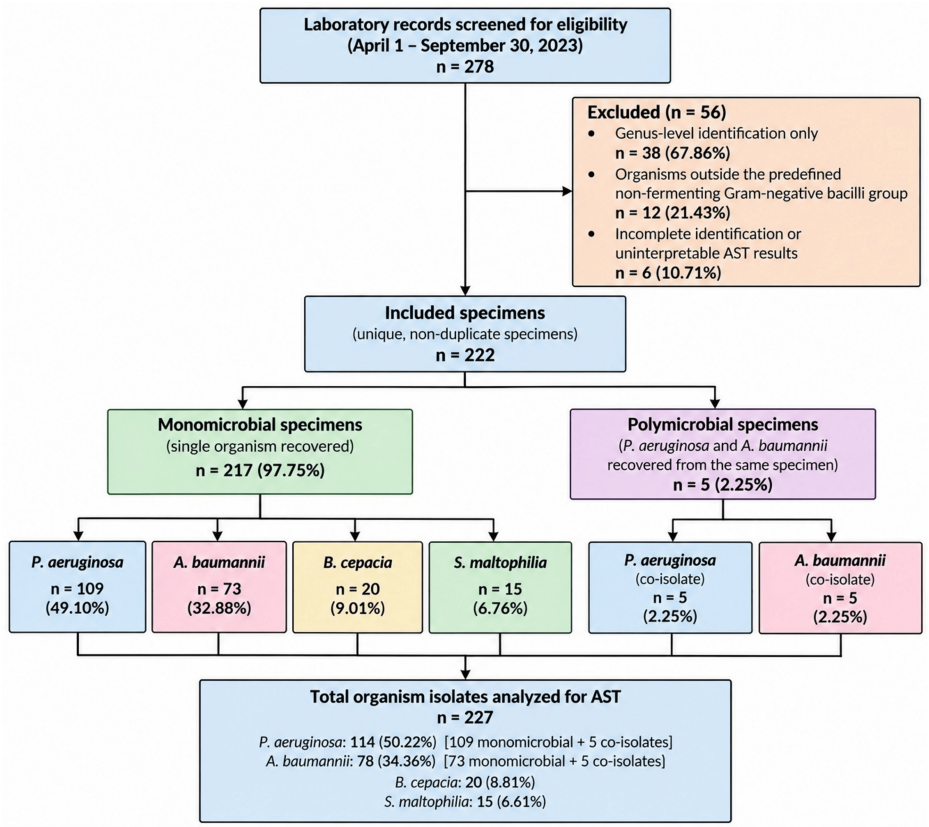


FIGURE 1: Flow Diagram of Laboratory Record Screening, Isolate Selection, and Organism Distribution for Antimicrobial Susceptibility Testing

A total of 278 laboratory records from April 1 to September 30, 2023, were screened for eligibility. Fifty-six records were excluded because of genus-level identification only (n = 38), identification of organisms outside the predefined study group (n = 12), or incomplete identification or uninterpretable antimicrobial susceptibility testing (AST) results (n = 6). The remaining 222 specimens comprised 217 monomicrobial and five polymicrobial specimens. The five polymicrobial specimens yielded both *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Overall, 227 organism isolates were analyzed for AST: 114 *P. aeruginosa*, 78 *A. baumannii*, 20 *Burkholderia cepacia* complex, and 15 *Stenotrophomonas maltophilia* isolates.

Isolation and identification of NFGNB

Clinical specimens were processed using standard microbiological techniques. Specimens were inoculated onto 5% sheep blood agar and MacConkey agar (and chocolate agar or CLED (cystine-lactose-electrolyte-deficient) agar for urine) and incubated aerobically at 35-37°C for 18-24 hours (extended up to 48 hours for slow-growing isolates). Pale, non-lactose-fermenting colonies on MacConkey agar were subjected to preliminary screening, including Gram staining (confirming Gram-negative bacilli), cytochrome oxidase testing, motility testing, and triple sugar iron (TSI) agar inoculation showing an alkaline/no-change (K/NC) non-fermentative reaction. Definite species-level identification was performed using the VITEK 2 Compact automated identification system (bioMérieux) with VITEK 2 GN identification cards.

Antimicrobial susceptibility testing

AST of the identified NFGNB was performed using the VITEK 2 automated system (bioMérieux). Bacterial suspensions were standardized to a 0.5 McFarland turbidity standard using a DensiCHEK Plus densitometer before card inoculation. Available card documentation included a GN 241 identification card and an AST-N406 AST card. However, the original laboratory records were insufficient to independently verify the specific AST card used for each organism, the complete antimicrobial panels tested, or the historical instrument software and configuration. The antimicrobial agents reported in the results reflect the available study records.

Interpretation of antimicrobial susceptibility results

The study documentation indicated that AST results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) Performance Standards for Antimicrobial Susceptibility Testing, 33rd

edition (M100-Ed33, 2023) [8,9]. However, the original instrument configuration and AST reports were unavailable for independent verification of the breakpoint implementation. Accordingly, the reported susceptibility categories should be interpreted in light of this documentation limitation.

Colistin susceptibility results were excluded from the organism-specific tables because CLSI interpretive criteria do not provide a conventional susceptible category for *P. aeruginosa* and *A. baumannii*. In addition, *B. cepacia* complex and *S. maltophilia* are intrinsically resistant to colistin [8]. Confirmatory broth microdilution testing was not performed, and the original minimum inhibitory concentration (MIC) records were unavailable for independent verification.

Tigecycline susceptibility results were also excluded from the tables because CLSI M100-Ed33 does not provide organism-specific interpretive breakpoints for *A. baumannii*, *B. cepacia* complex, or *S. maltophilia* [8]. The available records did not permit independent verification of the manufacturer-specific interpretive criteria or the Advanced Expert System (AES) configuration used at the time of testing. The VITEK 2 automated system used for microbial identification and AST is described by the manufacturer [10].

Quality control

The available study records did not permit independent verification of the quality-control procedures performed during the study period, including the use of reference strains, testing frequency, and recorded acceptance results. Consequently, compliance with specified CLSI quality-control ranges could not be independently confirmed. This limitation should be considered when interpreting the antimicrobial susceptibility findings.

Ethical approval

The study was approved by the Sumandeep Vidyapeeth Institutional Ethics Committee (SVIEC) as a minimal-risk observational study (outward reference number: SVIEC/ON/MEDI/RP/DEC/22/9, dated December 21, 2022). The study was conducted in accordance with the approved study synopsis. Written informed consent was obtained from the participants, and confidentiality was maintained through anonymization of study data.

Results

A total of 278 consecutive NFGNB isolates were initially identified during the study period from April 1, 2023, to September 30, 2023. Of these, 56 (20.14%) isolates were excluded from the final analysis, comprising 38 (13.67%) isolates identified only to the genus level (where species-specific CLSI interpretive breakpoints could not be assigned), 12 (4.32%) isolates belonging to species outside the four predefined target organisms, and six (2.16%) isolates with incomplete identification or antimicrobial susceptibility records. Consequently, 222 (79.86%) consecutive non-duplicate clinical specimens met the inclusion criteria and were included in the final analysis.

Because manual laboratory requisition forms accompanying specimens from busy inpatient wards frequently omitted demographic entries, patient gender was recorded for only 35 of the 222 included specimens (15.77%), with the remaining 187 (84.23%) lacking gender data. Within the subset of 35 specimens with recorded gender, 26 (74.29%) originated from male patients and nine (25.71%) from female patients; however, given the high proportion of missing demographic records, this subset is not representative of the overall study cohort.

Among the 222 analyzed clinical specimens, 217 (97.75%) yielded monomicrobial growth of a single target NFGNB species, while five (2.25%) polymicrobial specimens yielded co-isolation of both *P. aeruginosa* and *A. baumannii*, resulting in a total of 227 target NFGNB organism isolates across the 222 specimens. *P. aeruginosa* was the predominant organism, isolated as a single pathogen in 109 (49.10%) specimens, followed by *A. baumannii* in 73 (32.88%), *B. cepacia* complex in 20 (9.01%), and *S. maltophilia* in 15 (6.76%) specimens (Table *1*). For species-specific antimicrobial susceptibility analyses, each organism recovered from the five polymicrobial specimens was evaluated independently alongside the monomicrobial isolates, yielding organism-specific denominators of 114 for *P. aeruginosa* (109 monomicrobial + 5 co-isolated) and 78 for *A. baumannii* (73 monomicrobial + 5 co-isolated) (Table *1*).

Variable	Number, n (%)
Screening and eligibility cohort (N = 278 initially identified isolates)	
Included clinical specimens in final analysis	222 (79.86)
Excluded isolates (total)	56 (20.14)
Identified only to the genus level	38 (13.67)
Species outside predefined target organisms	12 (4.32)
Incomplete identification or susceptibility records	6 (2.16)
Gender distribution among specimens with recorded gender (n = 35)*	
Male	26 (74.29)
Female	9 (25.71)
Total with recorded gender	35 (100.00)
Organism distribution among included clinical specimens (N = 222)	
Pseudomonas aeruginosa (monomicrobial)	109 (49.10)
Acinetobacter baumannii (monomicrobial)	73 (32.88)
Burkholderia cepacia complex	20 (9.01)
Stenotrophomonas maltophilia	15 (6.76)
Pseudomonas aeruginosa + *Acinetobacter baumannii* (co-isolated)**	5 (2.25)
Total included clinical specimens	222 (100.00)

TABLE 1: Screening Breakdown and Overall Microbiological Profile of NFGNB Isolates

Data are presented as numbers (percentages). *Gender was recorded on laboratory requisition slips for only 35 (15.77%) of the 222 included specimens (missing for 187 (84.23%) specimens) and therefore does not represent the overall study population. **The five polymicrobial specimens yielding both *P. aeruginosa* and *A. baumannii* contributed five isolates to each respective species, resulting in 227 total organism isolates across the 222 specimens (114 *P. aeruginosa* and 78 *A. baumannii* isolates evaluated in species-specific susceptibility analyses).

NFGNB: non-fermenting Gram-negative bacilli

Pus and wound swabs constituted the most frequent specimen source, accounting for 83 (37.39%) specimens, followed by blood with 30 (13.51%), sputum with 29 (13.06%), endotracheal secretions with 28 (12.61%), and urine with 22 (9.91%). Catheter and device tips (including endotracheal tube tips and central venous catheter tips) accounted for 15 (6.76%) specimens, while cerebrospinal fluid, ascitic fluid, and other specimens accounted for eight (3.60%), two (0.90%), and five (2.25%) specimens, respectively.

Regarding departmental distribution, source ward codes could not be reliably mapped to a specific clinical department for 64 (28.83%) specimens due to abbreviated or legacy unit codes on manual requisition forms, leaving 158 (71.17%) specimens with resolvable departmental attribution. The intensive care units (ICUs) represented the predominant hospital source, contributing 123 (55.41%) of all 222 specimens and 77.85% (123/158) of specimens with a known hospital department, followed by the surgery department with eight (3.60% of total; 5.06% of known departments) (Table *2*).

Variable	Number, n (%)
Specimen type	
Pus	83 (37.39)
Blood	30 (13.51)
Sputum	29 (13.06)
Endotracheal secretion	28 (12.61)
Urine	22 (9.91)
Catheter and device tips	15 (6.76)
Cerebrospinal fluid	8 (3.60)
Ascitic fluid	2 (0.90)
Other	5 (2.25)
Hospital department	
Intensive care unit	123 (55.41)
Surgery	8 (3.60)
Casualty	5 (2.25)
Skin	5 (2.25)
Obstetrics and gynecology	4 (1.80)
Orthopedics	4 (1.80)
Chemotherapy	3 (1.35)
Oncology	2 (0.90)
Ear, nose, and throat	2 (0.90)
Urology	1 (0.45)
Pediatric ward	1 (0.45)
Department not resolvable from source ward code	64 (28.83)
Total	222 (100)

TABLE 2: Distribution of NFGNB Isolates According to Specimen Type and Hospital Department

Data are presented as numbers (percentages). Departmental classification was based on the available source ward or department records; the department could not be reliably resolved from the source ward code for 64 specimens.

Percentages for hospital departments are calculated against the total cohort (N = 222). Among the 158 specimens with resolvable departmental codes, intensive care units accounted for 77.85% (123/158) and surgery accounted for 5.06% (8/158).

NFGNB: non-fermenting Gram-negative bacilli

Among *P. aeruginosa* isolates, the highest recorded susceptibility was observed to amikacin, with 57 (50.00%) isolates, followed by cefepime with 53 (46.49%). Cefoperazone-sulbactam and meropenem each showed susceptibility in 52 (45.61%) isolates. Susceptibility to piperacillin-tazobactam was observed in 49 (42.98%) isolates, while ceftazidime and imipenem each showed susceptibility in 46 (40.35%) isolates. Lower susceptibility was recorded for ciprofloxacin, with 44 (38.60%) isolates, levofloxacin, with 39 (34.21%), gentamicin, with 30 (26.32%), and aztreonam, with nine (7.89%). Colistin results are not reported as susceptible because the applicable CLSI criteria do not define a conventional susceptible category for *P. aeruginosa*, and the original MIC-level records were unavailable for independent verification (Table *3*).

Antimicrobial agent	Susceptible, n (%)
Piperacillin-tazobactam	49 (42.98)
Ceftazidime	46 (40.35)
Cefoperazone-sulbactam	52 (45.61)
Cefepime	53 (46.49)
Aztreonam	9 (7.89)
Imipenem	46 (40.35)
Meropenem	52 (45.61)
Amikacin	57 (50.00)
Gentamicin	30 (26.32)
Ciprofloxacin	44 (38.60)
Levofloxacin	39 (34.21)

TABLE 3: Antimicrobial Susceptibility Profile of Isolates of Pseudomonas aeruginosa

Data are presented as numbers (percentages) of isolates reported as susceptible in the available study dataset. The denominator is 114 *P. aeruginosa* isolates, including five recovered alongside *Acinetobacter baumannii* from polymicrobial specimens. Colistin is omitted from the susceptible-results column because a conventional Clinical and Laboratory Standards Institute (CLSI) susceptible category is not defined for this organism. Confirmatory broth microdilution was not performed, and the original minimum inhibitory concentration (MIC) records were unavailable for verification.

Among *A. baumannii* isolates, the highest recorded susceptibility was observed to minocycline, with 28 (35.90%) isolates, followed by trimethoprim-sulfamethoxazole with 19 (24.36%) and gentamicin with 13 (16.67%). Cefoperazone-sulbactam showed susceptibility in 12 (15.38%) isolates, while amikacin and ciprofloxacin each showed susceptibility in 11 (14.10%) isolates. Piperacillin-tazobactam and levofloxacin each showed susceptibility in nine (11.54%) isolates. The lowest recorded susceptibility was observed for ceftazidime, cefepime, imipenem, and meropenem, with eight (10.26%) isolates each. Tigecycline results are not reported because the applicable interpretive criteria could not be independently verified from the available records (Table *4*).

Antimicrobial agent	Susceptible, n (%)
Piperacillin-tazobactam	9 (11.54)
Ceftazidime	8 (10.26)
Cefoperazone-sulbactam	12 (15.38)
Cefepime	8 (10.26)
Imipenem	8 (10.26)
Meropenem	8 (10.26)
Amikacin	11 (14.10)
Gentamicin	13 (16.67)
Ciprofloxacin	11 (14.10)
Levofloxacin	9 (11.54)
Minocycline	28 (35.90)
Trimethoprim-sulfamethoxazole	19 (24.36)

TABLE 4: Antimicrobial Susceptibility Profile of Isolates of Acinetobacter baumannii

Data are presented as numbers (percentages) of isolates reported as susceptible in the available study dataset. The denominator is 78 *A. baumannii* isolates, including five recovered alongside *Pseudomonas aeruginosa* from polymicrobial specimens. Tigecycline is omitted because the organism-specific interpretive criteria used by the instrument could not be verified. Colistin is omitted from the susceptible-results column because a conventional Clinical and Laboratory Standards Institute (CLSI) susceptible category is not defined for this organism. Broth microdilution confirmation was not performed, and original minimum inhibitory concentration (MIC)-level records were unavailable for verification.

Among *B. cepacia* complex isolates, the highest recorded susceptibility was observed to meropenem, with 15 (75.00%) isolates, followed by ceftazidime with nine (45.00%) and trimethoprim-sulfamethoxazole with eight (40.00%). Among *S. maltophilia* isolates, minocycline showed the highest recorded susceptibility, with 13 (86.67%) isolates, followed by levofloxacin with seven (46.67%) and trimethoprim-sulfamethoxazole with six (40.00%). Categorical tigecycline and colistin results are not reported because the applicable interpretive criteria could not be independently verified or did not support a conventional susceptible classification. The recorded susceptibility patterns for both organisms are presented in Table *5*.

Antimicrobial agent	*Burkholderia cepacia* complex, n (%)	*Stenotrophomonas maltophilia*, n (%)
Piperacillin-tazobactam	3 (15.00)	Not tested
Ceftazidime	9 (45.00)	1 (6.67)
Cefoperazone-sulbactam	5 (25.00)	2 (13.33)
Cefepime	3 (15.00)	0 (0.00)
Aztreonam	0 (0.00)	Not tested
Imipenem	4 (20.00)	Not tested
Meropenem	15 (75.00)	Not tested
Ciprofloxacin	3 (15.00)	0 (0.00)
Levofloxacin	4 (20.00)	7 (46.67)
Minocycline	3 (15.00)	13 (86.67)
Trimethoprim-sulfamethoxazole	8 (40.00)	6 (40.00)
Total isolates	20	15

TABLE 5: Antimicrobial Susceptibility Profiles of Burkholderia cepacia Complex and Stenotrophomonas maltophilia Isolates

Data are presented as numbers (percentages) of isolates reported as susceptible in the available study dataset. The denominators are 20 *B. cepacia* complex isolates and 15 *S. maltophilia* isolates. “Not tested” indicates that the antimicrobial agent was not included in the recorded testing panel. Tigecycline is omitted because the applicable organism-specific interpretation criteria could not be verified. Colistin is not presented as a susceptible result; the original interpretation and minimum inhibitory concentration (MIC) records were unavailable for independent verification.

Discussion

The present study evaluated 222 consecutive clinical specimens yielding 227 target NFGNB organism isolates (including five polymicrobial specimens co-infected with both *P. aeruginosa and A. baumannii*). *P. aeruginosa* was the predominant organism (isolated as a single pathogen in 49.10% of specimens), followed by *A. baumannii* (32.88%), *B. cepacia complex* (9.01%), and *S. maltophilia* (6.76%). A study from Ethiopia similarly reported a high prevalence of *P. aeruginosa* and *A. baumannii*, along with substantial antimicrobial resistance and the detection of multiple resistance genes [11]. Likewise, a study from Sudan identified *Pseudomonas* species as the predominant NFGNB, with *P. aeruginosa* and *A. baumannii* representing major clinical isolates [12]. While the predominance of these two species in our cohort aligns broadly with findings from other hospital-based investigations, direct quantitative comparisons across studies should be interpreted cautiously due to differences in patient populations, specimen selection criteria, healthcare settings, study timeframes, and identification methodologies [11,12].

In the present study, the ICUs accounted for 55.41% (123/222) of all analyzed specimens and 77.85% (123/158) of specimens with resolvable ward codes. Pus was the most frequent specimen source (37.39%), followed by blood (13.51%), sputum (13.06%), endotracheal secretions (12.61%), urine (9.91%), and catheter/device tips (6.76%). Because a large proportion of specimens originated from ICU patients, the observed species distribution and high resistance rates likely reflect our tertiary-care hospital's critical-care case mix and specimen-sampling intensity rather than overall hospital-wide or community prevalence. Previous clinical studies investigating carbapenem-resistant Gram-negative sepsis have noted that NFGNB and ventilator-associated pneumonia are major sources of resistant infections and identified mechanical ventilation, prior carbapenem exposure, previous infection, and prolonged hospitalization as associated clinical risk factors [13]. Similarly, *S. maltophilia* has been highlighted as an opportunistic pathogen among critically ill patients in the context of prolonged ICU stay and broad-spectrum antimicrobial exposure [14]. However, because patient-level clinical variables were not assessed in our laboratory-based study, these factors cannot be confirmed as explanations for the observed ICU distribution.

Among the 114 *P. aeruginosa* isolates tested, susceptibility across the routine panel was moderate to low. Amikacin demonstrated the highest susceptibility rate (50.00%), followed by cefepime (46.49%), cefoperazone-sulbactam (45.61%), and meropenem (45.61%), while susceptibility to piperacillin-tazobactam was 42.98%. Susceptibility was lower for ciprofloxacin (38.60%), levofloxacin (34.21%), and gentamicin (26.32%), whereas only 7.89% of isolates were susceptible to aztreonam. The SENTRY Antimicrobial Surveillance Program (2016-2019), which evaluated respiratory isolates from patients

hospitalized with pneumonia, demonstrated substantial geographic variation in *P. aeruginosa* susceptibility to piperacillin-tazobactam and meropenem across Western Europe, Eastern Europe, and the United States [15]. Although comparisons with international surveillance networks must account for differences in specimen sources (respiratory-only versus mixed clinical specimens), testing periods, and interpretive standards (CLSI versus EUCAST), the divergence between global surveillance benchmarks and our cohort underscores the necessity of institution-specific susceptibility monitoring [15].

The susceptibility profile of *A. baumannii* (n = 78) was characterized by pronounced resistance to nearly all tested antimicrobial classes. Minocycline demonstrated the highest in vitro susceptibility (35.90%), followed by trimethoprim-sulfamethoxazole (24.36%), gentamicin (16.67%), and cefoperazone-sulbactam (15.38%), whereas only 10.26% of isolates were susceptible to imipenem and meropenem. This high level of carbapenem non-susceptibility parallels the elevated resistance rates reported for *A. baumannii* in Eastern Europe in the SENTRY surveillance study [15]. Similarly, an eight-year study from a Romanian tertiary-care hospital reported substantial resistance to carbapenems, fluoroquinolones, and aminoglycosides among *A. baumannii* bloodstream isolates [16]. Nevertheless, cross-study comparisons should be made with caution because the underlying patient populations, specimen types (bloodstream-only versus all clinical specimens), and study periods differed [15,16].

Among the less frequently isolated NFGNB, *B. cepacia* complex (n = 20) showed the highest susceptibility to meropenem (75.00%), ceftazidime (45.00%), and trimethoprim-sulfamethoxazole (40.00%), whereas *S. maltophilia* (n = 15) demonstrated the highest susceptibility to minocycline (86.67%), levofloxacin (46.67%), and trimethoprim-sulfamethoxazole (40.00%). A recent study of bloodstream infections caused by less common NFGNB similarly identified *S. maltophilia* as a notable opportunistic pathogen and described variable susceptibility to trimethoprim-sulfamethoxazole [17]. Furthermore, in vitro surveillance studies have demonstrated high activity of novel agents such as cefiderocol against resistant NFGNB, including *A. baumannii*, *P. aeruginosa*, *B. cepacia* complex, and *S. maltophilia* [18]. However, it is essential to distinguish between in vitro phenotypic susceptibility and in vivo clinical treatment effectiveness; phenotypic susceptibility data alone, whether for established agents like minocycline and trimethoprim-sulfamethoxazole or novel agents, should not be interpreted as standalone evidence of clinical efficacy without correlation to site of infection, pharmacokinetic/pharmacodynamic parameters, and patient clinical response.

Overall, the present study demonstrates considerable variation in phenotypic antimicrobial susceptibility among NFGNB, with particularly low susceptibility to first-line beta-lactams, carbapenems, fluoroquinolones, and aminoglycosides among *A. baumannii* and *P. aeruginosa*. The predominance of isolates from critical-care units and the observed resistance profiles highlight the value of ongoing institutional microbiological surveillance and species-level susceptibility testing. While these local surveillance data may assist in informing hospital antimicrobial stewardship and infection-control initiatives, they should not be used in isolation to dictate empirical therapy; clinical treatment decisions must rely on comprehensive bedside assessment and individual culture and susceptibility results.

Limitations

This study has several important methodological limitations. First, the single-center design and six-month surveillance period (April 1 to September 30, 2023) limit the generalizability of our findings to other healthcare settings or longer temporal trends. Because this was a laboratory-based study without bedside chart review, patient-level clinical data (such as age, comorbidities, prior antibiotic exposure, mechanical ventilation, length of stay, and clinical outcomes) were unavailable; consequently, clinical risk factors could not be assessed, and true infection could not be distinguished from colonization, particularly in respiratory, urine, and device-tip specimens. In addition, incomplete manual requisition forms resulted in missing gender data for 84.23% (187/222) of specimens and unresolved hospital department codes for 28.83% (64/222) of specimens. The exclusion of 56 of 278 initially identified isolates (including 38 identified only to the genus level without secondary confirmation) may introduce selection bias, and the small sample sizes for *B. cepacia* complex (n = 20) and *S. maltophilia* (n = 15) limit the precision of their susceptibility estimates. Furthermore, the study was restricted to phenotypic testing without molecular characterization of resistance genes (e.g., carbapenemases) or formal multidrug-resistant (MDR) classification.

The study also had limitations related to retrospective verification of AST. Original VITEK 2 configuration reports and archived AST result printouts from the study period were unavailable. Consequently, the historical software version, AES settings, organism-specific AST card assignments, and breakpoint implementation could not be independently confirmed. Colistin testing was not confirmed by reference broth microdilution, and the original MIC-level records were unavailable for verification. In addition, categorical tigecycline results for organisms without established CLSI interpretive breakpoints could not be validated against the manufacturer-specific criteria used at the time and were therefore omitted from the revised tables. The initial screening counts could not be independently checked against the original screening records. These limitations reduce the reproducibility and certainty of the antimicrobial susceptibility findings and warrant cautious interpretation.

Conclusions

P. aeruginosa and *A. baumannii* were the predominant NFGNB isolated in this six-month, single-center surveillance study. Both organisms demonstrated limited susceptibility to several commonly tested antimicrobial agents, particularly carbapenems among *A. baumannii* isolates. Susceptibility patterns varied across species, although the findings should be interpreted cautiously because the historical VITEK 2 configuration and breakpoint implementation could not be independently verified, and confirmatory broth microdilution testing for colistin was not performed. These results provide institution-specific phenotypic surveillance data but cannot establish clinical treatment effectiveness, causal associations, or population-level resistance trends and should not be generalized beyond the study setting. Continued local microbiological surveillance can support institutional infection-control and antimicrobial stewardship initiatives, alongside individualized clinical assessment and culture-directed treatment. Future multicenter studies incorporating verified susceptibility-testing procedures, reference broth microdilution where indicated, standardized resistance classifications, molecular characterization, and clinical outcome assessment are warranted.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

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Disclosures

Human subjects: Informed consent for treatment and open access publication was obtained or waived by all participants in this study. Sumandeep Vidyapeeth (Deemed to be University) Institutional Ethics Committee issued approval SVIEC/ON/MEDI/RP/DEC/22/9. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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