

Prevalence and Antibigram of Non-Fermenting Gram-Negative Bacilli Isolates Obtained from Various Clinical Samples at A Tertiary Care Hospital

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Abstract

Background: Non-fermenting Gram-negative bacilli (NFGNB) are increasingly recognized as important healthcare-associated pathogens because of their ability to survive in hospital environments and develop resistance to multiple antimicrobial agents. This study aimed to determine the prevalence of NFGNB isolated from various clinical specimens and assess their antimicrobial susceptibility pattern at a tertiary care hospital. **Material and Methods:** A descriptive study was conducted in the Department of Microbiology, GMERS Medical College and attached General Hospital, Gandhinagar, Gujarat, from January to December 2024. A total of 8,060 clinical specimens were processed using standard microbiological methods. NFGNB were identified by conventional biochemical tests, and antimicrobial susceptibility testing was performed according to CLSI 2024 guidelines. **Results:** Of 8,060 specimens, 1,900 (23.57%) showed bacterial growth, and 285 NFGNB were isolated, giving an overall prevalence of 3.53%. *Pseudomonas aeruginosa* (58.24%) was the predominant isolate, followed by *Acinetobacter baumannii* (31.92%). Most isolates were from hospitalized patients (82.45%), and pus-related specimens were the commonest source (60.70%). *P. aeruginosa* showed high susceptibility to piperacillin-tazobactam and aztreonam (89.75% each) and cefepime (89.15%). In contrast, *A. baumannii* showed considerably lower susceptibility to most antibiotics, including imipenem (21.97%) and meropenem (25.27%). **Conclusion:** NFGNB are important healthcare-associated pathogens, with *P. aeruginosa* and *A. baumannii* being the predominant isolates. The high antimicrobial resistance observed in *A. baumannii* highlights the need for regular surveillance of local antibiograms, rational antibiotic use, antimicrobial stewardship and effective infection-control practices.

Keywords: Non-fermenting Gram-negative bacilli; *Pseudomonas aeruginosa*; *Acinetobacter baumannii*; antimicrobial susceptibility; antibiogram; antimicrobial resistance; healthcare-associated infections.

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INTRODUCTION

Non-fermenting Gram-negative bacilli (NFGNB) are a diverse group of organisms that either do not use carbohydrates as a source of energy or utilize them through oxidative pathways.^[1] They are widely distributed in the environment as saprophytes, while some may also exist as commensals in the human gut.^[2] Once considered organisms of limited clinical significance, NFGNB have now emerged as important opportunistic and healthcare-associated pathogens.^[3-7]

These organisms are frequently encountered in hospital environments and have a remarkable ability to survive on various surfaces and medical equipment. They have been isolated from devices such as ventilators and humidifiers, as well as from mattresses and the skin of healthcare workers.^[8] Their ability to persist in the hospital environment, along with resistance to several disinfectants, facilitates transmission between patients through contaminated equipment, fomites and the hands of healthcare personnel.^[2,3] Consequently, NFGNB have become increasingly associated with device-related and other healthcare-associated infections.

NFGNB account for approximately 12–16% of bacterial isolates recovered in clinical microbiology laboratories. They are associated with a wide range of infections, including septicemia, pneumonia, urinary tract infections, surgical site and wound infections, and osteomyelitis.^[5] The clinically important members of this group include *Pseudomonas* spp., *Acinetobacter* spp., *Alcaligenes* spp., *Stenotrophomonas maltophilia* and *Burkholderia cepacia* complex. Among these, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are the most frequently encountered human pathogens, whereas infections caused by other NFGNB are relatively less common.^[6]

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The growing antimicrobial resistance among NFGNB is a major clinical concern. Their intrinsic resistance to several antimicrobial agents, ability to colonize hospital surfaces and capacity to acquire additional resistance mechanisms make infections caused by these organisms particularly difficult to treat.^[7] Injudicious and empirical use of broad-spectrum antibiotics may further contribute to the selection and spread of resistant strains.^[5] Prolonged hospitalization, exposure to broad-spectrum antibiotics and underlying host factors also play an important role in infections caused by these organisms and their clinical outcomes.^[9]

Multidrug resistance among NFGNB is increasingly being reported, with resistance mechanisms including the production of extended-spectrum β -lactamases (ESBLs) and metallo- β -lactamases (MBLs).^[4] Since *P. aeruginosa* and *A. baumannii* are among the most frequently isolated NFGNB, the emergence of carbapenem resistance in these organisms is of particular concern.^[10] The antimicrobial susceptibility pattern of NFGNB may also vary between hospitals and geographical regions, making knowledge of the local resistance profile important for appropriate antimicrobial therapy. Therefore, the present study was undertaken to determine the prevalence of non-fermenting Gram-negative bacilli isolated from various clinical specimens and to assess their antimicrobial susceptibility pattern at a tertiary care hospital.

MATERIALS AND METHODS

This descriptive study was conducted in the Department of Microbiology, GMERS Medical College and attached General Hospital, Gandhinagar, Gujarat, over a period of one year, from 1 January 2024 to 31 December 2024, after obtaining approval from the Institutional Ethics Committee. A total of 8,060 clinical specimens received in the bacteriology laboratory during the study period and fulfilling the inclusion criteria were processed. Of these, 285 patients with culture-positive NFGNB isolates constituted the study population. Patients from all age groups and both sexes attending the outpatient department (OPD) or admitted to various wards and intensive care units (ICUs) were included. Relevant demographic and clinical details were recorded using a predesigned proforma. Clinical specimens included blood, urine, sputum, pus/pus aspirate, wound swabs, ear swabs, endotracheal secretions, bronchoalveolar lavage (BAL), cerebrospinal fluid (CSF), pleural fluid, ascitic fluid and other body fluids received in the microbiology laboratory. Stool, tissue, vaginal swabs, urethral swabs, throat swabs, conjunctival/corneal swabs, endotracheal tube tips, catheter tips and umbilical venous catheter tips were excluded. Samples not fulfilling standard specimen-acceptance criteria were also excluded.

All specimens were collected and processed according to standard microbiological procedures. Samples received in the laboratory were assessed for appropriate labelling,

container, specimen volume, collection time, leakage and completeness of the laboratory request form before processing. Following culture, suspected non-fermenting Gram-negative bacilli were identified on the basis of colony morphology, Gram-staining characteristics and conventional biochemical reactions. Identification included appropriate tests such as oxidase, motility, triple sugar iron (TSI) agar reaction, oxidative-fermentative (O/F) test, citrate utilization, urease, indole, amino-acid decarboxylase/dihydrolase reactions and other organism-specific tests. The isolates were identified to genus/species level wherever feasible.

The principal NFGNB identified included *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia* and *Burkholderia cepacia* complex, along with other *Pseudomonas* and *Acinetobacter* species. The identification algorithm differentiated the organisms using characteristics such as oxidase reaction, motility, growth at 42°C, pigment production and relevant biochemical reactions.

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing (AST) of the NFGNB isolates was performed on Mueller-Hinton agar by the Kirby-Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI), 34th edition, 2024 guidelines. A bacterial suspension equivalent to a 0.5 McFarland standard was prepared and inoculated uniformly onto Mueller-Hinton agar. After application of appropriate antimicrobial discs, plates were incubated at 37°C for 18–24 hours. Zones of inhibition were measured and isolates were categorized as susceptible, intermediate or resistant according to CLSI interpretative criteria. The antimicrobial panel was selected according to the NFGNB species and applicable CLSI recommendations. Colistin minimum inhibitory concentration (MIC) was determined by the broth microdilution method. For *S. maltophilia*, MICs for ceftazidime and ticarcillin-clavulanate were determined using the E-test, while for *B. cepacia* complex, MICs for ceftazidime, levofloxacin, meropenem, ticarcillin-clavulanate, minocycline and cotrimoxazole were determined by E-test.

Quality-control procedures were performed according to CLSI recommendations. *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were used as control strains. Commercially available culture media, reagents and antibiotic discs from Hi-Media Laboratories Pvt. Ltd., Mumbai, India, were used during the study. Data were compiled in a master chart and analysed using descriptive statistics. Results were expressed as frequencies, percentages and ratios.

RESULTS

During the one-year study period, 8,060 clinical specimens were processed. Bacterial growth was obtained in 1,900 (23.57%) specimens, of which 285 isolates were identified as non-fermenting Gram-negative bacilli (NFGNB), accounting for 15.0% of culture-positive specimens. The overall prevalence of NFGNB among all specimens processed was 3.53%.

Table 1: Overall prevalence and distribution of NFGNB isolates

Parameter	Number	%
Total clinical specimens processed	8,060	100
Culture-positive specimens	1,900	23.57

NFGNB isolates among culture-positive specimens	285/1,900	15.00
Overall prevalence of NFGNB	285/8,060	3.53
Male patients among NFGNB-positive cases	167/285	58.59
Female patients among NFGNB-positive cases	118/285	41.40
OPD isolates	50/285	17.54
IPD ward isolates	171/285	60.00
ICU isolates	64/285	22.45

Among the 285 NFGNB-positive patients, 58.59% were male and 41.40% were female. The largest proportion of isolates was obtained from patients aged 21-30 years (16.14%), followed by 41-50 years (15.43%) and 31-40

years (13.33%). Most NFGNB isolates were recovered from hospitalized patients 82.45%, 22.4% were from ICUs and 60.00% from other inpatient wards.

Table 2: Species-wise distribution of NFGNB according to patient location

NFGNB species	Total n	%	ICU	IPD ward	OPD
<i>Pseudomonas aeruginosa</i>	166	58.24	13	113	40
<i>Acinetobacter baumannii</i>	91	31.92	39	48	4
<i>Acinetobacter</i> spp.	10	3.50	1	6	3
<i>Stenotrophomonas maltophilia</i>	7	2.45	5	2	0
<i>Burkholderia cepacia</i> complex	4	1.40	4	0	0
<i>Pseudomonas</i> spp. other than <i>P. aeruginosa</i>	7	2.45	2	2	3
Total	285	100	64	171	50

Pseudomonas aeruginosa was the predominant isolate (58.24%), followed by *Acinetobacter baumannii* (31.92%). *Acinetobacter* spp. other than *A. baumannii* accounted for 3.50% isolates, while *Stenotrophomonas maltophilia* and *Pseudomonas* spp. other than *P. aeruginosa* each accounted

for 2.45% isolates. *Burkholderia cepacia* complex was the least frequently isolated organism (1.40%). Among ICU isolates, *A. baumannii* was the predominant NFGNB (39/64), followed by *P. aeruginosa* (13/64).

Table 3: Specimen-wise and species-wise distribution of NFGNB isolates

Specimen	<i>P. aeruginosa</i>	<i>A. baumannii</i>	<i>Acinetobacter</i> spp.	<i>B. cepacia</i> complex	<i>S. maltophilia</i>	Other <i>Pseudomonas</i> spp.	Total
Pus	127	36	3	0	1	3	170
Blood	8	33	4	4	4	2	55
Sputum	11	9	3	0	0	0	23
Urine	12	2	0	0	1	2	17
ET secretion	1	8	0	0	0	0	9
Pleural fluid	2	1	0	0	1	0	4
Ear discharge swab	3	0	0	0	0	0	3
Drain fluid	0	2	0	0	0	0	2
Abdominal fluid	1	0	0	0	0	0	1
BAL	1	0	0	0	0	0	1
Total	166	91	10	4	7	7	285

Pus-related specimens constituted the largest source of NFGNB. When pus from different sites and ear discharge swabs were grouped together, 60.70% isolates were recovered from this category, followed by blood (19.29%), respiratory specimens (11.57%), urine (5.96%) and other

body fluids (2.45%). *P. aeruginosa* was predominantly recovered from pus (76.50%), whereas *A. baumannii* was most frequently isolated from pus (39.56%) and blood (36.26%).

Table 4: Antimicrobial susceptibility pattern of *P. aeruginosa* and *A. baumannii*

Antibiotic	<i>P. aeruginosa</i> (n=166), susceptible n (%)	<i>A. baumannii</i> (n=91), susceptible n (%)
Ampicillin-sulbactam	NT	53 (58.24)
Ceftazidime	139 (83.73)	11 (12.08)
Cefepime	148 (89.15)	23 (25.27)
Piperacillin-tazobactam	149 (89.75)	23 (25.27)
Tobramycin	143 (86.14)	32 (35.16)
Gentamicin	NT	31 (34.06)
Ciprofloxacin	123 (74.09)	22 (24.17)
Levofloxacin	128 (77.10)	28 (30.76)
Imipenem	139 (83.73)	20 (21.97)
Meropenem	139 (83.73)	23 (25.27)
Amikacin	10/12 (83.33)	28 (30.76)
Aztreonam	149 (89.75)	NT
Netilmicin	130 (78.31)	NT

Piperacillin	NT	15 (16.48)
Cotrimoxazole	NT	28 (30.76)
Ticarcillin-clavulanate	NT	51 (56.04)
Minocycline	NT	65 (71.42)
Doxycycline	NT	61 (67.03)
Cefotaxime	NT	15 (16.48)
Ceftriaxone	NT	14 (15.38)
Colistin (MIC-BMD)	166 (100)	91 (100)
Polymyxin B	166 (100)	91 (100)

P. aeruginosa showed 100% susceptibility to colistin and polymyxin B. High susceptibility was also observed to piperacillin-tazobactam and aztreonam (89.75% each), cefepime (89.15%), tobramycin (86.14%), and ceftazidime, imipenem and meropenem (83.73% each). Susceptibility to ciprofloxacin was comparatively lower (74.09%). *A. baumannii* demonstrated substantially lower susceptibility to most routinely tested antimicrobials. The highest susceptibility, apart from colistin and polymyxin B (100% each), was observed with minocycline (71.42%), doxycycline (67.03%), ampicillin-sulbactam (58.24%) and ticarcillin-clavulanate (56.04%). Susceptibility to carbapenems was low (imipenem 21.97% and meropenem 25.27%), and ceftazidime showed the lowest susceptibility (12.08%). Among the less frequently isolated NFGNB, *Acinetobacter* spp. other than *A. baumannii* (n=10) showed 100% susceptibility to colistin and polymyxin B, followed by ampicillin-sulbactam (70%), minocycline and ticarcillin-clavulanate (60% each), and doxycycline (50%). *Stenotrophomonas maltophilia* (n=7) showed 100% susceptibility to levofloxacin, minocycline and ticarcillin-clavulanate, while susceptibility to cotrimoxazole and ceftazidime was 42.85% each. *Burkholderia cepacia* complex (n=4) showed 100% susceptibility to levofloxacin, minocycline and ticarcillin-clavulanate; susceptibility to ceftazidime and meropenem was 75% each and to cotrimoxazole 50%. *B. cepacia* complex was reported as intrinsically resistant to colistin and polymyxin B.

DISCUSSION

In the present study, 8,060 clinical specimens were processed, of which 1,900 (23.57%) showed bacterial growth. Other studies have reported culture positivity rates of around 35%.^[11,12] Of the culture-positive specimens in our study, 285 yielded non-fermenting Gram-negative bacilli (NFGNB), accounting for 15% of all culture-positive specimens. The overall prevalence of NFGNB among all specimens processed was 3.53%. Comparable isolation rates have been reported in previous studies, ranging from 3.5% to 5.1%.^[7,13-16] The variation in prevalence across studies may reflect differences in patient populations, types of specimens processed, hospital settings and local epidemiological patterns.

NFGNB were more frequently isolated from male patients (58.59%) than from females (41.40%). A similar male predominance was reported by Ridhima et al,^[17] with 69.7% of isolates recovered from males and 30.3% from females. Male predominance has also been observed in studies by Kalidas et al,^[18] and Aamal et al.^[19] This difference may be related to variations in healthcare utilization, underlying

illnesses, exposure to trauma or surgical procedures, or the overall patient profile of the hospital.

The highest proportion of NFGNB isolates was observed among patients aged 21–30 years (16.14%), followed by those aged 41–50 years and 31–40 years. A large majority of isolates (82.45%) were recovered from hospitalized patients, whereas only 17.54% were obtained from patients attending the outpatient department. Overall, 22.45% of isolates were from ICU patients. This predominance among hospitalized patients reinforces the importance of NFGNB as healthcare-associated pathogens. Prolonged hospital stay, exposure to broad-spectrum antibiotics, invasive procedures and devices, severe underlying illnesses, and cross-transmission within the hospital environment may all contribute to their occurrence among inpatients. Juyal et al,^[20] and Patel et al,^[12] have similarly reported a substantial burden of NFGNB among hospitalized and ICU patients.

Pseudomonas aeruginosa was the predominant NFGNB in the present study, accounting for 58.24% of isolates, followed by *Acinetobacter baumannii* (31.92%). The predominance of *P. aeruginosa* may be related to its ability to survive in moist hospital environments, colonize medical devices, form biofilms and persist under adverse conditions. These characteristics, together with its capacity to acquire antimicrobial resistance, contribute to its clinical importance.

Although *A. baumannii* was the second most common organism overall, it was the predominant NFGNB among ICU isolates. Of the ICU isolates, 39 were *A. baumannii*, compared with 13 *P. aeruginosa*. This finding is noteworthy because *A. baumannii* is particularly well adapted to the ICU environment and can survive for prolonged periods on hospital surfaces and equipment. Critically ill patients are also frequently exposed to mechanical ventilation, vascular and urinary catheters, multiple invasive procedures and broad-spectrum antibiotics, which may facilitate colonization and subsequent infection. Thus, while *P. aeruginosa* predominated overall, the greater contribution of *A. baumannii* in the ICU suggests that the epidemiology of NFGNB may differ considerably across different areas of the hospital.

Pus-related specimens were the most common source of NFGNB, accounting for 60.70% of isolates, followed by blood (19.29%), respiratory specimens (11.57%), urine (5.96%) and other body fluids (2.45%). The predominance of pus specimens highlights the important role of NFGNB in wound and soft-tissue infections, postoperative infections and surgical-site infections. Repeated wound manipulation, dressing procedures, invasive interventions, exposure to the hospital environment and prior antimicrobial use may contribute to the recovery of these organisms from such specimens.

Among *P. aeruginosa* isolates, 76.50% were recovered from

pus, indicating a strong association with wound and suppurative infections. The distribution of *A. baumannii*, however, was more varied, with 39.56% of isolates recovered from pus and 36.26% from blood, while respiratory specimens also contributed to its isolation. This distribution highlights the broader clinical spectrum of *A. baumannii*, particularly its importance in serious infections among hospitalized and critically ill patients.

The antimicrobial susceptibility pattern of *P. aeruginosa* was relatively favourable compared with that of *A. baumannii*. All *P. aeruginosa* isolates were susceptible to colistin and polymyxin B. High susceptibility was also observed to piperacillin-tazobactam and aztreonam (89.75% each), cefepime (89.15%), tobramycin (86.14%), and ceftazidime, imipenem and meropenem (83.73% each). Susceptibility to ciprofloxacin (74.09%) and levofloxacin (77.10%) was comparatively lower. The preserved activity of several antipseudomonal agents is encouraging; nevertheless, resistance to commonly used antibiotics indicates that treatment should preferably be guided by antimicrobial susceptibility testing rather than relying solely on empirical therapy.

Previous studies have also reported varying degrees of antimicrobial resistance among *P. aeruginosa* isolates. Sadhana et al,^[21] and Bimla and Rekha et al,^[22] reported resistance rates of approximately 28% and 25.3%, respectively, for the antibiotics assessed in their studies. In the present study, amikacin resistance among the urinary isolates tested was 16.66%, while Sarkar et al,^[23] and Bimla and Rekha et al,^[22] reported higher resistance rates of 40.9% and 39.3%, respectively. Netilmicin resistance in the present study was 21.68%, compared with 37% reported by Sadhana et al,^[21] These differences underline the variability in antimicrobial susceptibility patterns across institutions and geographical regions.

Piperacillin-tazobactam and aztreonam showed particularly good activity against *P. aeruginosa* in the present study, with resistance of only 10.24% to each agent. Patel et al,^[12] and Kalidas et al,^[18] reported higher resistance rates of 24.1% and 46.5%, respectively. Such variation may reflect differences in antimicrobial prescribing practices, selection pressure, patient characteristics and local resistance patterns.

In contrast to *P. aeruginosa*, *A. baumannii* demonstrated considerably greater resistance to commonly used antimicrobial agents. Although all isolates were susceptible to colistin and polymyxin B, susceptibility to other agents was substantially lower. Minocycline showed the highest susceptibility (71.42%), followed by doxycycline (67.03%), ampicillin-sulbactam (58.24%) and ticarcillin-clavulanate (56.04%). Of particular concern was the low susceptibility to carbapenems, with only 21.97% of isolates susceptible to imipenem and 25.27% to meropenem.

The high level of carbapenem resistance observed among *A. baumannii* isolates is clinically important, as carbapenems have traditionally been among the major therapeutic options for severe infections caused by multidrug-resistant Gram-negative organisms. Jaggi et al,^[24] and Kalidas et al,^[18] have also reported considerable resistance to carbapenems, while

Dash et al,^[25] reported resistance rates of 22% to meropenem and 19% to imipenem. Sinha and Srinivasa et al,^[26] reported carbapenem resistance in 50% of *Acinetobacter* isolates. The even higher resistance observed in the present study is concerning and may reflect local antimicrobial selection pressure, acquisition of resistance mechanisms and the ability of *A. baumannii* to persist and spread within the hospital environment.

CONCLUSION

The present study highlights the clinical importance of non-fermenting Gram-negative bacilli in a tertiary care hospital. *Pseudomonas aeruginosa* was the most frequently isolated NFGNB, followed by *Acinetobacter baumannii*. Most isolates were recovered from hospitalized patients, with pus, blood and respiratory specimens being the major sources, reflecting the important role of these organisms in healthcare-associated infections. The antimicrobial susceptibility patterns differed considerably between the two predominant organisms. *P. aeruginosa* retained good susceptibility to several commonly used antipseudomonal agents, including piperacillin-tazobactam, cefepime, aztreonam, carbapenems and tobramycin. In contrast, *A. baumannii* showed high resistance to most of the antibiotics tested, with particularly low susceptibility to imipenem and meropenem. Colistin and polymyxin B remained effective against the major NFGNB isolates tested.

The variation in susceptibility patterns among different NFGNB highlights the importance of accurate identification and regular monitoring of the local antibiogram to guide appropriate antimicrobial therapy. The high level of resistance observed in *A. baumannii* is particularly concerning and reinforces the need for judicious antibiotic use, effective antimicrobial stewardship and strict infection-control practices to limit the emergence and spread of multidrug-resistant NFGNB in healthcare settings.

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Conflicts of interest

There are no conflicts of interest.

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