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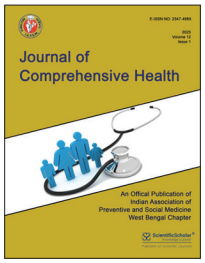

Original Article

A Study on the Magnitude of Non-Fermenter Gram-Negative Bacilli and their Antibiotic Susceptibility Pattern in Clinical Samples from a Tertiary Care Hospital, Kolkata

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ABSTRACT

Background: Non-fermenting Gram-negative bacilli (NFGNB) were initially considered non-pathogenic contaminants but now have emerged as major pathogens because of increased isolation from clinical samples and emerging resistance to common antibacterial agents.

Objectives: The study aims to determine the magnitude of NFGNB in clinical samples received at the Department of Microbiology, Nil Ratan Sircar Medical College, Kolkata, for a period of one year.

Material and Methods: The samples were processed according to standard procedures; for identification and antibiotic susceptibility test, Vitek 2 Compact (Biomerieux©) was used.

Results: Out of 725 bacterial isolates, 169 (23.31%) NFGNB were identified during the entire study period. *Pseudomonas* spp. (n = 84) was isolated most frequently, followed by *Acinetobacter* spp. (n = 73), *Burkholderia cepacia* (n = 7), and *Stenotrophomonas maltophilia* (n = 5). Both *Pseudomonas* spp. and *Acinetobacter* spp. showed greater susceptibility to piperacillin-tazobactam and poor susceptibility against cefepime.

Conclusion: It is necessary to identify these drug resistant pathogens in healthcare setup. The antibiotic susceptibility pattern of these pathogens needs to be monitored to guide clinicians in better patient care and management by initiating proper antibiotic therapy.

Keywords: Infection control, Acinetobacter infections, *Pseudomonas aeruginosa*, Cross-infection, Drug resistance

INTRODUCTION WITH OBJECTIVES

Organisms that are aerobic, non-spore-forming, and Gram-negative rod and either do not take carbohydrates as their energy source or utilize them by various metabolic pathways except fermentation are known as Non-fermenting Gram-negative bacilli (NFGNB).[1] They are saprophytic in nature but can cause significant infections particularly healthcare-associated infection (HCAI) in hospitalized patients, immunocompromised hosts, for example, patients with hematological malignancies.[2] NFGNB are known to account for about 15% of all bacterial isolates from a clinical microbiology laboratory.[3] These organisms are intrinsically resistant to commonly used disinfectants and their tendency to colonize on various surfaces have been pivotal in their

emergence as important pathogens of HCAI.[4] At present, *Pseudomonas* spp. and *Acinetobacter* spp. are the most commonly isolated non-fermenters which are pathogenic to humans. Infections caused by other species of NFGNB,e.g., *Burkholderia cepacia* and *Stenotrophomonas maltophilia*, are relatively infrequent.[5,6] Majority of infections caused by these organisms are secondary to patients' pre-existing conditions, e.g., burns, prolonged antimicrobial therapy, immunosuppressive therapy, and old age, etc.[7] Recent studies have shown that *Pseudomonas aeruginosa* is the second most common cause of HCAI pneumonia and ventilator-associated pneumonia.[8,9] NFGNB shows resistance to a wide range of commonly used antibiotics by several mechanisms, e.g., by producing beta-lactamases, efflux pumps mediated, and penicillin-binding protein site mutation.[10]

Due to the increased isolation of NFGNB and their resistance against common antibacterial agents, this study was designed to determine the magnitude of NFGNB in clinical samples and their susceptibility pattern in our hospital.

Aims and objectives

The objectives of the study were as follows:

1. To determine the magnitude of NFGNB among all clinical isolates
2. To determine the antibiotic susceptibility pattern of NFGNB isolated from clinical samples.

MATERIAL AND METHODS

The study was conducted for a period of one year between January 1 and December 31, 2020, in the Department of Microbiology, Nil Ratan Sircar Medical College, Kolkata. The samples received at the Department of Microbiology from various wards, intensive care unit and outdoors were processed. The samples received for processing were blood, body fluids, urine, sputum, endotracheal tube secretions, and central and peripheral line tips. Samples were inoculated into Blood agar and MacConkey agar and incubated at 37°C overnight at ambient air. NFGNB were isolated and identified through standard microbiological techniques (colony morphology, Gram staining and motility, and biochemical tests) and by the automated system Vitek 2 Compact (Biomerieux©). Antibiotic susceptibility test was done by both Vitek 2 Compact and Kirby Bauer disc diffusion method following CLSI guidelines 2020.[11] *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control organisms during the study.[12] The results were a analyzed, and interpretation was represented graphically in the form of various tables, pie charts, and bar diagrams.

RESULTS

Out of a total of 725 bacterial isolates, 169 NFGNB were isolated with an isolation rate of 23.31%. Of this 169 NFGNB, 84 isolates (49.7%) were *Pseudomonas* spp., 73 isolates (43.2%) were *Acinetobacter* spp., seven isolates were *B. cepacia* (4.14%), and five isolates were *S. maltophilia* (2.96%) [Figure 1].

Males were more commonly affected n = 115 (68%) as compared to females n = 54 (32%) [Figure 2].

Most of the NFGNB were isolated from pus (40.23%), followed by urine (20.71%), sputum (8.28%), body fluids (7.69%) and central and peripheral line tip (7.1%) [Table 1].

Most isolates of NFGNB were from the department of surgery 60 (35.5%), followed by pediatrics 32 (18.93%), orthopedics 20 (11.83%), hematology 15 (8.87%), chest 12 (7.1%), urology 8 (4.73%) medicine 7 (4.14%), gynecology and obstetrics 6 (3.55%), critical care 4 (2.36%), ENT 3 (1.77%), and endocrine 2 (1.18%) [Figure 3].

Antibiotic susceptibility profile of *Pseudomonas* spp. showed higher resistant pattern against cefepime, levofloxacin, and ticarcillin-clavulanic acid, and organisms were better susceptible to cefoperazone-sulbactam, piperacillin-tazobactam, and meropenem [Figure 4].

Among *Acinetobacter* spp. resistance was more against ceftazidime, levofloxacin, and ticarcillin-clavulanic acid,

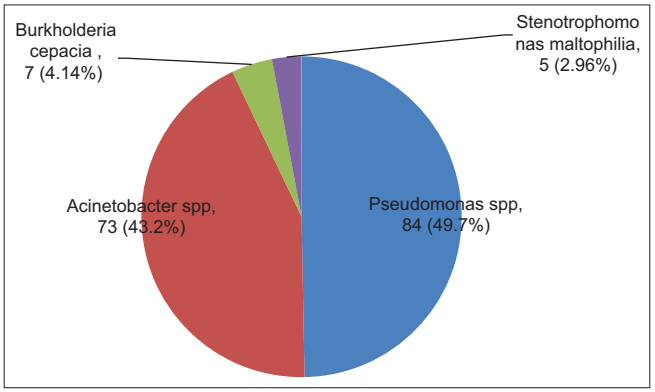

Figure 1: Pie diagram showing the distribution of non-fermenting Gram-negative bacilli into the type of isolated organism.

Table 1: The Distribution of types of samples.

S. No.	Source of sample	Number	Percentage
1.	Body fluid	13	7.69
2.	Blood	8	4.73
3.	Oral swab	2	1.18
4.	Urine	35	20.71
5.	Tissue sample	3	1.77
6.	Sputum	14	8.28
7.	Pus	68	40.23
8.	Et tube aspirate	9	5.32
9.	Cerebrospinal fluid	5	2.95
10.	Central line tip	12	7.1

Et: Endotracheal

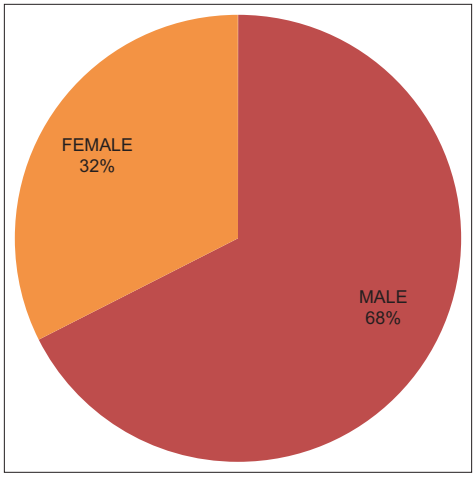

Figure 2: Pie diagram showing gender distribution of isolated non-fermenting Gram-negative bacilli.

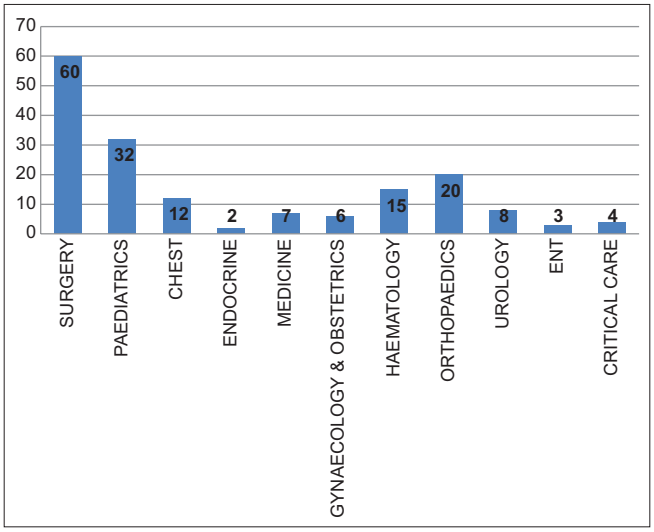

Figure 3: Bar diagram ward-wise distribution of isolated non-fermenting Gram-negative bacilli.

and better susceptibility was recorded against doripenem, meropenem, and piperacillin-tazobactam [Figure 5].

B. cepacia showed higher resistance against ticarcillin-clavulanic acid (82.16%) and better susceptibility to meropenem 75.29%. *S. maltophilia* showed better susceptibility against levofloxacin (92.64%), piperacillin-tazobactam (78.31%), minocycline (88.95%), and co-trimoxazole (85.7%).

DISCUSSION

In our study, out of 725 bacterial isolates, 169 isolates were NFGNB, with a proportion of 23.31%. This finding is similar to a study conducted by Vijaya *et al.*[12] (21.8%) and higher than a study conducted by Shastry *et al.*[13] (18.1%) and Kaur *et al.*[14] (16.5%) but lower than by a study done by Deb *et al.*[6] (29.6%) from the same institute. In the present study, the isolation rate of NFGNB out of the total samples was 68% males and 32% females, which is similar to the Jayapriya *et al.*[15] studies, which has reported NFGNB isolates from males 71% and females 29%.

Pseudomonas spp. (49.7%) and *Acinetobacter* spp. (43.2%) were the common isolates among NFGNB from pus, urine, and body fluids in samples; Deb S et al.[6] also showed a higher proportion of *Pseudomonas* spp. (52.8%) and *Acinetobacter* spp. (39.3%) isolated from clinical specimens. In our study, the isolation rate of NFGNB from Pus is 42.03% which is lower than Malini *et al.*[16] (62.17%) study. Isolation of NFGNB from urine samples is 21.01% in our study, which is higher than Benanchinmardi *et al.* (11%) and Malini *et al.*[16,17]

In our study, a greater proportion of *Pseudomonas* spp. (87%) *Acinetobacter* spp. (86.30%) were susceptible to piperacillin-tazobactam, but in a study by Shastri *et al.*,[13] only 75% of *Pseudomonas* spp. and 56.5% of *Acinetobacter* spp. were susceptible. Susceptibility of

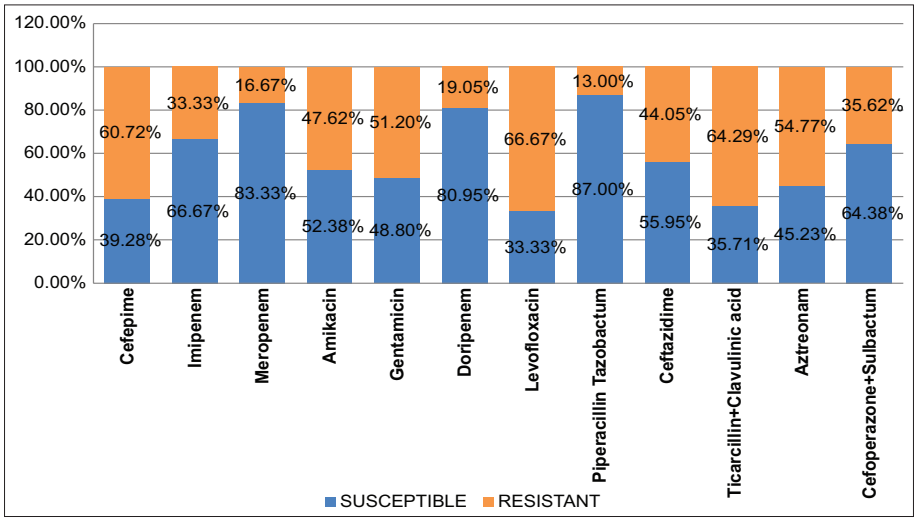

Figure 4: Bar diagram showing susceptibility pattern in blue color and resistant pattern in green color for *Pseudomonas* spp.

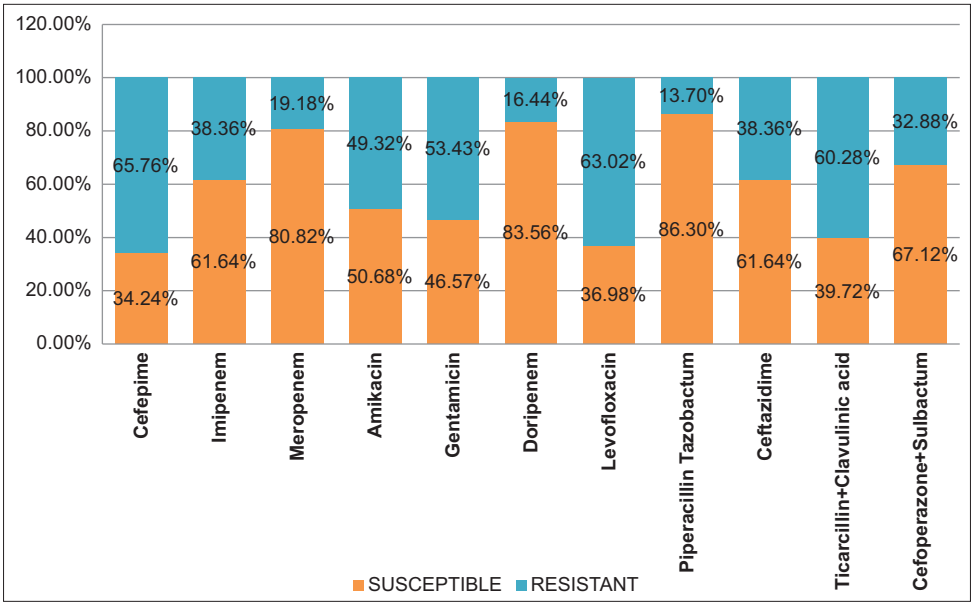

Figure 5: Bar diagram showing susceptibility pattern in orange color and resistant pattern in blue color for *Acinetobacter* spp.

Pseudomonas spp. and *Acinetobacter* spp. against meropenem were 83.33% and 80.82%, respectively, which was concordant with the findings of Maniyan *et al.*,[18] they showed that 79.6% of *Pseudomonas* spp. and 75% of *Acinetobacter* spp. were susceptible against meropenem.

Pseudomonas spp. and *Acinetobacter* spp. were least effective against cefepime (60.72% and 65.76% resistant) and levofloxacin (66.67% and 63.02%) that this finding is similar to the finding by Deb *et al.*,[6] they showed 97.1% NFGNB resistant against cefepime and 71% NFGNB resistant to levofloxacin.

Susceptibility profile of other NFGNB, *B. cepacia* showed higher susceptibility to meropenem, and *S. maltophilia* was susceptible to levofloxacin, piperacillin-tazobactam, and minocycline in greater proportion, and these findings are similar to findings by Gautam *et al.*,[19] Roy *et al.*,[20] and Namaji *et al.*[21]

CONCLUSION

This study highlights the significant presence of NFGNB in clinical samples with a notable proportion of pathogens such as *Pseudomonas* spp. and *Acinetobacter* spp. emphasizing the need for heightened awareness and surveillance in health-care settings. The antibiotic susceptibility patterns observed in this study unveil a concerning level of resistance among NFGNB, posing challenges to effective treatment strategies. The emergence of multidrug-resistant strains underscores the urgency of implementing stringent antimicrobial stewardship programs to curb the further spread of resistance. Antibiotic susceptibility patterns in our study are also crucial for clinicians to make informed decisions regarding empirical and targeted antibiotic therapy, ultimately contributing to improved patient outcomes. This study also highlights the importance of ongoing surveillance and continuous monitoring of antibiotic resistance patterns to adapt therapeutic approaches and mitigate the impact of resistant strains on patient outcomes.

Limitation of the study

1. Molecular mechanism of resistance pattern among NFGNB could not be detected
2. As this study was conducted during the COVID-19 pandemic, a smaller number of samples were received for processing as compared to the pre-COVID-19 period.

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Ethical approval

The Institutional Review Board approval is not required.

Declaration of patient consent

Patient's consent not required as there were no patients in this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript, and no images were manipulated using AI.

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