Section: Microbiology

Original Research Article

A STUDY OF THE ISOLATION OF NON-FERMENTING GRAM-NEGATIVE BACILLI AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN IN VARIOUS CLINICAL SAMPLES FROM A TERTIARY CARE HOSPITAL IN NORTHEAST INDIA

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Received : *15/05/2025*
Received in revised form : *03/07/2025*
Accepted : *22/07/2025*

Keywords:
Pseudomonas aeruginosa, Acinetobacter baumannii, Antibiogram, Drug resistance, Antimicrobial stewardship.

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DOI: 10.47009/jamp.2025.7.5.79

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2025; 7 (5); 399-403

ABSTRACT

Background: The Non-fermenting Gram-negative bacilli (NFGNB) which were once considered as contaminants have now emerged as important pathogens of nosocomial infections due to increased isolation and emergence of resistance to commonly used antimicrobial agents. The study aims to identify the NFGNB and to ascertain the antibiogram profiles of NFGNB isolates obtained from all the clinical samples received in the Department of Microbiology, Jawaharlal Nehru Institute of Medical Sciences, Imphal, Manipur over a period of three years (January 2022 to December 2024). **Materials and Methods:** All clinical samples received from various wards, intensive care units (ICUs), outpatient departments (OPDs) and accident & trauma care centre (ATC) were processed which include urine, sputum, tracheal aspirate, endotracheal tube, bronchoalveolar lavage (BAL), central and peripheral line tips, Foley's catheter, pus, swabs, blood, sterile body fluids, CSF and stool. The samples were inoculated into blood agar and MacConkey agar or CLED agar and incubated at 37°C overnight aerobically. NFGNB were isolated and identified by standard microbiological techniques like colony morphology, pigment production, Gram staining, Hanging drop motility, oxidase test, catalase test, Hugh-Leifson oxidative fermentative test for glucose, nitrate reduction test, indole test, citrate utilization test, urease test, lysine and ornithine decarboxylation test and arginine dihydrolase test. Antibiotic susceptibility test was done by Kirby Bauer disc diffusion method using commercially available disc (Hi-media). **Result:** Out of the total 50,533 clinical samples, 9246 (18.3%) were culture positive. Out of the 9246 culture positive samples, 1137(12.3%) NFGNB were isolated. Pseudomonas aeruginosa accounts for 963(84.7%) and Acinetobacter baumannii 174 (15.3%). No other NFGNB were isolated. Males were predominantly affected 730 (64%) while females were 407(36%). Majority of the isolates were from urine 283 (24.9%). Pseudomonas aeruginosa was highly susceptible to Piperacillin/Tazobactam (87.8%) while Acinetobacter baumannii to Amikacin (81.8%). **Conclusion:** It is crucial to identify these bacteria which are emerging as major pathogens of nosocomial infections. Active surveillance, strict infection control measures along with robust antimicrobial stewardship program should be carried out for controlling the multidrug resistance.

INTRODUCTION

The Non-fermenting Gram-negative bacilli (NFGNB) are organisms that are aerobic, non-sporing bacilli that do not utilize carbohydrates for their energy source or break it down metabolically rather than by fermentation.[1]

They are saprophytic in nature but can cause significant infections particularly healthcare-associated infection (HCAI) in hospitalized patients as well as immunocompromised patients.[2]

About 15% of bacterial isolates from clinical samples are NFGNB.[3]

NFGNB show resistance to a wide range of commonly used antibiotics by several mechanisms including innate resistance and by producing beta-lactamases, efflux pumps mediated and penicillin-binding protein site mutation.[4,5]

They are also intrinsically resistant to disinfectants used commonly and have tendency to colonise on various surfaces which have been crucial in their emergence as important pathogens of HCAI.[6]

Presently, Pseudomonas spp. and Acinetobacter spp. are the commonly isolated non-fermenters pathogenic to humans while infections caused by other NFGNB species are less frequent.[7,8]

On account of the increased isolation of NFGNB and their resistance against common antimicrobial agents, this study was conducted to evaluate the extent of NFGNB isolated in clinical samples and their susceptibility pattern in our hospital.

Aims and objectives:

The objectives of the study were:

1. To identify the NFGNB from all the clinical samples
2. To determine the antibiogram of NFGNB isolated

MATERIALS AND METHODS

The study was conducted retrospectively in the Department of Microbiology, Jawaharlal Nehru Institute of Medical Sciences, Imphal, Manipur over a period of 3 years extending from January 2022 to December 2024.

All clinical samples received from various wards, intensive care units (ICUs), outpatient departments (OPDs) and accident & trauma care centre (ATC) were processed which include urine, sputum, tracheal aspirate, endotracheal tube, bronchoalveolar lavage (BAL), central and peripheral line tips, Foley's catheter, pus, swabs, blood, sterile body fluids, CSF and stool.

The samples were inoculated into blood agar and MacConkey agar or CLED agar and incubated at 37°C overnight aerobically. NFGNB were isolated and identified by standard microbiological techniques like colony morphology, pigment production, Gram staining, Hanging drop motility, oxidase test, catalase test, Hugh-Leifson oxidative fermentative test for glucose, nitrate reduction test, indole test, citrate utilization test, urease test, lysine and ornithine decarboxylation test and arginine dihydrolase test. Antibiotic susceptibility test (AST) was done by Kirby Bauer disc diffusion method using commercially available disc (Hi-media).The different antimicrobials used were Gentamicin (10µg), Amikacin (30µg), Ceftazidime (30µg), Ceftriaxone (30µg), Piperacillin/Tazobactam (100µg/10µg), Imipenem (10µg), Meropenem (10µg), Ciprofloxacin (5µg), Trimethoprim/Sulfamethoxazole(1.25/23.75µg), Ampicillin/Sulbactam(10 µg /10 µg), Tetracycline (30 µg), Minocycline (30µg), Aztreonam(30µg) and Netilmicin (30 µg). The AST was interpreted following CLSI guidelines 2024. Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 were used as control strains during the study.[9]

Inclusion criteria

All the clinical samples sent to the Bacteriological section from various wards, OPDs, ICUs and ATC were included.

Exclusion criteria

Samples that were contaminated.

Statistical analysis: The results were analysed using Microsoft Excel Sheet and graphically represented in the form of tables, pie charts and percentages.

RESULTS

Out of the total 50,533 clinical samples, 9246(18.3%) were culture positive. Out of the 9246 culture positive samples, 1137(12.3%) NFGNB were isolated. Pseudomonas aeruginosa accounts for 963(84.7%) and Acinetobacter baumannii 174 (15.3%). No other NFGNB were isolated.

Males were predominantly affected 730 (64%) while females were 407(36%). [Figure 1]

The majority of the NFGNB were isolated from urine 283 (24.9%) followed by tracheal aspirate 227 (19.9%), endotracheal tube 192 (16.9%), sputum 165(14.5%), swabs 147 (12.9%), Foley's catheter 82(7.2%), sterile body fluid 21(1.8%), blood 15(1.3%) and stool 5 (0.4%) respectively [Table 1].

Most of the NFGNB isolated were from ICUs 489(43%) followed by wards 433 (38.1%), OPDs 206(18.1%) and ATC 9 (0.8%) respectively. [Figure 2]

The antibiotic susceptibility profile of Pseudomonas aeruginosa showed high susceptibility to Piperacillin/ Tazobactam (87.8%), Imipenem (78.5%), Meropenem (78.4%), Amikacin (69.9%), Gentamicin (63.6%), Ciprofloxacin (62.9%) and least susceptibility to Netilmicin (53.8%), Ceftazidime (45.8%), Ceftriaxone (45.8%) and Aztreonam (45.6%) respectively. [Table 2]

Acinetobacter baumannii isolates were more susceptible to Amikacin (81.8%), Piperacillin/ Tazobactam (72.7%), Gentamicin (69.6%), Imipenem (62.7%), Ciprofloxacin (52.2%) and least susceptibility to Tetracycline (46.3%), Minocycline (36.8%), Trimethoprim/Sulfamethoxazole (31.8%), Ampicillin/Sulbactam (28.6%) and Ceftazidime (21.4%) respectively. [Table 3]

ISSN (O): 2687-5365; ISSN (P): 2753-6556

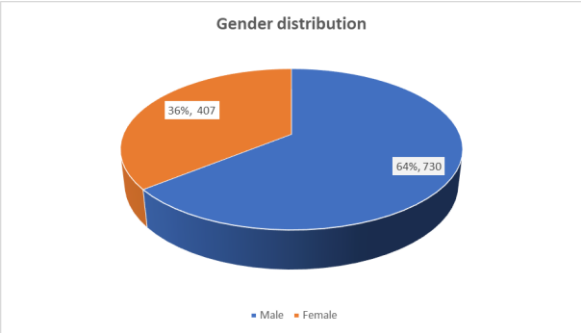

Figure 1: Pie chart showing gender distribution of NFGNB isolates

Table 1: Distribution of sample types

Sample source	Number	Percentage (%)
Urine	283	24.9
Tracheal aspirate	227	19.9
Endotracheal tube	192	16.9
Sputum	165	14.5
Swab	147	12.9
Foley's catheter	82	7.2
Sterile body fluid	21	1.8
Blood	15	1.3
Stool	5	0.4
Total	1137	100

Table 2:Antibiotic sensitivity pattern of isolated Pseudomonas aeruginosa

Antibiotic	Sensitivity	Percentage (%)
Piperacillin/Tazobactam	677 (N=771)	87.8
Imipenem	409 (N=521)	78.5
Meropenem	429 (N=547)	78.4
Amikacin	130 (N=186)	69.9
Gentamicin	112 (N=176)	63.6
Ciprofloxacin	550 (N=874)	62.9
Netilmicin	247 (N=459)	53.8
Ceftazidime	303 (N=662)	45.8
Ceftriaxone	66 (N=144)	45.8
Aztreonam	226 (N=496)	45.6

Table 3:Antibiotic sensitivity pattern of isolated Acinetobacter baumannii

Antibiotic	Sensitivity	Percentage (%)
Amikacin	27 (N=33)	81.8
Piperacillin/Tazobactam	80 (N=110)	72.7
Gentamicin	48 (N=69)	69.6
Imipenem	64 (N=102)	62.7
Ciprofloxacin	70 (N=134)	52.2
Tetracycline	38 (N=82)	46.3
Minocycline	21 (N=57)	36.8
Trimethoprim/Sulfamethoxazole	27 (N=85)	31.8
Ampicillin/Sulbactam	12 (N=42)	28.6
Ceftazidime	9 (N=42)	21.4

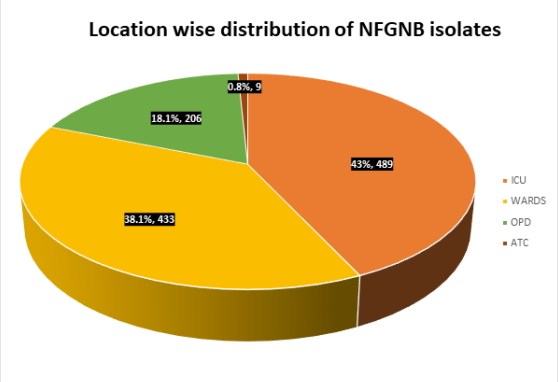

Figure 2: Pie chart showing location wise distribution of NFGNB isolates

DISCUSSION

In our study, out of the total 50,533 clinical samples,9246(18.3%) were culture positive. Out of the 9246 culture positive samples, 1137 (12.3%) were NFGNB. In studies done by Rit et al,[10] and Benachinmardi et al,[11] isolation rates of 12.8% and 10% were reported respectively which is in concordance with our study.

The most common NFGNB isolated in our study was Pseudomonas aeruginosa (84.7%,963/1137) followed by Acinetobacter baumannii (15.3%,174/1137) which is similar to results by Praveetha C et al,[12] where predominant isolate was Pseudomonas aeruginosa (65%) followed by Acinetobacter baumannii (22.54%) and by Ranjan R

ISSN (O): 2687-5365; ISSN (P): 2753-6556

et al,[4] who isolated P. aeruginosa 59.6% and A.baumannii (44.18%). A study by Malini et al,[13] found P. aeruginosa as the most common isolate accounting for 104/189 (53.8%) isolates, followed by A. baumannii (43/189, 22.2%). In another study by Rit et al,[10] P. aeruginosa was the predominant isolate (101/201, 50.24%), followed by A. baumannii (50/201, 24.8%).

In our study, there is male predominance (64%) followed by females with 36% which is similar to study conducted by Choudhary et al,[14] (males 68%, females 29%), Jayapriya et al,[15] (males71%, females 29%) and Praveetha C et al,[12] (59.55 % males, 40.45% females).

The greatest number of isolates in our study were from urine (24.9%) which is similar to studies conducted by Ranjan R et al,[4] (34.1%) and Sarkar M et al,[16] (29.4%) while other studies done by Praveetha C et al,[12] Soni et al,[17] Benachinmardi et al,[11] found pus/swab samples to be the most common clinical sample.

In the present study, Pseudomonas aeruginosa was found to be more susceptible to Piperacillin/ Tazobactam (87.8%) which is similar to study conducted by Choudhary et al,[14] (87%) and Shastri et al,[18] (75%) while least susceptibility was shown to drugs like Aztreonam (45.6%), Ceftazidime (45.8%) and Ceftriaxone (45.8%). A study done by Sarkar M et al,[16] showed susceptibility to Piperacillin/Tazobactam combination was 38.15% while Gokale S et al,[19] showed P. aeruginosa had good susceptibility to Meropenem (96.2%) followed by Ciprofloxacin (50%) and Amikacin (49.5%).

Acinetobacter baumannii in our study showed 81.8% susceptibility to Amikacin and 72.7% to Piperacillin/Tazobactam and least susceptibilities to Ampicillin/Sulbactam (28.6%) and Ceftazidime (21.4%). Choudhury et al,[14] in their study found Acinetobacter spp. were 86.30% susceptible to Piperacillin/Tazobactam. However, study by Gokale S et al,[19] reported highest susceptibility of A. baumannii to Meropenem (96.2%) and 45% susceptibility to Ciprofloxacin.

CONCLUSION

In conclusion, NFGNB which were once considered as contaminants are now an important pathogen of nosocomial infections. In our study, the notable presence of NFGNB particularly Pseudomonas aeruginosa and Acinetobacter baumannii in the clinical samples demand for a heightened clinical awareness and multidisciplinary approach. The variable susceptibility patterns to various antimicrobials pose a threat to effective treatment strategies for curbing the infections. The integration of active surveillance, strict infection control measures and a robust antimicrobial stewardship program should be carried out for controlling the emergence of multidrug resistance.

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ISSN (O): 2687-5365; ISSN (P): 2753-6556