VOLUME - 13, ISSUE - 06, JUNE - 2024 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Clinical Microbiology

DETECTION OF BETA-LACTAMASE PRODUCING GRAM-NEGATIVE NONFERMENTERS IN CRITICAL CARE UNIT OF A TERTIARY CARE HOSPITAL OF EASTERN INDIA.

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ABSTRACT At present times, aerobic nonfermenting gram negative bacilli (NFGNB) are associated with life threatening infections in patients of critical care units and re-emerging as multidrug resistant (MDR) pathogens which is a major factor for patients' outcome. **Objective:** To isolate and identify gram negative nonfermenters phenotypically from the Critical Care Unit patients' samples and to determine their antibiogram profile with beta-lactamase production by Vitek 2 compact system (bioMérieux). **Methodology:** - After collection, samples were processed by automated microbiological procedure. Antibiotic susceptibility of the isolated organisms was determined according to CLSI guidelines. Beta-lactamase production by NFGNB were detected by Vitek 2 compact system (bioMérieux). Then the data were recorded in a Microsoft Excel Spreadsheet and were analyzed according to the appropriate statistical tests. **Result:** Total nine species of NFGNB were identified which included Pseudomonas aeruginosa (46.96%), followed by Acinetobacter baumannii (25.75%) and Burkholderia cepacia complex (9.09%). 32.25% of isolated strains of Pseudomonas aeruginosa were producing carbapenemase, 9.67% were producing AmpC beta-lactamase and 12.90% were producing extended spectrum beta-lactamases. 59% of isolated strains of Acinetobacter baumannii were producing carbapenemase and 6% were producing AmpC beta-lactamase. 59% of isolated strains of Acinetobacter baumannii were multidrug resistant organism (MDRO). 33% of isolated strains of Burkholderia cepacia complex were MDRO. **Conclusion:** Most of the isolated NFGNB are MDRO and are producing beta-lactamases. On the other hand, these microorganisms have a huge potential to survive in hospital environment. So proper implementation of infection control procedures and antibiotic stewardship is needed to combat with healthcare associated infections.

KEYWORDS : NFGNB, Beta-lactamase production, MDRO.

INTRODUCTION:

Aerobic non-fermenting Gram-negative bacilli (NFGNB) are taxonomically diverse group of nonsporing bacilli that either do not utilize glucose as a source of energy or utilize it oxidatively.[1] They are essentially saprophytes, and up until recently, they were thought to either contaminants or commensals of little.[2,3] However, recent literature review shows that these organisms are now associated with life-threatening infections such as septicaemia, pneumonia, urinary tract infection, meningitis, surgical site infection, ventilator associated pneumonia (VAP), wound infection, etc. among ICU patients.[4] They account for around 15% of all bacterial isolates from clinical samples.[5]

Infection is the most common presentation among hospitalized patients of intensive care unit (ICU), and in many instances, is determining factor for patient outcomes.[6,7] Healthcare associated infections (HCAIs), in particular, are the major risks associated with critically ill patients of ICU, due to the reduced host defences, frequent use of invasive medical devices, administration of multiple drugs, cross transmission of pathogens among patients and staffs, and inadequate infection control procedures.[8,9] According to a large surveillance study, more than 70% of critically ill patients receive an antimicrobial drug during their ICU stay either for prophylaxis or for therapy.[6] Nevertheless, in the recent years, therapeutic drugs are being progressively ineffective against bacterial infections, threatening the success of routine treatment.[10] The major consequences of this problem are increased patient morbidity, mortality, health care related expenses and treatment failure.[11,12]

Pseudomonas aeruginosa, Acinetobacter baumannii, Strenotrophomonas maltophilia, and, less frequently, members of the *Burkholderia cepacia* group, are the species that NFGNB are most concerned about.[13] Except *P.aeruginosa* the NFGNB are most often cause nosocomial infections in immune-compromised Patients.[14] The major concerning factor is multidrug resistant property of this organisms which severely limits the treatment options.

One of the main risk factors for the emergence of infections with resistance is prior antibiotic usage. For the purpose of treating infections, preventing the emergence of resistance, and reducing hospital acquired cross infection, bacterial aetiologies and infection patterns should be monitored. Knowledge of susceptibility patterns are helpful in selecting empirical therapy and improving the likelihood of a satisfactory outcome for the patient.[15] The object of this study is to examine antimicrobial susceptibility pattern of NFGNB isolates to commonly used drug in CCU patients at a tertiary care hospital of West Bengal.

MATERIALS AND METHODS

Study Type- Institutional based observation study with cross section design.

Study Design-cross-sectional descriptive design.

Study Setting And Timelines- The study was carried out in bacteriology room under Department of Microbiology of Bankura Sammilani Medical College and Hospital, Bankura, West Bengal with time frame of one and a half year from acceptance of synopsis.

Place Of Study-

Department of Microbiology, Bankura Sammilani Medical College & Hospital, Bankura.

Sample size- calculated sample size for this study is around 143.

Inclusion Criteria

i. Patients: male or female admitted in Critical Care Unit of BSMC&H.
ii. Age group: 12 years onwards
iii. Informed written consent

Exclusion Criteria

i. Known seropositive patient (HIV, HBV, HCV)
ii. Patients with known COVID 19 infections.
iii. Terminally ill patient where any procedure may cause detrimental effect to the patient.

Study Variables:

i. Age
ii. Sex (male/female)
iii. Residence (urban/rural)
iv. Relevant clinical history
v. Duration of illness
vi. Medication history

Methodology:

Following information will be collected from the patient about

1. Relevant clinical history, relevant test reports.
2. Ongoing medication history.

Blood (10ml) should be collected aseptically in automated blood culture bottle. Other clinical samples (e.g., urine, pus, wound swab, sputum, pleural fluid etc.) should be collected aseptically in sterile container. All the samples should be collected with proper labelling. Sample should be disposed to the department of Microbiology without any delay. The sample should be received carefully after checking the details.

Samples will be inoculated onto feasible agar media and blood samples will be incubated in BACT/ALERT/ 3D/60 and after giving positive signal from automated blood culture bottle, subculture to be done in suitable media. After overnight incubation, colony characteristics of different bacteria shall be identified by colony size, shape, colour, sugar fermentation status etc. and confirmed by Gram staining, motility, and other conventional biochemical tests.

Identified pure bacterial growth is inoculated in Mueller-Hinton agar medium for antibiotic sensitivity pattern of the bacteria by disk diffusion method. For automated identification and antibiotic susceptibility testing gram stain will be done from pure culture. As per gram stain findings the organism will be introduced to the automated system. By automated system identification and antibiotic susceptibility pattern with minimum inhibitory concentration (MIC) of the organism will be done.

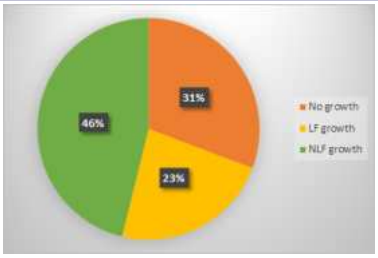
Statistical Analysis Plan:

Data will be compiled in Microsoft (MS) excel sheet and then analyzed by appropriate statistical methods. Data display will be done by the help of tables and various charts.

RESULT:

In this study a total of 143 different clinical samples were collected from patients admitted in CCU above 12 years of age. After sample collection, those were processed according to standard guidelines.

Figure no 1: Distribution of study subjects according to culture findings



(LF= lactose fermenting, NLF= non-lactose fermenting)

Figure no. 1 revealed that out of 143 samples 66 samples (46.15%) showed NLF growth.

Table no 1: Distribution of study subjects according to NLF (non-lactose fermenting) growth on culture (n=66)

Name of Sample	Number of samples	Percentage (%)
ET aspirate	1	1.51
Foley's catheter tip	2	3.03
Urine	10	15.15
Pus	12	18.18
Blood	14	21.21
Sputum	27	40.90
Total	66	100

Table no. 1 showed that the isolation rate of NFGNB was highest from sputum sample (40.90%) followed by blood (21.21%), pus (18.18%) and urine (1.51%).

Table no 2: Distribution of study subjects according to NLF (non-lactose fermenting) growth on culture (n=66)

Name of organism	Numbers of isolates	Percentage (%)
Acinetobacter junii	1	1.51
Pseudomonas fluroscence	2	3.03
Pseudomonas putida	2	3.03
Sphingomonas paucimobilis	2	3.03
Strenotrophomonas maltophilia	2	3.03
Acinetobacter lowffii complex	3	4.54
Burkholderia cepacia complex	6	9.09
Acinetobacter baumannii	17	25.75
Pseudomonas aeruginosa	31	46.96
Total	66	100

Table no. 2 revealed that from total isolates, nine different NFGNB were identified which included *Pseudomonas aeruginosa* (46.96%), followed by *Acinetobacter baumannii* (25.75%), *Burkholderia cepacia complex* (9.09%), *Acinetobacter lowffii complex* (4.54%), *Strenotrophomonas maltophilia* (3.03%), *Sphingomonas paucimobilis* (3.03%), *Pseudomonas putida* (3.03%), *Pseudomonas fluroscence* (3.03%) and *Acinetobacter junii* (1.51%).

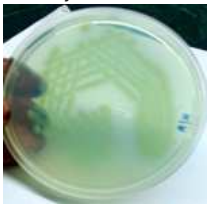


Figure 2: Colonies of *Pseudomonas aeruginosa* on nutrient agar.

Figure 3: Biochemical test for identification of *Pseudomonas aeruginosa*.

Table no 3: Distribution of non-fermentative gram-negative bacteria in different clinical specimens (n=66)

Name of Isolates	Urine	Sputum	Blood	Pus	ET aspirate	Foley's catheter tip
Acinetobacter junii	0	0	1	0	0	0
Pseudomonas fluroscence	0	1	0	1	0	0
Pseudomonas putida	0	2	0	0	0	0
Sphingomonas paucimobilis	0	0	0	2	0	0
Strenotrophomonas maltophilia	0	0	1	1	0	0
Acinetobacter lowffii complex	0	1	1	0	1	0
Burkholderia cepacia complex	0	3	2	1	0	0
Acinetobacter baumannii	2	8	3	3	0	1
Pseudomonas aeruginosa	8	12	6	4	0	1
Total	10	27	14	12	1	2

Figure 4: Non-lactose fermenting colonies of *Strenotrophomonas maltophilia* on MacConkey agar.

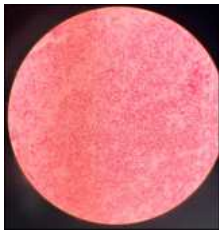


Figure 5: Gram stain from NLF colonies of *Strenotrophomonas maltophilia* shows gram negative bacilli arranged haphazardly.

Table no 4: Distribution of beta-lactamase production by *Pseudomonas aeruginosa* (n=31)

Type of beta-lactamase	Number of isolates	Percentage (%)
ESBL	4	12.90
AmpC beta-lactamase	3	9.67
Carbapenemase	10	32.25
None	14	45.16
Total	31	100

(ESBL= Extended spectrum beta-lactamase)

Table no. 4 showed that 32.25% of isolated strains of *Pseudomonas aeruginosa* were producing carbapenemase, 9.67% were producing AmpC beta-lactamase and 12.90% were producing ESBL.

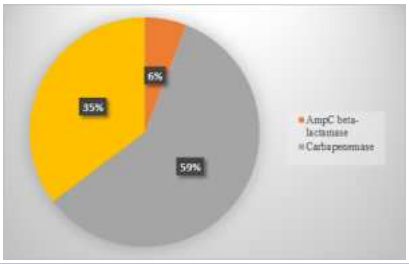


Figure no 6: Distribution of beta-lactamase production by *Acinetobacter baumannii* (n=17)

59% of isolated strains of *Acinetobacter baumannii* were producing carbapenemase and 6% were producing AmpC beta-lactamase.

Table no 5: Distribution of multidrug resistant strains of *Acinetobacter baumannii* (n=17)

MDRO	Number of isolates	Percentage (%)
Yes	10	59
No	7	41
+Total	17	100

Table no. 5 showed that 59% of isolated strains of *Acinetobacter baumannii* were multidrug resistant organism (MDRO).

Table no 6: Distribution of beta-lactamase production by *Burkholderia cepacia* complex (n=6)

Type of beta-lactamase	Number of isolates	Percentage (%)
ESBL	0	0
AmpC beta-lactamase	0	0
Carbapenemase	3	50
None	3	50
Total	6	100

Table no. 6 showed that 50% of isolated strains of *Burkholderia cepacia* complex were producing carbapenemase.

Table no 7: Distribution of multidrug resistant strains of *Burkholderia cepacia* complex (n=6)

MDRO	Number of isolates	Percentage (%)
Yes	2	33
No	4	67
Total	6	100

Table no. 7 showed that 33% of isolated strains of *Burkholderia cepacia* complex were multidrug resistant organism (MDRO).

Figure 7: Non-lactose fermenting colonies of *Burkholderia cepacia* complex on MacConkey agar.

DISCUSSION:

Healthcare associated infections (HAIs) caused by NFGNB are a major concern now a days, especially due to their capability of producing different beta-lactamases and being multi-drug resistant organisms.

In this study, most common NFGNB isolated was *Pseudomonas aeruginosa* (46.96%) followed by *Acinetobacter baumannii* (25.75%) which is similar to findings of Juyal et al[3] and Bhatnagar et al.[16] Next to that present study revealed an isolation rate of 9.09% for *Burkholderia cepacia* complex followed by *Acinetobacter lowffii complex* (4.54%), *Strenotrophomonas maltophilia* (3.03%), *Sphingomonas paucimobilis* (3.03%), *Pseudomonas putida* (3.03%), *Pseudomonas fluroscence* (3.03%) and *Acinetobacter junii* (1.51%). On the other hand, Yadav et al have found that most common NFGNB isolates was *Acinetobacter baumannii* (44.0%) followed by *Pseudomonas aeruginosa* (40.1%), *Burkholderia cepacia* complex (8.2%), and *Acinetobacter calcoaceticus* (2.7%). Sah et al have also found that most common NFGNB isolated was *Acinetobacter baumannii*.[17]

Isolated *Pseudomonas aeruginosa* strains were 100% sensitive to colistin. 70.96% strains were sensitive to amikacin, 74.19% strains were sensitive to gentamicin and 61.29% strains were susceptible to fluoroquinolones (e.g. levofloxacin and ciprofloxacin). 32.25% of isolated strains of *Pseudomonas aeruginosa* were producing carbapenemase, 9.67% were producing AmpC beta-lactamase and 12.90% were producing ESBL. 29% of isolated strains of *Pseudomonas aeruginosa* were MDRO.

94.11% strains of *Acinetobacter baumannii* were sensitive to colistin, 41.17% strains were sensitive to amikacin, 47.05% strains were sensitive to gentamicin and 41.17% strains were susceptible to fluoroquinolones (e.g. levofloxacin and ciprofloxacin). 59% of isolated strains of *Acinetobacter baumannii* were producing carbapenemase and 6% were producing AmpC beta-lactamase. 59% of isolated strains of *Acinetobacter baumannii* were MDRO.

100% strains of *Acinetobacter lowffii* were resistant to aztreonam, 66.66% strains were resistant to amikacin, 100% strains were resistant to ciprofloxacin and 66.66% strains were resistant to levofloxacin. 100% of isolated strains of *Acinetobacter lowffii* were producing carbapenemase. 67% of isolated strains of *Acinetobacter lowffii* were MDRO.

50% strains of *Burkholderia cepacia* complex were resistant to amikacin, 66.67% strains were resistant to gentamicin, 33.33% strains were resistant to ciprofloxacin and levofloxacin. All isolated strains were resistant to colistin due to intrinsic resistant. 50% of isolated strains of *Burkholderia cepacia* complex were producing carbapenemase. 33% of isolated strains of *Burkholderia cepacia* complex were MDRO.

CONCLUSION:

In ICU settings, NFGNB remains a significant pathogen, maintaining a consistent presence over recent decades. European surveys have highlighted its prominence in ICU-acquired infections, with recent multicenter studies in Belgium and Italy ranking it as the foremost Gram-negative species recovered. Various factors contribute to its prevalence, including patient conditions, invasive procedures, and microbial characteristics like virulence and drug resistance due to beta-lactamase production and by other mechanisms.

NFGNB once considered contaminants, are now recognized as important pathogens in healthcare-associated infections. Their multidrug resistance and ability to persist in hospital environments pose significant challenges. Surveillance and monitoring of antibiotic susceptibility patterns are crucial for effective management.

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