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Original Research Article

Characterization of Non Fermenting Gram Negative Bacilli and Determination of Their Antimicrobial Susceptibility Pattern

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Abstract

Background: Non-fermenting Gram negative bacilli (NFGNB) are capable of causing a variety of infections like bloodstream infections, skin and soft tissue infections including burn and surgical wound infections, endocarditis, meningitis and urinary tract infections. Furthermore, infections caused by NFGNB are not limited to hospital settings and cases involving otherwise healthy individuals of all age groups, occurring in community settings, following natural disasters and wars has been reported.

Material and Methods: This prospective observational study was conducted from January 2021 to June 2022 in the Microbiology laboratory in Katihar Medical College, Katihar.

120 consecutive isolates of NFGNB were identified by VITEK 2 Gram Negative identification card and susceptibility testing was performed using the same instrument.

Results: Out of 120 NFGNB 41.7% was *Pseudomonas aeruginosa*, 25.0% was *Acinetobacter baumannii*, 15.8% was *Burkholderia cepacia*, 6.7% was *Pseudomonas putida*, 3.3% was *Pseudomonas oleovorans* and *Alkaligenes* spp, *S. maltophillia* and *Sphingomonas paucimobili* were2.5% each. *P. aeruginosa* showed maximum susceptibility to levofloxacin 66% and maximum resistance to ceftazidime 62%, *Acinetobacter baumannii* showed maximum susceptibility to minocycline and maximum resistance to piperacillin/tazobactam whereas *Burkholderia cepacia* showed maximum sensitivity to trimethoprim/ sulfamethaxazole.

Conclusion: The present study gives us indication regarding the occurrence of NFGNB in Eastern Bihar. Isolation of non-fermenters and their antibiotic susceptibility pattern should be regarded with seriousness by Microbiology laboratories, in clinical practice and in clinical epidemiology because being resistant to multiple antibiotics, their prevalence not only limits treatment options but they also act as reservoir of drug resistance genes.

Keywords: Non Fermenting Gram negative bacilli, Antibiotic Susceptibility Pattern.

Introduction

Non-fermenting Gram negative bacilli (NFGNB) are a group of bacteria that grow only in the presence of oxygen and appear as uniformly stained Gram negative bacilli or coccobacilli. They metabolize glucose with the production of very less acidic product that too in the presence of oxygen. [1]

NFGNB are notorious pathogens with acquired antibiotic resistance that leaves few options for appropriate selection of antibiotics. [2] Most laboratories perform a few key biochemical tests on daily basis that misses out the emerging pathogens which have unique phenotypic characteristics. An extended panel of biochemical tests is required for their identification which is time consuming.

Plasmids facilitate the transfer of antibiotic resistant genes easily in these NFGNB.

Thus, there is a need for inclusion of these tests on a routine basis for identification of NFGNB. This in turn will help in throwing light on the prevalence of these organisms locally and raise awareness among the clinicians for rational use of antibiotics in critically ill patients who are infected with these pathogens. Extended spectrum beta-lactamase production and carbapenemase production are important mechanisms of resistance seen in NFGNB. [3]

Material and Methods

This hospital based prospective observational study was conducted to isolate and identify non-fermenting Gram negative bacilli from clinical samples received in the Microbiology department for routine culture and sensitivity and also to determine their antibiotic susceptibility pattern. The study was conducted from January 2021 to June 2022 in the Microbiology Laboratory of Katihar Medical College and Hospital, Katihar.

A total of 8217 clinical samples were received from patients admitted in various wards of the hospital including ICUs during the study period. Out of these 2974 samples showed growth of various bacteria and from amongst these 120 consecutive isolates of NFGNB were taken up for further study.

A proper history was taken from each patient with details of age, sex, occupation, residence, present and past history and history of antibiotic use.

All clinical samples were collected following standard sample collection procedure. [4]

For blood cultures BacT/ALERT BPA disposable culture bottles [Organon Teknika Corp. Durham, NC] containing 40ml of media and an internal sensor that detects carbon dioxide as an indicator of microbial growth was used. [5]

Urine samples were inoculated on blood agar and CLED medium by semi quantitative method using 4 mm [24 SWG] internal diameter standard loop. After overnight incubation at 37^0C, culture plates yielding bacterial counts of $\geq 10^5$ CFU/ml were considered as significant. The presence of the microorganisms in the blood culture bottle was indicated by the presence of CO_2 produced during metabolism of the bacteria. Subcultures were done from the bottles on chocolate agar [CA], blood agar [BA] and MacConkey agar [MA] plates and examined for any growth. All culture plates were incubated at 37ºC for 18 to 24 hrs. All other samples were also cultured on blood agar and MacConkey agar except sputum samples and body fluids which were also cultured on chocolate agar.

If there was presence of bacterial growth on BA, CLED, CA and MA they were processed for identification and characterization up to species level. [4]

Vitek 2 Gram Negative (Gn) Identification:

The VITEK 2 Compact (30 card capacity) system uses an advanced colorimetric methodology for organism identification and MIC based microdilution method for susceptibility testing. The VITEK 2 Gram-Negative identification card (GN) was used for the automated identification of non-fermenting Gram negative bacilli. The GN card is based on established biochemical methods and newly developed substrates measuring carbon source utilization and enzymatic activities. Final results were available in approximately 10 hours or less.

[6] The method used for antimicrobial susceptibility testing was doubling dilution technique for MIC based on microdilution method. A suspension of test organism was made in 3 ml of 0.45% sterile saline filled in unsensitized tube and adjusted with MacFarland *via* insertion into an optical block of the Densicheck Plus to get an acceptable reading between 0.5 – 0.63. In a second tube containing 3 ml of saline, 145 µl of diluted test organism was transferred. Then this tube was placed in the cassette with a susceptibility card. [7]

Results

Out of 8217 clinical samples received in the Department laboratory, only 2974 showed growth of various microorganisms. Out of the 2974 samples, 2874 showed growth of single organism and 100 showed polymicrobial growth. NFGNB grew in 567 samples, out of which 120 consecutive strains were taken up for further study. Out of the 120 isolates, 111 (92.5%) were isolated in pure culture while the rest were in combination with Gram positive or Gram negative bacteria.

NFGNB were predominantly isolated from age group 0-10 years (29.2%) followed by 41-50 years (13.3%). The overall male to female ratio was 1.5:1 (Table 1).

Table 1: Age and gender wise distribution of patients

Age group (yrs)	Female (%)	Male (%)	Total (%)
0-10	19 (39.5)	16 (22.2)	35 (29.2)
11-20	03 (6.3)	06 (8.3)	09 (7.5)
21-30	04 (8.3)	10 (13.9)	14 (11.6)
31-40	05 (10.4)	05 (6.9)	10 (8.3)
41-50	05 (10.4)	11(15.3)	16 (13.3)
51-60	08 (16.6)	07 (9.7)	15 (12.5)
61-70	03 (6.3)	10 (13.8)	13 (10.8)
>70	01(2.0)	07 (9.7)	08 (6.6)
Total	48 (40.0)	72 (60.0)	120

Maximum number of NFGNB were isolated from department of Paediatrics 26.7% (32/120) followed by Surgery 25.0% (30/120) and department of Medicine 23.3% (28/120). Most of the NFGNB were isolated from indoor patients 55.8% (67/120) as compared to outdoor patients 44.2% (53/120).

NFGNB were predominantly isolated from wound swabs 40 (33.3%) followed by blood 38 (31.7%) and urine 27 (25.2%).

Eight different species of NFGNB were identified, of which *P. aeruginosa* was the commonest 41.7% (50/120) followed by *Acinetobacter baumannii* 25.0 % (30/120) and *Burkholderia cepacia* 15.8% (19/120) (Table 2).

Table 2: NFGNB Isolated from Different Clinical Samples

Organism Isolated	Number	Percentage
Pseudomonas aeruginosa	50	41.7
Pseudomonas putida	08	6.7
Pseudomonas oleovorans	04	3.3
Acinetobacter baumannii	30	25.0
Burkholderia cepacia	19	15.8
Alkaligenes species	03	2.5
Stenotrophomonas maltophilia	03	2.5
Sphingomonas paucimobilis	03	2.5
Total	120	100

P. aeruginosa showed maximum sensitivity to levofloxacin 66% followed by minocycline 62%. Almost all strains showed intermediate sensitive to colistin 98%. Resistance to ceftazidime was 62% followed by aztreonam 48% and ciprofloxacin 46% (Table 3).

Table 3: Antimicrobial Susceptibility Pattern in *Pseudomonas aeruginosa*(n=50)

Name of Antibiotics	Sensitive (%)	Intermediate (%)	Resistant (%)
Levofloxacin	33(66.0)	05(10.0)	12(24.0)
Minocycline	31(62.0)	02(4.0)	17(34.0)
Ceftazidime	16(32.0)	03(6.0)	31(62.0)
Cefoperazone/sulbactam	30(60.0)	08(16.0)	12(24.0)
Ciprofloxacin	21(42.0)	06(12.0)	23(46.0)
Meropenem	25(50.0)	04(8.0)	21(42.0)
Imipenem	27(54.0)	03(6.0)	20(40.0)
Cefepime	22(44.0)	08(16.0)	20(40.0)
Aztreonam	20(40.0)	06(12.0)	24(48.0)
Amikacin	26(52.0)	02(4.0)	22(44.0)
Colistin	00(0.0)	49(98.0)	01(2.0)
Gentamycin	27(54.0)	03(6.0)	20(40.0)
Piperacillin/Tazobactam	23(46.0)	11(22.0)	16(32.0)

Acinetobacter baumannii showed maximum sensitivity to minocycline 43.3% followed by levofloxacin and trimethoprim/sulfamethoxazole 26.7% each, and ceftazidime and gentamycin 20.0%. Resistance to ciprofloxacin and piperacillin/tazobactam was 86.7%each followed by ceftazidime, meropenem, imipenem, cefipime and gentamicin 76.7%each and amikacin73.3% (Table 4).

Table 4: Antimicrobial Susceptibility Pattern in *Acinetobacter baumannii* (n=30)

Name of Antibiotics	Sensitive (%)	Intermediate (%)	Resistant (%)
Levofloxacin	08(26.7)	03(10.0)	19(63.3)
Minocycline	13(43.3)	01(3.3)	16(53.3)
Trimethoprim/Sulfamethoxazole	08(26.7)	01(3.3)	21(70.0)
Ceftazidime	06(20.0)	01(3.3)	23(76.7)
Cefoperazone/ sulbactam	05(16.7)	06(20.0)	19(63.3)
Ciprofloxacin	04(13.3)	00(00.0)	26(86.7)
Meropenem	05(16.7)	02(6.7)	23(76.7)
Imipenem	05(16.7)	02(6.7)	23(76.7)
Cefepime	02(6.7)	05(16.7)	23(76.7)
Amikacin	05(16.7)	03(10.0)	22(73.3)
Colistin	00(00.0)	28(93.3)	02(6.7)
Gentamycin	06(20.0)	01 (3.3)	23(76.7)
Piperacillin/Tazobactam	03(10.0)	01(3.3)	26(86.7)

Majority of *Burkholderia cepacia* strains showed sensitivity to trimethoprim/ sulfamethoxazole 84.2% followed by ceftazidime 79.0%, and minocycline 73.7%. Maximum resistance 100.0% was seen with colistin followed by amikacin 84.2% and gentamycin 68.4%.

On analysis of AES data of Vitek 2 Compact, various mechanisms of resistance to β-lactams was determined, 56.0% were found to be ESBL producers 8.5% strains

were carbapenamase producers and 42.0% strains showed impermeability to carbapenems. ESBLs, cephalosporinases and penicillinases were produced by 5 strains (10.0%).

Discussion

Out of 8217 clinical samples received in the department laboratory during the study period, only 36.2% showed growth of various microorganisms. NFGNB grew in 567 (19.0%) samples, out of which 120 consecutive strains were taken up for further study. Out of the 120 isolates, 111 (92.5%) were isolated in pure culture while the rest were in combination with Gram positive or Gram negative bacteria**.**

Patel et al (2010) reported culture positivity 48.3% in their study. NFGNB was isolated in 23.9% of samples. [1]

Isolation rate was found to be the most in the age group 0-10 years (29.2%) followed by 41-50 years (13.3%). The overall male to female ratio was 1.5:1. Wadhwa et al (2016) reported majority of patients were found in the age group of 45-60 years (35.4%) followed by age group 0-15 and 60-75 years 19.1% each.8Motbainor et al (2020) reported maximum number of patients in the age group >51 yrs (21.4%) followed by age group 21-30yrs (19.7%) and 1-10yrs (16.4%). [9]

Maximum number of NFGNB was isolated from Department of Paediatrics 26.7% followed by Surgery 25.0% and Medicine 23.3%.Most of the NFGNB were isolated from indoor patients 55.8% as compared to outdoor patients 44.2%.Yadav et al (2020) in their study found isolation rate of NFGNB to be 39.1% from ICU, 21.1% from Surgical and Medical wards, 6.5% from Orthopaedics and 4.9% from Paediatric wards. [10]

Among the 120 isolates, maximum number was from wound swab 33.3% followed by blood 31.7% and urine 25.2%. Patel et al in 2013 reported 58.6% NFGNB from wound swab, 10.8% from urine and6.9% from sputum. [1] Sharma et al (2014) reported 28.6% from wound swab, 23.7% from urine and 4.6% from blood. [2] These findings are similar with those of the present study.

In our study, 120 NFGNB were isolated which included eight different species, the most common being *P. aeruginosa* 41.7% followed by *Acinetobacter baumannii* 25.0% and *Burkholderia cepacia* 15.8%. Wadhwa et al (2016) reported 59.8% *A. baumannii,* 33.1% *P. aeruginosa*, 5.2% *Stenotrophomonas maltophilia* and 1.7% *Burkholderia cepacia* among the NFGNB isolated. [8]

Maximum number of *P. aeruginosa* strains was found sensitive to levofloxacin 66.0% and minocycline 62.0%. Resistance to ceftazidime was maximum 62.0% followed by aztreonam 48.0%. Yadav et al (2020) reported that strains of *P. aeruginosa* were found susceptible to imipenem 42.2%, meropenem 38.5%, levofloxacin 23.0% and cefepime15.5%. [10]

Maximum number of strains of *A. baumannii* was found sensitive to minocycline 43.3% followed by levofloxacin and trimethoprim/ sulfamethoxazole 26.7%. Resistance to ciprofloxacin and piperacillin/tazobactam was maximum 86.7% followed by ceftazidime, cefepime, cefeperazone/ sulbactum 76.7% each and amikacin 73.3%. Yadav et al (2020) reported that all strains were 100% susceptible to polymixin B and colistin sulfate followed by doxycycline 42.9% and amikacin 20.9%. [10]

Most strains of *Burkholderia cepacia* were found sensitive to trimethoprim/ sulfamethoxazole 84.2% followed by ceftazidime 79.0% and minocycline 73.7%. Maximum (100.0%) resistance was seen to colistin followed by amikacin (84.2%) and gentamycin (68.4%). Shukla et al (2018) reported maximum susceptibility to ceftazidime 72.1% followed by minocycline 55.8%. Maximum resistance was reported with ticarcillin/clavulanic acid

83.7% followed by cotrimoxazole 53.4%. [11,12]

Conclusion

The present study gives us indication regarding the occurrence of NFGNB in Eastern Bihar. Isolation of non-fermenters and their antibiotic susceptibility pattern should be regarded with seriousness in Microbiology laboratories as well as in clinical practice because these organisms, being resistant to multiple antibiotics, limits treatment options and also harbour drug resistance genes.

It has also been observed that majority of these NFGNB were from indoor patients which indicates that many of these strains may have been hospital acquired. The isolation and characterization of non-fermenters and determination of phenotypes is an essential aspect in clinical microbiology laboratories in order to determine the hospital epidemiology of these organisms thereby helping in implementation of preventive and control measures.

In the past it was a common practice to club these organisms under the broad umbrella of NFGNB without any attempts being made regarding characterization and speciation. It is very important to know the different genera and species that come under the umbrella term of NFGNB. These organisms are not only important in causing healthcare associated infections but also harbour antibiotic resistance genes, which may be both plasmid borne or chromosomal. The expression of these genes makes treatment difficult, at times no treatment options being available as in the case of extended drug resistance (XDR) and pan drug resistance (PDR). As they harbour plasmids encoding for drug resistance they are also responsible for both horizontal and vertical transfer of resistance genes leading to dissemination in other species and genera. Detection of resistance genes is a quick method to look for ESBL or carbapenemase producers; however, phenotypic determination of the production of these enzymes is very necessary as it categorically monitors the expression of resistance by these organisms. Determination of the antimicrobial susceptibility pattern is also very important because of the same reason. Biochemical media routinely used in the laboratories often fail to identify the organism up to the species level, the VITEK 2 compact helps in early and accurate identification of isolates.

References

1. Patel PH, Pethani JD and Rathod SD et al. Prevalence of nonfermenting Gram negative bacilli infection in tertiary care hospital in Ahmedabad, Gujarat. Ind J of Basic & Appld Med res. 2013;2(6): 608-613.
2. Sharma D, Vyas N and Sinha P et al. Non fermentative Gram-negative bacilli as nosocomial pathogens: Identification and antibiotic sensitivity in clinical samples of indoor patients. National J of Medical and Allied Sci.2014;3(2):101-105.
3. Sharan H, Katare N and Pandet A et al. Emergence of Hospital acquired carbapenem resistant non-Fermenters in teaching Institute. J of Clin and Diagn Res. for doctors. 2016;10(12): 20-23.
4. Winn WJ, Allen SJ Janda, W Koneman, Procop G Schreckenberg, P and Woods G. Guidelines for the Collection, Transport, Processing, Analysis and Reporting of Cultures from Specific Specimen Sources. In. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. Ed, Koneman EW. Lippincott Williams, Philadelphia, 2006; 83 – 104.
5. Thorpe TC, Wilson ML, Turner JE, DiGuiseppi JL, Willert M, Mirrett S, Reller LB. BacT/Alert: an automated colorimetric microbial detection system. J Clin Microbiol 1990 Jul; 28(7): 1608-12.

6. BioMeriux, Inc 100 Rodolphe Street Durham, North Carolina 27712 USA 2019. https://www.biomerieux-usa.com/vitek-2
7. Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Susceptibility Resring, 2020. 30th ed. Performance standards for antimicrobial susceptibility testing: Twenty-fourth informational supplement, M100-M2-A9, Wayne PA: Clinical and Laboratory Standards Institute.
8. Wadhwa R, Sharma Y and Upadhyay RP. Nosocomial infection by non-fermenting Gram-negative bacilli in tertiary care hospital: screening and cure. Int J Pharm and Pharm Sci. 2016; 8(3):274-277.
9. Motabainor H, Bereded F, Mulu W. Multi-drug resistance of bloodstream, urinary tract and surgical site nosocomial infection of Acinetobacter baumannii and pseudomonas aeruginosa among patients hospitalized at Felegehiwot referral hospital, Northwest Ethiopia: a cross-sectional study. BMC infectious Diseases. 2020; 20:1-11.
10. Yadav SK, Bhujel R and Mishra SK et al. Emergence of multidrug-resistant non-fermentative Gram-negative bacterial infections in hospitalized patients in a tertiary care center of Nepal. BMC Res Notes. 2020; 13:319-322.
11. Shukla R, Biliolikar AK Rani p et al. Antibiotic susceptibility pattern of Burkholderia cepacia complex from various clinical samples in a tertiary care center: A one-year prospective study. Med Sci Res. 2018; 6 (1): 1-5.
12. Dheyab Z. S. Clinically Important Yersinia: Minireview. Journal of Medical Research and Health Sciences, 2022; 5(10): 2295–2306.