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Original Research Article

An Observational Study to Identify the Non-Fermenters from Blood Specimens and Their Antimicrobial Susceptibility Pattern

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Abstract:

Aim: The aim of the present study was to assess frequency and antibiotic susceptibility pattern of non-fermenting gram-negative rods isolated from blood culture of patients**.**

Material & methods: The prospective study was conducted in the Department of Microbiology in between the duration of 1year. Blood Stream Infection (BSI) was defined as the isolation of a pathogen microorganism from >1 blood culture bottle. BSIs were classified as community- and hospital-acquired infections if detected within the first 48 h of hospitalization, or after 48 h of hospitalization, respectively.

Results: Total 500 NFGNB were isolated from 3455 culture positive clinical samples accounting for an isolation rate of 14.47%. Urine was the most common specimen (30%) followed by pus (26%), blood (16%), sputum (12%), tracheal aspirate (8%) and remaining 8% included other samples. Acinetobacter baumannii was the predominant isolate, 260 (52%) followed by Pseudomonas aeruginosa 200 (40%) and Burkholderia cepacia complex (BCC) 25 (5%). Burkholderia pseudomallei, Acinetobacter lwoffii and Stenotrophomonas maltophilia altogether accounted for 3%. Among the NFGNB isolated, A. baumannii showed highest sensitivity to gentamicin (61.53%) and lowest sensitivity to ceftriaxone (22.30%). P. aeruginosa was mostly sensitive to amikacin (84%) but least sensitive to ceftriaxone (28%). B. cepacia complex, B. pseudomallei and S. maltophilia showed 100% susceptibility to cotrimoxazole. A. lwoffii showed sensitivity to most of the antibiotics. A. baumannii and P. aeruginosa were mostly sensitive to gentamicin and amikacin and least sensitive to ceftriaxone.

Conclusion: This study underlines the need to identify NFGNB in tertiary care hospitals and to monitor their susceptibility pattern to guide the clinician for better care and management of patients. Improved antibiotic stewardship and strict infection control measures especially hand washing need to be implemented to prevent emergence and spread of multidrug resistant NFGNB in health care settings.

Keywords: Acinetobacter Sp, Antibiotic susceptibility, Nosocomial pathogens, non-fermenters

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Introduction

Non fermenting Gram-Negative Bacilli (NFGNB) are aerobic, non-spore forming organisms that either do not use carbohydrates as a source of energy (or) degrade them through metabolic pathways other than fermentation. [1-3] This heterogeneous group includes organisms like Pseudomonas sp, Acinetobacter sp, Alkaligene spp, Stenotrophomonas maltophilia, Burkholderia cepacian complex, etc. [4] These bacteria occur as saprophytes in the environment and also found as commensals in the human gut. [2] These are ubiquitous in nature particularly in soil and water. NFGNB are known to account for about 15% of all bacterial isolates from a clinical microbiology laboratory. [3]

Non fermenting Gram-Negative Bacilli cause various infections including wound infections, urinary tract infections, meningitis, pneumonia, septicemia, osteomyelitis, etc. [5] The non-fermentative gram-negative bacilli (NFGNB) are a group of aerobic, non-spore-forming bacilli that either do not use carbohydrates as a source of energy or degrade them through metabolic pathways other than fermentation. [6] They are widely distributed in nature as saprophytes or as commensals and act as opportunistic pathogens for man. [7] Increasing frequency from clinical specimens in a higher proportion of hospitalized patients suffering from illnesses like urinary tract infection, ventilator associated pneumonia, surgical site infection and septicaemia. [2,8]

NFGNBs are known to colonize initially and then subsequently invade the otherwise normally sterile site through trauma. It has been noted that disruption of natural barriers is an important route of entry of infections. [9,10] Rates of colonization increase in hospitalized patients particularly in those who have been hospitalized for extended periods or / and have received broad spectrum antimicrobial therapy/chemotherapy. [11] Most of the non-fermenters cause nosocomial blood stream infections particularly in debilitated and immunocompromised hosts and are usually multidrug resistant.

There are very few studies from India wherein the various NFGNB, isolated from patients' samples, have been identified and their clinical significance assessed. Hence, the present study was therefore taken to identify the non-fermenters from blood specimens and to determine their antimicrobial susceptibility pattern.

Material & Methods

The prospective study was conducted in the Department of Microbiology, ANM Medical College and Hospital, Gaya, Bihar, India in between the duration of 1year. Blood Stream Infection (BSI) was defined as the isolation of a pathogen microorganism from >1 blood culture bottle. BSIs were classified as community- and hospital-acquired infections if detected within the first 48 h of hospitalization, or after 48 h of hospitalization, respectively. The recovery of different species 72 h after the previous positive blood culture in a single patient was considered to be a distinct episode. Isolation of the same microorganism from a single patient was considered to be a single episode even if the culture was obtained after 72 h. Multiple bacteremic episodes in a single patient were considered to be distinct episodes, if separated by at least 7 days.

Sample Collection and Processing

Blood samples were collected from the patients before the administration of any antibiotic. For adults, after aseptic precautions, 5-10 mL of blood subsequently incubated in BacTAlert3D (Biomerieux, France), a fully automated blood culture system for detection of growth in blood culture. On getting a positive alarm, Gram stain were carried out on positive bottles, followed by sub culture on 5% sheep blood agar and MacConkey agar plates which were incubated aerobically at 37°C overnight for bacterial isolation. Isolates were identified by Vitek 2 Compact (Biomerieux).

Antimicrobial susceptibility testing was done with an automated microbiology system, Vitek 2 compact 60 system BioMerieux India®) and interpreted according to CLSI criteria. [12]

The patient data that were collected included age, sex, underlying diseases and risk factors. Quality control was performed by testing these same antimicrobials against reference strains of bacteria.

Statistical Analysis

Descriptive statistics were used to express overall results. Data were analyzed using SPSS 15.0. Categorical variables were evaluated by the chi-square test, and continuous variables were evaluated by the Mann Whitney U test and t-test.

Results

Table 1: NFGNB isolates obtained from various clinical specimens

NFGNB isolates	500 (14.47)
Others	2955 (85.53)

Total 500 NFGNB were isolated from 3455 culture positive clinical samples accounting for an isolation rate of 14.47%.

Table 2: Sample-wise distribution of NFGNB isolates

Samples	No. of NFGNB(n=500)	Percentage
Urine	150	30
Pus	130	26
Blood	80	16
Sputum	60	12
E.T. tube	40	8
Catheter Tip	10	2
CVP tip	10	2
Drain tip	6	1.2
Throat swab	6	1.2
Wound swab	4	0.8
Other body fluids	4	0.8

Urine was the most common specimen (30%) followed by pus (26%), blood (16%), sputum (12%), tracheal aspirate (8%) and remaining 8% included other samples.

Table 3: Prevalence of NFGNB isolates

Isolates	Number (n=500)	Percentage
A. baumannii	260	52
P. aeruginosa	200	40
B. cepacia complex	25	5
B. pseudomallei	8	1.6
A. lwoffii	5	1
S. maltophilia	2	0.4

Acinetobacter baumannii was the predominant isolate, 260 (52%) followed by Pseudomonas aeruginosa 200 (40%) and Burkholderia cepacia complex (BCC) 25 (5%). Burkholderia pseudomallei, Acinetobacter lwoffii and Stenotrophomonas maltophilia altogether accounted for 3%.

Table 4: Sensitivity pattern of nonfermenters to antimicrobial agents

Antimicrobials	*A. Baumannii* (%)	*P. Aeruginosa* (%)	*B. Cepacia*complex (%)	*B. Pseudomallei* (%)	*A. Lwoffii* (%)	*S. Maltophilia* (%)
Piperacillin/tazobactam 100/10 mcg	80 (30.76)	76 (38)	0	0	5 (100)	0
Ceftazidine 30 mcg	60 (23.07)	60 (30)	0	0	5 (100)	0
Ceftriaxone 30 mcg	58 (22.30)	56 (28)	0	0	5 (100)	0
Cefepime 30 mcg	84 (32.30)	68 (34)	0	0	5 (100)	0
Amikacin 30 mcg	131 (50.38)	168 (84)	0	0	5 (100)	0
Gentamicin 10 mcg	160 (61.53)	150 (75)	0	0	5 (100)	0
Ciprofloxacin 5 mcg	150 (57.69)	144 (72)	0	0	1 (20)	0
Cotrimoxazole 25 mcg	148 (56.92)	140 (70)	25 (100)	8 (100)	3 (100)	2 (100)
Meropenem 10 mcg	146 (56.15)	130 (65)	12 (48)	6 (75)	3 (100)	0

Among the NFGNB isolated, A. baumannii showed highest sensitivity to gentamicin (61.53%) and lowest sensitivity to ceftriaxone (22.30%). P. aeruginosa was mostly sensitive to amikacin (84%) but least sensitive to ceftriaxone (28%). B. cepacia complex, B. pseudomallei and S. maltophilia showed 100% susceptibility to cotrimoxazole. A. lwoffii showed sensitivity to most of the antibiotics. A. baumannii and P. aeruginosa were mostly sensitive to gentamicin and amikacin and least sensitive to ceftriaxone.

Discussion

Nonfermenting gram-negative bacilli (NFGNB) are a taxonomically diverse group of aerobic, nonsporing, bacilli that either do not utilize glucose as a source of energy or utilize it oxidatively. [6] They occur as saprophytes in the environment and some are also found as commensals in the human gut. [13,14]

Total 500 NFGNB were isolated from 3455 culture positive clinical samples accounting for an isolation rate of 14.47%. Urine was the most common specimen (30%) followed by pus (26%), blood (16%), sputum (12%), tracheal aspirate (8%) and remaining 8% included other samples. Nevertheless, in many studies, NFGNB were most commonly isolated from pus. [2,15] Acinetobacter baumannii was the predominant isolate, 260 (52%) followed by Pseudomonas aeruginosa 200 (40%) and Burkholderia cepacia complex (BCC) 25 (5%). Burkholderia pseudomallei, Acinetobacter lwoffii and Stenotrophomonas maltophilia altogether accounted for 3%. Among the NFGNB isolated, A. baumannii showed highest sensitivity to gentamicin (61.53%) and lowest sensitivity to ceftriaxone (22.30%). These results corroborated well with the studies of Goel V et al, where, A. baumannii (48.78%) was the most commonly isolated pathogen followed by P. aeruginosa (37.71%). [16] According to Samanta P et al, the isolation rate of Acinetobacter species was 66%, and Pseudomonas species was 26%. However, in other studies, the most common isolate was P. aeruginosa, followed by A. baumannii. [15,17-19] In our study, prevalence of A. baumannii was more in high risk areas, possibly due to increased colonisation of A. baumannii in hospital environment, including humidifiers, nebulizers, anaesthetic equipments, ventilators, healthcare workers etc. causing nosocomial opportunistic infections in patients with severe underlying illnesses. [16,18]

In the study of Jayanthi S et al, isolation rate for P. aeruginosa was 41.2%, followed by Acinetobacter species (26.29%). [17] Upgade A et al, reported 43% Pseudomonas spp. followed by Acinetobacter spp. 21%.[20] P. aeruginosa was mostly sensitive to amikacin (84%) but least sensitive to ceftriaxone (28%). B. cepacia complex, B. pseudomallei and S. maltophilia showed 100% susceptibility to cotrimoxazole. A. lwoffii showed sensitivity to most of the antibiotics. A. baumannii and P. aeruginosa were mostly sensitive to gentamicin and amikacin and least sensitive to ceftriaxone. Gokale

S et al, showed highest susceptibility to meropenem (96.2%) and 45% susceptibility to ciprofloxacin for A. baumannii. In the study of Gokale S et al, P. aeruginosa showed good sensitivity to meropenem (96.2%), followed by ciprofloxacin (50%) and amikacin (49.5%). [2]

In recent years, due to the liberal and empirical use of antibiotics, NFGNB have emerged as important healthcare-associated pathogens. They have been incriminated in infections, such as, septicemia, meningitis, pneumonia, urinary tract infections (UTI), and surgical site infections (SSI). [14] NFGNB are innately resistant to many antibiotics and are known to produce extended spectrum ß-lactamases and metallo ß-lactamases. [14,21]

Conclusion

This study underlines the need to identify NFGNB in tertiary care hospitals and to monitor their susceptibility pattern to guide the clinician for better care and management of patients. Improved antibiotic stewardship and strict infection control measures especially hand washing need to be implemented to prevent emergence and spread of multidrug resistant NFGNB in health care settings.

References

1. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC. Diagnostic microbiology. The nonfermentative gram-negative bacilli. Philedelphia: Lippincott-Raven Publishers. 1997:253-320.
2. Gokale SK, Metgud SC. Characterization and antibiotic sensitivity pattern of non-fermenting Gram-negative bacilli from various clinical samples in a tertiary care hospital, Belgaum. J Pharm Biomed Sci. 2012;17(14):1-5.
3. Benachinmardi KK, Padmavathy M, Malini J, Naveneeth BV. Prevalence of non-fermenting Gram-negative bacilli and their in vitro susceptibility pattern at a tertiary care teaching hospital. Journal of the scientific society. 2014 Sep 1;41(3):162-6.
4. Kang CI, Kim SH, Park WB, Lee KD, Kim HB, Kim EC, Oh MD, Choe KW. Bloodstream infections caused by antibiotic-resistant gram-negative bacilli: risk factors for mortality and impact of inappropriate initial antimicrobial therapy on outcome. Antimicrobial agents and chemotherapy. 2005 Feb;49(2):760-6.
5. Nagoba BS, Deshmukh SR, Gude UG, Gomashe AV, Wadher BJ. In vitro susceptibility of Pseudomonas aeruginosa to different antibiotics. Indian Journal of Medical Microbiology. 1997; 15:185-6.
6. Winn W Jr, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P, et al., editors. Nonfermenting Gram negative bacilli. In: Koneman's Color Atlas and textbook of Diagnostic Microbiology, 6th ed. USA: Lippincott Williams and Wilkins Company; 2006. p. 305-91.
7. Mandell GL, Bennett JE, Dolin RA. Mandell. Douglas, and Bennett's principles and practice of infectious diseases, 7thed, Churchill Livingstone 2010.
8. Malini A, Deepa EK, Gokul BN, Prasad SR. Nonfermenting gram-negative bacilli infections in a tertiary care hospital in Kolar, Karnataka. Journal of laboratory physicians. 2009 Jul;1(02):062-6.
9. Yasodhara P, Shyamala S. Identification and characterization of non fermenters from clinical specimens. Indian Journal of Medical Microbiology. 1997; 15:195-8.
10. Kielhofner M, Atmar RL, Hamill RJ, Musher DM. Life-threatening Pseudomonas aeruginosa infections in patients with human immunodeficiency virus infection. Clin Infect Dis. 1992; 14(2):403-11.
11. Quinn JP. Clinical problems posed by multiresistant nonfermenting gram-negative pathogens. Clinical infectious diseases. 1998 Aug 1;27(Supplement_1):S117-24.
12. CLSI. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. Twenty-Fourth Informational Supplement CLSI Document M100-S24, Wayne, PA. 2014.
13. Steinberg JP, Rio DC. Other Gram negative and Gram variable bacilli. In: Mandell GL, Bennett JE, Dolin R, editors. Principles and Practice of Infectious diseases, 6th ed. vol. 2. Philadelphia, USA: Elsevier Publication; 2005. p. 2751-68.
14. Gales AC, Jones RN, Forward KR, Linares J, Sader HS, Verhoef J. Emerging importance of multidrug-resistant Acinetobacter species and Stenotrophomonas maltophilia as pathogens in seriously ill patients: geographic patterns, epidemiological features, and trends in the SENTRY Antimicrobial Surveillance Program (1997–1999). Clinical Infectious Diseases. 2001 May 15;32(Supplement_2):S104-13.
15. Benachinmardi KK, Padmavathy M, Malini J, Naveneeth BV. Prevalence of non-fermenting Gram-negative bacilli and their in vitro susceptibility pattern at a tertiary care teaching hospital. J Sci Soc. 2014;41(3):162.
16. Goel V, Hogade SA, Karadesai SG. Prevalence of extended-spectrum beta-lactamases, AmpC beta-lactamase, and metallo-beta-lactamase producing pseudomonas aeruginosa and Acinetobacter baumannii in an intensive care unit in a tertiary care hospital. J Sci Soc. 2013; 40(1):28.
17. Jayanthi S, Jeya M. Clinical distribution and antibiotic resistance pattern of nonfermenting Gram negative bacilli. Int J Pharm Bio Sci. 2012;3(1):487-94.

18. Samanta P, Gautam V, Thapar R, Ray P. Emerging resistance of non-fermenting gram-negative bacilli in a tertiary care centre. Indian J Pathol Microbiol. 2011;54(3):666.
19. Bhargava D, Kar S, Saha M. Prevalence of non-fermentative gram-negative bacilli infection in tertiary care hospital in Birgunj, Nepal. Int J Curr Microbiol App Sci. 2015;4(7):301-7.
20. Upgade A, Prabhu N, Gopi V, Soundararajan N. Current status of antibiotic resistant non-fermentative gram-negative bacilli among nosocomial infections. Adv Appl Sci Res. 2012; 3(2):738-42.
21. Rubin SJ, Granato PA, Wasilauskas BL. Glucose nonfermenting Gram negative bacteria. In: Lennette EH, Balows A, Hausler WJ Jr, Shadomy HJ, editors. Manual of Clinical Microbiology, 4th ed. Washington, D.C: American Society for Microbiology; 1985. p. 330-49.