Section: Microbiology

Original Research Article

NON-FERMENTING GRAM NEGATIVE BACTERIA: A STUDY ON THEIR PREVALENCE AND ANTI-MICROBIAL SUSCEPTIBILITY PATTERN AMONG PATIENTS ADMITTED IN A TERTIARY CARE HOSPITAL OF BIHAR

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Abstract

Background: Nonfermenting gram-negative bacilli (NFGNB) are a taxonomically diverse group of aerobic, non-sporing, bacilli that either do not utilize glucose as a source of energy or utilize it oxidatively. The aim of the present study was to isolate and identify NFGNB from clinical samples and to assess prevalence and antimicrobial susceptibility profiles in a tertiary care hospital of Eastern India. **Materials and Methods:** An observational study with cross-sectional design was conducted between January 2022 to December 2022 (1 year) in the Department of Microbiology, JLNMCH, Bhagalpur, and Bihar. All the clinical samples including urine, pus, blood, wound swab and body fluids were received in the laboratory and inoculated on blood and Mac-Conkey agar or CLED agar and incubated aerobically at 37°C for 18 to 24 hours. The isolates which were non-lactose fermenting and showed alkaline change (K/NC) reaction in triple sugar iron agar media were provisionally considered as NFGNB. They were further identified using standard protocols for identification, like gram staining for morphology, hanging drop for motility, pigment production, oxidase test, catalase test etc. Antimicrobial susceptibility test was performed by Kirby-Bauer disc diffusion method using commercially available disc (Hi-Media). Statistical analysis was done by using Excel and SPSS V21.**Result:**Total 332 NFGNB were isolated from 2157 culture positive clinical samples accounting for an isolation rate of 15.4%. Urine was the most common specimen obtained followed by pus and blood. [Figure 1] Acinetobacter baumannii was the predominant isolate followed by Pseudomonas aeruginosa and Burkholderia cepacian complex. [Figure 2] Among the NFGNB isolated, A. baumannii showed highest sensitivity to gentamicin and lowest sensitivity to ceftriaxone.P. aeruginosa was mostly sensitive to amikacin but least sensitive to ceftriaxone. **Conclusion:** Since, these organisms have great potential to survive in hospital environment, improved antibiotic stewardship and infection control measures will be needed to prevent the emergence and spread of drug resistant NFGNB in healthcare settings.

INTRODUCTION

Nonfermenting gram-negative bacilli (NFGNB) are a taxonomically diverse group of aerobic, non-sporing, bacilli that either do not utilize glucose as a source of energy or utilize it oxidatively.[1] They occur as saprophytes in the environment and some are also found as commensals in the human gut.[2,3] NFGNB are known to account for about 15% of all bacterial isolates from a clinical microbiology laboratory.[4] 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).[3] NFGNB are innately resistant to many antibiotics and are known to produce extended spectrum ß-lactamases and metallo ß-lactamases. Non-fermenters are now resistant to many routinely used antibiotics and even to cephalosporins and carbapenems. Resistance compromises treatment,

prolongs hospital stay, increases mortality and healthcare costs.[3-6]

The aim of the present study was to isolate and identify NFGNB from clinical samples and to assess prevalence and antimicrobial susceptibility profiles in a tertiary care hospital of Eastern India.

MATERIALS AND METHODS

An observational study with cross-sectional design was conducted between January 2022 to December 2022 (1 year) in the Department of Microbiology, JLNMCH, Bhagalpur, and Bihar. All the clinical samples including urine, pus, blood, wound swab and body fluids were received in the laboratory and inoculated on blood and Mac-Conkey agar or CLED agar and incubated aerobically at 37°C for 18 to 24 hours. The isolates which were non-lactose fermenting and showed alkaline change (K/NC) reaction in triple sugar iron agar media were provisionally considered as NFGNB. They were further identified using standard protocols for identification, like gram staining for morphology, hanging drop for motility, pigment production, oxidase test, catalase test, Hugh-Leifson oxidative fermentative test for glucose, lactose, sucrose, maltose and mannitol, nitrate reduction test, indole test, citrate utilization test, urease test, utilization of 10% lactose, lysine and ornithine decarboxylation, arginine dehydrolation, growth at 42°C and 44°C.[1] The clinical significance of isolated NFGNB was assessed retrospectively by analyzing the case sheets for relevant laboratory and clinical criteria. Laboratory criteria included the presence of pus cells along with gram-negative bacilli in the stained smear from the sample, isolation of the same organism from a repeat sample, leukocytosis, and relevant radiological evidence. The clinical criteria included the presence of risk factors such as underlying diseases (diabetes mellitus, chronic renal failure, malignancy, cystic fibrosis, pneumonia and other immunosuppressive conditions), presence of intravenous or urinary catheters, duration of stay in intensive care unit (ICU), mechanical ventilation and recent surgery.[7,8] Antimicrobial susceptibility test was performed 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/tazobactum(100µg/10µg), imipenem (10µg), meropenem (10µg), ciprofloxacin (5µg), and cotrimoxazole (25µg). The results were interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 were used as control strains.[9]

Statistical analysis was done by using Excel and SPSS V21. The result of this analysis was used for comparison of data and to finalize the study results. P-value was determined to evaluate the levels of significance using Excel and SPSS ver. 20.0, p-value of < 0.05 was considered to be significant.

RESULTS

Total 332 NFGNB were isolated from 2157 culture positive clinical samples accounting for an isolation rate of 15.4%. Urine was the most common specimen obtained followed by pus and blood. [Figure 1] Acinetobacter baumannii was the predominant isolate followed by Pseudomonas aeruginosa and Burkholderia cepacian complex. [Figure 2] Among the NFGNB isolated, A. baumannii showed highest sensitivity to gentamicin and lowest sensitivity to ceftriaxone.P. aeruginosa was mostly sensitive to amikacinbut least sensitive to ceftriaxone. B. cepaciacomplex, 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 togentamicin and amikacin and least sensitive to ceftriaxone.[Table 1].

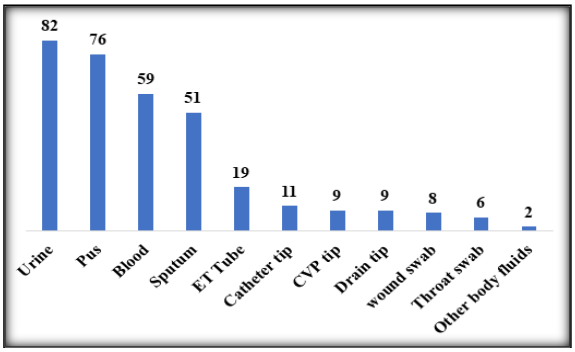

Figure 1: Distribution of cases based on type of sample obtained.

Table 1: Sensitivity pattern of isolated NFGNB to various antimicrobial agents

Antimicrobial	Isolated NFGNB (N)					
	Baumannii(173)	Aeruginosa(133)	B.Cepacia complex(19)	B.Pseudomallei(4)	A.Lwoffii(2)	S.Maltophilia(1)
Pipeacillin/Tazobactum	57 (32.9%)	53 (39.8%)	0	0	2 (100%)	0
Ceftazidime	38 (21.9%)	41 (30.8%)	0	0	2 (100%)	0
Ceftraixone	43 (24.9%)	41 (30.8%)	0	0	2 (100%)	0
Cefipime	51 (29.4%)	45 (33.8%)	0	0	2 (100%)	0
Amikacin	91 (52.6%)	112 (84.2%)	0	0	2 (100%)	0
Gentamycin	118 (68.2%)	101 (75.9%)	0	0	2 (100%)	0
Ciprofloxacin	112 (64.7%)	101 (75.9%)	0	0	1 (50%)	0

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Cotrimoxazole	121 (69.9%)	-	19 (100%)	4 (100%)	2 (100%)	1 (100%)
Meropenam	121 (69.9%)	92 (69.2%)	11 (57.9%)	1 (75%)	2 (100%)	0

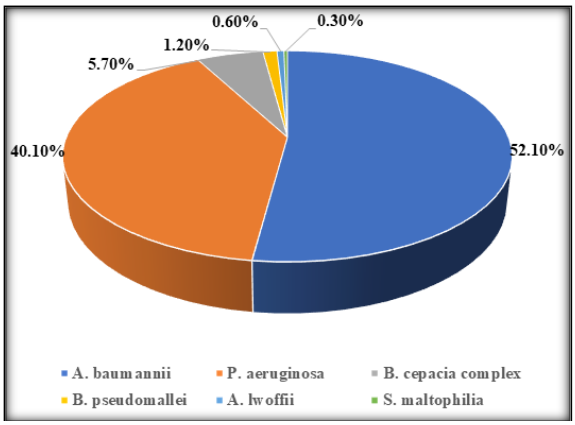

Figure 2: Pie distribution of cases based on isolated NFGNB

DISCUSSION

Nonfermentative gram-negative bacilli are ubiquitous in environment. They used to be considered as contaminants or commensals in the past. They have now emerged as important healthcare-associated and opportunistic pathogens due to their frequent isolation from clinical materials and their association with various diseases. In the present study, the isolation rate of NFGNB from clinical samples was 15.4%. This was parallel to the results of a study from Kolkata by Rit K et al, where NFGNB were isolated in 12.18% of clinical samples.[10] However, the prevalence of nonfermenters varies greatly from time to time and place to place. A study from Amritsar reported a very high isolation rate of 45.9% whereas; it was 3.58% in a study from Bangalore and 5.2% in another study from Chennai. In a study from Saudi Arabia NFGNB isolation rate was 16%.[11-14]

In the present study, NFGNB were most frequently isolated from urine samples, followed by pus. Nevertheless, in many studies, NFGNB were most commonly isolated from pus.[12] According to a study by Shobha KL et al, nonfermenters were emerging as an important cause of urinary tract infections (9.44%).[15] Frequent isolation of NFGNB from urine and pus samples in this study, could be attributed to the increase in number of critically ill, hospitalized patients requiring urinary tract catheterization and other instrumentations. Prolonged hospital stay, bed sores, burns, open wounds, surgical site infections, diabetes, malignancies and several underlying illnesses made these patients more vulnerable to NFGNB infections.

In this study, A. baumannii was the most common species isolated, accounting for 52.1%, followed by P. aeruginosa (40.1%) and B. cepacia complex (5.7%). Rest was constituted by A. lwoffii, B. pseudomallei and S. maltophilia together. 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.[12,13,17,18]

A. baumannii showed highest susceptibility to gentamicin and lowest susceptibility to ceftriaxone. However, Gokale S et al, showed highest susceptibility to meropenem (96.2%) and 45% susceptibility to ciprofloxacin for A. baumannii.[19]

P. aeruginosa showed highest susceptibility to amikacin, but least susceptibility to ceftriaxone. 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%).[19]

CONCLUSION

NFGNB are now emerging as important pathogens causing a wide range of nosocomial infections. Identification and monitoring of their susceptibility profiles are essential due to their variable sensitivity patterns and to help in proper management of the infections caused by them. Clinicians must be updated with the prevalence and antimicrobial susceptibility pattern of the circulating pathogens in their healthcare settings. Appropriate antimicrobials should be used for empiric therapy. Since, these organisms have great potential to survive in hospital environment, improved antibiotic stewardship and infection control measures will be needed to prevent the emergence and spread of drug resistant NFGNB in healthcare settings.

REFERENCES

1. Winn W Jr, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P, et al., editors. In: Koneman'sColor Atlas and textbook of Diagnostic Microbiology. 6th ed. USA: Lippincott Williams and Wilkins Company; 2006. Nonfermenting Gram negative bacilli; pp. 305–91.
2. Steinberg JP, Rio DC. 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. pp. 2751–68.
3. 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) Clin Infect Dis. 2001;32:104–13.
4. 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. pp. 330–49.
5. Memish ZA, Shibl AM, Kambal AM, Ohaly YA, Ishaq A, Livermore DM. Antimicrobial resistance among non-fermenting Gram-negative bacteria in Saudi Arabia. J AntimicrobChemother. 2012;67(7):1701-5.

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6. Slama TG. Gram-negative antibiotic resistance: there is a price to pay. Crit. Care. 2008;21;12(4):1.
7. Meharwal SK, Taneja N, Sharma SK,Sharma M. Complicated nosocomial UTI caused by nonfermenters. Indian J Urol. 2002; 18(2):123,
8. Hill EB, Henry DA, Speert DP. Pseudomonas. In: Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA. Manual of Clinical Microbiology, vol. 1, 9th ed. Washington, D.C: American Society for Microbiology; 2007. p. 734-48.
9. Wayne PA. Clinical and Laboratory Standards Institute: Performance standards for antimicrobial susceptibility testing: Twenty-fourth informational supplement, M100-S24. Clinical and Laboratory Standards Institute (CLSI). 2014;34(1).
10. Rit K, Nag F, Raj HJ, Maity PK. Prevalence and Susceptibility Profiles of Non-fermentative Gramnegative Bacilli Infection in a Tertiary Care Hospital of Eastern India. Int J Clin Pract. 2013;24(5):451-5.
11. Sidhu S, Arora U, Devi P. Prevalence of nonfermentative gram negative bacilli in seriously ill patients with bacteraemia. JK Sci. 2010;12(4):168-71
12. 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.
13. Jayanthi S, Jeya M. Clinical distribution and antibiotic resistance pattern of nonfermenting Gram negative bacilli. Int J Pharm Bio Sci. 2012;3(1):487-
14. Eltahawy AT, Khalaf RM. Antibiotic resistance among gram-negative non-fermentative bacteria at a teaching hospital in Saudi Arabia. J Chemother. 2017 Jan 1;13(3):260-4.
15. KL S, Rao GG, Kukkamalla AM. Prevalence of non-fermenters in urinary tract infections in a tertiary care hospital. Webmed Central Microbiol.2018;2(1):WMC001464.
16. Goel V, Hogade SA, Karadesai SG. Prevalence of extended-spectrum beta-lactamases, AmpCbetalactamase, and metallo-beta-lactamase producingPseudomonas aeruginosa and Acinetobacter baumannii in an intensive care unit in a tertiary care hospital. J Sci Soc. 2019;40(1):28.
17. Samanta P, Gautam V, Thapar R, Ray P. Emerging resistance of non-fermenting gram negative bacilli in a tertiary care centre. Indian J PatholMicrobiol. 2020;54(3):666.
18. Bhargava D, Kar S, Saha M. Prevalence of nonfermentative gram negative bacilli infection in tertiary care hospital in Birgunj, Nepal. Int J CurrMicrobiol App Sci. 2021;4(7):301-7.
19. Gokale SK, Metgud SC. Characterization and antibiotic sensitivity pattern of nonfermenting gram negative bacilli from various clinical samples in a tertiary care hospital, Belgaum. J Pharm Biomed Sci. 2022;17(17).

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