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Original Research Article

ISOLATION AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF NON-FERMENTING GRAM NEGATIVE BACILLI (NFGNB) FROM VARIOUS CLINICAL SAMPLES

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Abstract

Background: 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. **Materials and Methods:** An observational study with cross-sectional design was conducted between October 2022 to January 2023 in the Department Of Microbiology, Bhagwan Mahavir Institute of Medical Sciences, Pawapuri, Nalanda, 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. 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. 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:** Out of 1110 clinical samples, cultures were positive in 701 samples. Out of 701 culture positive samples, 129 (18.4%) yielded NFGNB. The mean of our study participants was found to be 42.22 ± 12.46 years, with a male: female ratio 2.6:1. P. aeruginosa was isolated in 77/129 (59.6%) samples, followed by A. Baumannii (57/129, (44.18%), Burkholderia pseudomallei 2/129, 1.5%), A. Lwoffi (1/129, 0.7%), and Stenotrophomonas maltophilia were rarely isolated, accounting together for 1. % of the isolates. Overall, most of the NFGNB isolates were susceptible to polymyxin B (95%), imipenem (90%) and cefoperazone + sulbactam (55%). **Conclusion:** Proper screening of non-fermenters in nosocomial settings, regular assessment of their antibiotic susceptibility profiles and judicious use of antibiotics are suggested for effective management of the infections caused by them and limiting the emergence of multidrug resistance.

INTRODUCTION

Non-fermenting 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]

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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 October 2022 to January 2023 in the Department Of Microbiology, Bhagwan Mahavir Institute of Medical Sciences, Pawapuri, Nalanda, 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/K) 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

Out of 1110 clinical samples, cultures were positive in 701 samples. Out of 701 culture positive samples, 129 (18.4%) yielded NFGNB. The mean of our study participants was found to be 42.22 ± 12.46 years, with a male: female ratio 2.6:1. P. aeruginosa was isolated in 77/129 (59.6%) samples, followed by A. Baumannii (57/129, (44.18%), Burkholderia pseudomallei 2/129, 1.5%), A. Lwoffi (1/129, 0.7%), and Stenotrophomonas maltophilia were rarely isolated, accounting together for 1.% of the isolates.

Clinical sources of various NFGNB isolates are shown in Figure 1. Out of 129 clinical samples positive for NFGNB, pus swab accounted for 25 (19.37%) samples, followed by urine culture 44 (34.1%), blood culture 17 (13.17%), sputum culture 25 (19.17%), tracheal swab 10 (7.7%). and endotracheal tube, 21 (16.27%) .

Clinico-microbiological correlation of NFGNB isolates in our study is shown.

Overall, most of the NFGNB isolates were susceptible to polymyxin B (95%), imipenem (90%) and cefoperazone + sulbactam (55%). Percentage antibiotic susceptibility of the various isolates is shown in [Table 1].

Table 1: Distribution of cases based on type of sample obtained

Sample	%
Pus	25(19.37)
Urine	44(34.1)
Blood culture	17(13.17)
Sputum	25(19.17)
Trachea	10(7.7)
Endotracheal tube	21(16.27)

Table 2: Sensitivity pattern of isolated NFGNB to various antimicrobial agents

Antimicrobial	Sensitivity pattern Isolated NFGNB (n=129)				
	P. aeruginosa (77)	A. baumannii (57)	B. Pseudomallei (2)	A. Lwoffii (2)	S. Maltophilia (1)
Pipeacillin/Tazobactum	52 (69.5%)	24 (44.0%)	0	2 (100%)	0
Ceftazidime	38 (49.6%)	22 (38.0%)	0	2 (100%)	0
Ceftraixone	120 (26.3%)	17 (32.0%)	0	2 (100%)	0

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Cefipime	38 (50.6%)	19 (36.0%)	0	2 (100%)	0
Amoxicillin + clavulanic acid	34 (44.6%)	17 (32.6%)	0	1 (50%)	1 (100%)
Amikacin	40 (54.7%)	20 (42.0%)	0	2 (100%)	0
Gentamycin	42 (57.4%)	30(57.0%)	0	2 (100%)	0
Ciprofloxacin	50 (66.8%)	21 (43.0%)	0	1 (50%)	1(100)
Ofloxacin	29 (39.45)	114 (26.95)	2 (50%)	0	0
Norfloxacin	24 (30.8%)	9 (17.7%)	1 (25%)	0	0
Cotrimoxazole	22 (29.1%)	-	3(100%)	2 (100%)	1 (100%)
Meropenam	44 (57.7%)	28 (54.0%)	1 (75%)	2 (100%)	0
Imipenem	65 (88.3%)	44 (84.8)	2 (70%)	2 (100%)	0
Polymyxin B	77(100%)	10(18.7%)	0	0	0
Cefoperazone + sulbactam	38 (51.8%)	39(75s.6%)	1 (50%)	100%	100%

DISCUSSION

NFGNB, which were only considered to be contaminants in the past, have now emerged as important nosocomial pathogens.[2] In our study, isolation rate of NFGNB was 18.4%, which is in parallel to other studies by Rit et al,[5] and Benachinmardi et al,[10] that reported isolation rates of 12.8% and 10%, respectively. The most common NFGNB isolated in our study was P. aeruginosa (59.6%), followed by A. baumannii (44.18%) which is similar to the results obtained by Malini et al,[2] who reported P. aeruginosa as the most common isolate accounting for 104/189 (53.8%) isolates, followed by A. baumannii (43/189, 22.2%).[2] Similarly, the study done by Rit et al,[5] also found P. aeruginosa to be the predominant isolate (101/201, 50.24%), followed by A. baumannii (50/201, 24.8%). Other Gram negative

Non-fermenters such as S. Maltophilia that were rarely isolated by us (1%) vary from study to study both in terms of their genera and prevalence. However, their role as opportunistic pathogens in immunocompromised and debilitated individuals has been invariably established.[11]

In our study, the highest number of isolates was isolated from urine (34.1%), which is in accordance with the observations made by Rit et al,[5] and Gokale and Metgud,[12] who also reported Urine and pus swabs as the source of maximum percentage of the isolates i.e., 27.86% and 58.4%, respectively. As evident from Figure 1, NFGNB were majorly found to cause urinary tract infections (35.95%) and would infections (22.9%).

P. aeruginosa isolates in our study were found to be most susceptible to polymyxin B (100%), which is not routinely used to treat infections caused by P. aeruginosa and is only tried as a last resort in case of severe multidrug resistant Gram-negative bacterial infections.[13] Nearly 87.3% of the P. aeruginosa isolates were found to be sensitive to imipenem. Similarly, Malini et al,[2] and Rit et al,[5] documented 94.2% and 91.08% susceptibility to imipenem, respectively. In contrast with the studies done by Benachinmardi et al,[10] and Naqvi et al,[14] that showed higher susceptibility to quinolones, only 64.8%, 37.4% and 24.9% of P. aeruginosa isolates in the present study showed susceptibility to the quinolones such as ciprofloxacin, ofloxacin and norfloxacin, respectively. In our study, P. aeruginosa showed least susceptibility to cefepime (48.6%) and amoxicillin + clavulanic acid (48.6%).

Almost 23.2% of the isolates of P. aeruginosa in our study were labelled as multidrug resistant (MDRPA), comparable to the findings of Jayakumar and Appalaraju who reported 22% isolation rate of MDRPA in their study.[15] About 100% of the MDRPA isolates were found to be susceptible to polymyxin B, which is similar to the results obtained by Ramakrishnan et al. who also reported 100% susceptibility to imipenem.[16] Nearly 70.1% of the MDRPA isolates in our study showed resistance to imipenem, which is usually the preferred therapeutic choice for treating the infections caused by them. As carbapenems are a potent antimicrobial weapon against MDRPA, this bacterium has developed resistance even against this group of drugs by producing metallo-beta-lactamases (carbapenemase).[17] Goossens,[18] and Ramakrishnan et al,[16] showed 44.9% and 40% resistance of MDRPA isolates to imipenem in their studies, respectively Imipenem resistance in MDRPA may possibly be influenced by the amount and duration of utilisation of the antibiotic used to treat these infections.

Isolates of A. baumannii in our study showed maximum susceptibility to imipenem (82.8%), followed by cefoperazone + sulbactam (74.6%). Results obtained by other studies show variable results. Rit et al. documented 90% and 16% susceptibility of A. baumannii isolates to imipenem and cefoperazone + sulbactam, respectively.[5] Tunyapanit et al. have reported 100% susceptibility to imipenem and 47% susceptibility to cefoperazone + sulbactam in A. Baumannii isolates.[19] Highest resistance amongst these isolates in our study was recorded against aztreonam (susceptibility = 17.1%). Similarly, Juyal et al,[20] reported least susceptibility of A. baumannii isolates to aztreonam (8.33%) in their study.

A total of 33 (67.41%) of A. baumannii isolates showed multidrug resistance (MDRAB) in the present study which is in accordance with Cai et al. who reported 72.23% prevalence of MDRAB isolates.[21] Fortunately, MDRAB isolates in our study showed good susceptibility to imipenem (87.5%), which is usually the most common therapeutic choice for MDRAB bacteraemia.[22] This is, however, in contrast with the findings of Tunyapanit et al,[19] and Cai et al,[21] who

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documented only 12% and 9.27% susceptibility to imipenem, respectively. Nearly 55.45% of the MDRAB isolates in our study were found to be susceptible to cefoperazone + sulbactam, which is comparable to Tunyapanit et al. who reported 47% susceptibility to cefoperazone + sulbactam combination.[19]

B. Pseudomallei were the third most commonly isolated NFGNB (3%) in our study. Sidhu et al. reported a prevalence of 2.31%.[23] The isolate of B. Pseudomallei showed maximum susceptibility (70%) to imipenem and ciprofloxacin. Sidhu et al. reported 100%, and 75% susceptibility of B. Pseudomallei isolates to imipenem and ciprofloxacin, respectively, in their study.[23] There is a lack of substantial data regarding the prevalence and antibiotic susceptibility profile of B. Pseudomallei due to its limited pathogenic role and rare isolation. S. maltophilia showed high resistance to almost most of the antibiotics tested for susceptibility. In our study, A. Lwofi was isolated from urine culture and showed maximum (100%) susceptibility to imipenem, in accordance with Sidhu et al. who also reported 100% susceptibility to imipenem.[23] Similarly, in the study done by Rit et al., B. cepacia isolates showed excellent susceptibility to imipenem (92.85%).[5] Therefore, it can be inferred that Imipenem offers excellent therapeutic effect in infections caused by A Lwofi, which is known to be resistant to many first-line therapeutics of choice against serious pseudomonal infections, such as beta-lactam drugs, polymyxin B and aminoglycosides.[24]

S. maltophilia, isolated from a pus swab, showed 100% susceptibility to some of the antibiotics, notably ciprofloxacin. Similar were the results obtained by Malini et al,[2] and Chawla et al,[11] who reported 100% and 93.3% susceptibility of S. maltophilia to ciprofloxacin, respectively. S. Maltophilia was found to be 100% resistant to majority of the antibiotics in our study, including imipenem, which could be attributed to the production of a zinc-dependent β-lactamase by this bacterium.[25]

CONCLUSION

Our study showed a significantly high prevalence of NFGNB, the most common being P. aeruginosa and A. baumannii. P. aeruginosa isolates showed good susceptibility to polymyxin B and imipenem whereas the isolates of A. baumannii showed good susceptibility to imipenem and cefoperazone + sulbactam. Isolation of MDRPA and MDRAB in the present study raises the concern of rapidly emerging antibiotic resistance in this group of bacteria in our region. Proper screening of non-fermenters in nosocomial settings, regular assessment of their antibiotic susceptibility profiles and judicious use of antibiotics are suggested for effective management of the infections caused by them and limiting the emergence of multidrug resistance.

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 non-fermenting 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.
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 non-fermenting 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.

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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 non-fermenting gram negative bacilli from various clinical samples in a tertiary care hospital, Belgaum. J Pharm Biomed Sci. 2022;17(17).
20. Juyal D, Prakash R, Shankarnarayan SA, Sharma M, Negi V, Sharma N. Prevalence of non-fermenting gram-negative bacilli and their in vitro susceptibility pattern in a tertiary care hospital of Uttarakhand: A study from foothills of Himalayas. Saudi J Health Sci 2013;2:108-12.
21. Cai XF, Sun JM, Bao LS, Li WB. Risk factors and antibiotic resistance of pneumonia caused by multidrug resistant Acinetobacter baumannii in pediatric Intensive Care Unit. World J Emerg Med 2020;3:202-7.
22. Kuo LC, Lai CC, Liao CH, Hsu CK, Chang YL, Chang CY, et al. Multidrug-resistant Acinetobacter baumannii bacteraemia: Clinical features, antimicrobial therapy and outcome. Clin Microbiol Infect 2020;13:196-8.
23. Sidhu S, Arora U, Devi P. Prevalence of nonfermentative gram-negative bacilli in seriously ill patients with bacteraemia. JK Sci 2010;12:168-71.
24. Govan JR, Brown AR, Jones AM. Evolving epidemiology of Pseudomonas aeruginosa and the Burkholderia cepacia complex in cystic fibrosis lung infection. Future Microbiol 2022;2:153-64.
25. Paton R, Miles RS, Amyes SG. Biochemical properties of inducible beta-lactamases produced from Xanthomonas maltophilia. Antimicrob Agents Chemother 2023;38:2143-9.

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