ORIGINAL RESEARCH PAPER
Volume - 11 | Issue - 11 | November - 2022 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

A STUDY OF NONFERMENTING GRAM NEGATIVE BACILLI ISOLATED FROM VARIOUS CLINICAL SAMPLES AND THEIR ANTIBIOTIC SENSITIVITY PATTERNS IN A TERTIARY CARE TEACHING HOSPITAL, CENTRAL GUJARAT

Microbiology

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ABSTRACT

Background: Nonfermentative gram-negative bacilli (NFGNB) have emerged as a major concern for nosocomial infections. Aerobic NFGNB are associated with life-threatening infections and emerging as multi drug resistant nosocomial pathogens. Aim of this study was to isolate and identify NFGNB in all the clinical samples and to determine their antibiotic susceptibility pattern. **Methods:** The cross sectional study was carried out over a period of 5 months. Clinical samples were processed by conventional bacteriological methods for isolation and identification. Susceptibility testing was done by Kirby-Bauer disc diffusion method as per Clinical and Laboratory Standard Institute. **Results:** 830 nonfermenters (10.4%) were isolated from 2603 culture positive clinical samples. Out of these nonfermenters , most were Acinetobacter spp. (61.8%) followed by Pseudomonas spp. (37.1%), Burkholderia spp. (1.1%) . **Conclusions:** Timely identification of nonfermenters and monitoring their susceptibility patterns will help in proper management of infections caused by them.

KEYWORDS

Acinetobacter spp., Nonfermenters, Pseudomonas spp., Burkholderia spp.

INTRODUCTION:

The Nonfermentative gram-negative bacilli (NFGNB) such as Pseudomonas spp., Acinetobacter spp., Burkholderia spp. 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.[1] They are widely distributed in nature as saprophytes or as commensals and act as opportunistic pathogens for man.[2] Nonfermenters are being recovered with 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.[3,4] The predisposing factors for such infections are diabetes mellitus, chronic renal failure, malignancy, osteomyelitis, 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.[5,6,7] They can also be recovered from hospital environment and have the potential to spread from patient-to-patient via fomites or the hands of health care personnel, commonly causing device related infections. [8]

Development of resistance in non-fermenters are multifactorial. Factors involved are mutations in genes encoding porins, efflux pump mechanisms, penicillin binding proteins, chromosomal beta lactamases.[9] NFGNB are innately resistant to many antibiotics and have been documented to produce extended spectrum β-lactamases and metallo-β-lactamases.[10] Nonfermenters are now resistant to many routinely used antibiotics and even to cephalosporins and carbapenems. Carbapenem resistance among Pseudomonas and Acinetobacter is of major concern.[11]

Resistance compromises treatment, prolongs hospital stay, increases mortality and healthcare costs.[12,13] Resistance to antibiotics among the NFGNB may vary widely from place to place even within a given geographical area but those isolated from the hospital settings are often multidrug resistant. Because of these variations a surveillance of the nosocomial pathogens for resistant organisms in a particular hospital set up is needed in order to guide appropriate selection of empirical therapy and to develop an antibiotic policy. [14] Success of antimicrobial therapy depends on the appropriateness of the choice of antibiotics that should be used on the basis of prior knowledge of the susceptibility pattern of the agent.[9] Therefore the aim of the present study was to isolate and identify NFGNB from clinical samples and their antimicrobial susceptibility profiles in a tertiary care teaching hospital of Central Gujarat, India.

METHODS:

This cross sectional study was conducted from January 2022 to May 2022 in the Department of Microbiology, tertiary care teaching hospital, Central Gujarat. A total of 7982 clinical samples including urine, pus, blood, wound swab and body fluids were received & Processed according to standard clinical laboratory practices. 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.[1]

The antibiotic used for NFGNB isolates were polymyxin B (300 µg), meropenem (10 µg), imipenem (10 µg), cefepime (30 µg), doxycyclin (30 µg), amikacin (30 µg), levofloxacin (5 µg), ceftriaxone (30 µg), piperacillin+tazobactum (100/10 µg), ceftazidime (30 µg) & ceftazidime+clavulanic acid (30/10 µg), cotrimoxazole (trimethoprim / sulphamethoxazole 1.25/23.75 µg) & gentamycin (10 µg) . Antimicrobial susceptibility test was performed by Kirby-Bauer disc diffusion method as per Clinical Laboratory Standard Institute guidelines (CLSI M100)[15].

RESULTS:

A total of 830 NFGNB were isolated from 2603 culture positive clinical samples accounting for an isolation rate of 31.9% (Figure 1).

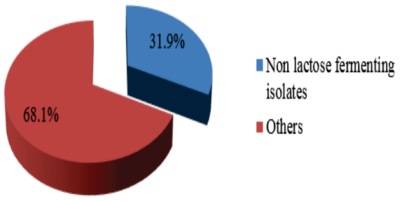

Figure 1: NFGNB isolates obtained from various clinical specimens.

Age limit ranged from day 0 to 70 years, males were 477 and females were 353 with Male to female ratio of 1.35:1. Maximum number of isolates were observed in the age Group of 51-60 years and 31-40 years. The age and sex distribution is shown in Figure 2.

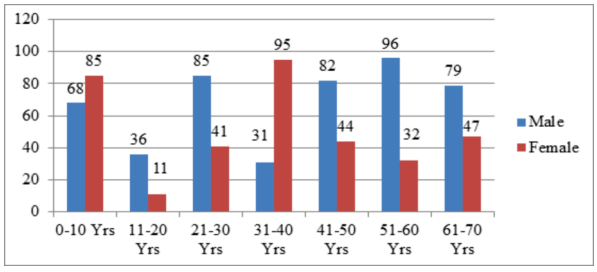

Figure 2: Bar diagram showing age and sex distribution of Non fermenting gram negative isolates

Wound swab was the most common specimen (41.32%) followed by body fluids (15.06%), sputum (13.85%), Blood (9.03%), E.T. tube (8.69%), Urine (4.21%) , Other (3.51%), Trecheostomy tube (3.37%) & UVC tip (0.96%). (Table 1).

Table 1: Sample-wise distribution of NFGN isolates.

Samples	No. of NFGNB (n=830)	Percentage
Wound swab	343	41.32%
Body fluids	125	15.06%
Sputum	115	13.85%
Blood	75	9.03%
ET tube	72	8.69%
Urine	35	4.21%
Other	29	3.51%
Trecheostomy tube	28	3.37%
UVC tip	8	0.96%

Acinetobacter spp. was the predominant isolate 513 (61.8%) followed by Pseudomonas spp. 308 (37.1%) and Burkholderia spp. 9 (1.1%). (Table 2).

Table 2: Prevalence of NFGN isolates.

Isolates	Number (n=830)	Percentage
Acinetobacter spp.	513	61.8%
Pseudomonas spp.	308	37.1%
Burkholderia spp.	9	1.1%

Among the NFGN isolates, Acinetobacter spp. showed highest sensitivity to Polymyxin B (98.9%) and lowest sensitivity to Amikacin (13%). Pseudomonas spp. showed highest sensitivity to Meropenem (90%) but lowest sensitivity to Ceftazidime (10%). Burkholderia spp. showed 100% susceptibility to Cotrimoxazole. Out of 308 Pseudomonas spp. isolates 64 (20.7%) were labeled as multidrug resistant. About 100% of the multidrug resistant pseudomonas spp. isolates were found to be susceptible to polymyxin B & 28 (43.7%) isolates showed susceptibility to Meropenem.

Table 3 : Sensitivity pattern of nonfermenters to antimicrobial agents.

Drug	Acinetobacter spp.	Pseudomonas spp.	Burkholderia spp.
Polymyxin B	98.9%	89%	-
Meropenem	92.3%	90%	84.2%
Imipenem	89.1%	-	-
Cefepime	72%	76%	-
Ceftazidime	-	10%	31.4%
Doxycycline	68%	14.4%	22%
Amikacin	13%	31%	-
Levofloxacin	76.9%	32%	58%
Ceftriaxone	23.1%	28.6%	-
Gentamycin	-	38.1%	-
Cotrimoxazole	-	-	100%
Piperacillin+Tazobactum	78.3%	48%	-

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 , out of 2603 culture positive samples 830 NFGNB were isloted so that isolation rate of NFGNB was 31.9% which is different from studies done by Bruno et al[16] ,Malini et al [3], where the isolation rate was 2.18% and 3.58% respectively . Nonfermenters have gained importance as causative agents of nosocomial infections having intrinsic as well as acquired resistance to different classes of drugs used frequently in clinical practice by the medical practitioners.[17]

Acinetobacter spp. being the commonest isolate followed by Pseudomonas spp. from high risk areas.[18] In our study, prevalence of Acinetobacter spp was more in high risk areas, possibly due to increased colonisation of Acinetobacter spp in hospital environment, including humidifiers, nebulizers, anaesthetic equipments, ventilators, healthcare workers etc. causing nosocomial opportunistic infections in patients with severe underlying illnesses.[18,19] In other clinical areas, Pseudomonas spp. was the commonest isolate , followed by Acinetobacter spp. Most of the isolates were from surgery and orthopaedic wards, where patients with road traffic accidents, burn, open wounds, abscesses, and surgical site infections were frequently admitted.

The world - wide resistance pattern of nosocomial pathogens shows wide variation from country to country and within the same country over a period of time.[20] Proper identification and detection of antibiotic sensitivity pattern is of great value because of high intrinsic resistance of different NFGN isolates to different antimicrobial agents. Therefore, various international authorities emphasize that every hospital should have antibiotic policy of its own.[21]

CONCLUSION :

To conclude, despite earlier being regarded as contaminants, NFGN isolates are now emerging as important pathogens causing a wide range of nosocomial infections. Identification of NFGN isolates 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. The interplay between multidrug resistant pathogens and the increasing number of immunocompromised patients pose a challenge for the microbiologists and clinicians likewise. 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 NFGN isolates in healthcare settings.

REFERENCES:

1. Winn W Jr, Allen S, Janda W, Koneman E, Prcop G, Schreckenberger P, et al, Koneman's Atlas and Textbook of Diagnostic Microbiology, 6thed, USA, Lippincott, William's and Wilkin's Company, 2006.
2. Mandell GL, Bennett JE, Dolin RA, Mandell. Douglas, and Bennett's principles and practice of infectious diseases, 7thed, Churchill Livingstone 2010.
3. Malini A, Deepa EK, Gokul BN, Prasad SR. Nonfermenting gram-negative bacilli infections in a tertiary care hospital in Kolar, Karnataka. J. Lab Physicians. 2009;1(2):62.
4. 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. 2012 ; 17(17).
5. Meharwal SK, Taneja N, Sharma SK, Sharma M. Complicated nosocomial UTI caused by nonfermenters. Indian J Urol. 2002 ; 18(2) : 123.
6. 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.
7. Shrihari N, Iswary M. Characterization and Antibiotic Sensitivity Pattern of Nonfermenting Gram Negative Bacilli from various Clinical Samples at a Tertiary Care Hospital. Int. J. Curr. Microbiol. App. Sci (2019) 8(9): 1502-1508.
8. Chaudhary NK , Dhakal S. Current Challenges in Antibiotic resistance: Non-fermenting Gram negative bacilli with Special References to Pseudomonas species and Acinetobacter species. JMSCR 2021 ; 9(3) ; 42-48.

9. Kaur A, Gill1 AK, Sing S. Prevalence and antibiogram of nonfermenting gram negative bacilli isolates obtained from various clinical samples in a tertiary care hospital, Bathinda, Punjab, India. Int J Res Med Sci. 2018;6(4):1228-1234.
10. Grewal US, Bakshi R, Walia G, Shah PR. Antibiotic Susceptibility Profiles of Non-fermenting Gram-negative Bacilli at a Tertiary Care Hospital in Patiala, India. Nigerian Postgraduate Medical Journal ;2017;24(2):121-125.
11. Krishnan S, Santharam P , Shanmugavadivoo N, Usha B. Prevalence of Non-Fermenting Gram Negative Bacilli and their Antibiotic Sensitivity Pattern at a Tertiary Care Hospital in Tamilnadu, India. Int. J. Curr. Microbiol. App. Sci (2018) 7(2): 2751- 2758.
12. Memish ZA, Shibl AM, Kambal AM, Ohaly YA, Ishaq A, Livermore DM. Antimicrobial resistance among non-fermenting Gram-negative bacteria in Saudi Arabia. J Antimicrob Chemother. 2012;67(7):1701-5.
13. Slama TG. Gram-negative antibiotic resistance: there is a price to pay. Crit. Care. 2008;21;12(4):1
14. Syiemiong B , Devi KM, Damrolien S , Devi KS. Antimicrobial resistance pattern among non-fermenting gram negative bacilli isolated from clinical samples in a tertiary care hospital in india. Int. J. Adv. Res. ; 2018;6(4), 1084-1088
15. Clinical and Laboratory Standards Institute (CLSI) M100. Performance standards for antimicrobial susceptibility testing 31st edition. CLSI 2021.
16. Bruno D, Nishino MK, Priore WN, Remus PR, do Carmo AA, Stefanello VB, et al. Prevalence of Gram negative nonfermenters patients in Porto Alegre RS. Journal Brasileiro de Patologia e Medicina Laboratorial 2011;47:529-34
17. Sharma AK , Aparajita A , Kumar M , Prasad A. Prevalence of Non Fermenting Gram Negative Bacilli with Their in vitro Antibiotic Sensitivity Profile in RIMS, Ranchi. Int J Med Res Prof. 2019 May; 5(3); 105-10.
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. Prashanth K, Badrinath S. In vitro susceptibility pattern of Acinetobacter species to Commonly used cephalosporins, quinolones, and aminoglycosides. Indian J Med Microbiol 2004;22:97-103.
21. Juyal D, Prakash R, Shankarnarayan S A, Sharma M, Negi V, et al. Prevalence of non-fermenting Gram negative bacilli and their in vitro susceptibility pattern in a tertiary care hospital of Utterakhand: A study from foothills of Himalayas. Saudi J Health Sci 2013;2:108-12.