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INCIDENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF NON-FERMENTING GRAM NEGATIVE BACILLI: A STUDY IN TERTIARY CARE HOSPITAL

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ABSTRACT

Introduction: Nonfermentative gram-negative bacilli (NFGNB) have emerged as a major cause of health care associated infections. This study was undertaken to know the prevalence of nonfermenters isolated from different clinical samples along with their antibiotic susceptibility pattern.

Material and Methods: Conventional bacteriological methods were used for identification and antibiotic susceptibility testing of nonfermenters. Antibiotic susceptibility testing was performed by methods as recommended by Clinical Laboratory Standard Institute (CLSI).

Results: Out of the 120 isolates, the majority of the non-fermenters were isolated from blood 37 (30.83%), followed by pus 32 (26.67%), urine 20 (16.67%), CSF 16 (13.33%), Ascitic fluid 5 (4.17%), the rest of the isolates were from other clinical specimens. The most common isolate was genus Pseudomonas (94) and followed bygenus *Acinetobacter* (26), *A.baumanii* (22) and *A.lwoffii (*04) respectively A high level of antibiotic resistance was recorded for most of the first and second line drugs. Colistin, imipenem, pipercillin –tazobactam and amikacin were the drugs with maximum activity.

Conclusion: NFGNB though regarded as contaminants are important bacteria causing wide range of health care associated infections. Irrational use of powerful antibiotics for prolonged periods added to the compromised host conditions might be responsible for multi-drug resistance (MDR). Improved antibiotic usage and infection control measures will be needed to prevent or slow the emergence and spread of multi-drug resistant NFGNB in the healthcare setting.

KEYWORDS :

Introduction

Non-fermenting gram negative bacilli (NFGNB) are a group of 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] NFGNB are ubiquitous in nature. The guanine and cytosine (G+C) content of the DNA ranges from 57 to almost 70 mol%.[2] They are considered as environmental contaminants and most of them have emerged as important health care associated pathogens.[2] Which can cause opportunistic infections in immunocompromised individuals. These organisms also cause generalized infections like urinary tract infection, septicaemia, sub-acute bacterial endocarditis and etc.[3]

In the recent years due to liberal and empirical use of antibiotics, NFGNB have emerged as important health care associated pathogens. They have been incriminated in infections such as, septicemia, meningitis, pneumonia, urinary tract infection and surgical site infections.Unlike the Enterobacteriaceae the NFGNB do not fit conveniently into single family of well characterized genera, and correct taxonomic placement of many nonfermentive, gram negative bacilli remain unresolved.[5]

The major genera of NFGNB have been classified into atleast 22 families.[5] In addition, there are number of clinically important nonfermenters that are not yet assigned to a family and whose taxonomic position is still uncertain. In recent years, the problem is further compounded by the emergence of resistance to antimicrobial agents which are widely used against the non-fermenters, making them as an important healthcare associated pathogen.[6]

Pseudomonas aeruginosa, and *Acinetobacter baumanii* are one of the most common nonfermenter pathogen responsible for health care associated infection are intrinsically resistant to many antibiotics. It also shows an increasing pattern of resistance towards β-lactam antibiotics, especially by production of class C chromosomal β-lactamases.[7] NFGNB show resistance to a wide range of antibiotics, leading to serious infections. Multi-drug resistance (aminoglycosides, fluoroquinolones, uriedopenicillins and third generation cephalosporins) exhibited by *Acinetobacter* species and *Pseudomonas aeruginosa* pose a major clinical problem in treatment[7]

Material and Methods

The prospective study was conducted in the department of microbiology, Gajra Raja Medical College Gwalior. All the specimen received from the patient of the OPD and IPD of all departments of J.A. Group of Hospitals of Gajra Raja medical College, Gwalior during the period of one year. All the specimens were processed for isolation and identification according to standard microbiological techniques.A total of 732 clinical specimen were included in the study. All the specimen were inoculated on MacConkey agar and Blood agar, which were aerobically incubated for 24 hours at 37^{0}C . The plates were read the following day but extending to 48 hours if there are no bacterial growth within 24 hour. Isolated colonies were subjected to identification by colony morphology, gram staining, hanging drop preparation, oxidase test and standard biochemical test.[8]

Antimicrobial susceptibility testing and interpretation was determined by Kirby Bauer disc diffusion method on Muller Hinton agar (MHA) as per clinical laboratory standard institute (CLSI) guidlines.[9]

RESULTS

A total of 120 nonfermenters isolates were isolated from the 732 clinical samples accounting for an isolation rate of 16.39%. The clinical specimen were blood (30.83%), pus (26.67%), urine (16.67%), CSF (13.33%), ascitic fluid and throat swab (4.17%) respectively. The most common isolated organism was *Pseudomonas aeruginosa* 94 (78.33%) followed by *Acinetobacter baumannii* 22(18.33%) and *Acinetobacter lowffii* 04(3.33%).

We also found relationship between nonfermenting gram negative

bacilli and sex. The prevalence was higher in male (57.5%) compared to female (42.5%). The most frequent isolation in relation to age was noted in the age group 1-10 years followed by 11-20 years. The most active antibiotic against *P.aeruginosa* showed a sensitivity of 95.74% for colistin, 85.10 % for imipenem, 77.66% for levofloxacin, 63.83% to ciprofloxacin and 60.64% to polymyxin B. *A. baumanii* showed a susceptibility of 50 % to ceftazidime, 50 % to ceftriaxone, 33.3% to cefepime and 33.3% to cefaperazone.

Table1. Admission of patients in various wards

Wards	Number of patients	Percentage
Surgery	46	38.33
Medicine	12	10
Obstetric and gynecology	8	6.67
Pediatrics	33	27.5
ENT	8	6.67
Orthopaedics	8	6.67
Neurosurgery	5	4.17

Table 2. Bacterial species isolated under each clinical infections

	Septicemia	Respiratory tract infection	Local infections.	Post OP infections	Urinary tract	C S O M	Post - trauma infections.	Gastro intestinal infections	Total
P.aeruginosa.	21	10	26	13	18	01	02	03	94
A.baumanii.	10	03	01	02	02	00	02	02	22
A.lwofii	02	00	01	00	00	00	01	00	04
Total	33	13	28	15	20	01	05	05	120

Table 3. Isolates Samplewise

S. No	Specimen	*P aeruginosa*		*A.baumanii*		*A.lwofii*	
		N	%	N	%	N	%
1	Pus	26	27.66	05	22.73	01	25
2	Ascitic fluid	03	3.20	02	9.09	00	00
3	Blood	25	26.60	10	45.45	02	50
4	Urine	18	19.15	02	9.09	00	00
5	Tracheal swab	03	3.20	00	00	00	00
6	CSF	13	13.83	02	9.09	01	25
7	Aural swab	01	1.06	00	00	00	00
8	Throat swab	05	5.32	01	4.55	00	00
	Total	94	100	22	100	04	100

Discussion

NFGNB are considered commensal or contaminants, their pathogenic potential has been well established by there frequent isolation with clinical isolates. In our study nonfermenters were isolated *P.aeruginosa* predominated followed by genus *Acinetobacter* resembling to study done by Zhang et al[10] , Memish et al[11] , Ruchita Mhajan et al[12] and Vijaya D *et al*[13].

NFGNB are known to cause infection in extremes age which was also seen in our study, 5% in the age group of 61 to 70 years and 13.33% in <1 year of age similar to study by Sorabh Singh Sambyal *et al*[14] and Sachdev *et al*[15], which could be due to subnormal immune system.

The total number of representative samples was 57.5% males and 42.5% females which are similar to study of Sorabh Singh Sambyal *et al*[14]

In our study a total of 38.33% were admitted in Surgery ward and in Paediatrics 27.5%. where as in Algun *et al*[16] study it was 47.2%.other patients were admitted to Orthopaedics, ENT,OBG and Neurosurgery.

In our study majority of NFGNB were isolated from blood sample, while earlier studies done by Mishra et al[17], TT Kitch et al[18], and P.Yasodhara et al[19] shown that NFGNBs have been most commonly isolated from pus sample.

P. aeruginosa were the most common isolate from Local infection like cellulitis, diabetic foot, burns in our study which was similar to studies done by Resmi .Rajan et al[20]and H Wisplinghoff et al[21].

P.aeruginosa as the main etiological agent responsible for 21.67% local infections in our study , however it was higher in a studies by Yashodhara et al[19] 66.95%, 9,66%in Mishra et al[17] study 2, 89.9% Resmi Rajan et al[20] ,72.5% in Cristane et al[22] study.

The differences in the percentages of various parameters may be due to the variation in the sample size. Infection at burns site is because of injury associated with breakdown of normal skin, immune defects and selection of antibiotics with inadequate coverage for this pathogen [23]

In our study, patients who had been catheterized for >72 hrs, post operative urinary tract infection was common with *Pseudomonas aeruginosa* and *A.baumanii*. *P.aeruginosa* and *A.baumanii*, both are known to cause chronic & recurrent Urinary tract infection and often Multi drug resistant[23]

The most common organism causing respiratory tract infection was Ps.aeruginosa 8.33% followed by A.baumanii 2.5%.

NFGNB displays a wide and variable spectrum of antibiotic sensitivity. There is no antibiotic to which all strains are susceptible[15].

It showed sensitivity of 53.3% to Piperacillin tazobactum in our study and the sensitivity ranged from 40% to 85% in other studies[20,24,25] . Piperacillin tazobactam is a preferred drug for treating NFGNB .

P. aeruginosa show 55.32% sensitivity to piperacillin tazobactum in our study which correlates with Ruchita mahajan et al[12] .

A. baumanii showed sensitivity of 50% to Piperacillin in our study where as it was 30%in Wong Fu et al[26].study and 40% in a study by Taneja et al[27]. A.baumanii showed a resistance of 50% resistance to Piperacillin tazobactam combination in our study, while in a study by Jawad et al[28] it was (46.1%) .

NFGNB showed resistance of 47.5% to Ceftazdime, 56.67% Cefaperazone sulbactum, 59.17% Ceftriaxone which are commonly used by the clinicians in our hospital. *P.aeruginosa* showed sensitivity of 47.87% in our study to Cefoperazone sulbactum. In Krishna Prakash et al[24]. study it was 67%and 98.2%. in a study by Resmi Rajan et al[20].

In our study NFGNBs showed 60% resistance to Norfloxacin and 43.33% to ciprofloxacin in our study. *P.aeruginosa* shows 36.17% resistance to ciprofloxacin in our study, in various other studies it ranged from 12.5% to 83%.[16,24,25,26,27] NFGNBs showed a good sensitivity to Amikacin 58.33% in our study. *P. aeruginosa* showed sensitivity 58.51% in our study which similar to other studies by Krishna Prakash et al[24] and Taneja et al[27]. Gentamycin showed 56.67% sensitivity in our study. In a study by Taneja et al[27] it showed 61.2%and 73.5% in Resmi Rajan et al[20] .

NFGNB showed an overall 15.83% resistance to Imipenem in our study, in a study by Taneja et al[27] it showed 36% . *Pseudomonas aeruginosa* showed 14.90% resistance to imipenem in our study, studies in India and Abroad showed a range of 11.8% to 81.5%[20,25,27] .

In our study the following risk factor were observed during study i.e. Catherisation (Urinary/ IV),prolong hospital stay , ICU ,surgery, diabetic, preexisting respiratory illness, trauma, neonate, immunosuppression due to malignancy and burns

Conclusion -

Identification of NFGNB and monitoring their antibiotic susceptibility pattern are important for management of infection caused by them. Minimized use of available antimicrobial, regular antimicrobial susceptibility surveillance and strict infection control measures are required to contain this emerging antibiotic resistance among NFGNB.

References

1. Malini A, Deepa E.K, Gokul B.N, Prasad S.R "Non-fermenting Gram Negative bacilli infections in a Tertiary care hospital in Kolar, Karnataka" J Lab Physicians 2009; 1(2) : 62-6.
2. Hoiby Niels, Ciofu Oana and Bjarnsholt Thomas .Pseudomonas. In: Jorgensen J H, Pfaller M A, Carroll K C, Funke G, Labdry M L,Richter S S, and Warnock D W, editors. Manual of Clinical Microbiology, 11th ed. Vol-1. Washington DC: ASM press; 2015. p.773-790.

3. Joseph NM, Sistla Sujatha et al " Reliability of Kirby Bauer disk diffusion method in detecting meropenem resistance among non fermenting Gram negative bacilli" Indian J Pathol Microbiol 2011 ;54(3) : 556- 60.
4. Manohar A, Chaterjee S, Mathai D, SARI study group. Detection and characterization of MBL producing P. aeruginosa. Indian J Med Microbiol 2010; 28 (3): 241-244.
5. The Nonfermentative Gram-Negative Bacilli . In: Procop G W, Church Deirdre L, Hall Geraldine S, Janda William M, Koneman Elmer W, Schreckenberger Paul C and Woods Gail L, editors. Koneman's Color Atlas and Textbook of Diagnostic Microbiology: 7th ed. Philadelphia: Lippincott Williams and Wilkins; 2006.p.316-431
6. Palleroni NJ. Family I. Pseudomonadaceae. In Krieg NR, Holt JG, eds. Bergys Manual of Systemic Bacteriology. Vol. 1. Baltimore, MD: Williams and Wilkins, 1984:141-219.
7. Sinha M, H. Srinivasa " Mechanisms of resistance to carbapenems in meropenem resistant Acinetobacter isolates from clinical samples" Indian J of Med Microbiol 2007;25(2):121-5.
8. Govan J.R.W. Pseudomonas, Stenotrophomonas, Burkholderia In: Collee JG, Fraser AG, Marimion BP, Simmons A, editors. Mackie and McCartney Practical Medical Microbiology: 14th ed. New York: Churchill Livingstone; 1996.p. 413-24
9. Clinical and Laboratory Standards Institute. 2014. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement (M100-S24); Table 2B; Vol. 34(1):58-67
10. Zhang C, Liang J, Liu P. Monitoring to drug resistance of non-fermenting gram-negative bacilli isolated from clinics in county hospital. Chin J Nosocomiol 2011;7:1432-3.
11. 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;67:1701-5.
12. Mahajan Dr. Ruchita, Dr. Neeraj, Dr. Sarika, Mahajan Dr. Bella. Isolation and Identification of Non Fermenting Gram Negative Bacilli in A Tertiary Care Hospital. Sch. J. App. Med. Sci., 2016; 4(3D):872-876.
13. Vijaya D, Kamala, Bavani S, Veena M. Prevalence of nonfermenters in clinical specimens. Indian J Med Sci 2000;54:87-91.
14. Sambyal S.S, Kaur Avneet, Soodan Puneet Singh, Mahajan Bella. Changing Antibiotic senstivity pattern in Gram Negative Nonfermenting Isolates: a Study in a Tertiary care Hospital. IOSR-JDMS;2015;5(14):129-133.
15. Sachdev HS,Deb M.Acinetobacter meningitis:case Report with review of Literature.Ind Paediatr 1980;17:551-555.
16. Algun U,Arisoy A,Gunduz T,Ozbakkaloglu B.The resistance of Pseudomonas aeruginosa strains to fluoroquinolone group of antibiotics.Ind J Med Microbiol 2004; 22:112-114.
17. Mishra E, Bhujwala RA, Shriniwas. NonFermenters in human infection. Indian J Med Res1986; 83:561-566.
18. Kitch TT,Jacobs MR,AppelbaumPC.Evaluation of the 4-hour RapidID Plus Method for Identificationof 345 Gram Negative Nonfermentative Rods.
19. Yashodhara P,Shyamala S.Identification and Characterization of NonFermenters From Clinical Specimens.Indian J Med Microbiol 1997; 15:195-197.
20. Rajan R, Saramma TI. Isolates of Pseudomonas aeruginosa from clinical specimens. J Acad clin Microbiol 2001; 3:11-15.
21. Wisplinghoff H, Perbix W, Seifert H. Risk factors for Nosocomial Bloodstream Infections Due to Acinetobacter baumanii:A Case Control study of Adult Burn Patients. Clin Infect Dis 1999; 28:59-66.
22. Cristane-Frosta CC,Moneira JLB. Frequency of Nonfermentative Gram-Negative Bacilli isolated from clinical materials of Pacients at Universidade federal Do Ceara Hospital Complex-Brazil.Rev Microbiol 1998;29:1343-45
23. Pollack M.Infections due to Pseudomonas species and related organism. In:Fauci, Braunwald, Isselbacher, Wilson,Martin,Kasper,Hauser, Longo,editors. Principles of Internal Medicine.18th edition vol.I, New York:Mc GrawHill 2005;943-950
24. Prakash KS, Chaudhary M, Kashyap B, Kumari T, Sharma VK. Imipenem Resistant Pseudomonas aeruginosa. A preliminary report. J Acad Clin Microbiol 2005; 7:27-30
25. Troillet N, Samore MH, Carmeli Y. Imipenem-Resistant Pseudomonas aeruginosa: Risk factors and Antibiotic Susceptiblity Patterns. Clin Infect Dis 1997; 25:1094-1098.
26. Fu W,Demei Z,Shi W,Fupin H,Yingyuan Z.The susceptibility of non-fermentative Gram-negative bacilli to cefperazone and sulbactam compared with other antibacterial agents.International J of Antimicrobiol Agents 2003;22:444-448.
27. Taneja N, Maharwal S,Sharma M. Imipenem Resistant in Nonfermenters causing Nosocomial Urinary Tract Infection. Ind J Med Sci 2003; 57:294-299.
28. Jawad A, Seifert H, Snelling AM, Heritage J, Hawkey PM. Survival of Acinetobacter baumanii on Dry surfaces: Comparison of Outbreak and Sporadic Isolates. J Clin Microbiol 1998; 36:1938-1941.