IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 18, Issue 12 Ser.12 (December. 2019), PP 51-55
www.iosrjournals.org

Characterization and Antibiotic Susceptibility Pattern of Nonfermenting Gram Negative Bacilli from Blood Stream Infections in Children in a Tertiary Care Hospital, Punjab

Dr.Atul kumar[1], Dr.Aroma oberoi[2], Mr.Ravi vashisth[3], Dr.(Col)V.K.Sharma[4,] Dr.(CoL) Paramjit singh[5]

1.Assistant Professor,Department of microbiology, Muzaffarnagar medical college,Muzaffarnagar
2.Professor & HOD,Department of microbiology, Christian medical college,Ludhiana
3. Assistant Professor,Department of microbiology, Muzaffarnagar medical college
4. Professor,Department of microbiology, Muzaffarnagar medical college
5. Professor & HOD,,Department of microbiology, Muzaffarnagar medical college
Corresponding Author: Dr. Atul Kumar, C-44, Faculty Campus, Muzaffarnagar Medical College, Muzaffarnagar,UP
Corresponding Author: Dr.Atul Kumar[1]

Abstract
Purpose: *Bacteraemia due to nonfermentative gram negative bacilli appears to be increasing in frequency particularly in hospitalized patients with severe underlying illness.*
Objectives: *Therefore purpose of this study was to characterize various nonfermenters and to know their antibiotic susceptibility pattern causing blood stream infections in children.*
Materials and Methods: *This retrospective study was conducted in the Microbiology department of a tertiary care hospital, in Punjab. A total of 2267 paediatrics blood cultures were received from suspected cases of septicaemia in the laboratory. All the blood samples were processed in the Microbiology Laboratory by RAPID BD BACTEC PLUS system and organisms were identified as per standard microbiological techniques. Antimicrobial susceptibility testing was done using Kirby Bauer disc diffusion method as per CLSI guidelines.*
Results: *Out of the 2267 samples from paediatrics patient's 187 (8.24%) blood cultures were positive. Gram negative bacilli were the predominant organism 96 (51.3%). Of these 32 (33.3%) were nonfermenters which were further characterized. Among the 32 NFGNB, Pseudomonas spp was the commonest isolate among the nonfermenters accounting for 71.8 % (23/32), followed by Acinetobacter spp 15.6% (5/32) and Shewanella putrefaciens 12.50% (4/32). These were 100% sensitive to Imipenam, Meropenam, Polymixin B and Colistin. It showed (87.50%) sensitivity to Chloramphenicol followed by Ciprofloxacin (84.38%), Piperacillin-Tazobactam (84.38%) and Amikacin (81.25%). The isolates showed least sensitivity to Ampicillin (12.50%).*
Conclusion: *P.aeruginosa and A.baumanii were the common NFGNB isolated in our study. Therefore identification of NFGNB and monitoring their susceptibility pattern are important for proper management of these infections caused by them.*
Key words: *Bacteraemia, Nonfermentative Gram Negative Bacilli*

Date of Submission: 18-12-2019 Date of Acceptance: 01-01-2020

I. Introduction

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[1].They occur as saprophytes in the environment and some are also found as commensals in the human gut[2,3].Nonfermentative gram negative bacilli (NFGNB), although frequently considered to be commensals or contaminants, but the pathogenic potential of these organisms has been established beyond doubt because of their frequent isolation from clinical specimens and their association with the disease[4]. Gram negative nonfermenting aerobic bacteria are being increasingly implicated in human disease. The complex physicochemical properties of these organisms necessitate a battery of tests for their precise identification.[5] In addition there is still much confusion regarding the taxonomic status of many of these nonfermenters. Identification of these nonfermenters has often being neglected[6]. In the recent years due to liberal and empirical use of antibiotics, NFGNB have emerged as important health care associated pathogens. NFGNB are innately resistant to many antibiotics and are known to produce extended spectrum β-Lactamases and metallo β- Lactamases[3,5,7,8]

II. Objectives

Therefore purpose of this study was to characterize various nonfermenters and to know their antibiotic susceptibility pattern causing blood stream infections in children.

III. Material & Methods

This retrospective study was conducted in the Microbiology Department of a tertiary care hospital in Punjab. A retrospective analysis was conducted from September 2012 to December 2013. During this period 2267 paediatrics blood cultures were received from suspected cases of septicaemia in the laboratory. All the blood samples were processed in the Microbiology Laboratory by RAPID BD BACTEC PLUS system and organisms were identified as per standard microbiological techniques[9]. Every positive vial flagged by the Bactec 9120 (Becton Dickinson, USA) was removed for sub culturing in the safety cabinet. The lid of the positive blood culture vial was disinfected with an alcohol swab and 1 ml of blood was aseptically removed from the vial and plated on blood agar, and MacConkey's agar. The Blood agar and MacConkey agar, incubated aerobically in incubator at 37˚C for 24 to 48 hours and then observed for growth of bacteria. All the plates showing growth were processed according to standard Microbiological techniques including gram staining[10]. All the gram negative bacilli/coccobacilli, oxidase positive or negative were inoculated on to triple sugar iron (TSI) medium. Organisms that failed to show any change, both in slant and butt of medium were considered nonfermenters. The isolates were further identified up to the species level based on motility, pigment production, enzyme production (catalase, urease, nitrate reductase)[11] and various biochemical tests included oxidative-fermentative test, growth at 42ºC, nitrate or nitrite reduction, gelatin liquefaction, arginine dihydrolase, lysine decarboxylase, citrate utilization, indole test, production of hydrogen sulphide and esculin hydrolysis. Antimicrobial susceptibility testing of all the isolates using Kirby-Bauer disc diffusion method[12] was carried out with disc containing,ampicillin(10µg), cotrimoxazole(25µg)**,** amikacin(10µg),cefotaxime(30µg), cefperazone(30µg), ciprofloxacin(5µg), piperacillin-tazobactam (100/10 µg), and imipenem (30 µg) and the results were interpreted as per clinical and laboratory standards institute (CLSI) recommendation[13].

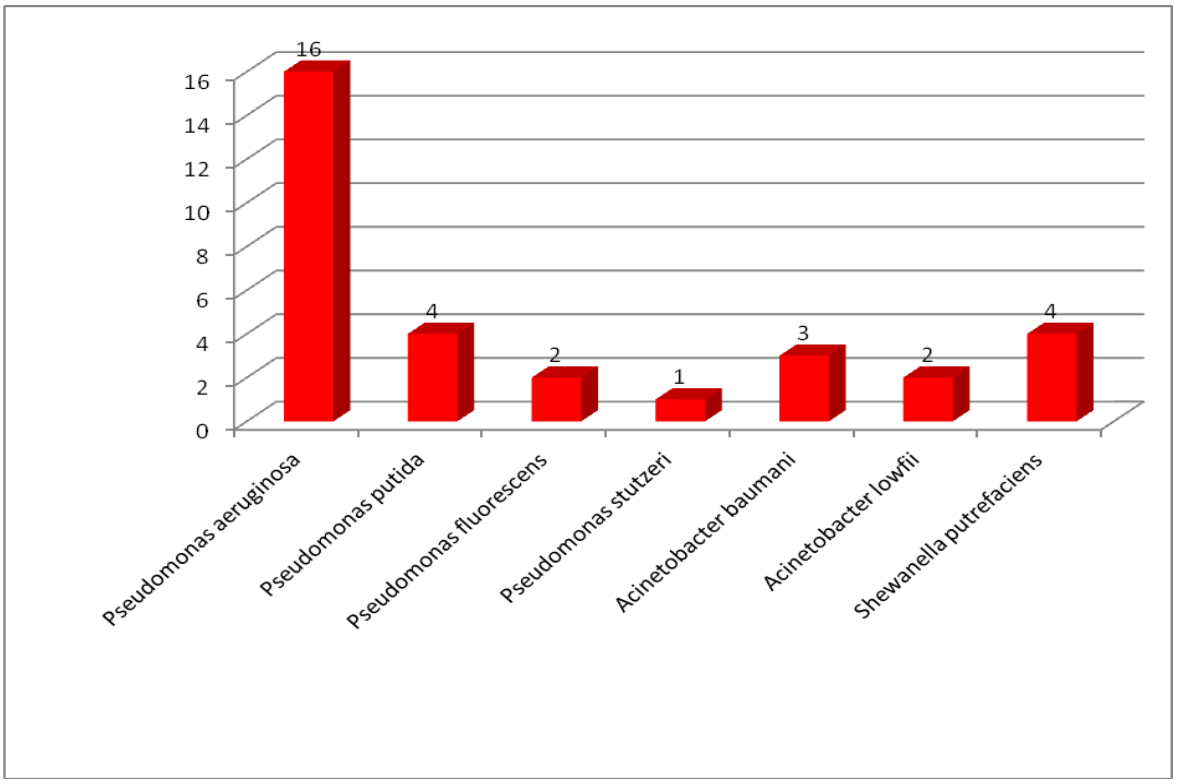
IV. Results:

Out of the 2267 samples from paediatrics patient's 187 (8.24%) blood cultures were positive. Gram negative bacilli were the predominant organism 96 (51.3%). Of these 32 (33.3%) were nonfermenters which were further characterized. Among the 32 NFGNB, Pseudomonas aeruginosa was the commonest isolate among the nonfermenters accounting for 50.0% (16/32), followed by Pseudomonas putida 12.50% (4/32), Shewanella putrefaciens 12.50% (4/32), Acinetobacter baumanii 9.38% (3/32), Acinetobacter lwofii 6.25% (2/32), Pseudomonas fluorescens 6.25% (2/32) and Pseudomonas stutzeri 3.12% (1/32). These were 100% sensitive to Imipenam, Meropenam, Polymixin B and Colistin. It showed (87.50%) sensitivity to Chloramphenicol followed by Ciprofloxacin (84.38%), Piperacillin-Tazobactam (84.38%) and Amikacin (81.25%). The isolates showed least sensitivity to Ampicillin (12.50%).

Table 1: Nonfermentative gram negative bacilli in blood culture isolates

Si.no	Isolate	Number
1.	Pseudomonas aeruginosa	16(50.0)
2.	Pseudomonas putida	4(12.50)
3.	Pseudomonas fluorescens	2(6.25)
4.	Pseudomonas stutzeri	1(3.12)
5.	Acinetobacter baumani	3(9.38)
6.	Acinetobacter lowfii	2(6.25)
7.	Shewanella putrefaciens	4(12.50)
	TOTAL	32(100)

Percentage in parentheses represent out of total isolates (n=32)



Graph 1: Nonfermentative gram negative bacilli in blood culture isolates

Table 2 : Antibiotic sensitivity pattern of nonfermenters

Antibiotic	**Sensitivity**
Ampiciline (amp)	4 (12.5)
Cotrimoxazole (cot)	19 (59.3)
Amikacin (amk)	26 (81.25)
Cefotaxime (ctx)	23 (71.8)
Cefperazone (cpz)	20 (62.5)
Pipracillin /tazo (pit)	27 (84.3)
Ciprofloxin (cip)	27 (84.3)
Imipenem (imp)	32 (100)
Meropenem (mrp)	30 (93.7)
Polymixin b(p)	32 (100)
Colistin (c)	32 (100)

Percentage in parentheses represent out of total isolates (n=32)

Table 3: Antibiotic sensitivity pattern of pseudomonas spp, acinetobacter spp and schwanella spp

Antibiotic	**Pseudomonas spp n (23)**	**Acinetobacter spp n(5)**	**Schwanella spp n(4)**
Ampiciline (amp)	2(8.69)	1(20)	1(25)
Cotrimoxazole (cot)	14(60.8)	1(20)	4(100)
Amikacin (amk)	23(100)	1(20)	2(50)
Cefotaxime (ctx)	20(86.9)	1(20)	2(50)
Cefperazone (cpz)	17(73.9)	1(20)	2(50)
Pipracillin /tazo (pit)	22(95.6)	2(40)	3(75)
Ciprofloxin (cip)	21(91.3)	2(40)	4(100)
Imipenem (imp)	23(100)	5(100)	4(1000
Meropenem (mrp)	23(100)	3(60)	4(100)
Polymixin b(p)	23(100)	5(100)	4(100)
Colistin (c)	23(100)	5(100)	4(100)

Percentage in parentheses represent out of isolates

V. Discussion:

In the present study, 32 (33.3%) strains of nonfermenters were isolated which is in accordance with the results of study done by Chang & Huang[14] isolated 31.62% of nonfermenters. Various workers have reported variable results in their studies. Rao & Shivananda [15] reported higher positivity rate of 66.88% while Seetha et

al[16] isolated 45% of nonfermenters. These variations might be because of the hospital infection control practices of that institute. Pseudomonas aeruginosa is an opportunistic pathogen which causes severe infections in patients with immune deficiency and newborns. The present study identified 11.50% (23/200) Pseudomonas spp and 2.50 %(5/200) Acinetobacter spp. Similar results were seen in a retrospective study conducted by (Asghar AH).[17] Nonfermenters are resistant to a large number of commonly used antibiotics. In our study they are 100% sensitive to Imipenam, Meropenam, Polymixin B and Colistin. Most of the strains showed sensitivity to Chloramphenicol (87%) followed by Ciprofloxacin (84.3%), Piperacillin-Tazobactum (84.3%) and Amikacin (81.2%).The isolates showed least sensitivity to Ampicillin (12.5%). This contrasts with the antibiotic sensitivity pattern of isolates obtained from other studies[18-20], where 70-80% of the isolates were sensitive to Ciprofloxacin & Amikacin.

In the present study, Pseudomonas spp showed (90%) resistance to Ampicillin and (56%) to Aztreonam. They are (60%) sensitivity to Cotrimoxazole, Cefotaxime (86%), Cefperazone(73%), , Piperacillin/Tazobactam (95%), Ciprofloxacin (91%). (100%) isolates were sensitive to Amikacin, Gentamicin, Imipenam, Meropenam, Polymixin B and Colistin. (Karlowsky JA et al)[21] have shown that P. aeruginosa was highly susceptible to Amikacin **(**92.3%) and Piperacillin-Tazobactam (91%) similar to the present study.This contrasts with the antibiotic sensitivity pattern of isolates from other studies [18-20] where (70-80%) of the isolates were sensitive to Ciprofloxacin & Amikacin.

In our study the frequency of Acinetobacter species was 2.5% (5/200) among the culture positive blood samples. The total number of Acinetobacter species isolated during the study period was 5, out of which 3 were A.baumanii and 2 were A.Iwofii. Acinetobacter species were less frequent in blood samples as compared to other bacteria causing blood stream infections. A study conducted in paediatric intensive care unit (PICU) of a tertiary care teaching hospital reported (8.6%) frequency of Acinetobacter species isolated from blood samples.[22] Another study done in National Cheng Kung University, Tainan reported the similar result of 9.9% for Acinetobacter species.[23] The frequency of Acinetobacter in our study was lower than the other studies. This could be due to clean hospital environment and hygienic conditions and good infection control practices. In the present study, Acinetobacter spp showed (80%) resistance to Ampicillin, Cotrimoxazole, Amikacin, Cefotaxime and Cefperazone of the isolates were resistant to Piperacillin/Tazobactam and Ciprofloxacin. It showed (60%) sensitivity to Meropenam. 100% isolates were sensitive to Imipenam, Polymixin B and Colistin. This finding is in conformity with the observation of other workers in 2009 a study from Rohtak showed that the resistance of Acinetobacter to Meropenem had increased to 25.6%(Goel et al).[24] In the same study the resistance to Amikacin was (87.2%). Our study showed (100%) sensitivity to Polymyxin. However there have been reports of Polymyxin resistant Acinetobacter from Greece, Slovakia and other parts of Europe (Gales et al)[25], (Souli et al)[26]. Shewanella spp showed 75.0% resistance to Ampicillin. It showed 50.0% sensitivity to Amikacin, Cefotaxime and Cefperazone and 75% sensitive to Piperacillin/Tazobactam.. All the isolates were 100% sensitive to Cotrimoxazole, Ciprofloxacin, Imipenam, Meropenam, Polymixin B and Colistin. This contrasts with the antibiotic sensitivity pattern of isolates from other studies.

VI. Conclusions:

P.aeruginosa and A.baumanii were the commonest NFGNB isolated in our study. The different species of NFGNB have shown a varied sensitivity pattern in our study. Therefore identification of NFGNB and monitoring their susceptibility pattern are important for proper management of these infections caused by them. Our study highlights that it is essential to establish the clinical relevance of the NFGNB isolated, before they are considered as pathogens. This would avoid unnecessary usage of antibiotics and emergence of drug resistant strains.

Bibliography:

[1]. Koneman E, Allen S, Janda W, Schreckenberger P, Winn W. Colour Atlas and textbook of Diagnostic Microbiology. 6th Edition, Lippincott –Raven Publishers, Philadelphia, 2006.
[2]. Gardner, B Griffin, Swartz, Kunz. Nonfermentative gram negative bacilli of nosocomial interest. American J Med 1970 Jun;48:735-49.
[3]. Bergogne-Berezin E, Towner KJ. Acinetobacter spp. as Nosocomial Pathogens: Microbiological, Clinical and Epidemiological Features. Clin Microbiol Rev 1996 Apr; 9: 148-65.
[4]. Aliaga L, Mediavilla JD, Cobo F. A clinical index predicting mortality with Pseudomonas aeruginosa bacteremia. J Med Microbiol. 2002 Jul; 51: 615-9.
[5]. Mehta M, Punia JN, Joshi RM. Antibiotic resistance in Pseudomonas aeruginosa strains isolated from various clinical specimens-a retrospective study. Indian J Med Microbiol 2001; 19:232.
[6]. Nagoba BS, Deshmukh SR, Ulka G Gude et al. Invitro susceptibility of Pseudomonas aeruginosa to different antibiotics. Indian J Med Microbiol 1997, 15:185-186.
[7]. Mishra B, Bhujwala RA, Shrinivas. Non fermenters in human infections. Indian J Med Res 1986 Jun; 83:561-6.
[8]. Rubin SJ, Granato PA and Wasilaskas BL. Glucose nonfermenting gram negative bacteria. In: Lennette EH, Balwos A, Hausler Jr. WJ and Shadomy HJ, editors. Manual of clinical Microbiology. 4th edition, washington, D.C: American society for Microbiology;1985. 330-49.

[9]. Collee JG, Marr W. Culture of bacteria:Mackie and McCartney practical medical microbiology. Collee JG, Fraser AG, Marimon BP, Simmon A, editor.14th ed, New York:Churchill Livingstone;1996.113-129.
[10]. Cheesbrough M. Microbiology:in medical laboratory manual for tropical countries. ELBS ed. UK:University Press Cambridge;1991.26-58**.**
[11]. Pezzlo M. Aerobic bacteriology. In: Isenberg, HD, editors. Clinical Microbiology Procedures handbook. WashingtonDC:Am Soc Microbiol.1.19.1-1.20.47.
[12]. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial disk susceptibility tests;approved standard–11th edition. Wayne, PA: CLSI; 2012. CLSI document M02‐A11.
[13]. Clinical Laboratory Standards Institute. Performance standard for antimicrobial susceptibility testing.23th Information supplement.NCCLS 2013:M100‐S23.
[14]. Chang TC, Huang AYH. Rapid differentiation of fermentative from nonfermentative gram negative bacilli in positive blood cultures by an impendance method. J Clin Microbiol 2000; 38:3589-94
[15]. Rao PS, Shivananda PG. Bacteraemia due to non fermenting gram negative bacilli in immunocompromised patients. Ind J Med Microbiol 1993;11:95-99.
[16]. Seetha KS, Bairy I, Shivananda PG. Bacteraemia in high- risk patients. Ind J Med Sci 2002; 56: 391-96
[17]. Asghar AH. Frequency and antibiotic susceptibility patterns of bacterial pathogens isolated from septicemic patients in Makkah hospitals. Saudi Med J 2006;27:361-7.
[18]. Mehta M, Punia JN, Joshi RM. Antibiotic resistance in Pseudomonas aeruginosa strains isolated from various clinical specimens a retrospective study. Indian J Med Microbiol.2001;19:232.
[19]. Vijaya D, Kamala, Bhavani S, Veena M. Prevalence of nonfermenters in clinical specimens. Indian J Med Microbiol. 2000;54:87-91.
[20]. Mahapatra A, Samal B, Pattnaik D, Mishra S, Ghosh SK. Antimicrobial susceptibility pattern of clinical isolates of nonfermentative bacteria. Indian J Pathol Microbiol.2003;46:526-7
[21]. Karlowsky JA, Jones ME, Draghi DC, Thornsberry C, Sahm DF, Volturo GA. Prevalence and antimicrobial susceptibilities of bacteria isolated from blood cultures of hospitalized patients in the United States in 2002. Ann Clin Microbiol Antimicrob.2004;3:7
[22]. Singhi S, Ray P, Mathew JL, Jayashree M, Dhanalakshmi. Nosocomial blood stream infection in a pediatric intensive care unit. Indian J Pediatr.2008;75:25-30.
[23]. Lee N, Chang TC, Wu C, Chang C, Lee H, Chen P, et al. Clinical manifestations, antimicrobial therapy and prognostic factors of monomicrobial Acinetobacter baumanii complex bacteremia. J Infection.2010;61:219-7.
[24]. Goel N, Chaudhary U, Aggarwal R, Bala K. Antibiotic sensitivity pattern of gram negative bacilli isolated from the lower respiratory tract of ventilated patients in the intensive care unit. Indian J Crit Care Med.2009;13:148-51.
[25]. Gales AC, RN Jones, HS Sader. Global assessment of the antimicrobial activity of polymyxin B against 54731 clinical isolates of gram-negative bacilli:report from the sentry antimicrobial surveillance programme(2001-2004). Clin Microbiol Infect. 2006;12:315-21.
[26]. Souli M, Kontopidou FV, Koratzanis E, Antoniadou A, Giannitsioti E, Evangelopoulou P, et al. In vitro activity of tigecycline against multiple-drug resistant, including pan-resistant, gram-negative and gram-positive clinical isolates from Greek hospitals. Antimicrob Agents Chemother.2006;50:3166-9.

Dr.Atul Kumar. "Characterization and Antibiotic Susceptibility Pattern of Nonfermenting Gram Negative Bacilli from Blood Stream Infections in Children in a Tertiary Care Hospital, Punjab." IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), vol. 18, no. 12, 2019, pp 51-55.