ORIGINAL ARTICLE

Prevalence of Nonfermentative Gram Negative Bacilli In Seriously ill Patients With Bacteraemia

Shailpreet Sidhu, Usha Arora, Pushpa Devi

Abstract

Bacteraemia due to nonfermentative gram negative bacilli appears to be increasing in frequency particularly in hospitalized patients with severe underlying illness. A total of 159 (79.50%) organisms were isolated from blood cultures of 200 seriously ill patients. Out of these, 73 (45.91%) were nonfermentative gram negative bacilli. Pseudomonas aeruginosa was the commonest isolate (32.88%) followed by Acinetobacter spp. (23.28%) and Burkholderia cepacia (10.96%). Analysis of antimicrobial susceptibility testing showed multidrug resistant pattern with majority of the isolates being resistant to three or more drugs

Key Words

Bacteraemia, Nonfermentative Gram Negative Bacilli

Introduction

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 (1). These apparently heterogenous microorganisms have common traits of clinical importance that justify their inclusion and study in a single group. They can be recovered from hospital environment, commonly cause device related infections, are often resistant to disinfectants and have the potential to spread from patient to patient via fomites or the hands of medical personnel (2). Most of the nonfermenters cause nosocomial blood stream infections particularly in debilitated and immunocompromised hosts and are usually multidrug resistant (3). Serious infections due to this group of organisms are currently being reported with increasing frequency and make a significant contribution to in-hospital mortality (4).The present study was undertaken to isolate, identify, characterize and to find out the association of NFGNB with clinical condition of the patient. Antimicrobial resistance pattern of the isolates was also studied.

Material & Methods

A total of 200 seriously ill patients admitted in different wards of tertiary care hospital were studied over a period of one and a half year. Blood sample of all the patients was obtained aseptically, inoculated into brain heart infusion broth and incubated at 37^0C. Blind subculture on to sheep blood agar and MacConkey agar was performed after 24,72 hours and subsequently on the seventh day. The plates were incubated aerobically at 37^0C for 24 hours and growth was recorded. 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) and various biochemical tests included oxidative-fermentative test, growth at 42^0C, nitrate or nitrite reduction, gelatin liquefaction, arginine dihydrolase, lysine decarboxylase, citrate utilization, indole test, production of hydrogen sulphide and esculin hydrolysis. The isolates which could not be identified by conventional methods were subjected to RapID NF plus system (5) for their identification.

Antimicrobial susceptibility testing of all the isolates using Kirby-Bauer disc diffusion method was carried out with discs containing ciprofloxacin (1 g), piperacillin (30 g), ticarcillin (75 g), gentamicin (10 g) amikacin (30 g), tobramicin (10 g), cefoperazone (75 g), sulbactam-

From the Department of Microbiology Govt. Medical College, Amritsar Punjab India

Correspondence to : Dr. Usha Arora, Professor and Head, Department of Microbiology, Govt. Medical College, Amritsar- Punjab-India

cefoperazone (30-75 g), imipenem (10 g) and the results were interpreted as per clinical and laboratory standards institute (CLSI) recommendation (6).

Results

A total of 159 (79.50%) organisms were isolated and of these, 73 (45.91%) were nonfermenters which were further characterized. All the patients with bacteraemia due to NFGNB had some predisposing underlying disease or condition (*Table 1*). Regarding invasive devices, 41 (56.16%) patients had peripheral venous and 23 (31.50%) had central venous line placed. Urinary catheter and endotracheal tube were found in 29 (39.72%) and 12 (16.43%) cases respectively. All but 9 (87.67%) patients had fever and 11 (15.06%) presented with severe sepsis. Forty nine (67.12%) patients had history of prior antibiotic intake and 9 (12.32%) were seen to be neutropenic. A total of 16 species of nonfermenters were isolated (*Table 2*). Pseudomonas aeruginosa was the commonest isolate (32.88%) followed by Acinetobacter spp. (23.28%) and Burkholderia cepacia (10.96%). Amongst 73 strains of NFGNB, 41 (56.18%) were isolated from intensive care unit (ICU) whereas 32 (43.85%) were from various other wards of hospital. Antimicrobial resistance pattern of the isolates is shown in *Table 3*. Most of the strains (76.71%) were resistant to 3 or more drugs. Only 6.85% of the isolates were found to be sensitive to all the drugs.

Seventeen (23.29%) patients with nonfermentative bacteraemia died during the hospital stay and of these 14 (82.35%) were admitted in ICU.

Discussion

In the present study, 73 (45.91%) strains of nonfermenters were isolated which is in accordance with the results of study done by Seetha *et al* (7). Various workers have reported variable results in their studies. Rao & Shivananda (8) reported higher positivity rate of 66.88% while Chang & Huang (9) isolated 31.62% of nonfermenters. These variations might be because of the hospital infection control practices of that institute. All the cases with nonfermentative bacteraemia were immunosuppressed due to presence of one or the other underlying condition or disease (*Table 1*). As reported previously by various workers (4,8) , nosocomial nonfermentative bacteraemia is usually presented in patients with severe underlying diseases. The frequent use of invasive devices in the form of peripheral venous catheter (56.16%), urinary catheter (39.72%), central venous catheter (31.50%) and endotracheal tube (6.43%) were found in most of the patients.These devices probably could have acted as a source of infection in these patients. Vidal *et al* (4) also emphasized that NF bacteraemia is often associated with invasive devices and found 60% of the patients had central line and 17.6% had urinary catheter inserted. In the present study, forty nine (67.12%) patients gave history of prior antibiotic usage. Other workers (4,10) also found prior antibiotic therapy as an important risk factor and reported it in 61.1% and 81.0% of the patients respectively. One more study analyzed risk factors for the acquisition of multidrug resistance and prior exposure to broad spectrum antimicrobials was found to be one of the contributing factors (11). Twelve percent of the patients were seen to be neutropenic and of these 55.55% had underlying malignancies. It has been observed that acute neutropenia caused by cancer chemotherapy is more likely to be associated with increased risk of infections (12). P. aeruginosa was found to be the most common isolate (32.88%) followed by Acinetobacter spp (23.28%). Other workers (7,8) also found these two organisms as predominant pathogens and their results correlate well with those presented in our study. Nonfermenters other than P. aeruginosa and Acinetobacter spp. have also been isolated (*Table-2*). These organisms have recently emerged as important cause of morbidity and mortality especially in compromised patients. Bacteraemia caused by these unusual pathogens has been reported in multiple studies (13,14). More than half of the strains (56.18%) were isolated from ICU which is comparatively higher than the results of Enoch *et al* (15) who isolated 43.20% of nonfermenters among ICU patients. One more study conducted on two ICUs reported it to be 43.80% and 38.90% respectively (16). Debilitated condition of the patient, invasive diagnostic and therapeutic procedures and more importantly use of contaminated fluids or life support equipment in ICU could be the source of infection. On observing the antimicrobial resistance pattern, most of the isolates were seen to be resistant to 3 or more drugs (*Table 3*). Various antibiogram studies with NFGNB also showed multidrug resistant pattern (8,17). Maximum resistance was seen with gentamicin (72.60%) followed by tobramicin (63.01%) which is comparable with the findings of other study in which it is reported to be 58.82% and 55.88% respectively (18). Veenu *et al* (17) have also shown gentamicin to be the least effective drug with 50.0% to 70.3% isolates being resistant to it. These differences in rate of drug resistance might be because of the variations in type of antimicrobials being prescribed by the clinicians. Imipenem was found to be the most effective drug (5.47% resistance) followed by sulbactam-cefoperazone (21.91% resistance). Workers have reported sulbactam-cefoperazone and carbapenems as the most effective drugs against

Table 1. Distribution Of Cases With Nf Bacteraemia According To Underlying Condition

Underlying condition	Number	Percentage
Surgery/Instrumentation	18	24.65
Pneumonia	7	9.59
Steroid therapy	6	8.22
Diabetes mellitus	6	8.22
Renal failure	5	6.85
Neonatal sepsis	5	6.85
Wounds/trauma	5	6.85
Burns	4	5.48
Carcinoma	4	5.48
Septic arthritis	3	4.11
Malnutrition	3	4.11
Transplantation	3	4.11
Leukemia	2	2.74
Liver diseases	2	2.74
Total	**73**	**100.0**

Table 2. Species Distribution of Nonfermentative Gram Negative Bacilli

Species	Number	Percentage
Pseudomonas aeruginosa	24	32.88
P. fluorescens	1	1.37
P. putida	2	2.74
P. stutzeri	4	5.48
Burkholderia cepacia	8	10.96
Brevundimonas vesicularis	1	1.37
Brevundimonas diminuta	1	1.37
Ochrobactrum anthropi	1	1.37
Acinetobacter baumannii	11	15.06
Acinetobacter lwoffii	6	8.22
Stenotrophomonas maltophilia	4	5.48
Alcaligenes faecalis	4	5.48
Achromobacter xylosoxidans subspp. *xylosoxidans*	3	4.11
Shewanella putrefaciens	1	1.37
Roseomonas spp.	1	1.37
Moraxella lacunata	1	1.37
Total	73	100.0

Table 3. Antimicrobial Resistance Pattern of Various Isolates

Isolate	Cf	G	To	Ak	Ti	P	Cp	Sb-Cp	I
P. aeruginosa (n=24)	14 (58.33%)	19 (79.16%)	16 (66.66%)	9 (37.50%)	16 (66.66%)	11 (45.83%)	11 (45.83%)	7 (29.16%)	3 (12.50%)
P. fluorescens (n=1)	-	-	-	-	-	-	-	-	-
P. putida (n=2)	1 (50.0%)	1 (50.0%)	1 (50.0%)	1 (50.0%)	1 (50.0%)	-	1 (50.0%)	-	-
P. stutzeri (n=4)	2 (50.0%)	3 (75.0%)	3 (75.0%)	1 (25.0%)	2 (50.0%)	1 (25.0%)	2 (50.0%)	1 (25.0%)	-
[1]*B. cepacia* (n=8)	2 (25.0%)	6 (75.0%)	5 (62.50%)	3 (37.50%)	5 (62.50%)	3 (37.50%)	4 (50.0%)	2 (25.0%)	-
[2]*B. vesicularis* (n=1)	-	-	-	-	-	-	-	-	-
[2]*B. diminuta* (n=1)	1 (100.0%)	-	1 (100.0%)	-	1 (100.0%)	1 (100.0%)	-	-	-
O. anthropi (n=1)	-	1 (100.0%)	1 (100.0%)	-	1 (100.0%)	1 (100.0%)	1 (100.0%)	-	-
[1]*A. baumannii* (n=11)	8 (78.72%)	9 (81.88%)	6 (54.54%)	3 (54.54%)	7 (63.63%)	6 (54.54%)	4 (36.36%)	2 (18.18%)	1 (9.09%)
[1]*A. lwoffii* (n=6)	3 (50.0%)	3 (50.0%)	2 (33.33%)	1 (16.66%)	3 (50.0%)	2 (33.33%)	1 (16.66%)	-	-
[1]*S. maltophilia* (n=4)	2 (50.0%)	4 (100.0%)	3 (75.0%)	3 (75.0%)	2 (50.0%)	1 (25.0%)	3 (75.0%)	1 (25.0%)	-
[2]*A. faecalis* (n=4)	1 (25.0%)	3 (75.0%)	3 (75.0%)	2 (50.0%)	2 (50.0%)	1 (25.0%)	2 (50.0%)	1 (25.0%)	-
**A.xylosoxidans* (n=3)	-	2 (66.66%)	3 (100.0%)	1 (33.33%)	1 (33.33%)	1 (33.33%)	1 (33.33%)	1 (33.33%)	-
[2]*S.Putrefaciens* (n=1)	-	1 (100.0%)	1 (100.0%)	-	-	-	-	-	-
Roseomonas spp.(n=1)	-	-	-	-	-	-	-	-	-
M. lacunata (n=1)	-	1 (100.0%)	1 (100.0%)	-	-	1 (100.0%)	1 (100.0%)	1 (100.0%)	-

1Acinetobacter, 2Alcaligenes, 1Burkholderia, , 2Brevundimonas,, 1Stenotrophomonas,2Shewanella, *Achromobacter xylosoxidans subspp. xylosoxidans
Cf-Ciprofloxacin; G-Gentamicin; To-Tobramicin; Ak-Amikacin; Ti-Ticarcillin; P-Piperacillin; Cp-Cefoperazone; Sb-Cp - Sulbactam-cefoperazone; I-Imipenem

nonfermenters (19,20). In the present study, the outcome of interest was in-hospital mortality. Significant mortality trend was seen in ICU patients. It appears that patients who could not survive were those who were in a critical condition because of a severe underlying disease, underwent one or the other invasive procedures or because of prolonged stay in ICU. The patient's clinical condition was so impaired that some of them died even with correct antibiotic therapy.

Conclusion

Nonfermentative gram negative bacilli are emerging as important opportunistic pathogens and are resistant to commonly used antimicrobials. For each of these organisms, underlying host factors were strongly associated with outcome. The interplay between these multidrug resistant pathogens and the increasing number of immunocompromised patients pose a challenge for the microbiologists and clinicians likewise. Early diagnosis and institution of empirical therapy based on recent antibiogram of the institute would reduce mortality and improve patient management. More importantly, these organisms have great potential to survive in hospital environment, so effective methods of sterilization and infection control measures should be implemented.

The present study has sufficient power to identify factors, organisms and antibiotics influencing the outcome of infection which will help us to understand the role of these organisms in human disease process better. However, use of additional features and large study sample would have enhanced the value of study and may have provided greater insight into a possible link.

References

1. Arora U, Aggarwal A, Sofat S. Nonfermenters in human infections. *Ind J Pathol Microbiol* 2003; 46: 265-67
2. Quinn JP. Clinical problems posed by multiresistant nonfermenting gram negative pathogens. *Clin Infect Dis* 1998; 27: 117-24
3. Mcgrowan JE, Moellering RC, Fishman N. Resistance in nonfermenting gram negative bacteria. *Am J Infect Control* 2006; 34: 29-37
4. Vidal F, Mensa J, Almela M, *et al*. Bacteraemia in adults due to glucose nonfermentative gram negative bacilli other than Pseudomonas aeruginosa. *Q J Med* 2003; 96: 227-34
5. Koneman EW, Allen SD, Janda WM, Winn WC, Procop GW, Schreckenberger PC, Woods GL. The nonfermentive gram negative bacilli. In: Color atlas and textbook of Diagnostic Microbiology. 6th ed., J.B.Lippincott Co., Philadelphia 2006.pp. 367-73.
6. Wayne PA. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: Fifteenth informational supplement. Approved Standard 2005; M7-A6
7. Seetha KS, Bairy I, Shivananda PG. Bacteraemia in high-risk patients. *Ind J Med Sci* 2002; 56: 391-96
8. Rao PS, Shivananda PG. Bacteraemia due to non fermenting gram negative bacilli in immunocompromised patients. *Ind J Med Microbiol* 1993;11:95-99
9. 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
10. Seifert H, Strate A, Pulverer G. Nosocomial bacteraemia due to Acinetobacter baumannii: Clinical features, epidemiology and predictors of mortality. *Internal Med J* 2005; 35: 599-603
11. Paterson DL. Looking for risk factors for the acquisition of antibiotic resistance: A 21st century approach. *Clin Infect Dis* 2002; 34: 1564-49
12. Steven M, Holland, John IG. Disorders of granulocytes and monocytes. In: Fauci A, Braunwald E, Isselbacher KJ, Wilson JD, Martin JB, Kasper DL, Hauser SL, Longo DL, Harrison's Principles of Internal Medicine. 14th ed., Vol 1., McGraw Hill, New York 1998.pp. 351-59
13. Martino R, Martinez C, Pericas R, Salazar R, Sola C, Brunat S, Sureda A, Albos DA. Bacteraemia due to glucose nonfermenting gram negative bacilli in patients with haematological neoplasias and solid tumors. *Eur J Clin Microbiol Infect Dis* 1996; 15: 610-15
14. Gales AC, Jones RN, Andrade SS, Sader HS. Antimicrobial susceptibility pattern of unusual nonfermentative gram negative bacilli isolated from Latin America: Report from sentry antimicrobial surveillance program. *Mem Inst Oswaldo Cruz Rio De Janeiro* 2005; 100 : 671-7
15. Enoch DA, Simpson AJ, Kibbler CC. Predictive value of isolating Pseudomonas aeruginosa from aerobic and anaerobic blood culture bottles. *J Med Microbiol* 2004; 53: 1151-54
16. Wroblewska MM, Rudnicka J, Marchel H, Luczak M. Multidrug resistant bacteria isolated from patients hospitalized in intensive care units. *Int J Antimicrob Agents* 2006; 27 : 285-89
17. Veenu, Sikka R, Arora DR. Isolation and susceptibility pattern of nonfermenting gram negative bacilli from clinical samples. *Ind J Med Microbol* 1998; 17: 14-8
18. Endimiani A, Luzzaro F, Tamborini A, *et al*. Identification and antimicrobial susceptibility testing of clinical isolates of nonfermenting gram negative bacteria by the phoenix automated microbiology system. *Microbiologica* 2002; 25: 323-29
19. Wang H, Chen MJ. Changes of antimicrobial resistance among nonfermenting gram negative bacilli isolated from intensive care units from 1994 to 2001 in China. *Zhongua Yi Xue Za Zhi* 2003; 85: 385-90
20. Laconis JP, Pitkin DH, Sheikh W, Nadler HL. Comparison of antibacterial activities of meropenem and six other antimicrobials against Pseudomonas aeruginosa isolates from North American studies and clinical trials. *Clin Infect Dis* 1997; 24 : 191-96