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Original Research Article

Spectrum of non-fermenting gram negative bacilli isolated from patients with blood stream infections in a tertiary care hospital in North India

Asifa Nazir*, Bushra Yousuf Peerzada, Ifshana Sana

Department of Microbiology, Government Medical College, Srinagar, Jammu and Kashmir, India

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***Correspondence:**
Dr. Asifa Nazir,
E-mail: asifanazir@gmail.com

ABSTRACT

Background: Non-fermenting Gram-negative bacilli (NFGNB) are emerging as important causes of blood stream infections (BSI) and they are a major cause of morbidity and mortality worldwide. High intrinsic resistance of NFGNB to antimicrobial compounds makes the treatment of BSIs caused by them difficult and expensive. The aim of this study was to assess frequency and antibiotic susceptibility pattern of non-fermenting gram-negative rods isolated from blood culture of patients.
Methods: A total of 3016 blood samples were received in the Department of Microbiology during the study period. All samples were processed according to standard microbiological procedures. Blood culture was done by automated blood culture system, (BacT/Alert) and identification and antibiotic susceptibility of non-fermenting gram negative bacilli was done by VITEK2 Compact System.
Results: A total of 120 NFGNB were identified out of which the most common non-fermenters isolated were *Acinetobacter sp.* (95) followed by *Pseudomonas aeruginosa* (11), *Burkholderia cepacia* (09) *Sternotrophomonas maltophilia* (03) and *Sphingomonas sp.* (02). Most of the non -fermenters were multi drug resistant showing a high level of antibiotic resistance to most of the first- and second-line drugs. The most effective drugs were colistin and tigecycline.
Conclusions: This study underlines the need to identify NFGNB in tertiary care hospitals and to monitor their susceptibility pattern to guide the clinician for better care and management of patients. Improved antibiotic stewardship and strict infection control measures especially hand washing need to be implemented to prevent emergence and spread of multidrug resistant NFGNB in health care settings.

Keywords: *Acinetobacter sp,* Antibiotic susceptibility, Nosocomial pathogens, Non-fermenters

INTRODUCTION

Bacteremia is defined as the presence of viable bacterial agent in the bloodstream and is diagnosed in daily clinical practice with the use of blood cultures. It is associated with significant healthcare costs and mortality, with a case-fatality rate as high as 30%.[1,2] Bacteraemia due to gram-negative bacilli is a significant problem in both hospitalized and community-dwelling patients. These organisms pose serious therapeutic problems because of the increasing incidence of multidrug resistance.[3] Infections caused by non-fermentative gram-negative bacteria (NFGNB) constitute an emerging

problem in nosocomial setting, especially in an immunocompromised host.

Non-fermenting Gram negative bacilli (NFGNB) are ubiquitous, heterogeneous, aerobic, non-sporing bacteria, saprophytic in nature. They do not utilize carbohydrate as a source of energy by fermentation and hence termed as 'Non-fermenters'.[4]

This heterogeneous group includes organisms like *Pseudomonas sp*, *Acinetobacter sp*, Alkaligene spp, *Stenotrophomonas maltophilia*, *Burkholderia cepacian* complex, etc.[5] NFGNB are very problematic because of their ubiquitous distributions in the environment and their antimicrobial resistance patterns.[6] Although frequently considered as contaminants, most of them have emerged as important nosocomial pathogens causing opportunistic infections in immunocompromised hosts. NFGNB are known to account for about 15% of all bacterial isolates from a clinical microbiology laboratory.[7]

NFGNBs are known to colonize initially and then subsequently invade the otherwise normally sterile site through trauma. It has been noted that disruption of natural barriers is an important route of entry of infections.[8,9]

Rates of colonization increase in hospitalized patients particularly in those who have been hospitalized for extended periods or / and have received broad spectrum antimicrobial therapy/chemotherapy.[10] Most of the non-fermenters cause nosocomial blood stream infections particularly in debilitated and immunocompromised hosts and are usually multidrug resistant. Data from the Surveillance and Control of Pathogens of Epidemiological importance (SCOPE) study revealed that approximately one-fourth of gram-negative bacteraemia were attributed to NFGNB.[11]

The present study was therefore taken to identify the non-fermenters from blood specimens and to determine their antimicrobial susceptibility pattern.

METHODS

Setting

The prospective study was conducted in the Department of Microbiology, Government Medical College, Srinagar, India, from November 2017 to October 2018.

Blood Stream Infection (BSI) was defined as the isolation of a pathogen microorganism from >1 blood culture bottle. BSIs were classified as community- and hospital-acquired infections if detected within the first 48 h of hospitalization, or after 48 h of hospitalization, respectively. The recovery of different species 72 h after the previous positive blood culture in a single patient was considered to be a distinct episode. Isolation of the same microorganism from a single patient was considered to be a single episode even if the culture was obtained after 72 h. Multiple bacteremic episodes in a single patient were considered to be distinct episodes, if separated by at least 7 days.

Sample collection and processing

Blood samples were collected from the patients before the administration of any antibiotic. For adults, after aseptic precautions, 5-10 mL of blood subsequently incubated in BacTAlert3D (Biomerieux, France), a fully automated blood culture system for detection of growth in blood culture. On getting a positive alarm, Gram stain were carried out on positive bottles, followed by sub culture on 5% sheep blood agar and MacConkey agar plates which were incubated aerobically at 37°C overnight for bacterial isolation. Isolates were identified by Vitek 2 Compact (Biomerieux). Antimicrobial susceptibility testing was done with an automated microbiology system, Vitek 2 compact 60 system BioMerieux India®) and interpreted according to CLSI criteria.[12]

The patient data that were collected included age, sex, underlying diseases and risk factors. Quality control was performed by testing these same antimicrobials against reference strains of bacteria.

Statistical analysis

Descriptive statistics were used to express overall results. Data were analyzed using SPSS 15.0. Categorical variables were evaluated by the chi-square test, and continuous variables were evaluated by the Mann-Whitney U test and t-test.

RESULTS

A total of 3016 consecutive blood samples were received in the Department of Microbiology during the study period. Non - Fermenter gram negative bacilli (NFGNB) were isolated from 120 patients. The most common non-fermenter isolated was *Acinetobacter sp* (95) followed by *Pseudomonas aeruginosa* (11), *Burkholderia cepacia* (09) *Sternotrophomonas maltophilia* (03) and *Sphingomonas sp.* (02) as shown in Table 1.

Table 1: Species distribution of Non-fermenter gram negative isolates (NFGNB) from blood culture (n=120).

Species	Number	Percentage
Acinetobacter baumannii	88	73.3%
Acinetobacter lwoffii	07	5.8%
Pseudomonas aeruginosa	11	9.1%
Burkholderia cepacia	09	7.5%
Stenotrophomonas maltophilia	03	2.5%
Sphingomonas sp.	02	1.6%

Amongst 120 strains of NFGNB, 91 (75. 8%) were isolated from intensive care unit (ICU) whereas 29(24.16%) were from various other wards of hospital. Most of the ICU patients with bacteraemia due to NFGNB had some predisposing underlying disease or condition as shown in Table 2.

Table 2: Predisposing risks factors associated with NFGNB isolation.

Underlying risk factor	Number	Percentage
Prolonged antibiotic, steroid therapy	84	70%
Instrumentation, surgery	23	19.1%
Prematurity	55	45.8%
Wounds /trauma	12	10%
Malignancies	08	6.6%
Diabetes mellitus	08	6.6%

Maximum number of cases were observed in the age group less than 10 years (34.1%) followed by age group >60 years (32.5%). The age distribution is shown in Figure 1.

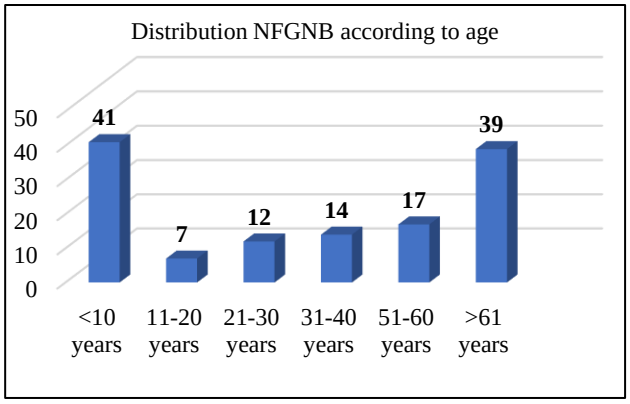

Figure 1: Age distribution of non -fermenter gram negative bacilli.

Males were more commonly affected than females with male to female ratio of 1.3:1.

NFGNB demonstrated high resistance to many groups of antimicrobial drugs. Most of the strains (78.71%) were resistant to 3 or more drugs. Only 7.85% of the isolates were found to be sensitive to all the drugs. The antibiotic sensitivity pattern of the isolates of *Acinetobacter sp* is shown in Figure 2. *Acinetobacter strains* were highly resistant to most of the tested antibiotics; all strains exhibited reduced susceptibility to imipenem, ceftriaxone, and amikacin. However, all the strains were sensitive to colistin. The most effective drug against *P. aeruginosa* was colistin followed by amikacin, gentamicin and ciprofloxacin. *S. maltophilia* showed 100% susceptibility for ciprofloxacin and cotrimoxazole but was completely resistant to imipenem.

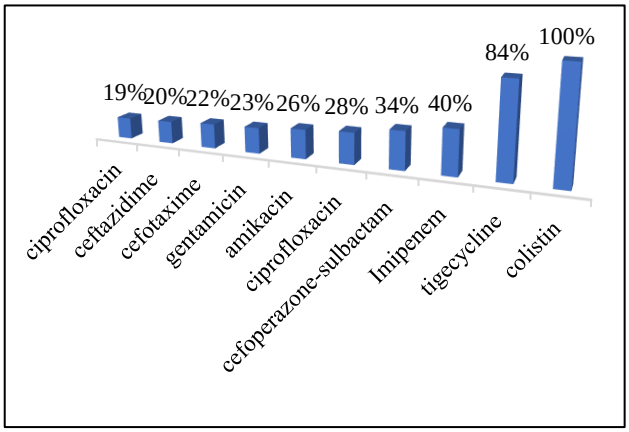

Figure 2: Antibiotic sensitivity pattern of *Acinetobacter sp*. (n=95).

DISCUSSION

Non-fermentative gram-negative bacilli are ubiquitous in environment. NFGNB were considered to be a contaminant in past but 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.[10,13]

The present study was undertaken to evaluate the prevalence of Non-fermenting gram negative bacilli in causing bloodstream infections especially in hospitalized patients and to know their antibiotic sensitivity. Out of 3016 blood cultures performed at the hospital from November 2017 to October 2018, 35% (1064/3016) tested positive for bacterial culture and 11.2% (120/1064, 10%) of these positive cultures grew NFGNB. Various workers have reported variable results in their studies. Rao et al, reported higher positivity rate of 66.88%, while Chang et al, isolated 31.62% of nonfermenters.[14,15] These differences might be because of the different hospital infection control practices in different institutes.

Amongst 120 strains of NFGNB, 91 (75.8%) were isolated from intensive care unit (ICU) whereas 29 (24.16%) were from various other wards of hospital These results are comparatively higher than the results of Enoch et al, who isolated 43.20% of non-fermenters among ICU patients. Another study conducted on two ICUs reported the incidence of NFGNB to be 43.80% and 38.90% respectively.[16,17] The results are however in concordance with studies conducted by Juyal et al, and Jayapriya et al, who reported isolation rate of NFGNB isolates from ICU samples to be 67% and 58% respectively.[18,19] Prolonged hospital stay, instrumentation, prematurity (in case of neonates) burns, open wounds, surgical site infections, diabetes, malignancies and several underlying illnesses made these patients more vulnerable to NFGNB infections.

Of 120 NFGNB, the most commonly isolated NFGNB in the present study was *Acinetobacter sp*. (79.1%) followed by *Pseudomonas sp*. (9.1%), *Burkholderia cepacia*, (7.5%), *S. maltophilia* (2.5%) and *Sphingomonas sp.* (1.6%). This correlates with the findings of Samantha et al in 2011, 20 who also found that *Acinetobacter sp*. (66%) followed by *Pseudomonas sp*. (26%) were the most commonly isolated NFGNB in their study. These findings also correlate with studies of other workers who found these two organisms to predominant pathogens in their study on non-fermenters.[21,22]

With respect to age, high prevalence of NFGNB was noted among patients in the age group less than 10 years (34.1%) followed by age group >60 years (32.5%). This indicates that comorbidities that develop with age likely influence the invasiveness of NFGNB and also due to weakened immune system at extremes of age.

Because of high intrinsic resistance of different NFGNB to different antimicrobial agents, the value of proper identification and resistance testing is of foremost importance in a given setup to guide appropriate selection of empiric therapy. On observing the antimicrobial resistance pattern most of the isolates were seen to be resistant to 3 or more drugs. Isolates of *Acinetobacter sp*. were susceptible to colistin (100%) and tigecycline (84%) but showed reduced sensitivity to imipenem (40%) cefoperazone-sulbactam combination (34%), ciprofloxacin (28%), amikacin (26%), gentamicin (23%), cefotaxime (22%), and ceftazidime (20%). These results are similar to various other studies.[18-20] The antimicrobial susceptibility pattern of *Pseudomonas* aeruginosa showed 100% sensitivity to colistin and *polymyxin* B, followed by piperacillin-tazobactum 52%, amikacin 51.3%, imipenem 35%, ceftazidime 34%, ciprofloxacin and ofloxacin 22.6%, and gentamycin 22%. This is in concordance with reports of other authors where MDR in *P. aeruginosa* has been reported.[23-24]

S. maltophilia showed 100% susceptibility for ciprofloxacin and cotrimoxazole but was completely resistant to imipenem, amikacin and most of the other drugs. *S. maltophilia* is intrinsically resistant to most β-lactams, including carbapenems.[25]

In the present study, *B. cepacia* showed very good sensitivity towards levofloxacin (75%), minocycline (70%) imipenem (66.6%) meropenem (67.7%). All isolates were sensitive to cotrimoxazole. *B. cepacia* is intrinsically resistant to many β-lactam drugs, aminoglycosides, colistin and polymixin B.[25] Limited sensitivity of *B. cepacia* isolates to imipenem and meropenem is significant as it is one of the first line drugs against *Pseudomonas sp*.[26]

NFGNB are rapidly emerging as nosocomial pathogens causing opportunistic infections in immunocompromised hosts. *Acinetobacter sp*, and *Pseudomonas aeruginosa* being the most common pathogens. Multi drug resistance is being commonly observed among NFGNB.[27,28] This study demonstrated higher prevalence of drug resistance among the NFGNB especially of *Acinetobacter sp.* isolates towards majority of the antibiotics. It is, therefore, important to document resistance among NFGNB, especially the carbapenem resistance, as these strains are often the cause of outbreaks in the ICU's and can limit therapeutic option due to the high degree of multi drug resistance. These organisms are also responsible for spreading resistance to other susceptible bacteria by horizontal gene transfer.

CONCLUSION

With the alarming increase in multidrug resistance in gram-negative bacteria especially non-fermenters, rendering many antimicrobial agents ineffective, it is important for clinicians to remain updated with prevalence and antimicrobial susceptibility pattern of the circulating pathogens in the selection of the antimicrobials to be used for empiric therapy. The polymyxins (polymyxin B and colistin) are increasingly used clinically as our last viable therapeutic option. Their use should be limited to exceptional conditions. More importantly these organisms have great potential to survive in hospital environment so improved antibiotic stewardship and infection-control measures will be needed to prevent or slow the emergence and spread of multidrug-resistant NFGNB in the healthcare setting.

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