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BACTERIOLOGICAL PROFILE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF NON-FERMENTING GRAM-NEGATIVE BACTERIAL BLOOD STREAM INFECTIONS

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ABSTRACT

Introduction: Non-fermenting Gram-negative bacilli (NFGNB) have emerged as a major nosocomial pathogens and many of them are intrinsically resistant to multiple antibiotics. This study was undertaken to determine the prevalence and susceptibility pattern of nonfermenters isolated from blood samples in patients with clinically suspected blood stream infections.

Methods: All 102 blood isolates of NFGNBs were identified by conventional bacteriological methods and susceptibility testing was performed as per Clinical Laboratory Standard Institute (CLSI) standards.

Results: Overall prevalence of NFGNBs isolation from blood specimen was 12.2%. *P. aeruginosa* and *Acinetobacter* species were the commonest isolates (73.5%). *P. aeruginosa* isolates were highly susceptible to imipenem (93%), meropenem (89.7%), and ceftazidime (82.8%), gentamicin (86.2%) and *Acinetobacter* spp. showed high rate of resistance to most of the tested antimicrobial agents. Intrinsically resistance to polymyxin B was seen among *Burkholderia pseudomallei (8.8%)* and *B.cepacia* (4.9%) isolates.

Conclusion: Accurate species identification and close monitoring of antimicrobial susceptibility patterns of NFGNBs is the corner stone for the proper management of the infections caused by them.

KEYWORDS : Nonfermentative gram-negative bacilli (NFGNB), prevalence, *Acinetobacter baumannii, Pseudomonas aeruginosa*, multidrug resistant

INTRODUCTION:

Non-fermenting gram negative bacilli (NFGNB) are a heterogeneous group of bacteria that do not have the ability to ferment carbohydrates; but utilize glucose oxidatively as a source of energy. 1,2 They account for 15-20% of bacterial isolates reported from most of the clinical microbiology laboratories. The predominant human pathogenic species are *Pseudomonas aeruginosa, Acinetobacter baumannii, Stenotrophomonas maltophilia* and, *Burkholderia cepacia* complex; they account for more than 75% of all clinical isolates of aerobic non-fermenters.3,4

These organisms are generally saprophytic, but they are ubiquitous in nature in the hospital environment and they may be isolated from humidifiers, ventilator machines, mattresses, and other equipment, as well as they colonize the skin of the healthcare workers. They cause opportunistic infections in seriously ill, hospitalized and immunocompromised patients, patients on mechanical ventilation and indwelling catheters, and undergoing invasive diagnostic and therapeutic procedures. They are commonly associated with urinary tract infections, ventilator associated pneumonia, surgical site infections and bacteremias.4,5

Blood stream infections caused by NFGNBs may lead to morbidity and mortality, due to difficult to identify by the conventional microbiological methods. They are often multidrug resistant, and many reports have shown an increasing trend of resistance to oxyimino-cephalosporins and carbapenems over the last two decades.4 Resistance may lead to treatment failure and in turn, leading to increased mortality, extended hospital stays and greater healthcare costs.6,7 These bacteria are of greater clinical and epidemiological relevance and their accurate diagnosis is first step towards the appropriate management of blood stream infections caused by them, due high intrinsic resistance to many commonly used anti-microbial agents.8

Considering the high morbidity and mortality rate of sepsis caused by NFGNB and difficulties in their identification as well in prescription of adequate antimicrobial agents for management, it is important to know the prevalence of these infections, bacteriological profile, and their antibiogram, so that appropriate antimicrobial agents can be used for management of these cases.

Aim of the study was to document the prevalence, the bacteriological profile and antimicrobial susceptibility pattern of non-fermenting gram-negative bacteria causing blood stream infections in patients admitted to a tertiary care hospital.

METHODS:

A descriptive retrospective study was conducted after obtaining a waiver of consent from Institute Ethics Committee and necessary permission from the hospital management. Retrospective data of all patients admitted in various wards of a tertiary care center, diagnosed with Non fermenting gram-negative bacterial sepsis by positive blood culture during the period from January 2016 to December 2017, were collected and analyzed from the hospital and laboratory records. A total of 102 patients were included in the study and the demographic details, clinical diagnosis, antimicrobial susceptibility reports were recorded from laboratory work registers. Repeat isolates from the same patients, and patients with incomplete case records were excluded from this study.

All the bacterial isolates were identified by colony morphology and standard biochemical tests by manual methods.9 The isolates, which could not be identified were further tested by VITEK 2 microbial identification system. All of the isolates were tested by the following antimicrobial agents by the standard Kirby–Bauer disc diffusion technique: amoxicillin + clavulanate (20/10 µg), piperacillin/tazobactam (100/10 µg), gentamicin (10 µg), amikacin (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), imipenem (10 µg), meropenem (10 µg), polymyxin B (300 Units). Susceptibility reports were recorded as per the Clinical Laboratory Standard Institute (CLSI) recommendation.10 the Data were entered in Microsoft excel sheet and descriptive statistics such as frequencies, percentages and standard deviation were analyzed.

RESULTS:

Out of 7,356 clinically suspected patients with blood stream infections admitted to a tertiary care hospital over a period of two years, 846 (11.5%) were blood culture positive; out of which 102 (12%) were positive for non-fermenting gram-negative bacteria. Maximum patients (35 [34.3%]) were 40 to 60 years, followed by >60 years (31[30.4]), and around 10% patients belong to the age group of less than one year. There were more number of male patients (68), with male to female ratio was 2:1. The majority of patients (55.5%), stayed in the hospital for ≤10 days, with mean length of hospital stay was 9.8 ± 5.56 days. In our study, death was recorded in six (5.7%) patients. Type

2 diabetes mellitus was the major associated risk factor (33 [32.2%]), and 28 (27.5%) were on urinary catheters.

Out of total of 102 patients, majority (44.1%) were clinically diagnosed to have sepsis and septic shock, followed by 14.7% fever for evaluation; 11.8% were admitted after RTA with subarachnoid hemorrhage and fever, 9.8% with respiratory tract diseases, 7.8% with renal diseases, 6.9% had skin and soft tissue infections, and 4.9% had decompensated liver diseases.

Out of total of 102 NFGNB isolates, polymicrobial isolation was recorded from 4 cases; Distribution of the bacteriological profile among patients with positive blood culture with NFGNB has been shown in Figure 1. Thirteen (12.7%) of other NFGNB isolates we identified by Vitek 2 microbial ID system as follows: *Ralstonia pickettii* (four), *Elizabethkingia meningoseptica* (two) and one each of *Roseomonas gilardii, Chryseobacterium gleum, Comamonas testosterone, Pandoraea sputorum, Sphingomonas paucimobilis, Achromobacter xylosoxidans* and *Pseudomonas stutzeri.*

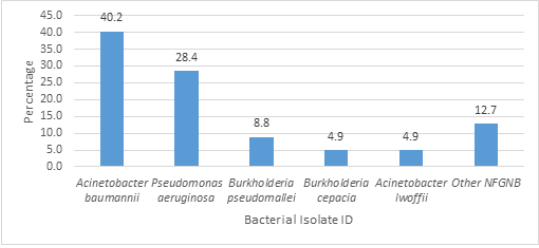

Figure 1: Distribution of bacterial profile of NFGNBs among patients with positive blood culture.

The overall antimicrobial resistance rate observed among 102 non fermenting gram negative bacterial isolates in the decreasing order was as follows: amoxicillin + clavulanate (Ac, 56.5%), amikacin (Ak, 56%), gentamicin (G, 48%), ciprofloxacin (Cf, 44%), piperacillin + tazobactam (Pt, 43%), meropenem (Me, 34%), ceftazidime (Ca, 28%), imipenem (I, 27%), and polymyxin B (Pb, 20%) as shown in figure 2. Susceptibility pattern of Pseudomonas aeruginosa (n=29) and Acinetobacter spp. (n=46) has been shown in table 1.

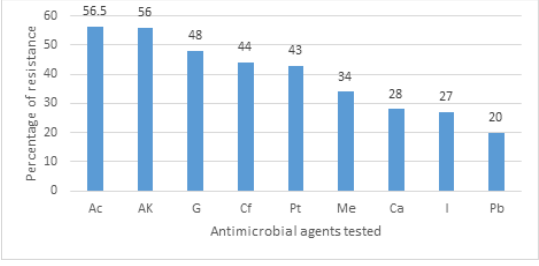

Figure 2: Antimicrobial agents tested for NFGNB isolates and their resistance pattern

Table1: Susceptibility pattern of *Pseudomonas aeruginosa* (n=29) and *Acinetobacter* spp. (n=46)

	***Acinetobacter* spp (n=46)**		***Pseudomonas aeruginosa* (n=29)**	
	No. isolated	% susceptibility	No. isolated	% susceptibility
Ceftazidime	12	26.1	24	82.8
Ciprofloxacin	15	32.6	23	79.3
Gentamicin	16	34.8	25	86.2
Amikacin	15	32.6	22	75.9
Piperacillin/ tazobactam	15	32.6	24	82.8
Imipenem	16	34.8	27	93.1
Meropenem	14	30.4	26	89.7
Polymyxinm B	46	100.0	29	100.0

DISCUSSION:

Non fermenting gram negative bacteria, which were considered as only contaminants in the past, have emerged as important human pathogens, especially in hospital settings. Their identification has become possible due to the invention of rapid newer microbial identification systems such as Vitek2 and matrix MALDI-TOF biotypers. 11 In our study, isolation rate of NFGNB from blood sample was 12%, which is in parallel to other studies by Bhargava D et al.5 and Grewal US et al. 12 We had 3 isolates (2.9%) from neonatal cases of sepsis and multidrug resistant *Acinetobacter baumannii* was isolated from all these three cases; which indicates the probability of nosocomial origin.13 NFGNBs were commonly isolated in wound infections resulting from road traffic accidents and skin and soft tissues infections including chronic ulcers.14 In our study the clinical conditions associated with NFGNB isolation were sepsis, RTA with subarachnoid hemorrhage, respiratory tract diseases, chronic kidney diseases, and skin and soft tissue infections.

P. aeruginosa and *Acinetobacter* species are the most common isolates from the clinical specimen, including blood also. In our study, *P. aeruginosa* and *Acinetobacter* species together accounted for 75/102 isolates (73.5%). *Acinetobacter* species was the commonest isolate 46 (45.1%), followed by *Pseudomonas aeruginosa*, 29 (28.4%). Similar observations have observed by in study by Oliveira MEF et al., where they showed prevalence of 55% for *Acinetobacter* spp. and 18% for *Pseudomonas* sp. 15; In contrast to these findings most of the other studies have reported *Pseudomonas* spp. as the commonest NFGNB from clinical isolates.2,14

High rate of resistance was observed for amoxicillin + clavulanate (56.5%), amikacin (56%), gentamicin (48%), ciprofloxacin (44%), piperacillin + tazobactam (43%), and high rate of susceptibility was observed for meropenem (34%), ceftazidime (28%), imipenem (27%), and polymyxin B (20%). However, the resistance to polymyxin B was seen only among *Burkholderia pseudomallei* (8.8%), *Burkholderia cepacia* complex (4.9%), *Ralstonia pickettii* (3.9%), and *Elizabethkingia meningoseptica* (1.9%) isolates; which are intrinsically resistant to polymyxin B and all other NFGNBs were uniformly susceptible to polymyxin B. Hence species identification is vital before selecting any antimicrobial agent for treatment with NFGNB infections. 16

In our study, *P. aeruginosa* isolates were highly susceptible to imipenem (93%), meropenem (89.7%), ceftazidime (82.8%), and gentamicin (86.2%). Similar observations were made by RIt K et al, and Oliveira MEF et al. 14,15; However, in contrast to these findings, in a study from Bangalore high rate of resistance was observed among *P. aeruginosa* isolates.17 However *Acinetobacter* spp. isolates, showed very high rate of resistance to most of the tested antimicrobial agents, and 17 out of 46 isolates were MDR, and sensitive only to polymixin B. Most of these patients were admitted to ICU for more than ten days and had prolonged exposure to multiple antibiotics. Similar observations were also done by Grewal U et al., and Benachinmardi K etal. 12,17 The difference in susceptibility pattern could be due to variations in type of antimicrobial agents being prescribed by the treating physicians or may be due to differences in the patient population under study. Higher rates of resistance may be due to mutant selection and resistance transfer from indiscriminate and over use of antibiotics.14

CONCLUSION:

There are variations in the susceptibility pattern of NFGNBs; hence, accurate species identification and close monitoring of their antimicrobial susceptibility patterns are corner stone for the proper management of the infection caused by them. It is also necessary to establish the clinical relevance of these isolates while reporting from clinical specimens, to avoid unnecessary over use of antibiotics and emergence of drug-resistant strains. In addition, there is urgent need of greater attention to hospital infection control practices and surveillance systems, to control infections by these multi drug resistant microorganisms.

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