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Research Article

NON-FERMENTING GRAM-NEGATIVE BACTERIA IN BLOOD CULTURE: A MENACE IN INTENSIVE CARE UNIT SETTINGS

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ABSTRACT

Objective: To know the prevalence of NFGNB isolated from blood culture specimens and their antibiotic sensitivity pattern in intensive care units.

Methods: A total of 3393 blood samples during 1 year were received from patients admitted in various ICUs. 5–7 mL blood was aseptically collected and added in BACTEC bottles and then subsequently incubated in BD BACTEC™ (FX40) fluorescent series instrument for up to 5 days. After incubation period, positive samples were processed for gram stain and subsequently sub-cultured on blood agar and MacConkey agar. These plates were incubated at 37°C for 24 h. Further identification and antimicrobial susceptibility testing of NFGNB were carried out by Vitek-2 Compact (Biomerieux India) as per the standard operating procedures.

Results: Out of 3393 samples 696 samples showed growth, out of which 96 (13.79%) were Gram-positive cocci, 36 (5.17%) were *Candida* spp., and 564 (81.03%) were Gram-negative bacilli (GNB). Among 564 GNB, 453(80.31%) were lactose fermenter and 111 (19.68%) were non-lactose fermenters. One (0.53%) isolate of *Aeromonas hydrophila* was excluded from this study. Among 110 NFGNB, *Acinetobacter baumannii* complex (41.66%) was the most predominant followed by *Pseudomonas aeruginosa* (32.72%). Amikacin was the most sensitive drug for all the NFGNB isolates followed by Piperacillin/Tazobactam. *Stenotrophomonas maltophilia* showed excellent susceptibility to minocycline (83.33%) followed by ceftazidime (66.66%). *Burkholderia cepacia* showed good susceptibility to Trimethoprim/Sulfamethoxazole.

Conclusion: Increasing antimicrobial resistance in NFGNB and their intrinsic or acquired resistance to many antibiotics makes them more lethal. It is therefore recommended to have quality guidelines on the "rational use of antibiotics" which need to be implemented strictly.

Keywords: Non-fermenting Gram-negative bacilli, Blood culture, Intensive care unit.

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INTRODUCTION

Blood stream infection (BSI) is a challenging problem as it causes prolonged patient hospitalization, increased health-care costs, and mortality rates, especially when the patients are admitted in intensive care units (ICUs) [1]. BSI represents about 15% of all nosocomial infections and affects approximately 1% of all hospitalized patients [2]. BSIs are caused by wide variety of organisms (Gram-positive or Gram-negative), but isolates can be fungal also [3]. BSIs caused by non-fermenting Gram-negative bacteria (NFGNB) are an emerging serious issue in hospital settings, especially in an immune-compromised patients or individuals with invasive devices and patients with hematological malignancies [4]. Non-fermenter Gram-negative bacteria (NFGNB) are a taxonomically diverse group of aerobic, non-sporing bacilli that either do not utilize glucose as a source of energy or utilize it oxidatively [5]. NFGNB have varied isolation rate, ranging from 2.2% to 45.9%. More than 120 species of NFGNB are classified as pathogenic. *Pseudomonas aeruginosa, Acinetobacter baumanii, Stenotrophomonas maltophilia, Sphingomonas paucimobilis, and Aeromonas hydrophila* are the most commonly isolated non-fermenting pathogenic for humans known for causing BSIs, Infections caused by other species are relatively infrequent [6,7]. BSIs caused by multidrug-resistant non-fermenting Gram-negative bacteria (NFGNB) constitute a serious problem for ICU patients throughout the world [8]. Carbapenemase producing *A. baumanii* and *P. aeruginosa* causing BSI poses a threat in treatment and thus leads to a high burden of mortality rates [9].

Therefore, this study was conducted to know about the prevalence of NFGNB isolated from blood samples and their antibiotic sensitivity pattern in ICUs.

METHODS

The present cross-sectional study (March 2022–March 2023) was conducted on blood samples received from various ICU (like medicine, surgery, obstetrics and gynecology, gastroenterology, neonatal, neurosurgery, and cardiac) clinically suspected with bacteremia/septicemia in Department of Microbiology, MMIMSR, Mullana, India. The ethical clearance was taken from the Institutional Ethical Committee.

NFGNBs isolated from blood samples were recruited in this study. Blood samples received from wards and all isolates other than NFGNB were excluded from this study.

Sample processing

Total 3393 blood samples were received from patients admitted in various ICUs. 5–7 mL blood was aseptically collected and added in BACTEC bottles and subsequently incubated in BD BACTEC™ (FX40) fluorescent series instrument for up to 5 days. After incubation period, positive samples were processed for gram staining and subsequently subcultured on blood agar and MacConkey agar. These plates were then incubated at 37°C for 24 h. Further identification and antimicrobial susceptibility testing of NFGNB was carried out by Vitek-2 Compact (Biomerieux India) as per the standard operating procedures.

Identification of bacteria and antibiotic sensitivity testing

From the isolated colonies grown on the media, a bacterial suspension was prepared in 3 ml of sterile saline (aqueous 0.45–0.50% NaCl, pH=4.5–7.0) in a 12 × 75 mm clear plastic (polystyrene) test tube. The turbidity of the suspension was adjusted to a McFarland standard of 0.5 with the help of Densichek Plus (Biomerieux India). The time gap between the preparation of inoculum and filling of the card was <30 min. Identification with the VITEK-2 compact system was performed using a Gram-negative (GN) card and Antibiotic sensitivity testing was performed using N406 card as per CLSI guidelines and manufacturer's instructions [10,11].

Quality control

The Vitek-2 Compact machine was validated using the standard strains as per the manufacturer's instructions. These include *P. aeruginosa* (ATCC 25923), *A. baumanii* (ATCC 19606), *Stentotrophomonas maltophila* (ATCC 13637), and *Sphingomonas paucimobilis* (ATCC 29837) from HiMedia, India.

RESULTS

During the study period, a total of 3393 consecutive blood samples with clinically suspected cases of septicemia from various ICUs were received. Out of which, 696 (20.51%) flagged positive by BD BACTEC™ (FX40), while the remaining 2697 (79.49%) blood samples were incubated for upto 5 days and declared sterile. Out of 3393 samples, the majority of the samples were received from Medicine ICU with a positivity rate of 32.60%, followed by surgery ICU with a positivity rate of 22.90%, (Table 1). A total of 696 strains were tested in VITEK-2 Compact system for identification and their susceptibility pattern. Out of 696 (20.51%) samples, 96 (13.79%) were Gram-positive cocci (GPC), 36 (5.17%) were Candida spp., and 564 (81.03%) were Gram-negative bacilli (GNB). Out of 564 GNB, 453(80.31%) were lactose fermenter and 111(19.68%) were non-lactose fermenter, (Table 2). GPC, Candida spp., and one isolate of *A. hydrophila* (NFGNB) were excluded from this study. Out of 110 NFGNB, *Acinetobacter baumannii* Complex (41.66%) was predominant followed by *P. aeruginosa* (32.72%), *S. maltophilia* (16.36%), *S. paucimobilis* (5.45%), and *Burkholderia cepacia* (4.54%). Overall, Amikacin was the most sensitive drug for all the NFGNB isolates followed by Piperacillin/Tazobactam. However, the isolate of *S. maltophilia* showed excellent susceptibility to minocycline (83.33%) followed by ceftazidime (66.66%), isolate of *B. cepacia* showed good susceptibility to Trimethoprim/Sulfamethoxazole (Table 3).

DISCUSSION

Non-fermenting Gram-negative bacilli (NFGNB) are among the most important causes of BSI worldwide resulting in increased morbidity and mortality in patients. High intrinsic resistance of NFGNB to antimicrobials further compounds the challenges encountered in the treatment of BSIs caused by them [12]. A total of 3393 consecutive blood samples were received from various ICUs in the department of microbiology during the study period. In the present study, blood culture positivity rate in various ICUs was 20.51%. This is in accordance with the study done by Meshram *et al*. in the Central India, in which positivity rate was estimated to be 17.18% [13]. However Wattal *et al*. in North India reported low positivity rate in ICUs as 12.7% [14]. These differences might be because of the different hospital infection control practices in different institutes. Our study revealed, out of 3393 samples, majority of the samples were received from Medicine ICU 966 (28.47%) with high positivity rate (32.60%) followed by surgery ICU 825 (24.31%) positivity rate (22.90%), obstetrics and gynecology ICU 669 (19.71%), positivity rate (11.65%) and gastroenterology ICU 498 (14.67%), and positivity rate (11.44%). Similar study done by author Kaur *et al.* in India reported that majority of samples were received from medicine ICU followed by surgery ICU with high positivity rate [9]. BSIs are potentially the most fatal and costly. Patients admitted to ICUs have an even higher risk of nosocomial BSIs than those in other type of units [15]. Out of 696 positive cultures, GNB accounted for 564 (81.03%), followed by GPC 96 (13.79%) and Candida spp. 36 (5.17%). A total of 111 NFGNB isolates were obtained out of total 564 GNB isolates, accounting for the prevalence rate of 19.68%**.** Similar results were reported by author Chang and Huang from Taiwan who recorded prevalence rate of NFGNB as 22.4% [16]. The isolation rate of NFGNB in BSI in ICUs varies from hospital to hospital. In a study conducted by Nazir *et al*. in North India illustrated that the isolation rate of NFGNB in ICUs was 75.8% [17]. In the present study, *A. baumannii* complex. was the predominant pathogen out of all non-fermenter with 45 (41.66%) isolates followed by *P. aeruginosa* 36 (32.72%) and *Stenotropho monasmaltophilia* 18 (16.36%). This correlates with other study conducted by Rattanaumpawan *et al*. from Thailand, in which major causative pathogens were *A. baumanii* (32.7%) followed by *P. aeruginosa* (27.8%) and *S. monasmaltophilia* (5.4%) [3]. However, other study conducted by Varghese *et al.* in Karnantaka, India, the most predominant pathogen in their study was *Acinetobacter* spp. followed by *Pseudomonas* spp. and *B. cepacia* [18]. Frequent isolation of NFGNB from blood samples in the study could be attributed to the increase in number of critically ill, hospitalized patients requiring invasive procedures, prolonged hospital stay, surgical site infections, diabetes, malignancies, and several underlying illnesses which made these patients more endangered to NFGNB infections [19]. In our study, *P. aeruginosa*, *A. baumanii* complex, and *S. paucimobilis* showed good susceptibility to Amikacin followed by Piperacillin/Tazobactam. These results were comparable with Juyal *et al.* in India, in which Amikacin and Piperacillin/Tazobactam were the most sensitive drug

Table 1: Sample-wise distribution and positivity rate in various ICUs

ICU	Total no. of samples received (S)	No. of positive samples by BACTEC™ (FX40)	Positivity rate (P/S) (%)
Medicine	966	315	32.60
Surgery	825	189	22.90
Obstetrics and Gynecology	669	78	11.65
Gastroenterology	498	57	11.44
Neonatal	309	30	9.70
Neurosurgery	78	18	23.07
Cardiac	48	9	18.75

ICUs: Intensive care unit

Table 2: Percentage of organisms causing BSI.

No. of culture positive	GPC (%)	GNB or cocco-bacilli (n=564)		*Candida* spp. (%)
		Lactose fermenter	Non-lactose fermenter	
696	96 (13.79%)	453 (80.31%)	111 (19.68%)	36 (5.17%)

GNB: Gram-negative bacilli, GPC: Gram-positive cocci, BSI: Blood stream infection

Table 3: Susceptibility pattern of different non-fermenting Gram-negative bacilli (n=110)

Antimicrobial agents	*Acinetobacter baumanii* (%) n=45	*Pseudomonas aeruginosa* (%) n=36	*Stenotrophomonas maltophilia* (%) n=18	*Sphingomonas paucimobilis* (%) n=6	*Burkholderia cepacia* (%) n=5
Cefepime	12 (26.66%)	9 (25.0%)	NT	3 (50%)	NT
Piperacillin/Tazobactam	27 (60.0%)	30 (83.33%)	NT	6 (100%)	NT
Ceftazidime	9 (20.0%)	9 (25.0%)	12 (66.66%)	6 (100%)	0 (0%)
Ticarcillin/Clavulanic acid	NT	NT	9 (50.0%)	6 (100%)	3 (100%)
Ciprofloxacin	12 (26.66%)	12 (33.33%)	NT	6 (100%)	NT
Levofloxacin	21 (46.66%)	15 (41.66%)	9 (50.0%)	3 (50%)	3 (100%)
Amikacin	33 (73.33%)	33 (91.66%)	NT	3 (50%)	NT
Gentamicin	12 ((26.66%)	21 (58.33%)	NT	3 (50%)	NT
Imipenem	24 (53.33%)	27 (75.0%)	NT	NT	NT
Meropenem	24 (53.33%)	27 (75.0%)	NT	NT	3 (100%)
Minocycline	15 (33.33%)	NT	15 (83.33%)	6 (100%)	3 (100%)
Trimethoprim/Sulfamethoxazole	15 (33.33%)	NT	9 (50.0%)	6 (100%)	3 (100%)
Chloramphenicol	NT	NT	NT	NT	3 (100%)
Azteronam	NT	NT	NT	6 (100%)	NT

against *P. aeruginosa* and *A. baumanii* [20]. In another study conducted by Simgamsetty *et al.* in India, Imipenem and Meropenem were the most sensitive drug against NFGNB [12]. In our study, isolates of NFGNB showed least susceptibility to Ciprofloxaxin and Ceftazidime. Similar results were also reported by author Nazir *et al*. North India, in which Ciprofloxacin and ceftazidime showed least susceptibility against NFGNB [17]. However, *S. monasmaltophilia* showed excellent susceptibility to minocycline (83.33%) followed by ceftazidime (66.66%). Kim *et al.* reported that patients under a definitive regimen with quinolone it was (32%) and with trimethoprim it was (24.8%) [21]. Among NFGNB, *S. onasmaltophilia* has been reported to be the third most commonly isolated pathogen after *Acinetobacter* spp. and *P. aeruginosa. S. onasmaltophilia* bacteraemia is associated with high mortality rates. The previous studies have reported that risk factors associated with mortality for *S. onasmaltophilia* are intrinsic resistant to many antibiotics such as beta-lactams and aminoglycosides, and these antibiotics are widely used in the treatment of nosocomial bacteremia. Very few antibiotics such as trimethoprim-sulfamethoxazole, levofloxacin, and minocycline were effective on *S. onasmaltophilia* infections, and the national committee for clinical laboratory standards recommended antimicrobial susceptibility for *S. Onasmaltophilia,* that is, minocycline, levofloxacin, trimethoprim-sulfamethoxazole, and ceftazidime [11,22,23]. Isolate of *B. cepacia* showed good susceptibility to Trimethoprim/Sulfamethoxazole. Comparable to the finding of Kausar *et al.* India, in which Trimethoprim/Sulfamethoxazole was the most sensitive drug against *B. cepacia* [24]. Emergence of drug resistance in NFGNB is the major issue around the world threatening the life of patients. The pattern of antibiotic resistance varies from place to place, time to time, and depends on factors like self-medication and empirical use of antibiotics. It also depends on different hospital infection control practices in different institutions around the world.

CONCLUSION

Rate of isolation of NFGNB causing BSI was 19.68% and most commonly isolated bacteria was *A. baumannii* complex followed by *P. aeruginosa.* Most of the isolates were sensitive to amikacin followed Piperacillin-tazobactam. *S. maltophilia* showed excellent susceptibility to Minocycline. Trimethoprim-Sulfamethoxazole was the most sensitive drug against *B. cepacia.* The effectiveness of various antimicrobials is in jeopardy because the treatment that once worked no longer do so because of the increasing anti-microbial resistance among them. The present findings therefore reveal the importance of rationale use of antibiotics which is the need of an hour superadded by appropriate infection control approach.

Limitations

In the present study, evaluation of risk factors of outcomes of NFGNB infections in hospitalized patients was not done due to unavailability of sufficient data. Furthermore, molecular analysis and genetic mechanisms of drug resistance were not determined.

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CONFLICTS OF INTERESTS

All authors declare no conflicts of interest.

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