Achieving pre-eminence of antimicrobial resistance among non-fermenting Gram-negative bacilli causing septicemia in intensive care units: A single center study of a tertiary care hospital

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Abstract

Introduction **Bloodstream infections in the intensive care unit have always been a global healthcare challenge. The present study was conducted with the aim to evaluate the yearly trend of antibiotic resistance in non-fermenting Gram-negative bacilli (NFGNB) causing septicemia in intensive care units.**

Methods **Blood samples were collected from the patients admitted in various intensive care units and processed for isolation and identification of non-fermenting Gram-negative bacilli. The isolated bacterial strains were subjected to antibiotic susceptibility testing as per standard operating procedures.**

Results **Out of 3632 blood samples, 977 (26.9%) samples showed microbial growth, of which 10.1% were Gram positive cocci, 8.7% were Gram negative bacilli (Enterobacterales), 7% were NFGNB and 1% were *Candida* spp. Increasing resistance among *Acinetobacter baumannii* complex was observed to ceftazidime, cefepime, amikacin, ciprofloxacin, levofloxacin, meropenem and trimethoprim-sulfamethoxazole. Moreover, *Pseudomonas aeruginosa* strains were found to be associated with increased resistance to ciprofloxacin, levofloxacin, ceftazidime and meropenem. A substantial increase in resistance levels was observed among *Stenotrophomonas maltophilia* and *Sphingomonas paucimobilis* as well.**

Conclusions **An increasing trend of antimicrobial resistance in NFGNB envisages the worst consequences in ICUs in the coming years. Therefore, reviewing and strict implementation of the antimicrobial policies including 'rational use of antibiotics' is recommended.**

Keywords ***Acinetobacter baumannii, Pseudomonas aeruginosa, Stenotrophomonas maltophilia, Sphingomonas paucimobilis.***

Introduction

Non-fermenting Gram-negative bacilli (NFGNB) are taxonomically varied bacterial strains that either do not use carbohydrate as a source of energy or use it oxidatively.[1] NFGNB, in recent years, have emerged as significant pathogens for various infectious diseases. Moreover, a high fatality rate (35-50%) has been documented with NFGNB causing bloodstream infection (NFGNB-BSI) in intensive care units (ICUs).[2] The management of NFGNB in ICUs is always fraught with challenges owing to their intrinsic resistance potential.[3-5]

NFGNB are not only intrinsically resistant to several antibiotics but also have been shown to produce β-lactamases, enzymes which render bacteria resistant to various antibiotics.[6-7] The scientific literature already has multiple evidences of NFGNB isolates from various clinical samples

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which were found to be resistant to almost all routine antibiotics.[8-10]

The emergence of antibiotic resistance in NFGNB is primarily associated with horizontal gene transfer. However, there have been several contributing factors for spreading of multidrug resistant organisms (MDRO) especially in resource-limited countries. These factors include self-prescription, over-the-counter sales of antibiotics, lack of knowledge about the antibiotic resistance, failing in developing and implementing antibiotic policy etc.[11-13]

The present study was conducted to evaluate the yearly trend of antibiotic resistance in NFGNB causing septicemia in intensive care units.

Methods

A prospective study was carried out in the Department of Microbiology, Maharishi Markandeshwar Institute of Medical Sciences and Research, Mullana, between January 2020 to March 2022. The institute is attached with a 1140 bedded tertiary care hospital and is located at a distance of 218 kilometers from New Delhi. This hospital plays a significant role in the local public health system and provides medical facilities to a population of 7.7 million approximately. A study protocol was prepared and approval was taken from the Institutional Ethical Committee (IEC). Informed consent was taken from the participants (in case of critically ill patient, consent was taken from the guardian).

Determination of sample size

The sample size was calculated by using a single population proportion formula. The initial sample size was estimated to be 254 with 20.8% prevalence (chosen from a previous study), 95% confidence interval (z=1.96) and 5% margin of error (d=0.05).[14]

Inclusion and exclusion criteria

All consecutive blood samples received during the study period from various ICUs (Medicine, Surgery, Neurosurgery, Pediatric, Obstetrics & Gynecology and Burn) were included in this study while blood cultures received from different general and emergency wards were excluded from the study. Moreover, blood samples with NFGNB only were further processed and evaluated. No blood sample showed the growth of multiple NFGNB.

Sample collection and processing

A pair of two aerobic blood culture bottles were inoculated with 10 mL of blood from two different peripheral veins aseptically within an interval of 30-60 minutes and were incubated in automated blood culture system (BD BACTEC FX40, Becton Dickinson, USA). The inoculated blood culture bottles were received in the Microbiology lab and incubated for 5 days. The flagged positive blood culture bottles were further processed by doing subculture on Blood agar and MacConkey agar (HiMedia, India) and pure bacterial isolates were subjected to species identification and antimicrobial susceptibility testing.

Only those blood cultures in which both bottles were flagged positive were considered to be positive. The blood culture bottles in which a single bottle was flagged positive were considered contamination; blood cultures not flagged positive up to 5 days were considered negative.

Identification and antimicrobial susceptibility testing (AST)

The bacterial isolates were identified and simultaneously processed for antimicrobial susceptibility testing (AST) by VITEK-2 Compact (BioMérieux, India) using Gram Negative Identification (GN-ID) card and N406 AST card respectively. All identified NFGNB (*Acinetobacter baumannii* complex, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Sphingomonas paucimobilis)* were also processed manually for antimicrobial susceptibility testing as per CLSI and/or EUCAST guidelines.[7,15] All isolates of *Sphingomonas paucimobilis* were tested for AST by VITEK-2 Compact only.

The following antibiotics were tested in the present study: cephalosporins [3rd generation – ceftazidime (10 µg) (except *S. maltophilia*), 4th generation – cefepime (30 µg)], β-lactam combination agent [piperacillin-tazobactam

(100/10 µg), ticarcillin-clavulanic acid], monobactam [aztreonam], fluoroquinolones [ciprofloxacin (5 µg), levofloxacin (5 µg)], tetracycline [(minocycline (30 µg)], folate pathway antagonist [trimethoprim-sulfamethoxazole (1.25/23.75 µg)], aminoglycosides [(gentamicin (10 µg), amikacin (30 µg)], carbapenems [imipenem (10 µg), meropenem (10 µg)].

Quality control

Pseudomonas aeruginosa (ATCC 25923), *Acinetobacter baumannii* (ATCC 19606), *Stenotrophomonas maltophilia* (ATCC 13637) and *Sphingomonas paucimobilis* (ATCC 29837) from HiMedia, India, were utilized as reference strains for quality control in culture and susceptibility assays.

Detection of co-resistance and data analysis

Antimicrobials from the same class with identical resistance profiles in isolates of a given species were merged to investigate the co-resistance, and entries with missing data were excluded. The data was entered and processed in Microsoft Excel to identify co-resistance pattern and Chi-square test was used to calculate p-value.

Results

A total of 3632 blood samples from various ICUs were collected and processed from January 2020 to March 2022 (Figure 1). The majority of the samples were received from Medicine ICU and Surgery ICU followed by Neurosurgery and Pediatric ICUs. The combined blood culture positivity rate of all ICUs was 26.5%. The blood culture positivity rate was found to be 26.4% in 2020, 25.7% in 2021 and 29.9% in 2022 (between January to March 2022) – Table 1.

Due to fewer numbers of isolates, 6 strains of *Burkholderia cepacia* in 2020 and 1 strain of *Kocuria rosea* in 2021 were not included in this study. Therefore, a total of 249 non-Enterobacterales were processed for antimicrobial susceptibility testing – Table 2.

Acinetobacter baumannii complex showed increasing trend of resistance to ceftazidime (61.7% to 83.3%), cefepime (55.3% to 91.6%), amikacin (59.5% to 75%), ciprofloxacin (51.0% to 83.3%), levofloxacin (65.9% to 83.3%), meropenem (55.3% to 75%), and trimethoprim-sulfamethoxazole (53.1% to 75%) – Table 3.

Pseudomonas aeruginosa showed yearly increasing resistance to ciprofloxacin (30.3% to 50%), levofloxacin (30.3% to 50%), ceftazidime (33.3% to 50%) and meropenem (36.3% to 41.6%) – Table 3.

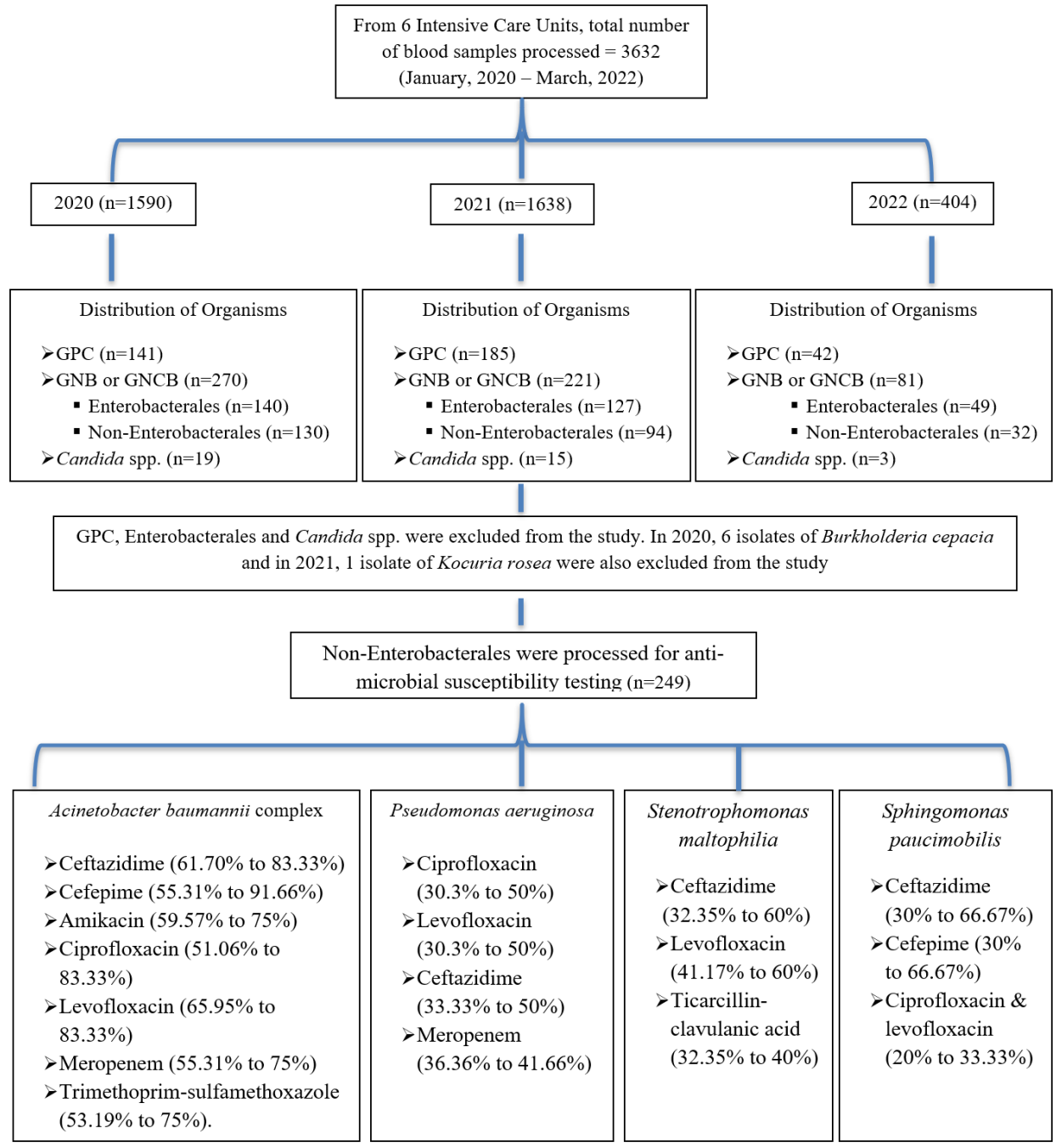
Stenotrophomonas maltophilia strains also were found to be associated with a substantial increase in antibiotic resistance to ceftazidime (32.3% to 60%), levofloxacin (41.1% to 60%) and ticarcillin-clavulanic acid (32.3% to 40%) – Table 3.

Sphingomonas paucimobilis showed rise in resistance to ceftazidime (30% to 66.6%), cefepime (30% to 66.6%), ciprofloxacin and levofloxacin (both 20% to 33.3%) – Table 3.

All NFGNB were observed for co-resistance against several combinations using cephalosporins, piperacillin-tazobactam, aminoglycosides, minocycline, fluroquinolones, trimethoprim-sulfamethoxazole and carbapenem – Table 4.

Discussion

Septicemia has always been considered a life-threatening ailment. In addition, the patients admitted to the ICUs are already immunocompromised and prone to several infections. In our study, a total of 3632 blood samples from various ICUs were received from January 2020 to March 2022, showing 26.8% positivity rate. The similar studies conducted in India by Chaturvedi P et al. (2021) and Grewal US et al. (2017) had reported positivity rates of 22.7% and 20.2%, respectively.[14-16] However, another study conducted in India by Kaur N et al. (2021) exhibited a comparatively low positive rate (17.1%).[17] Moreover, the studies conducted by Birru M et al. (2021) in Ethiopia and Orsini J et al. (2012) in the USA reported a low positivity rate of 12.6% and 9.8% respectively.[18,19] This data can be attributed to the fact that the limited resources and challenges of implementing policies contribute significantly in spreading infectious diseases. The study by Birru M et al. (2021) was also conducted in a low-income



GPC – Gram-positive cocci; **GNB** – Gram-negative bacilli; **GNCB** – Gram-negative coccobacilli.

Figure 1. Study workflow

Table 1. Sample wise distribution and positivity rate in various ICUs

	Intensive care unit																	
	Medicine			Surgery			Neurosurgery			Pediatric			Obstetrics and gynecology			Burn		
	2020	2021	2022	2020	2021	2022	2020	2021	2022	2020	2021	2022	2020	2021	2022	2020	2021	2022
Total no. of samples received (P)	835	875	137	283	313	84	121	150	59	175	161	70	151	131	51	25	8	3
No. of sample flagged positive by BACTEC (S)	262*	243	65@	72	84#	21	38	32	17	31	32#	9	13	28	8	4	2	1
Positivity rate (P/S) %	31.4	27.7	47.4	25.4	26.8	25	31.4	21.3	28.8	17.7	19.8	12.8	8.6	21.3	15.7	16	25	33.3

The following symbols in superscript show false positive flagging by BACTEC, which were not included in study:
*= 3 samples from Medicine ICU.
#= 2 samples from Surgery ICU and 3 samples from Pediatric ICU.
@= 1 Sample from Medicine ICU.

Table 2. Distribution of organisms causing blood stream infection (BSI)

Year	Total no. of blood samples processed (N=3632)	GPC* (n_1=368)	GNB or GNCB# (n_2=572)		*Candida* spp. (n_3=37)
			Enterobacterales ($n_{2.1}$=316)	Non-Enterobacterales ($n_{2.2}$=256)	
2020	1590	141 (8.8)	140 (8.8)	130 (8.1)	19 (0.5)
2021	1638	185 (11.3)	127 (7.7)	94 (5.7)	15 (0.9)
2022	404	42 (10.4)	49 (12.1)	32 (7.9)	3 (0.7)
Total positivity		10.1 %	8.7 %	7.0%	1 %
			15.7%		

GPC* – Gram positive cocci
GNB or GNCB# – Gram negative bacilli or Gram negative coccobacilli

Table 3. Year wise antibiotic resistance pattern of non-Enterobacterales isolated from blood in ICU (n_f=249)

	Acinetobacter baumannii complex			*Pseudomonas aeruginosa*			*Stenotrophomonas maltophilia*			*Sphingomonas paucimobilis*		
	2020 (n=47)	2021 (n=28)	2022 (n=12)	2020 (n=33)	2021 (n=43)	2022 (n=12)	2020 (n=34)	2021 (n=15)	2022 (n=5)	2020 (n=10)	2021 (n= 7)	2022 (n=3)
Piperacillin-tazobactam	35 (74.4)	22 (78.5)	9 (75)	12 (36.3)	29 (67.4)	6 (50)	NT	NT	NT	2 (20)	3 (42.8)	1 (33.3)
Ceftazidime	29 (61.7)	24 (85.7)	10 (83.3)	11 (33.3)	20 (46.5)	6 (50)	11 (32.3)	5 (33.3)	3 (60)	3 (30)	3 (42.8)	2 (66.6)
Cefepime	26(55.3)	24 (85.7)	11 (91.6)	12 (36.3)	17 (39.5)	4 (33.3)	NT	NT	NT	3 (30)	3 (42.8)	2 (66.6)
Imipenem	33 (70.2)	22 (78.5)	8 (66.6)	11 (33.3)	20 (46.5)	5 (41.6)	NT	NT	NT	NT	NT	NT
Meropenem	26 (55.3)	23 (82.1)	9 (75)	12 (36.3)	18 (41.8)	5 (41.6)	NT	NT	NT	NT	NT	NT
Gentamicin	35 (74.4)	21 (75)	9 (75)	14 (42.4)	19 (44.1)	5 (41.6)	NT	NT	NT	2 (20)	1 (14.2)	0 (0)
Amikacin	28 (59.5)	19 (67.8)	9 (75)	10 (30.3)	20 (46.5)	4 (33.3)	NT	NT	NT	2 (20)	1 (14.2)	0 (0)
Minocycline	16 (34)	9 (32.1)	5 (41.6)	NT	NT	NT	7 (20.5)	3(20)	1 (20)	0 (0)	0 (0)	0 (0)
Ciprofloxacin	24 (51)	22 (78.5)	10 (83.3)	10 (30.3)	16 (37.2)	6 (50)	NT	NT	NT	2 (20)	2 (28.5)	1 (33.3)
Levofloxacin	31 (65.9)	22 (78.5)	10 (83.3)	10 (30.3)	17 (39.5)	6 (50)	14 (41.1)	8 (53.3)	3 (60)	2 (20)	2 (28.5)	1 (33.3)
Trimethoprim-sulfamethoxazole	25 (53.2)	21 (75)	9 (75)	NT	NT	NT	8 (23.5)	7 (46.6)	1 (20)	1 (10)	0 (0)	0 (0)
Aztreonam	NT	NT	NT	NT	NT	NT	NT	NT	NT	5 (50)	3 (42.8)	1 (33.3)
Ticarcillin-clavulanic acid	NT	NT	NT	NT	NT	NT	11 (32.3)	4 (26.6)	2 (40)	2 (20)	3 (42.8)	1(33.3)

NT – not tested.

Table 4. Co-resistance in NFGNB isolated from blood (2020-2022)

Organism	Antimicrobial group/antibiotic 1	Number of isolates resistant to antimicrobial group 1 & 2/ Number of isolates resistant to antimicrobial group 1 (%)	Number of isolates resistant to antimicrobial group 1 & 2/ Number of isolates resistant to antimicrobial group 2 (%)	p-value (p<0.05)	Odds ratio (95% CI)	Antimicrobial group/antibiotic 2
Acinetobacter baumannii complex	Cephalosporins	51/61 (83.6)	51/66 (77.2)	0.767	0.9 (0.5-1.5)	Piperacillin-tazobactam
		49/61 (80.3)	49/60 (81.6)	0.951	1 (0.6-1.7)	Aminoglycosides
		21/61 (34.4)	21/30 (70)	0.062	2 (0.9-4.3)	Minocycline
		46/61 (75.4)	46/60 (76.6)	0.952	1 (0.6-1.7)	Fluoroquinolones
		42/61 (68.8)	42/55 (76.3)	0.718	1.1 (0.6-1.9)	Trimethoprim-sulfamethoxazole
		49/61 (80.3)	49/61 (80.3)	1	1 (0.5-1.7)	Carbapenems
	Piperacillin-tazobactam	48/66 (72.7)	48/60 (80)	0.725	1.1 (0.6-1.8)	Aminoglycosides
		23/66 (34.8)	23/30 (76.6)	0.032	0.4 (0.2- 0.9)	Minocycline
		45/66 (68.1)	45/60 (75)	0.730	1.1 (0.6-1.9)	Fluoroquinolones
		43/66 (65.1)	43/55 (78.1)	0.518	1.2 (0.6-2.1)	Trimethoprim-sulfamethoxazole
		51/66 (77.2)	51/61 (83.60)	0.767	1.1 (0.6-1.8)	Carbapenems
	Aminoglycosides	21/60 (35)	21/30 (70)	0.068	2 (0.9-4.2)	Minocycline
		45/60 (75)	45/60 (75)	1	1 (0.5-1.7)	Fluoroquinolones
		42/60 (70)	42/60 (70)	1	1 (0.5- 1.7)	Trimethoprim-sulfamethoxazole
		48/60 (80)	48/61 (78.6)	0.951	0.9 (0.5 – 1.6)	Carbapenems
	Minocycline	29/30 (96.6)	29/60 (48.3)	0.044	2 (1-3.9)	Fluoroquinolones
		18/30 (60)	18/60 (30)	0.084	0.5 (0.2 – 1.1)	Trimethoprim-sulfamethoxazole
		22/30 (73.3)	22/61 (36)	0.058	0.5 (0.2 – 1)	Carbapenems
	Fluoroquinolones	41/60 (68.3)	41/60 (68.3)	1	1 (0.5 – 1.7)	Trimethoprim-sulfamethoxazole
		45/60 (75)	45/61 (73.7)	0.952	0.9 (0.5 – 1.7)	Carbapenems
	Trimethoprim-sulfamethoxazole	43/60 (71.6)	43/61 (70.5)	0.953	0.9 (0.5 – 1.7)	Carbapenems
Pseudomonas aeruginosa	Cephalosporins	27/35 (77.1)	27/47 (57.4)	0.402	0.9 (0.5 – 1.8)	Piperacillin-tazobactam
		21/35 (60)	21/36 (58.3)	0.942	0.9 (0.4 – 2.1)	Aminoglycosides
		17/35 (48.5)	17/33 (51.5)	0.888	1.1 (0.4-2.4)	Fluoroquinolones
		17/35 (48.5)	17/36 (47.2)	0.946	0.9 (0.4 – 2.2)	Carbapenems

Organism	Antimicrobial group/antibiotic 1	Number of isolates resistant to antimicrobial group 1 & 2/ Number of isolates resistant to antimicrobial group 1 (%)	Number of isolates resistant to antimicrobial group 1 & 2/ Number of isolates resistant to antimicrobial group 2 (%)	p-value (p<0.05)	Odds ratio (95% CI)	Antimicrobial group/antibiotic 2
	Piperacillin-tazobactam	18/47 (38.3)	18/33 (54.5)	0.380	1.4 (0.6 – 3.1)	Fluoroquinolones
		23/47 (48.9)	23/36 (63.8)	0.469	1.3 (0.6 – 2.6)	Aminoglycosides
		31/47 (65.9)	31/36 (86.1)	0.428	1.3 (0.6 – 2.5)	Carbapenems
	Aminoglycosides	23/36 (63.8)	23/33 (69.7)	0.819	1.1 (0.5 – 2.3)	Fluoroquinolones
		22/36 (61.1)	22/36 (61.1)	1	1 (0.4 – 2.1)	Carbapenems
	Fluoroquinolones	17/33 (51.5)	17/36 (47.2)	0.835	0.9 (0.4 – 2)	Carbapenems
Stenotrophomonas maltophilia	Ceftazidime	13/19 (68.4)	13/17 (76.4)	0.829	1.1(0.4 – 3)	Ticarcillin-clavulanic acid
		12/19 (63.1)	12/16 (75)	0.746	1.1 (0.4 – 3.3)	Trimethoprim-sulfamethoxazole
		5/19 (26.3)	5/11 (45.4)	0.458	1.72 (0.4 – 7.3)	Minocycline
		16/19 (84.2)	16/25 (64)	0.556	0.7 (0.3 – 1.9)	Levofloxacin
	Ticarcillin-clavulanic acid	13/17 (76.4)	13/16 (81.2)	0.908	1.1 (0.3 – 2.9)	Trimethoprim-sulfamethoxazole
		6/17 (35.3)	6/11 (54.5)	0.521	1.5 (0.4 – 6)	Minocycline
		12/17 (70.5)	12/25 (48)	0.454	0.6 (0.2 – 1.8)	Levofloxacin
	Trimethoprim-sulfamethoxazole	7/16 (43.7)	7/11 (63.6)	0.571	1.4 (0.4 – 5.3)	Minocycline
		11/16 (68.7)	11/25 (44)	0.402	0.6 (0.2 – 1.8)	Levofloxacin
	Minocycline	11/11 (100)	11/25 (44)	0.142	0.4 (0.1 – 1.3)	Levofloxacin
Sphingomonas paucimobilis	β-lactam combination agents	2/6 (33.3)	2/8 (25)	0.800	0.7 (0.08 – 6.9)	Cephalosporins
		3/6 (50)	3/3 (100)	0.521	2 (0.2 – 16.6)	Aminoglycosides
		4/6 (66.6)	4/5 (80)	0.844	1.2 (0.2 – 7.4)	Fluoroquinolones
		3/6 (50)	3/9 (33.3)	0.676	0.6 (0.09 – 4.4)	Aztreonam
	Cephalosporins	1/8 (12.5)	1/8 (12.5)	1	1 (0.05 – 18.9)	Aminoglycosides
		4/8 (50)	4/5 (80)	0.604	1.6 (0.2 – 9.4)	Fluoroquinolones
		7/8 (87.5)	7/9 (77.7)	0.870	0.8 (0.2 – 3.6)	Aztreonam
	Aminoglycosides	5/8 (62.5)	5/5 (100)	0.581	1.6 (0.3 – 8.4)	Fluoroquinolones
		7/8 (87.5)	7/9 (77.7)	0.870	0.8 (0.2 – 3.6)	Aztreonam
	Fluoroquinolones	5/5 (100)	5/9 (55.5)	0.485	0.5 (0.1 – 2.9)	Aztreonam

The p-value for difference was calculated using the Chi-square test; p-value is significant at p<0.05.

country, but the low positivity rate might be due to the short study period.[18]

In the current study, the majority of samples were received from Medicine ICU (50.8%) followed by Surgery ICU (18.7%), Pediatric ICU (11.1%), Obstetrics & Gynecology ICU (9.1%) and Neurosurgery ICU (9.0%). The yearly trend of positivity rate was found to increase in Surgery ICU (25.4% to 26.8%), Pediatric ICU (17.7% to 19.8%) and Burn ICU (16% to 25% and 25% to 33.3%). A study conducted by Kaur N et al. (2021) in India reported that the majority of samples were received from Medicine ICU (58.7%) followed by Surgery ICU (23.5%) and Obstetrics & Gynecology ICU (12.3%).[17]

In 2020, 8.8% of Gram-positive cocci (GPC), 16.9% of GNB and 0.5% of *Candida* spp. were isolated from blood samples in the present study. Out of 16.9% of GNB, 8.8% were Enterobacterales and 8.1% were non-Enterobacterales. Further, 6 isolates of *Burkholderia cepacia* isolated in 2020 were excluded from this study. A study conducted by Gunasekaran SD et al. (2020) in United Arab Emirates showed 39.4% isolates as GPC, 36.8% as GNB and 15.7% as *Candida* spp.[11] Another study conducted by Bandy A et al. (2020) showed 62.2% GNB followed by 36.4% GPC.[20] The difference in results in the present study is associated with the fact the we calculated the number of isolates from all received samples in the respective year, whereas the abovementioned studies calculated the result using positive samples only.

In 2021, 11.3% were GPC, 13.4% were GNB and 0.9% were *Candida* spp. from all blood samples received. In non-Enterobacterales, one isolate of *Kocuria rosea* was not included in the current study. This rare bacterial isolate has also been reported earlier in India by Bala R et al. (2021).[21] In a study conducted by Birru M et al. (2021) in Ethiopia, 59.1% of isolates were GPC and 41.1% were GNB.[18] Another study by Chaturvedi P et al. (2021) in India showed 45.2% as GNB, 43.9% as GPC and 10.9% as *Candida* spp.[16] A study by Harte J et al. (2021) showed 30.7% as GPC and 69.2% as GNB.[22]

In 2022, 10.4% were GPC, 20% were GNB and 0.7% were *Candida* spp. from all blood samples received. In a study conducted by Oladele R et al. (2022) in Nigeria, 21.2% of isolates were *Candida* spp., 18.9% were *Klebsiella pneumoniae* and 15.6 % were *Acinetobacter baumannii*.[23] Another study conducted by Costescu Strachinaru DI et al. (2022) in Belgium showed 56.3% as GNB and 38.1% as GPC.[24]

The Gram-positive cocci, Enterobacterales and *Candida* spp. have always been the predominant organisms causing BSI but in the current study, only non-Enterobacterales (*Acinetobacter baumannii* complex, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia and Sphingomonas paucimobilis*) were included.

Clinical isolates of *Acinetobacter baumannii* were susceptible to antibiotics like ampicillin, gentamicin, chloramphenicol, and nalidixic acid in the 1970s. It became an important nosocomial pathogen in the late 1970s, owing to the widespread use of broad-spectrum antibiotics in hospitals.[25,26] In the current study, *Acinetobacter baumannii* complex showed the highest resistance to 3rd and 4th generation cephalosporins, β-lactam combination agent, fluoroquinolones and aminoglycosides. An increasing trend of antimicrobial resistance has also been noted in ceftazidime (2020-61.7%, 2021-85.7% and 2022-83.3%) and cefepime (2020-55.3%, 2021-85.7% and 2022-91.6%). Also, a yearly increasing resistance trend was seen in ciprofloxacin (2020-51%, 2021-78.5% and 2022-83.3%) and levofloxacin (2020-65.9%, 2021-78.5% and 2022-83.3%). The most effective antibiotic against *Acinetobacter baumannii* complex was found to be minocycline. The increasing AMR can be attributed to indiscriminate use of higher end drugs like carbapenems and aminoglycosides. Several studies conducted by Tjoa E et al. (2013) in Indonesia, Chaturvedi P et al. (2021) and Grewal US et al. (2017) in India reported higher resistance to 3rd and 4th generation cephalosporins, β-lactam combination agent, fluoroquinolones and aminoglycosides.[14,16,27] Another study conducted by Manyahi J et al. (2020) in Tanzania reported higher resistance to nearly all antibiotics.[28] A study conducted by

Bandy A et al. (2020) in Saudi Arabia reported *Acinetobacter baumannii* isolates as significantly resistant to gentamicin, 1st to 4th-generation cephalosporins, and carbapenems.[20] In a study conducted by Di Franco S et al. (2021), the scientific literature was searched comprehensively using several databases including PubMed, Embase, and Cochrane Library for bloodstream infections by multidrug resistant bacteria, which revealed very high resistance in *Acinetobacter baumannii* against 3rd and 4th generation cephalosporins, fluoroquinolones and carbapenems. However, minocycline was reported as the most sensitive antibiotic against *Acinetobacter baumannii* complex.[29]

Pseudomonas aeruginosa carries multiple virulence factors along with a genetic information for intrinsic as well as acquired resistance against a number of antibiotics.[8,30] In the current study, *Pseudomonas aeruginosa* strains were found to be less resistant as compared to *Acinetobacter baumannii* complex. The highest resistance was reported against 3rd generation cephalosporin and fluoroquinolones. A yearly increase in resistance was also reported in ceftazidime (2020-33.3%, 2021-46.5% and 2021-50%), fluoroquinolones [2020-30.3%, 2021-37.2-39.5% and 2022-50%], imipenem (2020-33.3%, 2021-46.5%) and meropenem (2020-36.3%, 2021-41.8% and 2022-41.6%). The most effective antibiotics were found to be cefepime and amikacin. In a study conducted by Manyahi J et al. (2020) in Tanzania, *Pseudomonas aeruginosa* was reported to be 70% resistant to ceftazidime, 46% resistant to meropenem and 64% resistant to ciprofloxacin.[28] Other studies by Kaur N et al. (2021) in India and Motbainor H et al. (2020) in Ethiopia also reported higher resistance to ceftazidime, meropenem and ciprofloxacin.[17,31] In the SENTRY Antimicrobial Surveillance Program, Pfaller MA et al. (2020) reported that 15.4% strains of *Pseudomonas aeruginosa* were multidrug resistant.[32] Another study conducted by Kim D. et al (2022) in South Korea showed that 12.3% isolates of *Pseudomonas aeruginosa* were resistant to piperacillin-tazobactam, approximately 13% of isolates were resistant to anti-pseudomonal drugs, and more than 20% of strains were resistant to carbapenems.[33]

Stenotrophomonas maltophilia poses a challenge to medical science to treat patients with serious infections appropriately because of the presence of its two intrinsic resistance mechanisms; inducible β-lactamase enzymes (L1) from Ambler class 'B' and inducible β-lactamase enzymes (L2) from Ambler class 'A' which render the bacteria resistant to nearly all β-lactam agents.[34,35] In our study, a yearly increase in antibiotic resistance in *Stenotrophomonas maltophilia* was seen in ceftazidime (2020-32.3%, 2021-33.3% and 2022-60%) and levofloxacin (2020-41.1%, 2021-53.3% and 2022-60%). However, minocycline was found to be the most effective antibiotic against *Stenotrophomonas maltophilia*. A retrospective database study conducted by Cai B et al. (2020) in the USA reported *Stenotrophomonas maltophilia* as 94.9% susceptible to trimethoprim-sulfamethoxazole, 83% susceptible to fluoroquinolones and 91.8% susceptible to minocycline.[36]

Sphingomonas spp. are found in the environment and are known to cause mostly nosocomial illnesses.[37] A study conducted by Toh SH et al. (2011) in Taiwan reported that 52.7% isolates of *Sphingomonas* spp. were community-acquired.[38] *Sphingomonas* spp. has a diverse range of resistance phenotypes, which contribute to resistance to a variety of antimicrobials. Therefore, every patient requires individualized and appropriate antimicrobial therapy.[39,40] In the current study, *Sphingomonas paucimobilis* isolates were found to be highly resistant to 3rd and 4th generation cephalosporins. However, there was no resistance found against gentamicin, amikacin, minocycline and trimethoprim-sulfamethoxazole. A study conducted by Bayram N et al. (2013) in Turkey reported higher resistance in *Sphingomonas paucimobilis* against 3rd generation cephalosporins.[41]

Multidrug resistance has now emerged as a major public health problem around the globe.[42] In the current study, almost all NFGNB strains showed resistance to the multiple combinations of antibiotics.

Significance of this study

This study emphasizes the yearly trend of antimicrobial resistance along with co-resistance of non-fermenting Gram-negative bacilli (*Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Sphingomonas paucimobilis*) causing septicemia in intensive care units.

Conclusions

The present study revealed the prevalence of bloodstream infection caused by NFGNB in ICU. Minocycline was found to be the most effective antibiotic against NFGNB, except for *Pseudomonas aeruginosa* which was highly sensitive to amikacin and cefepime, but the increasing trend of antibiotic resistance among all NFGNB is a matter of concern. The present study also revealed the resistance pattern of NFGNB to different combinations of antibiotics. The co-resistance was not found to be statistically significant to any combination of antibiotics, but the diverse resistance pattern to different combinations of antibiotics envisages the possibility of choosing the correct therapeutic antibiotic combination.

The limitation of this study starts with the inclusion of only NFGNB isolated from bloodstream infections in ICU while major pathogens like Gram-positive cocci (*Staphylococcus* spp., *Streptococcus pneumoniae* and *Enterococcus* spp.), Enterobacterales and *Candida* spp. were not included in this study. However, the present data significantly makes an addition to the limited literature available on NFGNB causing bloodstream infections in the ICU.

Further, the study did not reveal the resistance mechanisms among NFGNB at molecular level, which would have been a comprehensive scientific approach towards studying antibiotic resistance. However, the antibiogram of NFGNB strains may suffice to compare, review and modify the ongoing antimicrobial policies. Moreover, the antimicrobial data may help clinicians deciding their treatment approaches.

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