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Study of multidrug resistant nonfermenting gram-negative bacilli

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Abstract
Introduction: Non-fermenting gram-negative bacilli (NFGNB) are ubiquitous in the nature. They are considered as environmental contaminants and most of them have emerged as important no socomial pathogens. So this study was undertaken to identify the various NFGNB with its antimicrobial susceptibility pattern of specimens from patients admitted to Intensive care unit in Government Medical College and Hospital, Bettiah.
Methods: Total 107 NFGNB were identified during the study period of March 2014-Nov 2015. All isolates were identified using a standard conventional method like motility, oxidase production; oxidation-fermentation test etc. and failing to acidify butt in triple sugar iron medium. Antibiotic susceptibility testing was performed with the help of the Kirby-Bauer disc diffusion method and (Metallobetalactamase) MBL detection done by DDST and Modified Hodge test.
Result: P. aeruginosa (16%) was the commonest isolate obtained followed by A. baumannii (13.5%). NFGNB were maximum susceptible to imipenem (80.4%), piperacillin tazobactum (71.9%) and amikacin (52.3%). P. aeruginosa and A. baumannii were 90% and 60.6% sensitive imipenem and 85% and 54.5% to piperacilli-tazobactum. 19.6% MBL producers were obtained in all nonfermenters. Maximum MBL production was obtained in Acinetobacter spp. (39.4%) followed by S. maltophilia (20%) and P. aeruginosa (10.0%)
Interpretation & conclusion: Multidrug resistance infection in ICU is emerging; we also obtained 19.6% MBL producers in all NFGNB. Local antibiotic policy should be generated to prevent the emerging MDR in NFGNB.

Keywords: nonfermenters, intensive care unit, MDR, antibiotic sensitivity test

1. Introduction
Non-fermenting gram-negative bacilli (NFGNB) are ubiquitous in the environment and are a group of aerobic, non-sporing bacilli that either do not utilize glucose as a source of energy or utilize it oxidatively, positive cytochrome oxidase test and failure to grow on MacConkey agar.
[1] NFGNB is known to account for about 15% of all bacterial isolates from a clinical microbiology laboratory.
[2] The predominant species of concern are P. aeruginosa, A. baumannii, S. maltophilia and, less so, members of the B. cepacia group [3]. Except P. aeruginosa the NFGNB are most often cause nosocomial infections in immune-compromised patients like urinary tract infections (UTI), ventilator associated pneumonia (VAP), surgical site infections (SSTI) and bacteraemia [1]. NFGNB often are multidrug resistant, with resistance increasing to aminoglycosides, fluroquinolones, third generation cephalosporin's and carbapenems [3, 4].
As NFGNB are highly intrinsically resistant to different antimicrobial agents, its identification comes to the forefront. [5] Hence present study was undertaken to identify NFGNB from various clinical specimens from ICU infected patients and to detect its antimicrobial susceptibility pattern with multidrug resistance (MDR) pattern by detecting (metallobetalactamase) MBL production.

2. Material and Method
The study was carried out in Government Medical College and Hospital, Bettiah during duration of March 2014 to November 2015. Depending on sites of infections, various samples like ET aspirate, pus, blood, urine were collected and processed as per the standard guidelines [6, 7].
Inclusion criteria: All patients admitted in ICU and developed infections after 48 hours of admission to ICU were included in the study while those developed infection prior to ICU admission were excluded in the study [6].
The NFGNB were identified using characteristic features they assessed like morphology on Gram's stain, motility, pigment production, oxidase production, failure to acidify butt and slant in triple sugar iron agar, of test (Hugh-Leifson's medium), lysine decarboxylase test and gelatin liquefaction test [1, 7]. The isolated NFGNB were subjected to antimicrobial susceptibility testing by Kirby Bauer disc diffusion method as per CLSI 2011 guideline [8]. E. coli ATCC 25922, P. aeruginosa ATCC 27853 were used as the control strains. All NFGNB isolates found resistant to imipenem (I) were tested for metallo-β- lactamase (MBL) production by EDTA double disc synergy test (DDST) and Modified Hodge test [8, 9].
Test strains were adjusted to McFarland 0.5 standard & used to inoculate Mueller Hinton agar plates. Two discs of 10 µg imipenem and imipenem EDTA disc were placed on plate at 15mm distance. After overnight incubation, zone of imipenem with EDTA should be >7 mm than plain imipenem disc to consider test to be positive. (figure1)
1) Modified Hodge Test (10): Standard strain of E. coli ATCC 25922, at a turbidity of 0.5 McFarland standard, was used to swab inoculate the surface of Mueller Hinton agar plate. Test strain was heavily streaked from centre to plate periphery. After, the plate was allowed to stand for 15 minutes at room

temperature. 10µg imipenem disc was placed at centre and plate was incubated overnight. The presence of distorted inhibition zone was interpreted as a positive result for carbapenem hydrolysis screening. (Figure 2)

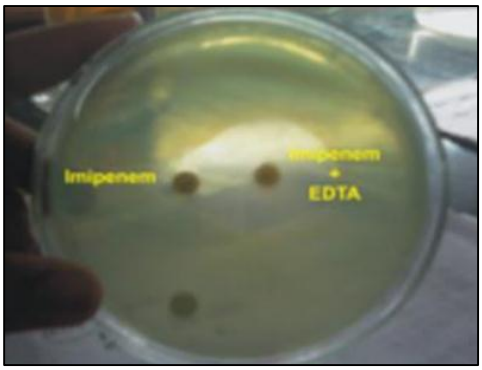

Fig 1: DDST with imipenam and imipenam EDTA

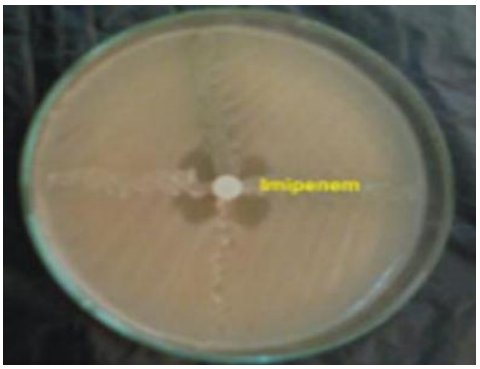

Fig 2: Hodge test

Table 1: Microbial Aetiology of the Varous Samples in ICU

Isolates	ET aspirate (n=151)	Blood culture (n=49)	Urine (n=27)	Pus (n=21)	CSF (n=2)	Total (n=250) (%)
Gram negative bacilli						**162 (64.8)**
E. coli	8	0	8	1	0	18 (7.2)
K. pneumoniae	23	1	1	4	0	29 (11.6)
K. oxytoca	1	0	0	0	0	1 (0.4)
C. freundii	2	0	0	2	0	5 (2.0)
E. cloacae	1	0	0	0	0	1 (0.4)
Pr. mirabilis	0	0	0	1	0	1 (0.4)
P. aeruginosa	36	3	1	0	0	40 (16.0)
P. stutzeri	10	0	0	0	0	10 (4.0)
A. baumanii	32	1	0	0	0	33 (13.5)
A. lwoffi	13	0	0	0	0	13 (5.2)
A. hemolyticus	0	1	0	0	0	1 (0.4)
S. maltophilia	0	0	0	10	0	10 (4.0)
Gram positive cocci						**19 (6)**
S. aureus	9	0	0	4	0	13 (5.2)
S. epidermidis	-	0	2	0	0	2 (0.8)
S. pneumoniae	2	0	0	0	0	2 (0.8)
E. faecalis	0	0	2	0	0	2 (0.8)
No growth	14	43	13	1	2	**73 (30.8)**

Table 2: antimicrobial sensitivity of nonfermenters (a=107)

Drugs	*A.baumanni* n=33 (%)	*A. lwoffi* n=13(%)	*A.hemolyticus* n=1(%)	*P. aeruginosa* n=40 (%)	*P. stutzeri* n=10 (%)	*S. maltophilia* n=10 (%)	Total n=107 (%)
	(n=47)						
Ceftazidime	2 (6.1)	4 (8.5)	0	13 (32.5)	8 (80.0)	3 (30.0)	30 (28.0)
Cefotaxime	2 (6.1)	2 (15.4)	1 (100)	10 (25.0)	6 (60.0)	2 (20.0)	23 (21.5)
Cefepime	3 (9.1)	5 (38.5)	1 (100)	14 (35.0)	8 (80.0)	3 (30)	34 (31.8)
Piperacillin	5 (15.2)	4 (8.5)	1 (100)	17 (42.5)	6 (60.0)	3 (30.0)	36 (33.6)
Piperacillin + tazobactum	18 (54.5)	10 (76.9)	1 (100)	34 (85.0)	9 (90.0)	5 (50.0)	77 (71.9)
Imipenem	20 (60.6)	11(84.6)	1 (100)	36 (90.0)	10 (100)	8 (80.0)	86 (80.4)
Gentamicin	6 (18.2)	3 (6.4)	1 (100)	19 (47.5)	7 (70.0)	0	36 (33.6)
Amikacin	14 (42.4)	6 (12.8)	1 (100)	26 (65.0)	9 (90.0)	0	56 (52.3)
Tobramycin	9 (27.3)	5 (38.5)	1 (100)	11 (27.5)	8 (80.0)	0	34 (31.8)
Ciprofloxacin	5 (15.2)	6 (12.8)	1 (100)	14 (35.0)	7 (70.0)	7 (70.0)	40 (37.4)
Cotrimoxazole						10 (100.0)	

Table 3: Distribution of MBL Producing Gram Negative Bacilli

Gram negative bacilli	MBL NFGNB (n=107)
P. aeruginosa (n=40)	4 (10.0%)
A. baumannii (n=33)	13 (39.4%)
A. lwoffi (n=13)	2 (23.07%)
S. maltophilia (n=10)	2 (20.0%)
P. stutzeri (n=10)	0
A. hemolyticus (n=1)	0
Total (%)	**21 (19.62%)**

Table 4: Distribution Imipenem Resistant Isolates Confirmed By DDST and Modified Hodge Test

Total no of isolates (n=21)	DDST with EDTA	Modified hodge test
21(90.5%)	Positive	Positive
2(9.5%)	Negative	Positive

3. Result

In our study, out of total 2529 ICU admissions, 250 patients were clinically diagnosed to have infections like VAP, UTI, SSTI and bacteremia. Of 250 patients various specimens were collected of which, total162 (60.8%) gram negative bacilli were isolated and in 73 (30.8%) isolates no growth was obtained (Table 1).

Out of these 162 isolates, 107 (42.8%) isolates were NFGNB. The majority of NFGNB were isolated from ET aspirates, pus and blood. (Fig 3). P. aeruginosa (16%) was the commonest isolate obtained followed by A. baumannii (13.5%), A. lwoffi (5.2%), S. maltophilia (4%), P. stutzeri (4%) and A. hemolyticus (0.4%) as shown in Fig 4. In our study, P. aeruginosa were 90% sensitive to imipenem, piperacilli-tazobactum (85%), amikacin (65%), cefipime (35%), ceftazidime (32.5%) and ciprofloxacin (35%). While isolated P. stutzeri showed 100%sensitivity to imipenem, 90% sensitivity to amikacin and piperacillin-tazobactum.

Sensitivity pattern in A. baumanii was imipenem (60.6%) sensitive followed by amikacin (42.2%), ciprofloxacin (15.2%) and for third generation cephalosporins sensitivity was 6.1%. Sensitivity of A. lwoffi to imipenem was 84.6%, piperacillintazobactum 76.9%. S. maltophilia were 100% sensitive to cotrimoxazole while 100% resistant to aminoglycosides and 20% resistant to imipenem. (Table-2) Of the 21 imipenem resistant isolates, 21 were positive by double disc synergy test, 21 were positive by Modified Hodge test. 2 isolates which were negative by DDST came positive by Modified Hodge test.

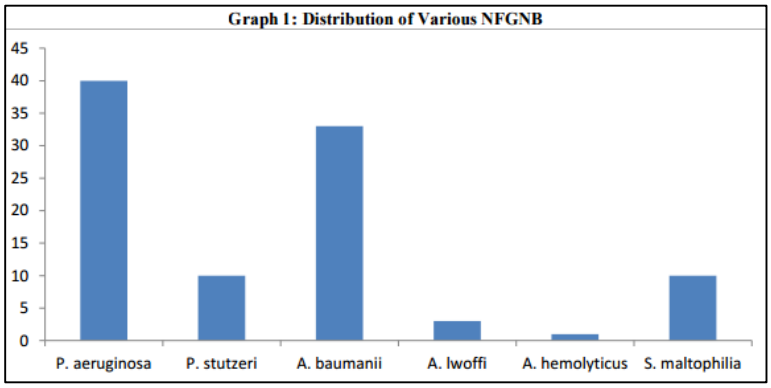

Fig 3

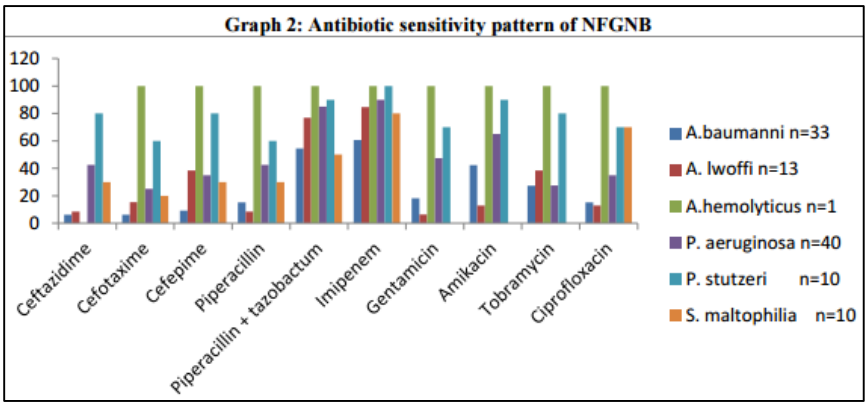

Fig 4

4. Discussion

NFGNB were considered to be a contaminant in past have now emerged as an important health care pathogen so its proper identification is important now a days [11] ICUs are the epicenters of outbreaks of MDR organisms which made treatment of ICU-acquired infections very difficult [12]. Several outbreaks were reported due to carbapenem resistant NFGNB with considerable rate of morbidity and mortality [13, 15].

Resistance pattern of P. aeruginosa in our study was similar to study conducted by Shehabi and Baadran [16] while resistance pattern of A. baumanii was similar to Jamshidi *et al* [17]. While that of S. maltophilia was comparable to study conducted by Patel PH etal [11]. MBL production in NFGNB has tremendous therapeutic consequences since these organisms also carry multidrug resistance genes and the only viable option remains the potentially toxic polymyxin B and colistin [13].

In our study, maximum MBL production was obtained in Acinetobacter spp. (39.4%) followed by S. maltophilia 20% and P. aeruginosa (10.0%) as shown in Table 3. These strains were found MBL producers by DDST with EDTA and Modified Hodge test. In our study we obtained 19.6% MBL producers in all non-fermenters (Table 3).

Our results are similar to study conducted by other authors Joseph *et al.* 18 and John *et al*. 19. Gopal Krishnan *et al* 20, De *et al*. 21 and Wattal *et al*, 22 had reported high MBL production in their studies. This study implicates the severity of MDR producing NFGNB which gives an idea of success or failure of infection control programs.

5. Conclusion

To conclude, P. aeruginosa and A. baumannii are the most common NFGNB isolated in our study. Emergence of MBLs

producing NFGNB in ICU is alarming therefore regular monitoring and documentation of imipenem resistance is necessary. Thus local data is required to help to formulate antibiotic policies and to nip any emerging outbreak early before, it leads to serious consequences.

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