

## STUDY OF MULTIDRUG RESISTANT NONFERMENTING GRAM-NEGATIVE BACILLI IN INTENSIVE CARE UNIT, NAGPUR

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### ABSTRACT

**Background and objectives:** Nonfermenting gram-negative bacilli (NFGNB) are ubiquitous in the nature. They are considered as environmental contaminants and most of them have emerged as important nosocomial 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 (ICU) in our hospital

**Methods:** Total 107 NFGNB were identified during the study period of September 2009-October 2011. 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 tazobactam (71.9%) and amikacin (52.3%). *P. aeruginosa* and *A. baumannii* were 90% and 60.6% sensitive imipenem and 85% and 54.5% to piperacillin-tazobactam. 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

### INTRODUCTION

Non-fermenting gram-negative bacilli (NFGNB) are ubiquitous in the environment and are a group of aerobic, non-spore-forming 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. <sup>(1)</sup> NFGNB is known to account for about 15% of all bacterial isolates from a clinical microbiology laboratory. <sup>(2)</sup> The predominant species of concern are *P. aeruginosa*, *A. baumannii*, *S. maltophilia* and, less so, members of the *B. cepacia* group. <sup>(3)</sup> 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 (SSI) and bacteraemia. <sup>(1)</sup> NFGNB often are multidrug resistant, with resistance increasing to aminoglycosides, fluoroquinolones, third generation cephalosporins and carbapenems. <sup>(3,4)</sup> As NFGNB are highly intrinsically resistant to different antimicrobial agents, its identification comes to the forefront. <sup>(5)</sup>

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.

### MATERIAL AND METHOD

The study was carried out in the Department of Microbiology, Indira Gandhi Government Medical College Nagpur in medical and surgical ICUs during duration of September 2009 to October 2011. Depending on sites of infections, various samples like ET aspirate, pus, blood, urine were collected and processed as per the standard guidelines. <sup>(6,7)</sup>

**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. <sup>(6)</sup>

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. <sup>(1,7)</sup> The isolated NFGNB were subjected to antimicrobial susceptibility testing by Kirby Bauer disc diffusion method as per CLSI 2011 guideline. <sup>(8)</sup> *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- $\beta$ -lactamase (MBL) production by EDTA double disc synergy test (DDST) and Modified Hodge test. <sup>(8,9)</sup>

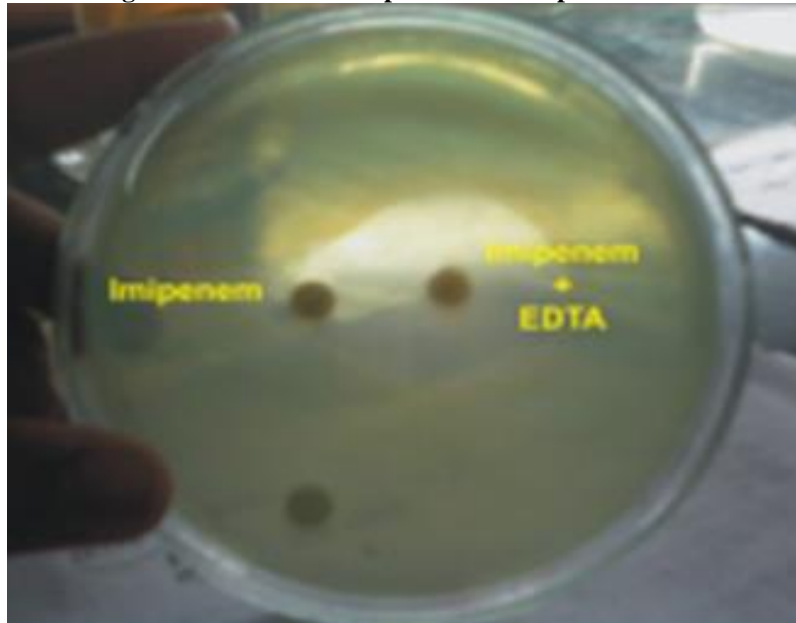
2) Double Disc Synergy Test with EDTA <sup>(9)</sup>

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<sup>(10)</sup>: 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)

**Figure 1: DDST with Imipenam and Imipenam EDTA**



**Figure 2: Hodge test**



**Table 1: Microbial aetiology of the various samples in ICU**

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

**Table 2. Antimicrobial sensitivity of nonfermenters (n=107)**

Drugs	<i>A.baumannii</i> n=33 (%)	<i>A. lwoffii</i> n=13(%)	<i>A.hemolyticus</i> n=1(%)	<i>P. aeruginosa</i> n=40 (%)	<i>P. stutzeri</i> n=10 (%)	<i>S. maltophilia</i> 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 + tazobactam	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)
<i>P. aeruginosa</i> (n=40)	4 (10.0%)
<i>A. baumannii</i> (n=33)	13 (39.4%)
<i>A. lwoffii</i> (n=13)	2 (23.07%)
<i>S. maltophilia</i> (n=10)	2 (20.0%)
<i>P. stutzeri</i> (n=10)	0
<i>A. hemolyticus</i> (n=1)	0
<b>Total (%)</b>	<b>21 (19.62%)</b>

**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
<b>21(90.5%)</b>	Positive	Positive
<b>2(9.5%)</b>	Negative	Positive

**RESULT**

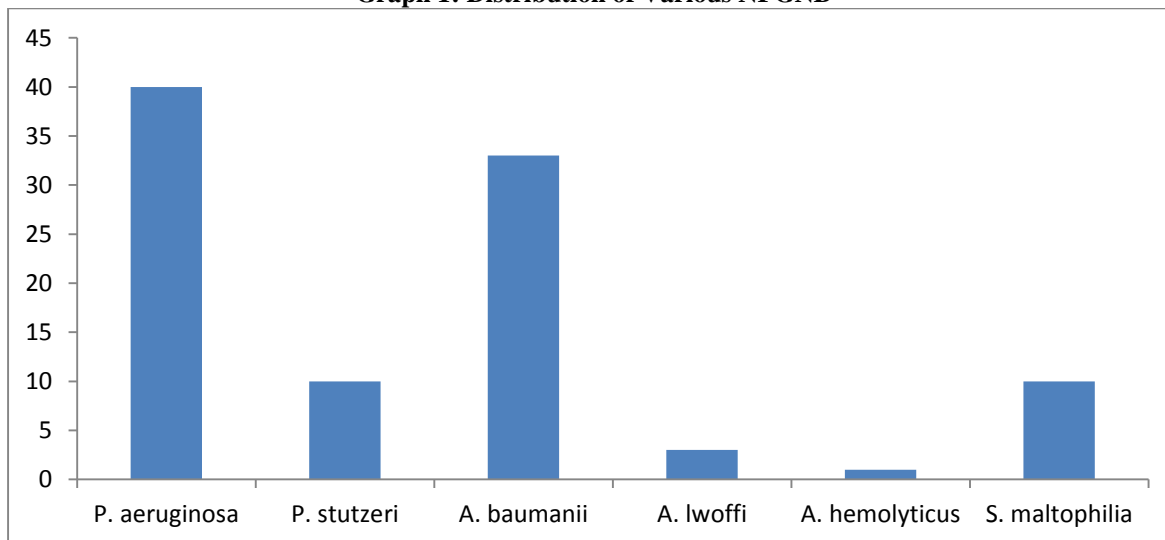
In our study, out of total 2529 ICU admissions, 250 patients were clinically diagnosed to have infections like VAP, UTI, SSTI and bacteremia. Of 250 patients various specimens were collected of which, total 162 (60.8%) gram negative bacilli were isolated and in 73 (30.8%) isolates no growth was obtained (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. (Graph 1).

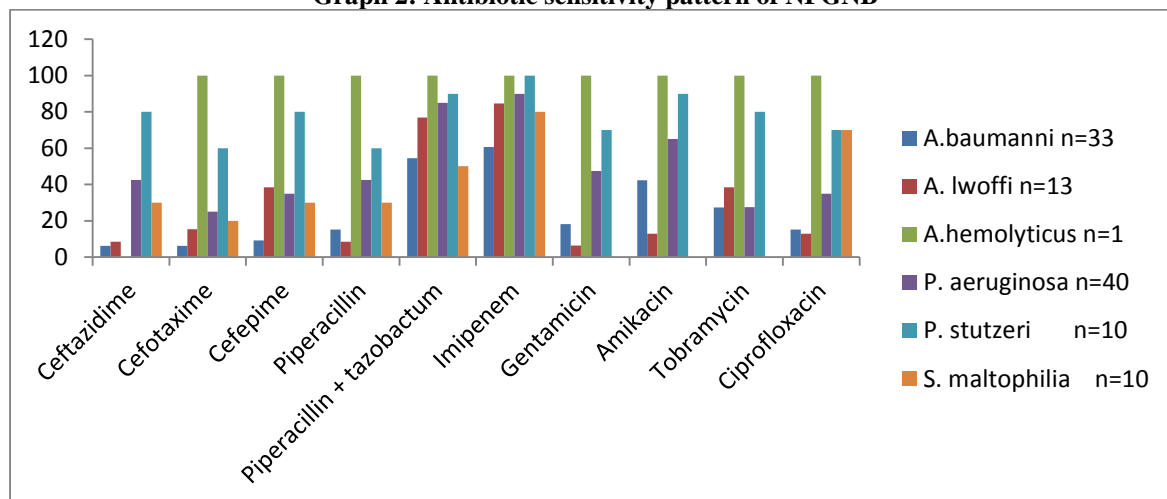
*P. aeruginosa* (16%) was the commonest isolate obtained followed by *A. baumannii* (13.5%), *A. lwoffii* (5.2%), *S. maltophilia* (4%), *P. stutzeri* (4%) and *A. hemolyticus* (0.4%) as shown in Graph 1. In our study, *P. aeruginosa* were 90% sensitive to imipenem, piperacilli-tazobactam (85%), amikacin (65%), cefipime (35%), ceftazidime (32.5%) and ciprofloxacin (35%). While isolated *P. stutzeri* showed 100% sensitivity to imipenem, 90% sensitivity to amikacin and piperacillin-tazobactam. Sensitivity pattern in *A. baumannii* 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. lwoffii* to imipenem was 84.6%, piperacillin-tazobactam 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.

Statistical analysis was done, chi square value was calculated which was 0.19, P value was not significant (<0.05) that means any of the test can be used.

**Graph 1: Distribution of Various NFGNB**

Graph 2: Antibiotic sensitivity pattern of NFGNB



## 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<sup>(11)</sup> ICUs are the epicenters of outbreaks of MDR organisms which made treatment of ICU-acquired infections very difficult.<sup>(12)</sup>

Several outbreaks were reported due to carbapenem resistant NFGNB with considerable rate of morbidity and mortality.<sup>(13,14,15)</sup> Resistance pattern of *P. aeruginosa* in our study was similar to study conducted by Shehabi and Baadran<sup>(16)</sup> while resistance pattern of *A. baumannii* was similar to Jamshidi et al.<sup>(17)</sup> While that of *S. maltophilia* was comparable to study conducted by Patel PH et al.<sup>(11)</sup>

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.<sup>(13)</sup> 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<sup>18</sup> and John et al<sup>19</sup>. Gopalkrishnan et al<sup>20</sup>, De et al<sup>21</sup> and Wattal et al,<sup>22</sup> 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.

## 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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