



IDENTIFICATION OF NON-FERMENTER GRAM NEGATIVE BACILLI (NFGNB) FROM VARIOUS CLINICAL ISOLATES WITH SPECIAL REFERENCE TO CARBAPENEM RESISTANCE IN VARIOUS ISOLATES.

Microbiology

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ABSTRACT

Non Fermenter Gram negative bacilli (NFGNB) has emerged as important hospital pathogens they are more significant as they are found to be multi drug resistant. Resistance to carbapenems is common among NFGNB.

AIMS & OBJECTIVES: To isolate & identify NFGNB from various clinical samples and to detect resistance to carbapenem in isolates resistant to Imipenem.

MATERIAL & METHOD: NFGNB isolated from various samples were speciated using standard tests. Isolates resistant to Imipenem were subjected to detection of MBLs (Metallo- β -lactamase) and Amp-C.

RESULTS: Out of 1566 samples received, NFGNB were 200. Among them 112 were *Pseudomonas aeruginosa* from which 31 were found to be resistant to Imipenem, of which 3 were MBL producer by Modified Hodge test while 4 were MBL producer by EDTA disc synergy test. Out of 200 NFGNB 71 were *Acinetobacter baumannii*, of which 23 were found to be resistant to Imipenem, of which 6 were MBL producer by Modified Hodge test, while 4 were seen to be MBL producer by EDTA disc synergy test. Nineteen isolates of *Acinetobacter baumannii* were found to be resistant to ceftioxin of which 6 were found to be Amp-C producer by Amp-c disc test. None of the *Pseudomonas aeruginosa* were Amp-C producer. Other NFGNB isolated were either sensitive to Imipenem or if resistant were not MBL or Amp-C producer.

KEYWORDS

Non fermenter gram negative bacilli, Metallo β lactamase, Amp-C, Modified Hodge test.

INTRODUCTION

Non fermenter Gram negative bacilli (NFGNB) are group of aerobic, non-spore forming organisms. They are ubiquitous in nature. Although they are commonly considered to be environmental contaminants, they have emerged as important nosocomial pathogens¹. This group includes various genera like *Pseudomonas*, *Acinetobacter*, *Stenotrophomonas*, *Sphingomonas*, *Burkholderia* etc^{2,3}.

These organisms are known to infect immune-compromised patients⁴, these organisms frequently colonize on various devices and fluids used for patient care and also widely distributed in hospital environment⁴. They cause various infections like ventilator associated pneumonia (VAP), urinary tract infection (UTI), Surgical site infection (SSI), Septicemia etc⁵.

Infections due to NFGNB are very difficult to treat as they are often multidrug resistant, important mechanisms of resistance seen in NFGNB are MBLs (Metallo- β -lactamase) and ESBLs (Extended spectrum β lactamases). Various mechanisms for carbapenem resistance includes mutation of porin channels of cell membrane, loss of outer membrane proteins, efflux mechanism⁵. These enzymes have high proteolytic activity against penicillins, cephalosporins and carbapenems. Resistance to carbapenems can be intrinsic or acquired. Acquired resistance by Class B MBLs and Class d serine carbapenamases are found commonly in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*⁶. These acquired MBL genes like VIM, GIM, SPM are clustered on mobile DNA elements that have potential to spread horizontally and spread rapidly to other species and may lead to outbreaks⁷. Therefore it is essential to regularly monitor and report carbapenem resistance as it will help in prevention of outbreak and control infections and also enable to formulate hospital antibiotic policy.

MATERIAL AND METHODS:

NFGNB isolated from various clinical samples like pus, blood, urine and respiratory specimen received for culture and sensitivity in Microbiology department at a tertiary care hospital in Solapur were included in the study. Study was conducted between December 2015 to July 2017. Clinical isolates of NFGNB from above foresaid samples were included in the study, while samples other than above foresaid and mixed growth of more than three types were excluded from the

study. NFGNB isolated from various samples were speciated using standard tests. Isolates resistant to Imipenem were subjected to detection of MBL by Modified Hodge test and EDTA combined disc test and Amp-C detection was done using Amp-c disc test.

Quality control strains were *Escherichia coli* ATCC 25922, for Modified Hodge test-Positive control- *Klebsiella pneumoniae* ATCC BAA-1705 and Negative control- *Klebsiella pneumoniae* ATCC BAA-1706.

Modified-Hodge test⁸

Lawn culture of *E.coli* ATCC 25922 was made from an overnight culture suspension adjusted to 0.5 MC Farland standards on Mueller Hinton agar. After drying the plate 10 μ g Imipenem disc was placed at the center and test strain was streaked from edge of disc to periphery of the plate. Plate was incubated at 37^o C for overnight. The presence of distorted zone of inhibition (clover leaf pattern) was interpreted as positive (Figure-1).

Combined Disc test⁸

Test organism was inoculated on Mueller Hinton agar and two 10 μ g Imipenem disc were placed, 10 μ l solution of EDTA (Ethylene diamine tetra acetic acid) was added to one of the disc, plate was incubated at 35^oC overnight. Metallo- β -lactamase positive result was considered if zone of inhibition of Imipenem+ EDTA disc was > 7mm than that of Imipenem disc alone (Figure-2).

Amp-C disc test⁸

Lawn culture of *E.coli* ATCC 25922 was made from an overnight culture suspension adjusted to 0.5 Mc Farland standards on Mueller Hinton agar. A 30 μ g Cefoxitin disc placed in center and a blank disc was moistened with sterile normal saline and few colonies of test organism were inoculated to the sterile disc, which was placed beside Cefoxitin disc almost touching it. Plate was incubated overnight at 37^oC. A flattening of zone of inhibition of Cefoxitin in the vicinity of disc containing test organism was interpreted as positive (Figure-3).

RESULTS

A total of 1566 samples like pus, blood, urine, respiratory secretions were received in Microbiology department for culture and sensitivity,

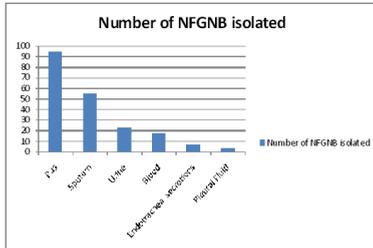
of which NFGNB isolates were 200(12.77%).

Majority of NFGNB isolates were from males which constituted 130 (65%) while 70 (35%) were from females.

Distribution of NFGNB according to clinical samples

Among 200(12.77%) NFGNB isolates 95 (47.5%) isolates were from pus followed by 55 (27.5%) isolates from sputum, 23(11.5%) isolates from urine, 17 (8.5%) isolates from blood, 7(3.5%) isolates from endotracheal secretions and 3 (1.5%) isolates from pleural fluid. As shown in graph-1

Graph-1 showing distribution of NFGNB according to clinical samples



Different isolates of NFGNB

Out of 200 NFGNB isolates, *Pseudomonas aeruginosa* were the commonest organism constituting 112(56%) of the total isolates, followed by *Acinetobacter baumannii* which constituted for 71 (35.5%), *Pseudomonas fluorescens*- 7 isolates (3.5%), *Acinetobacter lwoffii*- 6 isolates (3%), *Burkholderia cepacia complex*- 2 isolates (1%) and *Sphingomonas paucimobilis* – 2 isolates (1%).

Table-1: Distribution of non-fermenter isolates in various specimens:

Species	Sputum	Pleural fluid	Endotracheal secretion	Pus	Blood	Urine
<i>Pseudomonas aeruginosa</i>	25	03	04	65	02	13
<i>Pseudomonas fluorescens</i>	04	-	-	03	-	-
<i>Acinetobacter baumannii</i>	20	-	03	24	14	10
<i>Acinetobacter lwoffii</i>	02	-	-	03	01	-
<i>Burkholderia cepacia complex</i>	02	-	-	-	-	-
<i>Sphingomonas paucimobilis</i>	02	-	-	-	-	-
Total	55	03	07	95	17	23
Percentage (%)	18.77	1.5	3.5	47.5	8.5	11.5

NFGNB isolates showing MBL production

A total of 31(27.67%) isolates from 112 *Pseudomonas aeruginosa* were found to be resistant to imipenem of which 3(9.67%) were found to be MBL producer by modified Hodge test while 4(12.90%) isolates were found to be MBL producer by EDTA disc synergy test. one isolate of *Pseudomonas fluorescens* was resistant to imipenem however it was not found to be MBL producer by modified Hodge method and EDTA disc synergy method as shown in Table-2, Image 1 & 2

Out of 71 *Acinetobacter baumannii* isolates 23 (32.39%) were found to be imipenem resistant. From which 6 (26.08%) were found to be MBL producer by Modified Hodge test while 5 (21.73%) isolates were found to be MBL producer by EDTA disc synergy test as shown in Table-3.

Rest all other NFGNB isolated which were resistant to imipenem were non MBL producer and Amp-c producer, while those NFGNB which were found to be sensitive to imipenem no further testing was done

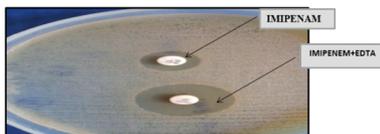


IMAGE-1 Combined disc test showing IE (Imipenem+EDTA) zone of inhibition >7mm as compared to zone of inhibition of imipenem alone suggestive of MBL production.

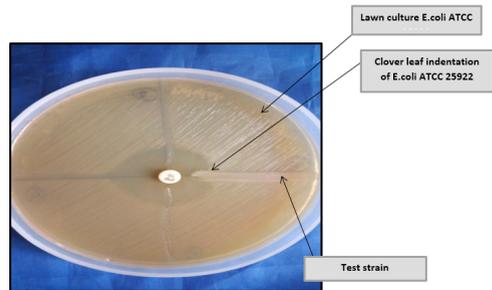


IMAGE-2 Modified Hodge test showing clover leaf pattern at the intersection of E.coli ATCC 25922 growing along the test organism

IMAGE-3 showing Amp-C Disk test

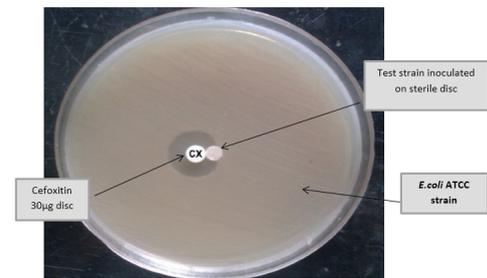


Table- 2: Percentage of MBL producing isolates of Pseudomonas aeruginosa

Test	Number of isolates (n=31)	Percentage (%) of MBL producers
Modified Hodge test	03	9.67
EDTA disc synergy test	04	12.90

Table-3: Percentage of MBL producing Acinetobacter baumannii

Test	Number of isolates (n=23)	Percentage (%) of MBL producers
Modified Hodge test	06	26.08
EDTA disc synergy test	05	21.73

Cefoxitin resistance and Amp-C detection

Imipenem and Cefoxitin resistance were tested for Amp-C detection, *Pseudomonas aeruginosa* resistant to imipenem and cefoxitin but none were Amp-C producer. However out of 71 isolates of *Acinetobacter baumannii* 23 isolates were found to be resistant to imipenem and 19 isolates were found to be resistant to cefoxitin but only 3 isolates were Amp-C producer as shown in Image-3. Other NFGNB which were resistant to imipenem were not Amp-C producer. While other NFGNB sensitive to imipenem and cefoxitin were not tested further.

DISCUSSION

NFGNB that were considered to be contaminants in the past have now emerged as an important health care associated pathogen¹. The multidrug resistant organisms have increased over the time which has led to increased economic burden, duration of hospital stay and increased mortality and morbidity.

In the present study out of 1566 clinical samples 200 NFGNB were isolated accounting to isolation rate of (12.77%). Studies done by various workers have shown variable isolation rates of NFGNB, Mahajan R et al⁹ reported 12.40% of NFGNB isolation rate. Juyal D et al¹⁰ reported 9.32%. Malini A et al³ reported NFGNB isolation rate of 4.5% which is lower than other studies, the difference in isolation rate can be attributed to difference in population and living conditions of the study group.

In present study males 130(65%) were more commonly affected with different infections caused by non-fermenter gram negative bacilli than females 70 (35%) which is comparable with the studies done by Usha Rani P et al¹¹ which reported males (64%) as compared to females (36%). Varaiya A et al¹² reported males (66.66%) while females (33.3%). Bohra R et al¹³ reported males (53.77%) while females (46.22%). The present study has comparable results with the studies done by various workers. Male predominance may be attributed to their more involvement in outdoor activities and therefore chances of exposure to infections are more likely as compared to females.

In the present study total 200(12.77%) NFGNB isolates were processed from various clinical samples, maximum NFGNB were isolated from pus sample 95 (47.5%) followed by sputum 55 (27.5%), urine 23 (11.5%), blood 17 (8.5%), endotracheal secretion 07 (3.5%) and pleural fluid 03 (1.5%). Similar results were seen in study done by Bhatnagar R et al¹⁴ in which maximum NFGNB isolates were from pus (49.20%) followed by sputum (19.84%). Malini A et al² also reported maximum NFGNB isolates from pus (61.90%) followed by sputum (12.69%). Present studies showed maximum NFGNB are isolated from pus sample and sputum which coincides with the studies done by various workers that shows predominantly NFGNB are isolated from pus and respiratory samples.

In the present study *Pseudomonas aeruginosa* 112(56%) were the commonest isolates followed by *Acinetobacter baumannii* 71 (35.5%). Other NFGNB which were isolated were *Pseudomonas fluorescens*-7 isolates (3.5%), *Acinetobacter lwoffii*- 6 isolates (3%), *Burkholderia cepacia complex* -2 isolates (1%) and *Sphingomonas paucimobilis* - 2 isolates (1%). Similar results were seen in the study done by Sharma D et al¹⁵ where *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated were (48.9%) and (33%) respectively. Mahajan R et al reported⁹ *Pseudomonas aeruginosa* (54.54%) while *Acinetobacter baumannii* (41.08%). However studies done by Sharma M et al¹⁵ have reported *Acinetobacter baumannii* to be most common isolate accounting to (50.4%) followed by *Pseudomonas aeruginosa*(38.1%). The present study is in concordance to the studies done by most of the workers. Where predominant NFGNB isolated were *Pseudomonas aeruginosa* followed by *Acinetobacter baumannii*.

Multi drug resistance is major problem so infection caused by them are very difficult to treat. Polymyxin class of antimicrobial drug has fairly consistent activity against all multi drug resistant strains of non-fermenters.

Among the *Pseudomonas aeruginosa* 31 (27.67%) isolates were resistant to imipenem. In the study done by Sharan H et al²⁰ imipenem resistance was (40%) in *Pseudomonas aeruginosa*. In another study done by Usha Rani P et al² (7.5%) of *Pseudomonas aeruginosa* isolates were resistant to carbapenems. Out of these 31 imipenem resistant isolates of *Pseudomonas aeruginosa*, 3 isolates (9.67%) were found to be MBL producer by modified Hodge test while *Pseudomonas aeruginosa*, EDTA disc synergy test showed 4 isolates (12.90%) of *Pseudomonas aeruginosa* to be MBL producers as shown in table-2. Navneeth et al¹⁶ reported (15.23%) isolates to be MBL producer. While Jayalakshmi S et al¹⁷ reported (33%) isolates to be MBL producers. Thipperudraswamy T et al¹⁰⁶ reported (10.58%) isolates to be MBL producer. Amjad A et al¹⁸ reported (30%) isolates to be MBL producer.

Among the 23 imipenem resistant isolates of *Acinetobacter baumannii*, 6 isolates (26.08%) were found to be MBL producer by modified Hodge test, while 5 isolates of *Acinetobacter baumannii* were found to be MBL producer by EDTA disc synergy test. Amjad A et al¹⁸ reported (12%) isolates of *Acinetobacter baumannii* to be MBL producer. While Shivprasad A et al¹⁹ reported (100%) isolates of *Acinetobacter baumannii* to be MBL producers.

CONCLUSION

NFGNB isolation from various clinical samples has proved their existence in all sites leading to range of disease. Multidrug resistance exhibited by NFGNB has become challenge in treating these infections. So judicious use of antibiotics by instituting antibiotic policy and following hospital infection control measures will help in effective management of patient care.

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