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A STUDY OF PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF NON FERMENTER GRAM NEGATIVE BACILLI ISOLATED FROM VARIOUS CLINICAL SAMPLES AT A TERTIARY CARE CENTER, JAIPUR

Medical Microbiology

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ABSTRACT

Introduction: Non-fermentative Gram-negative bacilli (NFGNB) are occasionally involved in infectious diseases pathology, but have shown resistance to multiple antibiotics and the capability to gain new resistance factors in the hospital environment. The present study was aimed to investigate the prevalence of Metallo-beta-lactamase (MBL) producing non-fermenters among the carbapenem-resistant isolates. **Methods:** A total of 200 NFGNB isolates from various clinical samples were analyzed by using different standard microbiological techniques and antimicrobial susceptibility using Kirby-Bauer method. MBL detection was performed by Modified Hodge test (MHT), imipenem-EDTA combined disc test, and modified Carbapenemase inactivation method (mCIM). **Results:** In our study, 200 NFGNB isolates recovered from various clinical samples. Pseudomonas aeruginosa was the most common NFGNB, accounting 112 (56%), followed by Acinetobacter baumannii 56 (28%), Burkholderia species 26 (13%) and other rare nonfermenter Gram negative bacilli 6 (3%). NFGNB isolates were maximally isolated from blood samples 73 (36.5%) followed by respiratory samples 46 (23%), urine 40 (20%), pus 37 (18.5%) and CSF 4 (2%). All the NFGNB isolates were susceptible to polymyxin B, colistin (100%), and cefoperazone + sulbactam (45.53%). Out of 112 Pseudomonas isolates, 77(68.75%) were imipenem resistant and of 56 Acinetobacter isolates, 35(62.5%) were imipenem resistant. Out of 77 imipenem resistant Pseudomonas 70 (90.9%) were MBL producers and out of 35 imipinem resistant Acinetobacter spp 32 (91.42%) were MBL producers. **Conclusion:** NFGNB are now emerging as organisms of nosocomial infections. The use of broad-spectrum antibiotics should be avoided and quick detection and efficient infection control measures are essential to prevent the further spread of MBLs to other Gram-negative bacilli. Detection of MBL production and rationale antibiotic usage are the most important factors which control the gradually increasing NFGNB related infections.

KEYWORDS

Non-fermenting Gram-negative bacilli, Carbapenems, Metallo-beta-lactamas

INTRODUCTION

Nonfermentative Gram-Negative bacilli (NFGNB) are a group of aerobic, non-spore forming, Gram-negative bacilli that either do not utilize carbohydrates as a source of energy or degrade them through metabolic pathways other than fermentation.[1]

NFGNB are broadly distributed in nature as saprophytes or as commensals and act as opportunistic pathogens for man.[2,3]

This heterogeneous group includes organisms like *Pseudomonas.*spp, *Acinetobacter* spp, *Burkholderia cepacia complex (BCC), Alkaligenes* spp, *Stenotrophomonas maltophilia, Sphingomonas paucimobilis* and others.[7]

In recent years due to liberal and empirical use of antibiotics, most of these organisms have become resistant to many routinely used antibiotics particular β-lactams including penicillins and cephalosporins.[11]

The Carbapenem group of antimicrobial agents play a vital role in the management of hospital-acquired Gram-negative infections particularly β- lactamases resistance bacteria, owing to their broad spectrum activity.[12] In recent years there is also emerging resistance to the carbapenem antimicrobial for nonfermenter.

Resistance to carbapenem group of antimicrobial agents in *P. aeruginosa, Acinetobacter spp.* is due to decreased outer membrane permeability, increased efflux system, alteration of penicillin-binding protein and carbapenem-hydrolyzing enzymes carbapenemases.[13,14,]

Carbapenemase enzymes belong to the β-lactamase group that hydrolyse the amide bond of the four-membered β-lactam ring is the primary resistance mechanism.

β-Lactamases are divided into four classes; the active-site serine β-lactamases (classes A, C and D) and the zinc-dependent or metallo-β-lactamases (MBLs; class B).

The emergence and dissemination of acquired Metallo-β-lactamases, encoded by genes carried on mobile DNA elements, among major Gram-negative pathogens, including members of the family Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter* species have made the situation more worrying. [15] Plasmid mediates MBL genes spread to other species of Gram-nagative bacilli.[18]

The emergence of metallo-beta-lactamase (MBL) producing strains, is a therapeutic challenge, as these enzymes possess high hydrolytic activity effective even against carbapenems (imipenem, meropenem).[20] Therefore rapid detection of Metallo β -lactamases production is necessary to modify therapy and to initiate effective infection control to prevent their dissemination.

Institution of appropriate treatment is necessary to reduce the morbidity and mortality due to these organisms in hospitalized patients.[21]

The present study was aimed to investigate magnitude of NFGNB isolated from clinical samples and to evaluate MBL production by the phenotypic method among these isolates.

MATERIAL AND METHODS:

The present study was conducted in the Bacteriology lab of the Department of Microbiology in SMS medical college, Jaipur over for one year period from July 2019 to June 2020. Samples received were processed as soon as possible. Samples like Urine, Sputum, Wound swab, Blood, Endotracheal tube, Body fluids, Pus, CSF, etc. were received from patients admitted in different wards, ICU, and OPD patients.

Antimicrobial susceptibility testing was done by using Kirby Bauer's disc diffusion method as per CLSI guidelines 2020 using a commercially available antimicrobial discs. *Escherichia coli* ATCC 25922 & *Pseudomonas aeruginosa* ATCC 27853 were used as control organisms during the study.

Antimicrobial susceptibility testing of *Pseudomonas spp* for colistin was done by using minimum inhibitory concentration (MIC) testing.

Imipenem resistance NFGNB isolates were futher processed for MBL production.

Phenotypic Detection of MBL Positive Isolates.

Modified Hodge Test[12,13]

This test was originally described by the CDC for Carbapenemase

detection. When the test strain produces the enzyme Carbapenemase, it allows the growth of a carbapenem susceptible strain (*Escherichia coli* ATCC25922) toward a carbapenem disc. The positive result is a characteristic of cloverleaf indentation. A lawn culture of 1:10 dilution of 0.5 McFarland's standard *Escherichia coli* (ATCC 25922) broth was done on a Mueller Hinton Agar plate. A 10 µg imipenem disc is placed in the center of the plate. Test strains showing resistance/ reduced susceptibility to imipenem were streaked from the edge of the disk to the periphery of the plate in four different directions. After overnight incubation, the plate was observed for the presence of a clover-leaf-shaped zone of inhibition which was interpreted as Modified Hodge test positive.

Combined Disc Synergy Test (CDST) [14,15]

An overnight broth culture of the test strain of 0.5 McFarland opacity standards was inoculated on a Mueller Hinton agar (MHA) plate. Two imipenem (10µg) and imipenem+EDTA (10µg +750µg) discs were placed on MHA at a distance of 21mm from center to center. Zone of inhibition around imipenem and imipenem+EDTA disc were recorded after 16 to 18 hours of incubation at 37^{0}C. Zone enhancement of at least 7 mm in the imipenem+EDTA disc compare to the imipenem disc was interpreted as positive for MBL production.

Modified Carbapenemase Inactivation Method (mCIM) [16,17]

This test was done for *Pseudomonas spp.* only. Tryptic soy broth (2 ml) was inoculated with overnight culture test organism and to this broth 10 ug imipenem disc was added and incubated at 37^{0}C for 4 hours. A lawn culture of *E.coli* ATCC 25922 was prepared on an MHA plate and after drying this plate, the imipenem disc removed from tryptic soy broth was placed on this plate. This plate was incubated at 37^{0}C for 24 hours and the zone of inhibition was measured and interpreted as follows:

Zone size(mm)	Results	Interpretation
6-15mm or pin point colonies within 16-18mm zone	Positive	Carbapenemase detected
>19 mm	Negative	Carbapenemase not detected
16-18 mm or >19 mm zone within pinpoint colonies	Indeterminate	Test inconclusive

RESULTS

The present study included 200 isolates of Non-Fermenting Gram-Negative Bacilli (NFGNB) isolated from various clinical samples received in the Department of Microbiology, SMS Medical College, and attached hospitals.

Out of 200 isolates, the majority of the isolates (64%) were from inpatient departments followed by ICU (23.5%). Isolates from the outpatient department accounted for only 12.5%.

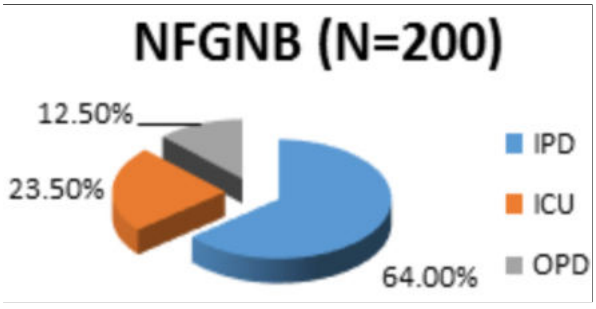

Figure No. 1: OPD/IPD /ICU Wise Distribution Of NFGNB

Out of these 200 NFGNB isolates, 133(66.5%) were isolated from male patients and 67(33.5%) from female patients.

Of these 200 NFGNB, 131 (65.5%) isolates from adult patients and 69 (34.5%) isolates from pediatric patients.

Among the 200 NFGNB, 112 isolates (56%) were identified as *Pseudomonas* species, 56 isolates (28%) were *Acinetobacter* species, 26 (13%) were *Burkholderia* spp. Other NFGNB isolated were *Stenotrophomonas spp., Sphingomonas spp*. 6 were each.

Most of the NFGNB were isolated from blood (36.5%) followed by respiratory sample (23%), urine (20%), pus (18.5%), CSF (2%). *Pseudomonas* species are mainly isolated from respiratory samples (34.82%) sample. *Acinetobacter* species are mainly isolated from blood (58.92%). *Burkholderia* strains were isolated from blood (26/200, 13%).

Table No. 1. Organisms Isolated (N=200)

NFGNB Isolates	Number	Percentage
Pseudomonas Spp	112	56%
Acinetobacter Spp	56	28%
Burkholderia Spp	26	13%
Others	6	3%
TOTAL	200	100%

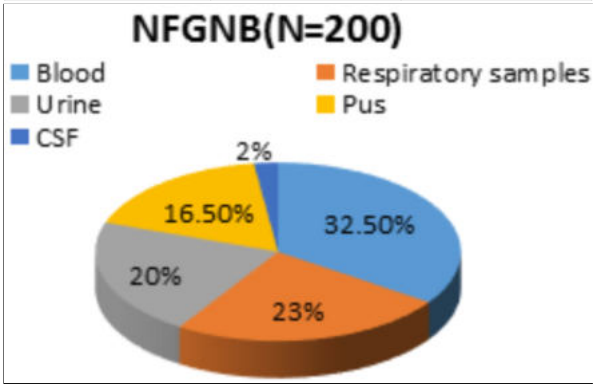

Figure No. 2. Sample Wise Distributions Of NFGNB (N=200)

Antimicrobial sensitivity testing showed that among the *Pseudomonas* species all the isolates (100%) were sensitive to colistin followed by cefoperazone-sulbactam (45.53%), tobramycin (44.64%), gentamicin (42.85%), imipenem (31.25%), and Piperacillin-Tazobactum (19.64%)

Among *Acinetobacter* species maximum susceptibility was observed to colistin and tigecycline (100% each) followed by minocycline (98.21%). The least susceptibility was seen against ceftazidime and cefotaxime 21.42% each.

For *Burkholderia* species, maximum susceptibility was observed with meropenem (88.46%) and least susceptibility with chloramphenicol (30.76%).

Out of 168 NFGNB (*Pseudomonas* spp. & *Acinetobacter* spp) isolate 82 (48.80%) were MDR (Multidrug resistance) and 40 (23.80%) isolates were possible XDR (Extended drug resistance). No isolates was found to be PDR (Pandrug resistance).

Out of 200 NFGNB, 168 isolates (112 *Pseudomonas* + 56 *Acinetobacter* spp) were analysed for imipenem resistance.

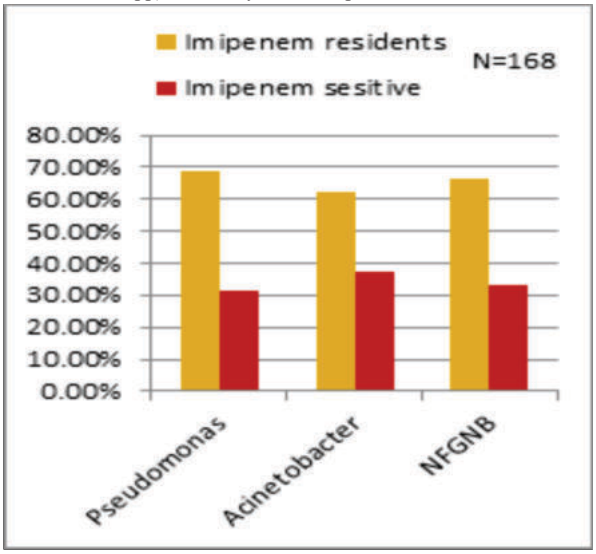

Figure No.3: Resistance To Imipenem Of NFGNB Isolates (N=168)

Imipenem resistant isolates further subjected to MBL detection by phenotypic methods including modified Hodge test (MHT), combined disc synergy test (CDST), modified Carbapenemase detection method (mCIM).

It was observed that out of 112 imipenem resistant NFGNB isolates 102(91.07%) were positive for MBL production.

Table No. 4: MBL Detection By Different Phenotypic Methods In 112 Imipenem Resistant Isolates

Test	MHT		CDST		MCIM	
	Positive	Negative	Positive	Negative	Positive	Negative
Pseudomonas (77)	67 (87.01%)	10 (12.98%)	70 (90.9%)	7 (9.09%)	70 (90.9%)	7 (9.09%)
Acinetobacter (35)	30 (85.71%)	5 (14.28%)	32 (91.42%)	3 (8.57%)	*	*

Among *Pseudomonas spp,* 90.9% were positive by mCIM and CDST while 87.01% were positive by MHT. *Acinetobacter* spp revealed 91.42 % positivity by CDST and 85.71% by MHT.

Table No.5 : Metallobetalactase Production In *Pseudomonas* And *Acinetobacter* Isolates (Positive By CDST And mCIM*)

Organism	Imipenem resistant isolates No.	MBL producer No.(%)	MBL Nonproducer No.(%)
Pseudomonas spp.	77	70 (90.9%)	7 (9.1%)
Acinetobacter spp.	35	32 (91.42%)	3 (8.57%)
Total	112	102 (91.07%)	10 (8.93%)

*mCIM test not recommended for *Acinetobacter* by CLSI guideline

Among imipenem resistant *Pseudomonas* 90.9% were MBL producer and among imipenem resistant *Acinetobacter* 91.42% were MBL producer.

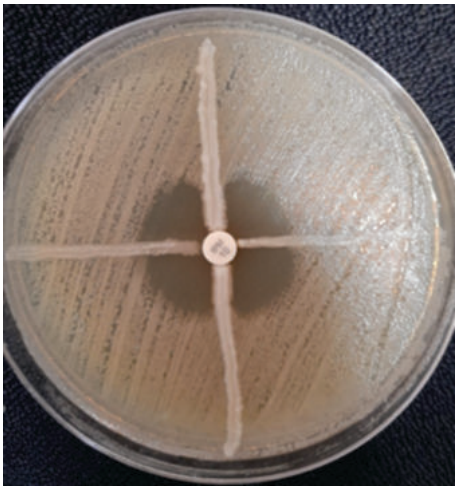

Image No. 1: Modified Hodge Test (MHT)

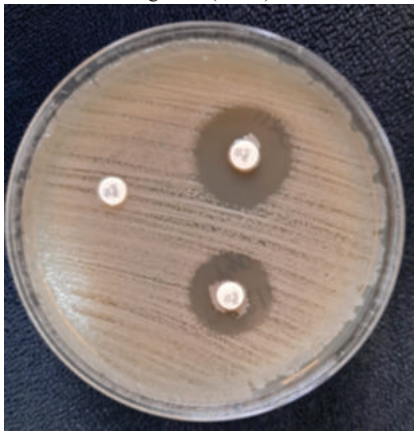

Image No. 2: Modified carbapenem inactivation method (mCIM test)

DISCUSSION

Non fermenter Gram Negative Bacilli are ubiquitous in nature. Although these organisms were considered as non-pathogenic or commensals or contaminants but in recent time the pathogenic potential of NFGNB has been known. (konman)

Present study was conducted on 200 clinical isolates of NFGNB. The most common NFGNB isolated in our study was *P. aeruginosa* (112, 56%), followed by *A. baumannii* (56, 26%) which is similar to the results obtained by Malini *et al*[18]. who reported *P. aeruginosa* as the most common isolate accounting for 104/189 (53.8%) isolates, followed by *A. baumannii* (43/189, 22.2%).

Further gender based distribution showed male preponderance (Male-66.5%, Female-33.5%), similarly male preponderance was also observed by Maniyan G. et al[19] (male-65.4%, female-34.5%).

In our study, the highest number of isolates were isolated from pus swabs (40%), which is in accordance with the observations made by Rit *et al*.[21] who also reported pus swabs as the source of maximum percentage of the isolates i.e., 27.86%.

NFGNB isolates are known pathogens for infections in hospitalized patients. In present study it was found that cases were recovered more from hospitalized patients IPD patients being 64% and ICU being 23.5% as compared to OPD 12.5%. These findings are comparable with the other studies done by Yeruva S. et al(OPD- 2.3%, IPD-23.8%) but Yeruva S. et al[44] reported more NFGNB isolates from ICU (69.0%). Out of 200 NFGNB, 168 isolates (112 *Pseudomonas* + 56 *Acinetobacter* spp) were analysed for imipenem resistance.

Out of 112 *Pseudomonas* isolates 77 (68.75%) were found to be imipenem resistant and among 56 *Acinetobacter* isolates 35 (62.5%) were imipenem resistant.

These isolates showing resistance to imipenem were further subjected to detection of MBL production.

MBL among imipenem resistant isolate can be detected by various phenotypic methods and molecular methods. Although PCR based molecular methods are gold standard for MBL detection, their use is mainly restricted to research purpose.

In our study various phenotypic methods including and mCIM (Modiied Carbapenemase Inactivation Method), CDST (Combined Disc Synergy test), MHT (Modified Hodge Test) were used for MBL detection.

Out of 77 imipenem resistant *Pseudomonas* 70 (90.9%) were found to be positive for MBL production by mCIM and CDST method and 67(87.01%) were positive by MHT method.

Out of 56 *Acinetobacter* spp 35 were imipenem resistant strains, of which 32 (91.42%) were positive by CDST and 30 (85.71%) were positive by Modified Hodge test. mCIM test was not performed for *Acinetobacter spp* as it was not recommended for *Acinetobacter* spp by CLSI.

In our study those imipenem resistant isolates positive by mCIM and CDST method were considered as MBL producers. Accordingly 99.9% (70/77) *Pseudomonas* isolates and 91.42% (32/35) *Acinetobacter* isolates were reported to be MBL producer. These findings are comparable with the other studies done by Pandya N. et al (2011) [22] and Verma N et al (2019) [20] reported 96.30% and 93.17% respectively.

CONCLUSION:

The occurrence of MBL-positive isolate in a hospital setting poses a therapeutic problem, as well as a serious concern for infection control management. The accurate identification and reporting of MBL-producing *P. aeruginosa* and *A.baumani* will aid infection control practitioners in preventing the spread of these multidrug-resistant isolates.

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Nil.

Conflicts Of Interest
There are no conflicts of interest

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