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Original Research Paper

Microbiology

PREVALANCE OF CARBAPENEMASE PRODUCING NON- FERMENTERS FROM VARIOUS CLINICAL ISOLATES AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Background: Aerobic non-fermenting Gram-negative bacilli (NFGNB) are non-sporing bacilli that either do not utilize glucose as a source of energy or utilize it oxidatively. The important members of the group include Pseudomonas aeruginosa, Acinetobacter baumannii, Acinetobacter lwoffii, Stenotrophomonas maltophilia, and Burkholderia cepacia. They cause various infections such as septicaemia, meningitis, pneumonia, urinary tract infections and surgical site infections (SSI). NFGNB pose significant challenges in health care settings because of their multiple, intrinsic, or acquired antibiotic resistance. Carbapenems are the drugs of last resort for the treatment of multi-drug resistant infections in non-fermenters. The rate of carbapenem resistance in NFGNB is gradually increasing worldwide that need to be considered seriously. **Objective:** The present study determines the prevalence of Carbapenemase producing NFGNB, isolated from various clinical specimens, in this geographical area by the phenotypic tests named Carba NP Test. **Materials And Methods:** This study was conducted at Department of Microbiology, Tertiary Care Centre from May 2019 to December 2019. A total of 3856 clinically significant isolates of Non-Fermenting Gram Negative Bacilli (NFGNB) were included in study obtained from clinical samples like urine, blood, pus, sputum, pleural fluid, peritoneal fluid,endotracheal aspirations, drain tip and CSF received for culture and sensitivity in department of microbiology from both outpatients and inpatients. **Results:** This study shows 30.86% NFGNB as Carbapenem resistant. Out of these 15.79% were Carbapenemase producers giving CarbaNP test results positive predominantly from Swab (45.21%), mostly received from Wards(55.85%). The most common Carbapenemase producing NFGNB was P.aeruginosa (56.38%) followed by A.baumannii (36.70%) with male(67.55%) prepordance in the study; most commonly in age group of 30-39yrs (19.14%).Carbapenemase producing NFGNB were resistant to maximum classes of antibiotics but they were 100% susceptible to Colistin. **Conclusion:** From present study it can be concluded that prevalence of Carbapenamase producing NFGNB holds a significant percentage. Detection of Carbapenemase producers has epidemiological significance for the prevention of further spread of carbapenem resistance in the society, which depends mostly on early detection of carriers producing these Carbapenemases. Therefore, this study can be helpful for early detection of carbapenem resistance and their transmission.

KEYWORDS : NFGNB, Multiple Drug Resistant, Carbapenemase, CarbaNP Test,

INTRODUCTION

Aerobic non-fermenting Gram-negative bacilli (NFGNB) are taxonomically diverse group of non-sporing bacilli that either do not utilize glucose as a source of energy or utilize it oxidatively[1]. They are ubiquitous in nature, found as saprophytes inhabiting soil or water and some are also found as commensals in human and animal gut[2]. NFGNB accounts for nearly 15 % of all gram-negative bacilli cultured from clinical specimens in a clinical microbiology laboratory[2,3]. The important members of the group include Pseudomonas aeruginosa, Acinetobacter baumannii, Acinetobacter lwoffii, Stenotrophomonas maltophilia and Burkholderia cepacia. They cause various infections such as septicaemia, meningitis, pneumonia, urinary tract infections and surgical site infections (SSI)[2].

The burden of resistance is presumably more due to the higher rate of empirical antimicrobial treatment than with the virulence of particular strains[4]. Carbapenems are the drugs of last resort for the treatment of multi-drug resistant infections in non-fermenters and other gram negative bacilli[5]. NFGNB are increasingly acquiring resistance to carbapenems[6]. Mechanism of carbapenem resistance in non-fermenting Gram-negative bacilli occurs by various mechanisms including Carbapenemase production, decrease permeability due to loss of porin channels, overexpression of efflux pump and changes in penicillin binding proteins[7].

The alarming level of carbapenem resistance has presented particular challenges for the management of a variety of infections caused by non-fermenters because of the low permeability of the outer bacterial membrane to several antibiotics, including, but not limited to, the carbapenems[8,9]. The dissemination of plasmids carrying resistant determinant genes from one species to another makes organisms refractory to the common antibiotics used in clinical practice. Continuous monitoring and rapid detection of these virulent organisms may check their spread and play a vital role in infection control[10]. World Health Organization (WHO) has categorized carbapenem resistant Pseudomonas aeruginosa and Acinetobacter baumannii as Priority 1- Critical organisms for the research and development of newer antibiotics[11].

MATERIAL AND METHODS

A total of 3856 clinically significant, consecutive, non-repetitive isolates of Non-Fermenting Gram Negative Bacilli (NFGNB) were included in this study. The isolates were obtained from heterogenous clinical samples like urine, blood, pus, sputum, pleural fluid, peritoneal fluid, endotracheal aspirations, drain tip and CSF received for culture and sensitivity in department of microbiology from both outpatients and inpatients of Tertiary Care Centre.

The specimens were inoculated onto Blood Agar and Mac Conkey agar and incubated over night at 37 degree. Isolated colonies were identified by Characteristic appearance on respective media, Gram staining and were confirmed by pattern of biochemical reactions using standard methods.

The antimicrobial susceptibility testing was performed with the help of the Kirby Bauer disc diffusion on Muller Hinton Agar plates.The result was interpreted as per the Clinical and Laboratory Standards Institute (CLSI) guidelines. Organisms showing resistance of one of the carbapenem drugs including imipenem and meropenem with susceptibility zones of <=23mm and 23mm were identified as carbapenem resistant and were subjected to one of the Carbapenemase detection Phenotypic screening test known as CarbaNP Test.

OBSERVATION AND RESULTS

A total of 24880 were received at laboratory from May 2019 to December 2019 . Out of these, 13134 samples were positive for culture and rest were either culture negative or had normal flora.Out of these, 10452 were Gram negative bacteria, 6596 (63.10%) were fermenters and 3856(36.89%) were Non-fermenters. Out of 3856 Non-fermenters , 1190(30.86%) were carbapenem resistant. Out of these 188 (15.79 %) isolates were Carbapenemase producing non fermenters giving Carba NP test positive.

Out of 188 CarbaNP positive non fermenters, 85(45.21%) were isolated from Swab , 60(31.91%) Urine, 21(11.17%) from Sputum, 5(2.65%) from Blood and rest 17(9.04%) from the various Fluids. The Sample wise distribution of carbapenamase producing NFGNB is shown in the table 1:

Table-1

Samples	Total	Percentage (%)
Swab	85	45.21
Urine	60	31.91
Sputum	21	11.17
Blood	5	2.65
ET secretion	7	3.72
Fluids		
• CSF	3	1.39
• Pus	2	1.06
• Body fluid	2	1.06
• Pleural fluid	10	0.53
• Drain	1	0.53
Tissue	1	0.53

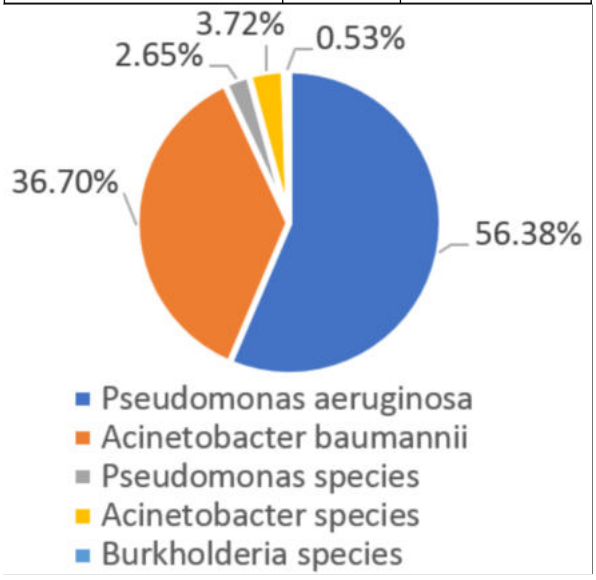

Figure-1: *Species-wise Distribution Of Carbapenamase Producing NFGNB*

Out of 188 samples , the highest number of isolates giving Carba NP test positive were of Pseudomonas aeruginosa 106 (56.38%) ,followed by Acinetobacter baumannii 69 (36.70 %).The rest comprises of Pseudomonas species , Acinetobacter species and Burkholderia species.

Carbapanamese producing non fermenters were isolated more from the males 127(67.55%) compared to Females 61 (32.44%). The age group 30-39 years has the highest percentage of isolates whereas the least number of isolates was in age group 0-9 years.

Among the 188 CarbaNP positive isolate , the highest number of isolates came from wards (55.85%) followed by ICU (38.82 %) and the least number were from OPD (5.31%).

Table-2

AGE GROUP (years)	NUMBER OF ISOLATES	PERCENTAGE (%)
0-9	8	4.25
10-19	27	14.36
20-29	31	16.48
30-39	36	19.14
40-49	18	9.57
50-59	29	15.42
60-69	25	13.29
70 and above	14	7.44

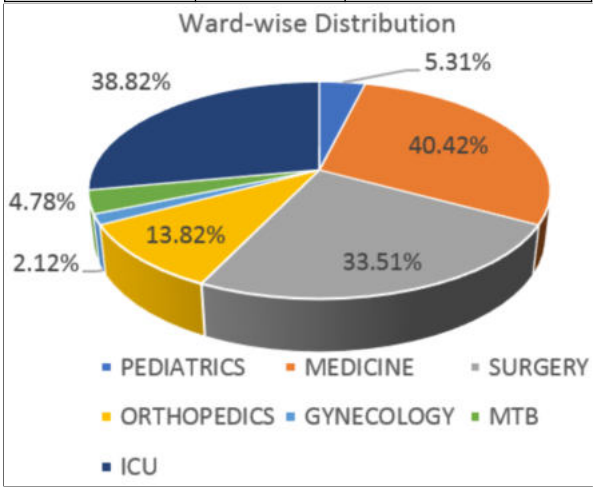

Figure 2: *Ward-wise Distribution Of Carbapenamase Producing Isolates*

All the Pseudomonas and Acinetobacter isolates showed no growth on Colistin Screen Agar and were found 100% sensitive. Other drug resistance pattern in the study are shown in the charts below.

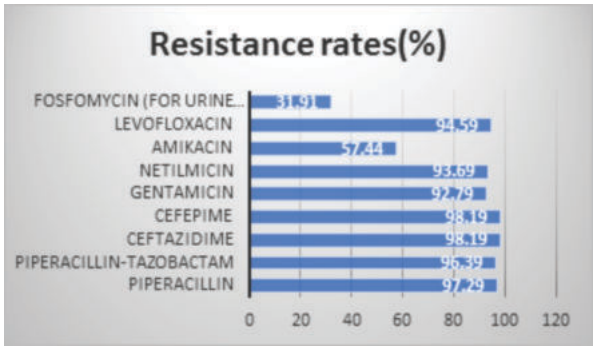

Chart-1: *Antimicrobial resistance rates in carbapenamase producing Pseudomonas*

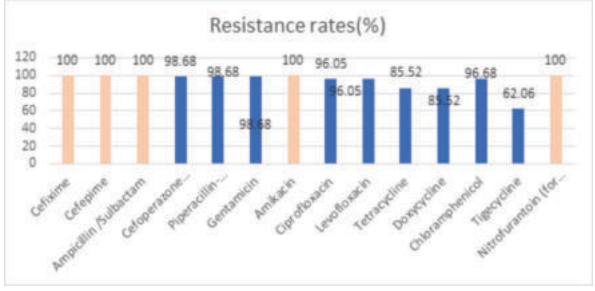

Chart-2: *Resistance rates in Carbapenemase producing Acinetobacter isolates*

DISCUSSION:

The isolation in the current study is in concordance with the study done by Barnali Kakati et al [12](27.04%). It is higher compared to studies like Vijaya D et al [13](21.80%) and Jane

Esther et al [14] (11.06%) which could be due to rise in the rate of Community and Healthcare Associated Infections in the recent years.

Out of 3856 NFGNB, 1190 (30.86%) isolates were Carbapenem Resistant.The prevalence of carbapenem resistant NFGNB in our study is 30.86% which is in concordance with R.P.R Suyambu et al [15](32%). It is much higher than the study done by Jane et al (8.7%) in 2015. This shows the increasing resistance of NFGNB for the Carbapenems.

Out of 1190 Carbapenem resistant NFGNB, 188 (15.79%) were Carbapenamase producers giving CarbaNP Test positive. Findings of the current study was in concordance with Jane Esther et al(15.39%)and slightly higher than Aishwarya JR et al [17] (13.33%).

The Carbapenamase producing NFGNB most commonly encountered in our study are Pseudomonas aeruginosa (56.38%) and Acinetobacter baumannii(36.70%). The current study results are similar to Jane Esther et al whereas the study done by R.P.R Suyambu shows higher isolation rates of Pseudomonas and lower isolation rates of Acinetobacter baumannii. The current showed prevalence of Carbapenamase producing NFGNB more in Males(67.55%) as compared to females (32.44%).Our study gave the similar results in Gender-wise distribution as Jane Esther et al(67%) and Barnali Kakati et al(77%).The current study showed maximum Carbapenamase production in age group 30-39yrs (19.14%) followed by 20-29yrs (16.48%).In our study maximum Carbapenamase producing NFGNB were isolated from Wards(55.85%) followed by ICU(38.82%) and least from the OPD(5.31%) which are in concordance with Benachimardi et al[18]. In our study more Isolates were from Medical wards (40.42%) followed by Surgical ward (33.51%) as compared to study done by Jane Esther et al which showed more prevalence in Surgical wards (61.5%). Also our study results are different from R.P.R Suyambu et al and Sidhu et al 2010 which shows maximum presence in ICU as compared to wards. In our study maximum Carbapenamase producing NFGNB were isolated from Swab (45.21%) followed by Urine (31.91%) and Sputum (11.17%). Studies like Jane Esther et al and others showed more isolates from Pus (40.4%) and R.P.R Suyambu et al and Sidhu et al 2010 showed more isolation from Respiratory Samples. These results may vary depending upon the sample type received in different hospital laboratories.

In Current study, Susceptibility result reported as 'Resistant' of Piperacillin 97.29%, Piperacillin-tazobactam 96.39%, Cephems 98.19%, Aminoglycosides 93.39%, Levofloxacin 94.59% and Fosfomycin 31.91% (for urine isolates) for Carbapenemase producing Pseudomonas aeruginosa. The Colistin showed 100% sensitivity. Burkholderia spp. showed 100% resistance to all the drugs tested including Colistin. This showed concordance with study done by Aishwarya JR et al showing 100% resistance to Cephems, almost 100% resistance to piperacillin and piperacillin-tazobactam. Aminoglycosides resistance is only 50% which is much lower than the current study. All the urine isolates showed 100% resistance for Nitrofurantoin in both the studies. Also Colistin showed 100% sensitivity in both the studies. In Current study, Susceptibility result reported as 'Resistant' of Beta-lactams 98.34%, Cephems (including cephalosporins 1,2,3 and 4) 100%, Aminoglycosides 98.34%, Tetracyclines 85.52%, Tigecycline 62.06%, nitrofurantoin 100% in urine isolates for Carbapenemase producing Acinetobacter baumannii. The Colistin showed 100% sensitivity. Maximum isolates of Acinetobacter showed multiple drug resistance. The study done by Barnali Kakati et al showed 86% resistance to Beta-lactams, 91% resistance to Cephems, 75.5% resistance to Aminoglycosides and 80% resistance to Tetracyclines which is slightly lower than the resistance shown in current study. Colistin is proving the most effective drug in the current scenario.

CONCLUSION

Present study was carried out with the aim to find the prevalence of Carbapenemase producing non-fermenters from various samples and detection of possible antimicrobial resistance in them.

Detection of Carbapenemase producers has epidemiological significance for the prevention of further spread of carbapenem resistance in the society, which depends mostly on early detection of carriers producing these Carbapenemases. Therefore, this study can be helpful for early detection of carbapenem resistance and their transmission. The phenotypic method named Carba NP test is reliable, inexpensive and easy screening tests, reducing remarkably the time for the first feedback information for clinical laboratories.

Among the limitations of Carba NP test, it can be mentioned that standardization regarding the concentration of reagents is necessary for proper interpretation and reproducibility. Inability to perform the recent modification of the test and molecular analysis of the strains by PCR would have enhanced the quality of this study, and can be mentioned as a limitation. In future, further analysis based on molecular techniques is needed for validation of the results of this study.

With the increasing acquired resistance among NFGNB to major classes of antimicrobial agents, colistin still hold hope with resistance of lower rates. Standard method of detection of such drug resistance, like micro broth dilution essays, should be adopted for susceptibility testing.

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