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RESEARCH ARTICLE

ANTIMICROBIAL RESISTANCE PATTERN AMONG NON-FERMENTING GRAM NEGATIVE BACILLI ISOLATED FROM CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL IN INDIA.

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Abstract

Background: Non-fermenting gram negative bacilli have emerged as important pathogens and their isolation is increasing among hospital acquired infections. Many are innately resistant to most antibiotics and often multidrug resistant posing difficulty in treatment. **Aim:** The aim of the study was to determine the prevalence of Non-fermenting gram negative bacilli isolated from various clinical samples and to evaluate their antimicrobial resistance pattern. **Materials and methods:** This was a retrospective study of Non-fermenting gram negative bacilli isolated from clinical samples over a 2-year period from July 2015 to June 2017. Non-fermenting gram negative bacilli were grown on Blood agar and MacConkey agar and identified by standard methods including gram staining, motility and appropriate biochemical tests. Antimicrobial susceptibility testing was done by Kirby Bauer disc diffusion method as per Clinical and Laboratory Standards Institute guidelines and resistance pattern was studied. **Results:** A total of 323 Non-fermenting gram negative bacilli were isolated from 10819 samples with *Pseudomonas* being the most common species followed by *Acinetobacter* species. *Pseudomonas* species showed high resistance to Piperacillin-Tazobactam (60.6%). *Acinetobacter* species have shown higher resistance to Meropenem (42.1%) and Ceftazidime (73.7%). **Conclusion:** *Acinetobacter* species have emerged as highly resistant microorganisms causing infections especially in the Intensive Care Unit settings. However resistance to Colistin was not seen.

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Introduction:-

Non-fermenting Gram-negative bacilli (NFGNB) or non-fermenters are a group of organisms that are widely distributed in nature as saprophytes or as commensals and pathogens for man, plants, animals and insects. They are aerobic gram negative bacilli that undergo oxidative breakdown of carbohydrate yielding relatively small amount of acid. Some of these are regular contaminants of the hospital environment and are increasingly recognized as opportunist pathogens associated with infections that range from bronchopneumonia to septicemia in immunocompromised patients. Predisposing factors include the presence of a prosthesis, endotracheal intubation,

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intravenous catheters and prior antibiotic therapy in a seriously ill patient in a hospital.¹ They exhibit high intrinsic resistance to many antibiotics at levels attainable in body tissues and as a result of liberal and empirical use of antibiotics most of these organisms have in recent times acquired resistance to many routinely used antibiotics.

Aims and objects:-

1. To determine the prevalence of Non-fermenting Gram negative bacilli isolated from various clinical samples
2. To evaluate their antimicrobial resistance pattern

Material and methods:-

This retrospective study was done at the department of Microbiology, Regional Institute of Medical Sciences, Imphal over a two year period from July 2015 to June 2017. The study participants were patients who were referred to our department for culture and antibiotic susceptibility testing from various locations including outpatient department (OPD), inpatient department or clinical wards and Intensive care units (ICU) and were found to be culture positive for non-fermenters. Clinical specimens such as sputum, pus, urine, blood, catheter tip and other body fluids sent for culture and sensitivity testing were inoculated on Blood agar and MacConkey agar and incubated aerobically at 37°C for 18–24 hours. Organisms showing growth were identified using standard identification methods and appropriate biochemical tests. Characters assessed include colony morphology, gram staining, motility, oxidase, catalase, indole, urease, nitrate reduction, citrate utilization and oxidation-fermentation reaction of glucose.¹ Few species were confirmed by automated Vitek 2 Compact system (Biomérieux).

Antibiotic susceptibility testing to Imipenem, Meropenem, Piperacillin-Tazobactam, Ceftazidime, Ceftazidime-Clavulanate, Cefepime, Gentamicin, Ciprofloxacin, Trimethoprim-sulfamethoxazole and Colistin was performed with the help of the Kirby–Bauer disc diffusion method using commercially available antibiotic discs (HiMedia) on Mueller–Hinton agar. Briefly, a 0.5 McFarland standard inoculum of a pure culture of the organism is prepared and a lawn culture is plated onto Mueller-Hinton agar. Antibiotic discs were placed onto the agar and the plates were incubated for 18-24 hours.¹ Interpretation was done according to the Clinical and Laboratory Standards Institute guidelines.¹³

Results:-

Of the 10819 clinical samples collected, 323 non-fermenters were isolated making an isolation rate of 3%. Of these 103(31.9%) isolates were from sputum, 93(28.8%) from urine, 67(20.7%) from pus, 38(11.8%) from tracheal aspirates, 10(3.1%) from blood, and 12(3.7%) from other samples like bronchoalveolar lavage (BAL) fluid, stool, catheter tip and other body fluids. *Pseudomonas* was the most common non-fermenter isolated (61.6%) followed by *Acinetobacter* (37.2%), 2 *Burkholderia cepacia* complex, 1 *Stenotrophomonas maltophilia* and 1 *Chryseobacterium indologenes*.

Among the *Pseudomonas* species, 34.7% were isolated from sputum, 26.6% from urine, 22.6% from pus, 12.6% from tracheal aspirate and 0.5% from blood. Majority of *Acinetobacter* species were isolated from urine (31.6%) followed by sputum (28.3%), pus (18.3%), tracheal aspirate (10.8%) and blood (5.8%). One isolate each for *Stenotrophomonas maltophilia* and *Chryseobacterium indologenes* were isolated from urine. Two species belonging to *Burkholderia cepacia* complex were isolated from blood (Table 1).

Table 1:-Non-fermenting gram negative bacilli isolated from various clinical samples

Organism	Sputum	Urine	Pus	Tracheal aspirate	Blood	Others*
<i>Pseudomonas</i> Species	69(34.7%)	53(26.6%)	45(22.6%)	25(12.6%)	1(0.5%)	6(3%)
<i>Acinetobacter</i> Species	34(28.3%)	38(31.6%)	22(18.3%)	13(10.8%)	7(5.8%)	6(2.5%)
<i>Burkholderia cepacia</i> complex					2(100%)	
<i>Stenotrophomonas maltophilia</i>		1(100%)				
<i>Chryseobacterium indologenes</i>		1(100%)				

*BAL, pleural fluid, ascitic fluid, catheter tip, stool

One hundred eighty eight isolates were from clinical wards, 82 from outpatient department and 53 from ICU settings. *Pseudomonas* species were isolated more frequently from clinical wards (58.8%) followed by outpatient department (24.6%) and ICUs (16.6%). Similarly *Acinetobacter* species isolated from ward, outpatient and ICUs were 66.7%, 27.5% and 15.8% respectively (Table 2).

Table 2:-NFGNB isolated from outpatient, inpatient and intensive care units

Organism	Location			Total(n)
	Outpatient	Inpatient	Intensive care unit	
<i>Pseudomonas</i> species	49(59.8%)	117(62.2%)	33(62.2%)	199
<i>Acinetobacter</i> species	33(40.2%)	68(36.2%)	19(35.8%)	120
<i>Burkholderia cepacia</i> complex		1(0.5%)	1(1.9%)	2
<i>Stenotrophomonas maltophilia</i>		1(0.5%)		1
<i>Chryseobacterium indologenes</i>		1(0.5%)		1
Total (n)	82	188	53	323

Resistance profile of *Pseudomonas* and *Acinetobacter* species is shown in Table 3. In the ICUs, 60.6% of *Pseudomonas* were resistant to Piperacillin-Tazobactam whereas *Acinetobacter* species showed higher resistance to Ceftazidime (73.7%), Meropenem (42.1%) as well as to Cotrimoxazole (57.9%) and Gentamicin (52.6%). About 64.7% of *Acinetobacter* species isolated from clinical wards were found to be resistant to Ceftazidime. However resistance to Colistin was not seen in both *Pseudomonas* and *Acinetobacter* species. Two isolates of *Burkholderia cepacia* complex were found to be resistant to Gentamicin and Ciprofloxacin (data not shown).

Table 3:-Antibiotic resistance pattern of *Pseudomonas* and *Acinetobacter* species isolated from various locations

Drugs	<i>Pseudomonas</i> species			<i>Acinetobacter</i> species		
	OPD (%)	Ward (%)	ICU (%)	OPD (%)	Ward (%)	ICU (%)
Imipenem	4.1	3.4	12.1	0	20.6	15.8
Meropenem	10.4	17.1	9.1	18.2	27.9	42.1
Piperacillin- tazobactam	18.7	22.2	60.6	12.1	25	31.6
Ceftazidime	20.8	37.6	24.2	33.3	64.7	73.7
Ceftazidime-clavulanic acid	10.4	18.8	30.3	33.3	72.1	57.9
Cefepime	6.3	12.8	12.1	3	13.2	15.8
Gentamicin	2	11.9	18.2	0	22.1	52.6
Ciprofloxacin	14.5	17.9	18.2	18.2	32.4	47.4
Colistin	0	0	0	0	0	0
Trimethoprim-sulfamethoxazole	-	-	-	21.2	47.1	57.9

Discussion:-

NFGNB are now considered as potential pathogens established by their frequent isolation from clinical samples and their association with a variety of infections.³ They are also known to cause nosocomial infections and outbreaks and resistance to antimicrobials has increased over the years.

A number of studies on NFGNB have reported a wide range of isolation rates. In the present study NFGNB were isolated in 3% of clinical samples and this was parallel to the results of studies by Bruno *et al* and Benachinmardi *et al* whose isolation rates were 2.18% and 3.58% respectively.^{6,7} On the other hand, Samanta *et al*, Vijaya *et al* and Sidhu *et al* have reported higher rate of isolation i.e., 10%, 21.80% and 45.9% respectively.^{2,8,9} Most of the isolates in our study were from sputum samples followed by urine, pus and tracheal isolates. It was observed that NFGNB isolated from clinical wards were much more than those isolated from OPD accounting for 58.2% of all NFGNB.

Pseudomonas species was the most common non-fermenter followed by *Acinetobacter* species and this is in concordance with most of other studies.^{5,11}

Resistance to antibiotics among the NFGNB may vary widely from place to place even within a given geographical area but those isolated from the hospital settings are often multidrug resistant. Because of these variations a surveillance of the nosocomial pathogens for resistant organisms in a particular hospital set up is needed in order to guide appropriate selection of empirical therapy and to develop an antibiotic policy.^{3,10}

In our study higher resistance to antibiotics among the non-fermenters was seen in the Intensive care units as these settings often harbor highly resistant pathogenic strains. Among the *Pseudomonas* species high level of resistance was seen for Piperacillin-Tazobactam (60.6%) and is probably due to the frequent use of this drug for empirical therapy. Low levels of resistance were observed for Cefepime (12.1%), Ciprofloxacin (18.2%) and Ceftazidime (24.2%) in contrast to other studies who reported 60-70% resistance to these antibiotics.^{5,11} Resistance to Gentamicin, though not very pronounced, was seen to be increasing in comparison to in-patient and outpatient. *Acinetobacter* species isolated from ICUs were highly resistant to most antibiotics like Ceftazidime, Meropenem, Co-trimoxazole and Gentamicin a similar finding reported by many researchers.^{3,5} These glucose non-fermenters have the ability to develop resistance rapidly to almost all available antibiotics and make them particularly dangerous in hospital settings. Resistance to Piperacillin-Tazobactam (31.6%) was much higher than those of in-patient and outpatient departments. Imipenem resistance was seen in 15.8% among ICUs and 20.6% among inpatient isolates with an overall resistance of 14.2%. Though Imipenem resistance in our study was lesser in comparison to studies by Taneja *et al* and Juyal *et al* who reported higher overall resistance of 36.4% and 30.54% respectively,^{3,12} but emerging resistance to this group of drug is of major concern because these are considered as drugs of last resort.

Therefore studying and analyzing resistance to antibiotics among NFGNB is important for every healthcare setting, as infections caused by such strains can significantly increase the morbidity especially among the high risk patients due to their resistance to multiple antibiotics. These organisms by virtue of their adaptation in the hospital environment can also transfer resistance to other susceptible bacteria by horizontal gene transfer.³

Conclusion:-

NFGNB are important bacteria causing a wide range of infections and nosocomial outbreaks. Increasing resistance to multiple antibiotics among NFGNB especially in health care facilities is of particular concern especially to Carbapenem group of drugs. It has been observed that Intensive care units are notorious for harboring such highly resistant microorganisms. Unhygienic practices in hospital, especially contaminated hands of health care workers, and warm hospital environment promote colonization by these multidrug resistant organisms. Hence proper and rational use of antibiotics and improved infection control measures coupled with surveillance will be of utmost importance to prevent and halt the progress of antimicrobial resistance among NFGNB in the healthcare setting.

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