

source of energy or utilize it by oxidative mechanism rather than fermentative pathway. Nonfermenters are most important cause of nosocomial infection. They have been incriminated in infections such as meningitis, septicemia, pneumonia, urinary tract infection, surgical site infections. Nonfermenting organisms are intrinsically resistant to many antibiotics and are known to produce ESBL and metallobetalactamases.

Objectives: 1. To isolate and identify nonfermenting gram negative bacilli (NFGNB) from various clinical specimens

2. To study the antimicrobial susceptibility pattern of isolated NFGNB

**Material and Method:** This is a cross sectional study carried out from the period of  $1^{st}$  Oct 2015 to  $30^{th}$  June 2017. All samples received from various clinical inpatient and outpatient departments were subjected to culture and sensitivity.

Selection criteria - Inclusion criteria-All isolates which do not acidify butt of TSI (Triple sugar iron) slant were processed.

Exclusion criteria- All isolate which acidify butt of TSI slant were excluded.

Nonfermenters were identified by standard microbiological technique. Antimicrobial susceptibility test was done by Kirby-bauer disc diffusion test.

**Result-** During the study period, total 228 (10.88%) nonfermenters were isolated. Maximum number of nonfermenters were isolated from miscellaneous specimen (28.08%) followed by sputum (27.64%), pus (22.80%), urine (13.15%), blood (4.83%), fluid (3.50%). *Pseudomonas aeruginosa* (61.84%) was the most commonly isolated nonfermenter. It was most susceptible to polymyxin B (78%) followed by tobramycin (64.53%), gentamicin (63.82%), ciprofloxacin (61.70%), meropenem (60.82%) and least susceptible to ticaracillin (10.63%). *A. baumannii* (5.28%) was the second most isolated NFGNB. It was most susceptible to gentamicin (75%) and the least susceptibility was reported to co-trimoxazole (25%).

**Conclusion-**NFGNB are emerging as an important nosocomial pathogen. Multiple drug resistance in nonfermenters limits therapeutic option and is making the treatment difficult. It is important to establish clinical significance of NFGNB before it is considered as pathogen. As they have potential to survive in the hospital environment, infection control practices need to be followed strictly.

KEYWORDS : Nonfermenters, Nosocomial infection, Multidrug resistant infection,

# INTRODUCTION

Nonfermenters are the heterogeneous group of gram negative bacilli that are aerobic, nonsporing which cannot utilize carbohydrates and therefore are not able to ferment carbohydrate. This group includes organisms like *Pseudomonas spp., Acinetobacter spp., Alcaligenes spp., Stenotrophomonas maltophilia, Burkholderia cepacia* complex. Currently *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are the most common isolated nonfermenter pathogens<sup>(1,2)</sup> Nonfermenters are ubiquitous in environment and are often considered as contaminant in clinical laboratory. But the pathogenic potential of these organisms has been established beyond doubt because they are frequently isolated from clinical specimens and associated with the diseases.<sup>(6)</sup> Nonfermenters account for about 15% of all bacterial isolates from clinical microbiology laboratory<sup>(4,5)</sup>

They are found as inhabitant of soil, water and plant.<sup>(6)</sup> In hospital environment, they may be recovered from instruments such as ventilator machine, humidifiers, mattresses and other equipments as well as from the skin of healthcare workers. All these organisms have potential to spread horizontally on fomites or the hands of healthcare worker. <sup>(2,7,8,9)</sup> They have ability to thrive in the environment with minimal nutrient and exhibit resistance to several antibiotic. These properties make nonfermenters as emerging nosocomial pathogen. <sup>(0,1)</sup> They are associated with infections such as bacteremia, meningitis, pneumonia, urinary tract infection, surgical site infections, wound infections, osteomyelitis etc. <sup>(12)</sup> Nonfermenters are of low virulence and often cause nosocomial infections in immunocompromised persons.<sup>(13)</sup>

Many risk factors for infection with nonfermenters have been identified. It includes immunosuppression (oncology patient on cytotoxic therapy/radiotherapy, organ transplant patients and even patient with AIDS), neutropenia, mechanical ventilation, cystic fibrosis, indwelling catheters, invasive diagnostic and therapeutic procedures. Treatment of infections with nonfermenters is difficult because of its virulence, intrinsic and acquired antibiotic resistance, and limited choice for effective antimicrobial agents.<sup>(14)</sup>

Members of NFGNB show resistance to most of commonly used antibiotics by several mechanisms. These includes antimicrobial inactivating enzymes, reduced access to bacterial targets and point mutations that change cellular functions.<sup>(15)</sup>

Considering all these issues, identification of NFGNB to species level has become important. Hence, this particular study was aimed at identifying and characterizing NFGNB isolated from various specimens.

## AIMSAND OBJECTIVES-

- 1. To isolate and identify the nonfermenting gram negative bacilli from various clinical specimens
- 2. To study their antimicrobial susceptibility pattern of isolated gram negative nonfermentative bacilli

# MATERIALAND METHOD

This was a cross sectional, prospective study carried from period of  $1^{st}$  Oct 2015 to  $30^{th}$  June 2017.

Approval was obtained from institutional ethical committee before the start of the study.

All samples (Pus, Sputum, Urine, Blood, Fluid, Miscellaneous which included gastric lavage, endotracheal secretion) received from various clinical inpatient and outpatient departments were subjected to culture and sensitivity.

**SELECTION CRITERIA** - Inclusion criteria- All isolate which do not acidify butt of TSI (Triple sugar iron) slant were processed.

**EXCLUSION CRITERIA-** All isolate which acidify butt of TSI slant were excluded Nonfermenters were identified by gram staining, cultural characteristics, motility, oxidase test, Oxidative fermentation (OF) test, citrate test, urease test, indole test nitrate reduction, gelatin liquefaction, esculin hydrolysis, decarboxylase test- Lysine, Ornithine, Arginine.

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Antimicrobial susceptibility test was done by Kirby-bauer disc diffusion test for following antibiotics- ceftazidime, gentamicin, tobramycin, piperacillin, piperacillin +tazobactum, aztreonam, cefepime, ciprofloxacin, meropenem, ticaracillin, co-trimoxazole, levofloxacin, tetracycline, amikacin, ampicillin + sulbactum

#### **RESULT-**

During the study period, total 4365 samples were processed. Out of which 1905 (43.64%) samples were culture positive.

From 1905 culture positive samples, 2094 isolates were obtained and out of 2094, 228 (10.88%) were nonfermenters.

#### FIG. no.1-Specimen wise distribution of nonfermenter



Maximum number of nonfermenters were isolated from miscellaneous group specimen (28.08%) followed by sputum (27.64%), pus (23%), urine (13%), blood (5%) and fluid (3%).

In our study, maximum number of nonfermenters were isolated from male patients (56.14%) as compared to female patients (43.85%).

### Table no.1-Sample wise distribution of nonfermenters species

Clinical specimen	Р	U	S	Mis	В	F	Total
P. aeruginosa(141)	36	20	46	32	1	6	141(61.84%)
Other P. species(51)	8	9	14	16	3	1	51(22.36%)
A. baumanii (12)	2	0	1	7	2	0	12(5.28%)
A. Lowefii (4)	2	0	1	0	1	0	4(1.75%)
A. Species(9)	2	1	0	4	2	0	9(3.94%)
Moraxella (4)	1	0	0	2	1	0	4(1.75%)
Burkholderia (3)	0	0	0	2	1	0	3(1.31%)
Stenotrophomonas	1	0	0	0	0	0	1(0.43%)
Maltophilia (1)							
Spingomonas paucimobilis (1)	0	0	0	1	0	0	1(0.43%)
Achromobacter(1)	0	0	0	0	0	1	1(0.43%)
Spingobacterium(1)	0	0	1	0	0		1(0.43%)
Total	52	30	63	64	11	8	228

Note: (P-Pus, U- Urine, Sp- Sputum, Mis- Miscellaneous, Bl-blood, Fl-Fluid)

# Table no. 2- Antimicrobial susceptibility pattern of nonfermenters

Antimicrobial drugs	P. aeruginosa (141)	Other Pseudo species	A. baumanii	A.species	A.loweffii	Moraxella	Burkholderia
Gentamicin	63.82%	62.74%	75%	33.33%	25%	100%	66.66%
Meropenem	60.82%	54.90%	66.66%	44.44%	25%	75%	66.66%
Ciprofloxacin	61.70%	43.13%	0%	0%	0%	50%	100%
Azetreonam	28.36%	37.25%	0%	0%	0%	25%	66.66%
Tobramycin	64.53%	58.82%	0%	0%	0%	75%	100%
Ceftazidime	39.71%	31.37%	66.66%	0%	25%	50%	66.66%
Piperacillin	23.40%	21.56%	50%	11.11%	50%	50%	33.33%
Cefepime	31.20%	31.37%	58.33%	11.11%	50%	75%	66.66%
Ticaracillin	10.63%	13.72%				0%	33.33%
Piperacillin+	34.04%	37.25%	41.66%	55.55%	25%	75%	66.66%
Tazobactum							
Poly B	78.01%	70.58%	0%	0%	0%	75%	0%
Co-trimoxazole	0%		25%	33.33%	0%	0%	0%

*P. aeruginosa* was the most commonly (61.84%) isolated nonfermenter. It was most susceptible to polymyxin B (78%) followed by tobramycin (64.53%), gentamicin (63.82%), ciprofloxacin (61.70%), meropenem (60.82%) and least susceptible to ticaracillin (10.63%).

Followed by *P*. aeruginosa, 22.36 % other pseudomonas spp. were isolated and 10.96% isolates were Acinetobacter species. Among *Acinetobacter spp.*, *A. baumannii* (5.28%) was the most common. *A.baumannii* was most susceptible to gentamicin (75%), and least susceptible to co-trimoxazole (25%).

Other species of nonfermenters isolated during the study includes *Moraxella* (1.75%), *Burkholderia* (1.31%), *Stenotrophomonas* maltophilia, Spingomonas paucimobilis, Achromobacter, Spingobacterium (0.43% each).

### **DISCUSSION-**

NFGNBs were considered as contaminants in the past but now they have been emerged as important major pathogenic organisms. Because of complex physiochemical properties, these organisms require a battery of test for their precise identification. In addition, there is still much confusion regarding the taxonomic status of many of these nonfermenters. Hence, identification of nonfermenters has often being neglected. <sup>(16)</sup> Because of widespread use of antibiotics and other chemotherapeutic agents, there is an increase in the frequency of infections such as bacteremia, meningitis, pneumonia, urinary tract infections, surgical site infections, wound infections, osteomyelitis.<sup>(17)</sup> This study was conducted to identify NFGNB and to study their antimicrobial susceptibility pattern. A Total of 228(10.88%) nonfermenters were isolated during the study period.

In the present study, percentage of isolation of Nonfermenting Gram

Negative Bacilli from various clinical specimens is as follows-Miscellaneous specimen (28.08%) [which included endotracheal tip, gastric lavage], sputum (27.64%), pus (22.80%), urine (13.15%), blood (4.83%), and fluid (3.50%).

*P. aeruginosa* (61.84%) was the most predominantly isolated nonfermenter followed by *Acinetobacter baumanii* (5.28%). Our results were comparable with the study by Shalini Gore<sup>(18)</sup> who reported 62.66% *Pseudomonas aeruginosa* followed by *Acinetobacter baumannii* (23.33%) from various clinical specimens.

In our study, *P. aeruginosa* was isolated from sputum (32.62%), pus (25.53%), miscellaneous (22.69%), urine (14.18%), fluid (4.25%), blood (0.70%). *P.aeruginosa* has potential to cause infection at any site of the body because of the wide array of virulence factors it produces, and also its ability to counteract the host defenses.<sup>(19)</sup>

Majority of *P. aeruginosa*, in our study were isolated from Medicine (36.87%) and Surgical (17.73%) wards .This emphasizes that prolonged hospital stay, antibiotic therapy, presence of intravenous and urinary catheters are the important risk factors for *Pseudomonas infection*. *P. aeruginosa* is an important nosocomial pathogen. Nosocomial infection rate is higher in ICU than elsewhere in hospitals, as ICUs are the hub of severely ill patients, who are more prone to opportunistic infection.<sup>(20)</sup>

Antimicrobial susceptibility pattern of *P. aeruginosa* in our study was given as below- polymyxin B (78%) followed by tobramycin (64.53%), gentamicin (63.82%), ciprofloxacin (61.70%) and meropenem (60.82%) and least susceptible to ticaracillin (10.63%)

There are several mechanisms which cause resistance to develope against *P. aeruginosa*. The resistance to Gentamicin is mainly due to N-acetylation of deoxystrepatamine moiety. Amino glycoside

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phosphoryl transferase causes inactivation of amino glycosides such as kanamycin, neomycin and streptomycin. Because of impermeability, uptake of aminoglycoside is reduced and hence resistance to amikacin, gentamicin, tobramycin is seen.<sup>(21</sup>

Acinetobacter baumannii is associated with variety of nosocomial infection such as catheter associated urinary tract infection, surgical site infection, catheter related blood stream infection, skin and soft tissue infection in hospitalized patients. Acinetobacter known to colonize the skin and the gastrointestinal tract of patients and also survives for longer periods in the hospital environment. This leads to enhanced opportunities for transmission between the patients.<sup>(1)</sup>

Most of the isolates of A. baumannii were from miscellaneous group of specimen (58.33%) followed by pus (16.66%), blood (16.66%), sputum (8.33%). The highest susceptibility was reported to gentamicin75% and the least was to co-trimoxazole 25%.

#### CONCLUSION-

Nonfermenting gram negative bacilli are emerging as an important nosocomial pathogen. They have been isolated from various clinical specimen and this has proved their role in wide range of diseases.<sup>(23)</sup> Prevalence of nonfermenters varies between different population and hence updates about prevalence and susceptibility pattern of nonfermenters is important for clinicians<sup>(24)</sup>

Multiple drug resistance in nonfermenters limits therapeutic options and making treatment difficult. ESBL and MBL production by these organism lead to high morbidity and mortality as we are left with only Colistin and Polymyxin B for treating infection. Further, these agents are potentially toxic.

It is also important to establish clinical significance of isolated NFGNB before it is considered as pathogen. This will avoid unnecessary usage of antibiotics and emergence of drug resistant strains.<sup>(25)</sup>. Since these organisms have potential to survive in hospital environment, infection control measures which includes equipment decontamination, strict attention to hand washing and isolation procedures are needed to prevent spread of multidrug resistant nonfermentative gram negative bacilli in healthcare setting.

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