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Changing Antibiotic sensitivity pattern in Gram Negative Nonfermenting Isolates: a Study in a Tertiary care Hospital

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Background: *Nonfermenting gram negative bacteria are a group of heterogenous,aerobic,nonsporing bacteria.They are saprophytic in nature and found as commensals in man and other animals.*
Objectives: *This study aims at isolation, identification and antibiotic susceptibility pattern of nonfermenting gram negative bacteria.*
Material and Methods*: 70 isolates from various age groups of both male and female patients were included in the study.The clinical specimens were collected under aseptic precautions and subjected to preliminary biochemical test for identification.*
Result: *In the present study Acinetobacter baumanii and Pseudomonas aeruginosa were isolated.These Nonfermenting gram negative bacteria were isolated from Pus/wound swab, Blood, CSF, Tissue, Endotracheal tips and showed variability in their antibiotic susceptibility results.*
Conclusion: *Nonfermenting gram negative bacilli are mainly seen in patients with serious underlying risk factors like prolonged stay in hospital, catheterization and various other health related infections.*
Keywords: *NFGNB: Non fermenting gram negative bacilli, Spp: Species, TSI: Triple sugar iron, NF: Non fermenters.*

I. Introduction

Nonfermenting Gram negative bacteria (NFGNB) are the potential microbes that are distributed widely in nature and have been isolated from soil, water and medical devices as well as from clinical specimens.[1]They occur as saprophytes in the environment and some are also found as commensals in the human gut.[2,3] Taxonomically, they are diverse group of aerobic, nonsporing bacteria that either donot utilize carbohydrates as a source of energy or degrade them through metabolic pathway than fermenting or utilizing it oxidatively. [1,4].Nonfermentative Gram negative bacteria(NFFGNB) are primarily opportunistic and account for about 15% of all bacterial isolates from a clinical microbiology laboratory. [5,6] Although frequently considered as contaminants in the past but their pathogenic potential has been proved beyond doubt by their frequent isolation from clinical material and their association with disease.[7] They are considered as major contaminants in hospital environment, so major cause of hospital acquired infection.[8] Antibiotic resistance among bacteria is becoming more and more serious problem throughout the world and emerges commonly when patients are treated with empiric antimicrobial drugs.[9,10] In the recent years due to liberal and empirical use of antibiotic, NFGNB have emerged as important health care associated pathogens. They have been incriminated in infections such as septicaemia, pneumonia and surgical site infections.[3] Nonfermentative Gram negative bacilli are innately resistant to many antibiotic and are known to produce extended spectrum B-lactamases and metallo B-lactamases.[3,5] Nonfermentative Gram negative bacteria are being encountered with increasing frequency as agents of opportunistic and very serious infections as well as nosocomial infections.They are primarily opportunistic pathogens which cause health care associated infections in majority of patients who are critically ill or immunocompromised.[4,6] Most commonly occurring nonfermentative gram negative bacilli are Pseudomonas aeruginosa, Acinetobacter, Strenotrophomonas, Alcaligens spp, Roseomonas spp, Sphingobacterium spp, Ralstonia spp, Shwenella spp.These bacterial species are occasionally isolated from hospital environment.[11,12] Study for monitoring antimicrobial resistance trends is an ongoing global antimicrobial surveillance program focused on clinical isolates. Hospital Antibiograms are commonly used to help guide empiric antimicrobial treatment and are important component of detecting and monitoring trends in antimicrobial resistance.This study was done with the objective to assess the clinical significance, evolution of the antimicrobial susceptibility patterns and to identify the various infections caused by non fermenting gram negative bacteria.

II. Material And Methods

The present study was a prospective study carried out for a period of four months in the bacteriology section at the Department of Microbiology, Government Medical College Jammu (J&K) during the period from

January 2013 to April 2013. A total of 70 Nonfermenters isolated from clinical samples such as pus/wound discharge, Blood, Tissue, Endotracheal tip, Cerebrospinal fluid were included in the study.

2.1Sample collection and Processing

Under aseptic precautions, clinical specimens were collected from the patients at the respective hospital wards and transported immediately in a sterile container to the microbiology laboratory.The samples were initially screened on routine media such as MacConkey agar, Blood agar, Chocolate agar and UTI agar for separation of nonfermenter organisms. The culture plates were then observed for growth of bacteria and were subjected to preliminary biochemical tests for identification. Futher processing was done by standard laboratory procedures. Antibiotic susceptibility testing was also done by inoculation on Muller Hilton agar and incubating the plates at 37^0c for 24hrs.

III. Results

This prospective study was carried out for a period of four months from January 2013 to April 2013. A total of 70 Nonfermenters isolated from clinical samples such as pus/wound discharge, Blood,Tissue, Endotracheal tip, Cerebrospinal fluid received in the department of Microbiology, G.M.C Jammu were included in the study.

Table 1:Biochemical characteristics of nonfermenters

Test	P.aeruginosa	A.baumanii	A.lwofii	Moraxella
Oxidase	Positive	Negative	Negative	Positive
Indole	Negative	Negative	Negative	Negative
Urease	Positive	Positive	Negative	Negative
TSI	K/NC	K/NC	K/NC	K/NC
Growth at 42^0c	Positive	Positive	Positive	Positive

The clinical significance of the NFGNB isolated was assessed prospectively by analyzing the case sheets for a combination of relevant laboratory and clinical criteria including the presence of pus cells along with gram negative bacilli in the stained smear from the sample, monomicrobial infection and repeated isolation of the same organism from samples collected at different intervals.

Table 2: Total number of clinical specimens (n=518)

Total number of clinical specimens	Nonfermenters isolated	Percentage
518	70	13.5

Out of 518 clinical specimens, Nonfermenters isolated were 70 (13.5%).

Table 3: Total no. of Nonfermenter isolated from different clinical specimens (n=70)

Specimen	Number	Percentage%
Blood	34	48.57
Pus	27	38.58
Endotracheal tip	6	8.58
Cerebrospinal fluid	2	2.85
Tissue	1	1.42

A total of 70 Nonfermenters were isolated from different clinical specimens. Maximum were isolated from Blood samples 34(48.57%) followed by Pus 27(38.58%), Endotracheal tip 6(8.58%), Cerebrospinal fluid 2(2.85%) and Tissue 1(1.42%).

Table 4 :**Organisms isolated from various clinical specimens (n=70)**

Isolate	Number	Percentage%
Acinetobacter baumanii	45	64.29
Pseudomonas aeruginosa	25	35.71

Acinetobacter baumanii was the commonest isolate accounting for 45 (64.28%) followed by Pseudomonas aeruginosa 25(35.71%). Majority of the non fermenter were isolated from pus and blood cultures.

Table 5: **Distribution rate of isolated Nonfermenters in clinical specimens (n=70)**

Specimen type	Acinetobacter baumanii	Pseudomonas aeruginosa
Pus	9	18
Blood	32	2
Tissue	0	1
Endotracheal tip	3	3
Cerebro spinal fluid	1	1

Out of the total NFGNB isolated from various clinical samples, Acinetobacter baumanii was isolated maximum from Blood samples 32 (45.71%), followed by Pus 9(12.85%), Endotracheal tip 3(4.28%) and Cerebrospinal fluid 1(1.42%). Pseudomonas aeruginosa was isolated maximum from Pus 18 (25.71%) followed by Endotracheal tip 3(4.28%), Blood 2(2.85%), Tissue 1(1.42%) and cerebrospinal fluid 1(1.42%). Majority of the nonfermenter were isolated from Blood and pus culture.

Table 6: Age wise distribution of patients (n=70)

Age in years	Number of cases	Percentage
<1	21	30%
1-10	12	17%
11-20	7	10%
21-30	5	7%
31-40	3	4%
41-50	10	14%
51-60	5	7%
61-70	3	4%
71-80	4	5%

Maximum cases were found in patients belonging to age group less than 1 year 21(30%) followed by age group 1-10 years 12 (17%) cases and 41-50 years 10(14%) cases.

Table 7: Gender wise distribution(n=40)

Gender	Number of cases	Percentage
Males	41	58.58%
Females	29	41.42%

Of the total number of representative samples, 41(58.58%) were males and 29(41.42%) were females.

Table 8 : Antibiotic sensitivity pattern of nonfermenters (n=70)

Antibiotics	Number	Percentage%
Piperacillin	6	8.57
Pipera-Tazobactum	34	48.57
Ceftazidine	22	31.42
Cefepime	26	37.14
Cefaperazone-Sulbactum	27	38.57
Imipenem	31	44.28
Amikacin	34	48.57
Gentamycin	27	38.57
Tobramycin	27	38.57
Ciprofloxacin	28	42.85
Colistin	1	1.42
Polymixin B	9	12.8
Bacitracin	17	24.2

Nonfermenters showed maximum sensitivity to Amikacin (48.57%), Piperacillin Tazobactum (48.57%), followed by Imipenum (44.28%), Ciprofloxacin (42.85%), Cefoperazone-Sulbactum (38.57%), Gentamycin (38.57%), Tobramycin (38.57%) respectively. The isolates showed least sensitivity to Colistin (1.42%), Piperacillin (8.57%) and Polymixin B (12.8%).

Table 9: Antibiotic Sensitivity pattern of Pseudomonas aeruginosa isolates n=25

Antibiotics	Sensitivity	Percentage%
Piperacillin	2	8
Pipera-Tazobactum	15	60
Ceftazidine	16	64
Cefepime	15	60
Cefaperazone-Sulbactum	14	56
Imipenem	15	60
Amikacin	14	56
Gentamycin	13	52
Tobramycin	8	32
Ciprofloxacin	13	52
Aztreonam	15	60
Colistin	1	4
Polymixin B	6	24
Bacitracin	8	32

Pseudomonas aeruginosa isolated in present study were sensitive to Ceftazidine (64%) followed by Piperacillin Tazobactum (60%), Cefepime (60%), Aztreonam (60%), and Imipenum (60%).

Table 10: Antibiotic Sensitivity pattern of Acinetobacter species (n=45)

Antibiotics	Sensitivity	Percentage %
Piperacillin	3	6.66
Pipera-Tazobactum	19	42.2
Ceftazidine	6	13.3
Ceftriaxone	11	24.4
Cefepime	16	35.5
Cefaperazone-Sulbactum	13	28.8
Imipenem	16	35.5
Amikacin	20	44.4
Gentamycin	17	37.7
Tobramycin	19	42.2
Ciprofloxacin	15	33.35
Cotrimoxazole	9	20.0
Colistin	0	0
Polymixin B	3	6.66
Tetracycline	0	0
Bacitracin	9	20.0

Acinetobacter species showed maximum sensitivity towards Amikacin (44.4%), followed by Tobramycin (42.4%), Pipera-Tazobactum (42.2%), Gentamycin (37.7%) and Imipenem (35.5%).

IV. Discussion

Non fermenting Gram negative aerobic bacteria are being increasingly associated with human disease. NFGNB considered to be contaminants in the past have now emerged as important major pathogenic organisms. Acinetobacter species are known to be the common nosocomial pathogens similarly to pseudomonas species.[13] The complex physiochemical properties of these organisms require series of tests foe their precise identification. In addition there is still much confusion regarding the taxonomic status of many of these nonfermenters. Identification of these nonfermenters has often being neglected. Therefore we intended to identify commonly encountered, clinically significant Nonfermenting Gram negative bacteria from clinical specimen along with their antibiotic sensitivity pattern.

This study was conducted during the period from January 2013 to April 2013. A total of 70 isolates were collected from different clinical samples which gave an alkaline/alkaline (K/K) reaction on Triple sugar iron agar. All these Non fermenting Gram negative bacteria were identified by a series of tests as per standard laboratory techniques.

In this study nonfermentars were isolated from Blood sample (34) followed by Pus samples (27), Endotracheal tips (6), Cerebrospinal fluid (2) , Tissue (1). Most of the isolates of NFGNB were from Blood samples. NFGNB were commonly involved in wound infections following burn and in chronic non healing ulcers. Antibiotics like Amikacin, Gentamicin, Piperacillin Tazobactum, Imipenem and Ciprofloxacin were used to treat these infections. Acinetobacter baumanii and Pseudomonas aeruginosa were common organisms incriminated in Blood, Pus and Endotracheal tips. Pseudomonas aeruginosa was the major pathogen causing burn wound infection. Acinetobacter baumanii was highest in blood.[14,15]

In general nonfermenters appear inert in the typical tests used for fermentative gram negative bacilli. Conventional sugar media used for fermentative bacteria do not support the growth of many nonfermenters and the acids produced are often too weak to convert the pH indicator. P.aeruginosa oxidize Glucose to produce acid.[15,16]

In present study most of the strain showed maximum sensitivity to Amikacin (48.57%), Piperacillin Tazobactum (48.57%), followed by Imipenum (44.28%), Ciprofloxacin (42.85%), Cefoperazone-Sulbactum (38.57%), Gentamycin (38.57%), Tobramycin (38.57%) respectively. The isolates showed least sensitivity to Colistin (1.42%), Piperacillin (8.57%) and Polymixin B (12.8%). Pseudomonas aeruginosa isolated in present study were sensitive to Ceftazidine (64%) followed by Piperacillin Tazobactum (60%), Cefepime (60%), Aztreonam (60%), and Imipenum (60%). Similarly Acinetobacter species showed sensitivity towards Tobramycin (42.4%), Amikacin (44.4%), Gentamycin (37.7%) and Imipenem (35.5%).[17]

In present study males were more infected than females. Most of our patients were from neonatal and paediatrics ward and from surgical wards with inappropriate antibiotic intake.[15,16]

V. Conclusion

Identification of NFGNB and monitoring their susceptibility pattern are important for proper management of infections caused by them. Present study highlights that it is essential to establish the clinical relevance of the NFGNB isolated, before they are considered as pathogens. This would avoid unnecessary usage of antibiotics and emergence of drug resistant strain.

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