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Current status of antibiotic resistant nonfermentative gram negative *bacilli* among nosocomial infections

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ABSTRACT

Nonfermentative gram negative bacilli (NFGNB) are considered as a major contaminants in hospital environment but now it make a threat alarm of emerging an healthcare associated infections. Most of the isolates of NFGNB are highly resistant to major antibiotics including carbapenes and beta lactum antibiotics. Prevalence and considering this possibilities, the present study interpreted NFGNB from clinical specimens. Batteries of 121 clinical specimens were included. All the specimens were identified by the classical Microbiological and Biochemical tests. The result showed that 64(43.%) isolates among 149 specimens supported Pseudomonas spp. followed by acenetobacter spp. 32(21%) and 55(36%) recognised as Sphingomonas. The antibiotic sensitivity assay showed 80% of resistance to major antibiotics included. Due to multi resistance observation of the isolates, its is found by more NFGNB constant survey of antibiotic sensitivity is essential to control and management of nosocomial infections.

Keywords: NFGNB, Threat alarm, niche organism, immunocompromised.

INTRODUCTION

Nonfermentative gram negative bacilli are the potential candidates that are distributed widely in nature and have been isolated from soil, water, and from medical devices as well as from clinical specimens .having ability to produce energy for cell function without fermentation of sugar. These are a group of aerobic non spore forming gram negative bacilli that either do not utilize carbohydrate as a source of energy or degrade them through metabolic pathway other than fermentation, commonly used by fermentative organisms.[1]

Over past decade nonfermenters have emerged as important opportunistic pathogens in increasing population of patients who are “niche organisms” or “niche pathogens” that primarily caused opportunistic health care associated infection in patients who are critically ill or immunocompromised.[2,3] The wide spread use of antibiotics and other chemotherapeutics agents in the treatment of diseases has a major role in the increased frequency of infection by these organisms because of the disruption of the normal flora.[3]

These NFGB (Nonfermentative Gram Negative Bacilli) are primarily opportunistic. MDR (Multiple Drug Resistance) is common and increasing day by day which make treatment of infection caused by the organism tedious. Gram negative non fermentative bacteria are less chemically active and less virulent than enteric pathogen. [4] The Dextrose non fermenting bacilli have been associated with human infection.[5] The Dextrose utilizing nonfermentative bacilli are catalase positive. Some species are able to grow anaerobically in presence of nitrate and many of produce water soluble pigments. [6] The glucose non fermenting gram negative bacilli, most often associated with human infections, having a characteristic smell, and are strictly aerobes that grow at 5 – 42^{0}C [7]. Interpreting the significance of the isolate from clinical specimens is often difficult because of wide spread distribution of the *Acenatobacter* and *Pseudomonas* in the nature and its ability to colonize on the healthy and damage tissue. During routine clinical microbiological work in labs, NFGNB other than *Pseudomonas* are not taken seriously as a pathogen mostly they are persuied for identification and are avoided as contaminants [8]. The most commonly occurring non fermentative gram negative bacilli are *Pseudomonas aeruginosa, Acinetobacter,*

Strenotrophomonas, S. maltophilia, Alcaligens spp., Flavomonas, Oryzihabitants, Sphingobacterium, Burkholderia spp., Cepacia, Acromobacter spp., Bordetella spp, Commamonas spp., Methylobacterium spp., Olizella spp., Ralstonia spp.,Psychrobacter spp., Roseomonas spp., Shwenella spp., Sphingobacterium spp., Elizabethkingia spp. These are occasionally been isolated from clinical specimens. [8, 9]

This study was undertaken to isolate and characterize prevalence of glucose nonfermenters species in clinical human specimens associated with hospital environment. Many members of this group are slow growing or require a special cultivation medium for growth. They Weakly produce acid metabolites, Hence can not be detected with test system routinely used with other groups of bacteria. The low rate of recovery in most clinical labs and almost endless shifting of nomenclature and reclassification of theses identification of nonfermenters. [10], Very few laboratories in India identify these organisms as a routine because non fermenters are slow growing and require the use of special culture media and biochemical test for their identification. It is hoped that this would be novel step in determining the role of organism in the infection. [11]

MATERIALS AND METHODS

Total 121 clinical specimens were isolated includes blood, sputum, stool, pus, throat swab, nasal swab, processed in Department of microbiology ,collected from hospital from outpatient Department. All 121 clinical samples were initially screened on routine media such as blood agar, Mac Conkey agar, for seperation nonfermenter organisms. As further isolation of nonfermenters the following steps were used for primary recognition of nonfermenters by Absence of acid production in TSI (Triple Sugar Iron). Absence and growth on Mac Conkey agar, especially for the glucosenonfermentative organism. [12,13,14,15,16] Distribution of samples were done as per the standard system includes, Blood sample-36, Sputum-16,Stool -22, Pus-25,Swab-22,[17,18,19,20]. Biochemical characterization by conventional methods includes Gelatinase, Starch hydrolysis, Urease, Nitrate reduction test, Indole test, H_2S production on KIA (Kligler Iron agar) agar, Growth on 6.5% NaCl. [21, 22, 23, 24, 25] Which were followed by sugar fermentation experiments with O/F media (Oxidative fermantative media). Antibiogram was determined using traditional method. The antimicrobial agents used in the study includes Amikacin, Ciprofloxacin, Co-trimoxazole, Gentamycin, Tetracycline etc. [31,32]Species differentiation done on the basis of glucose oxidation, Gelatin liquification, heamolysis etc.

RESULTS

Total 149 bacterial isolates obtained from 121 clinical specimens, Among 121 specimens 64(43.%) isolates supported *Pseudomonas spp.*, while 32(21%) supported *Acinetobacter spp* and 55(36%) recognised as *Sphingomonas spp.* Primary screening result shown in the Table 1. For Sugar fermentation results shown in the Table2.1,2.2, 2.3. The results are shown in Table 2.and most are found to be glucose nonfermenters. According to *konneman et.al* organisms were classified on biochemical tests. All were gram negative bacilli. Antibiogram assay showed that all among isolates of as NFGNB confirmed 80% of resistance to major antibiotics included

Table No.1 Showing Primary Screening Results

S. no.	Samples(No.)	Growth on MCA	Growth on TSI	Presence of Acid /gas
1.	Stool (22)	22% -ve	Colour change	Acid and gas
2.	Sputum (16)	90% +ve	No colour change	Not done
3.	Pus (25)	49% -ve	colour change	High acid/no gas
4.	Blood (36)	99% +ve	No colour change	Not done
5.	Swab (22).	90% -ve	colour change	High acid high gas

-ve = No growth *+ve = growth*

Table No.2.1 Biochemical test results shown by nonfermenters from Stool

Biochemical test	Stool specimens			
	NFG 1	NFG 2	NFG 3	NFG 4
Oxidase test	-Ve	+ve	+ve	+ve
Urease	-Ve	-Ve	-Ve	-Ve
KIA	-Ve	-Ve	-Ve	-Ve
Nitrate reduction test	-Ve	-Ve	-Ve	-Ve
Gelatinase test	-Ve	-Ve	-Ve	-Ve
Complete haemolysis	-Ve	+ve	+ve	+ve
MR-VP test	-Ve	-Ve	-Ve	-Ve
Sugar utilization test	Glucose	Maltose and mannitol	Glucose	Dextrose and fructose
Identified as	*Acinetobacter spp*	*Pseudomonas spp*	*Pseudomonas spp*	*Sphingomonas spp*

-Ve --- Negative +Ve--- Positive

Table No. 2.2 Biochemical test results shown by nonfermenters from Swab.

Biochemical Test	Swab specimens																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Oxidase test	-	+	-	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	-	+
Urease	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
KIA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nitrate reduction test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gelatinase test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Complete haemolysis	-	+	-	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	-	+
MR-VP test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sugar utilization test	G	D&F	G	G	D&F	G	G	D&F	G	D&F	D&F	G	M&M	G	M&M	M&M	G	G	G	M&M
Identified as	A	S	A	P	S	A	A	S	P	S	S	A	P	P	P	P	P	P	A	P

-Ve --- Negative +Ve--- Positive
G – Glucose, D& F – Dextrose and fructose, M&M – Maltose and mannitol
A – Acinetobacter, P– Pseudomonas, S– Sphingomonas

Table no 2.3 Biochemical test results shown by non-fermenters from Pus

Biochemical Test	Pus specimens											
	1	2	3	4	5	6	7	8	9	10	11	12
Oxidase test	-	+	+	+	+	+	-	+	+	+	+	-
Urease	-	-	-	-	-	-	-	-	-	-	-	-
KIA	-	-	-	-	-	-	-	-	-	-	-	-
Nitrate reduction test	-	-	-	-	-	-	-	-	-	-	-	-
Gelatinase test	-	-	-	-	-	-	-	-	-	-	-	-
Complete haemolysis	-	+	+	+	+	+	-	+	+	+	+	-
MR-VP test	-	-	-	-	-	-	-	-	-	-	-	-
Sugar utilization test	G	D&F	M&M	G	D&F	G	G	D&F	G	D&F	M&M	G
Identified as	A	S	P	P	S	P	A	S	P	S	P	A

-Ve --- Negative +Ve--- Positive
G – Glucose, D& F – Dextrose and fructose, M&M – Maltose and mannitol
A – Acinetobacter, P– Pseudomonas, S– Sphingomonas

Table: 3.1 Antibiograms of *Pseudomonas Spp.*

S. No.	Name of Antibiotic	Strength	Mean & error	Remarks
1.	Ampicillin	10mcg	6.1± 2.56	Sensitive
2.	Sulbactum	20mcg	12.3±1.90	Intermediate
3.	Amikacin	30 mcg	20.6±2.56	Sensitive
4.	Cefotaxim	30 mcg	11.06±1.00	**Resistant**
5.	Ciprofloxacin	5 mcg	13.0±1.09	**Resistant**
6.	Co-trimoxazole	25 mcg	12.6±0.95	Intermediate
7.	Gentamycin	5 mcg	11.0±1.95	**Resistant**
8.	Tetracycline	30 mcg	10.6±1.27	**Resistant**

Table: 3.2 Antibiograms of *Acinetobacter Spp.*

S. No.	Name of Antibiotic	Strength	Mean & error	Remarks
1.	Ampicillin	10mcg	5.1± 3.56	Sensitive
2.	Sulbactum	20mcg	10.3±0.90	Intermediate
3.	Amikacin	30 mcg	15.6±2.96	Resistant
4.	Cefotaxim	30 mcg	17.07±2.00	Resistant
5.	Ciprofloxacin	5 mcg	14.0±0.09	Resistant
6.	Co-trimoxazole	25 mcg	07.6±0.95	Intermediate
7.	Gentamycin	5 mcg	12.0±1.95	Resistant
8.	Tetracycline	30 mcg	09.6±1.11	Resistant

Table: 3.3 Antibiograms of *Sphingomonas Spp.*

S. No.	Name of Antibiotic	Strength	Mean & error	Remarks
1.	Ampicillin	10mcg	10.8± 2.33	Resistant
2.	Sulbactum	20mcg	10.3±0.90	Intermediate
3.	Amikacin	30 mcg	13.6±1.28	Sensitive
4.	Cefotaxim	30 mcg	19.07±03.45	Resistant
5.	Ciprofloxacin	5 mcg	17.0±0.06	Resistant
6.	Co-trimoxazole	25 mcg	05.6±0.79	Intermediate
7.	Gentamycin	5 mcg	13.0±1.44	Resistant
8.	Tetracycline	30 mcg	07.6±1.76	Resistant

DISCUSSION

In all 121 sample screened from different patients and six of them found to be not growing on primary media but growth on TSI media. results of differential growth yellow isolate designated as *Sphingomonas paucimobilis previously known as Pseudomonas paucimobilis(26)*

The other orange colour forming organism confirmed as *Brevendimonas vesicularis* by conventional source tracking method.*(26)*

Infections include bacteraemia/septicaemia caused by contaminated solutions, e.g. distilled water, haemodialysis fluid and sterile drug solutions. Cases of pseudo bacteraemia have been recorded in association with S. paucimobilis, as have many cases of unusual infections both invasive and severe, e.g. septic arthritis and osteomyelitis. No cases of death have been recorded in the literature related to *S. paucimobilis*. This review illustrates that *S. paucimobilis* is a more important pathogen than previously thought [27].Also Pseudomonas spp. and Acinetobacter shows a shocking results which is need to be pull on the floor to discuss in details.

In recent years, due to the liberal and empirical use of antibiotics, Non fermentative gram negative bacilli have emerged as important health care-associated pathogens. They have been incriminated in infections, such as septicemia, meningitis, pneumonia, urinary tract infections (UTI), and surgical site infections (SSI) [28]. now a days recent studies carried out on the urgent areas like identification of Non fermentative gram negative bacilli, and monitoring their susceptibility patterns, which is important for the proper management of the infections caused by them, and to highlights the fact that it is essential to establish the clinical relevance of the isolated Non fermentative gram negative bacilli, before they are considered as pathogens. This would avoid unnecessary usage of antibiotics and emergence of drug-resistant strains. [29]

This study reported nonfermenter specially *S. paucimobilis* which is having a lot of outbreaks recently reported in case of pediatric infections, neonatal intensive care units etc. can be isolated from various clinical specimens and distilled water too. [30] The fact that the nonfermenters are resistant to the commonly used antibiotics emphasises the importance of including tests for their isolation and identification schemes, which can focus on the prevalence and pathogenic role of these slow growing organisms.

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