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IDENTIFICATION AND CHARACTERIZATION OF NONFERMENTATIVE GRAM NEGATIVE BACILLI FROM VARIOUS CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL, JAIPUR.

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Abstract

Background: Non fermentative gram negative bacilli that were considered to be contaminants in the past have now emerged as important health care associated pathogens.

Aim: This study was undertaken to identify and characterize Nonfermenters isolated from various clinical samples.

Material and Methods: Present study was conducted in the department of Microbiology, National Institute of Medical Sciences (NIMS) Medical College and hospital, Jaipur from July 2013 to July 2014. A total 150 strains of Nonfermenters were isolated from 1200 various non repetitive clinical samples. The samples were processed and Non fermentative gram negative bacilli (NFGNB) identified as far as possible up to species level as per standard protocols.

Results: In the present study, 12.50% of incidence / isolation rate of Nonfermenters in various clinical samples was found. Out of 150 strains of Nonfermenter, Pseudomonas aeruginosa was the most common isolate 134 (89.33%) followed by Acinetobacter baumannii 16 (10.67%). 89 (59.33%) NFGNB were isolated from males and 61 (40.67%) were from females. They were mainly isolated from age group 21-30 Years. Higher rate of isolation of NFGNB were 85 (56.67%) from IPD and 38 (25.33%) from ICU patients. Among all clinical samples Pus and Wound Discharge yield maximum isolates of NFGNB i.e. 54 (36%) followed by Sputum 39 (26.0%).

Conclusion: NFGNB should not be ignored as mere contaminant but correlate clinically for its pathogenic potential and identified using standard protocol so as to institute appropriate and timely antibiotic coverage

Keywords: Identification, Nonfermenter, Pseudomonas, Acinetobacter

Introduction

Nonfermenters 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 pathways other than fermentation.[1]

Nonfermenters groups includes diverse organisms like Pseudomonas, Alcaligenes, Burkholderia, Moraxella, Strenotrophomonas, Flavobacter, Oligella, Flavimonas etc.[2]

They are found as saprophytes in nature, inhabitating soil and water and are also found as commensals in man and other animals. Though they are frequently isolated as incidental organisms, their increased frequency as pathogens can be attributed to the advent of antibiotics and patients with reduced immune responses.

They are commonly isolated from surgical site infections, UTI, wound infections, respiratory tract infections particularly VAP (Ventilator Associated Pneumonia), bacteremia, septicemia and osteomyelitis etc.

Pseudomonas aeruginosa a common isolates particularly from surgical site infections wound infections, burn and UTI a common cause of nosocomial infections.

Acinetobacter is a recently emerging potential pathogen in hospital setting particular in causing respiratory tract infections that is VAP (Ventilator Associated Pneumonia) and also commonly isolated from blood stream infections, meningitis, UTI, peritonitis etc.

Material and Methods

The present study was entitled as "Identification and Characterization of Nonfermentative gram negative bacilli from various clinical samples in a tertiary care hospital, Jaipur" Patients of all age groups and both sexes attending OPD, IPD and ICU of National Institute of Medical Science Hospital, Jaipur were included from July 2013 to July 2014.The present study was observational with a Cross Sectional design.

The present study was carried out on 150 of strains of Nonfermenters isolated from 1200 various non repetitive

clinical samples. Sample size was calculated at 95% confidence level assuming 10% incidence of NFGNB among isolates from various clinical samples of hospital Justified by other study.[3] The relative allowable error (precision) of 20% minimum 900 isolates were required for sample size. Specimens included were Pus, Wound discharge, Blood culture, Urine, Sputum, Ear discharge, Body fluid.

The organisms that grew as non fermenters on MacConkey agar and produced an alkaline reaction in triple sugar iron agar were provisionally considered to be nonfermentative gram negative bacilli and were stocked and stored at -20^0 C. Later on time to time these were revived and identified as per following protocol.

The clinical samples were processed immediately and cultured as per Standard protocol using battery of tests[1]

1. Cultural characters: Blood agar, MacConkey and Nutrient agar.
2. Pigment production: Blood agar and Nutrient agar
3. Morphology and Gram's stain
4. Motility : Hanging drop preparation.
5. Catalase test
6. Oxidase test
7. Indole, Methyl red, Voges Proskauer, Citrate utilization test, Urease test, and Triple sugar iron reaction.
8. Oxidation / fermentation (Hugh and Leifson's media) for glucose, lactose, xylose, mannitol and maltose.
9. Lysine, Ornithine decarboxylase and Arginine dihydrolase activity.
10. DNase test
11. ONPG tests
12. Esculin test.

Results

A total of 150 nonfermenters isolated from different clinical samples were identified and characterized. As shown in **Table 1:** Incidence of Nonfermenters isolates 12.50% from various clinical samples. **Table 2:** Most common isolate from clinical samples was Pseudomonas aeruginosa 89.33% followed by Acinetobacter baumannii about 10.67%. As shown in **Table 3:** The maximum number of NFGNB isolates 37/150 were from age group 21-30 followed by 51-60 Years. Out of 37 in age group 21-30 Years Nonfermenters 30 were Pseudomonas aeruginosa and 7 were Acinetobacter baumannii followed by age group 51-60 years in which 22 were Pseudomonas aeruginosa and 4 were Acinetobacter baumannii. In age group ≤10 years only14 Pseudomonas aeruginosa were isolated. **Table 4:** 83 Pseudomonas aeruginosa and 6 Acinetobacter baumannii isolated from 89 male patient and 51 Pseudomonas aeruginosa and 10 Acinetobacter baumannii isolated from 61 female patient. **Table 5:** show Maximum number of Pseudomonas aeruginosa isolated from IPD and minimum number from OPD patients, while maximum number of Acinetobacter baumannii were isolated from ICU followed by IPD Patients but it was not isolated from OPD patients. **Table 6:** show the maximum number of Pseudomonas aeruginosa was isolated from Pus and wound discharge while minimum from the Pleural fluid and CSF i.e. 1 and zero respectively. The maximum number of Acinetobacter baumannii was isolated from sputum while minimum from the CSF and Pleural fluid i.e. 1 and zero respectively.

Table 1: Incidence of Nonfermenters isolates from Clinical Samples

No. of samples tested	1200
No. of Isolates of Nonfermenters	150
Incidence	12.50%

Table 2: Spectrum of Nonfermenters Isolates from Clinical Samples (n=150)

Species	No.	%
Pseudomonas aeruginosa	134	89.33
Acinetobacter baumannii	16	10.67

Table 3: Age Wise Distribution of Nonfermenters

Age (Years)	Pseudomonas aeruginosa		Acinetobacter baumannii		Total	
	No.	%	No.	%	No.	%
≤10	14	100.00	0	0.00	14	100.00
11-20	21	100.00	0	0.00	21	100.00
21-30	30	81.08	7	18.92	37	100.00
31-40	14	82.35	3	17.65	17	100.00
41-50	16	94.12	1	5.88	17	100.00
51-60	22	84.62	4	15.38	26	100.00
>60	17	94.44	1	5.56	18	100.00
Total	134	89.33	16	10.67	150	100.00

Chi-square = 9.202 with 6 degrees of freedom; P = 0.163

Table 4: Sex Wise Distribution of Nonfermenters

	Pseudomonas aeruginosa		*Acinetobacter baumannii*		Total	
	No.	%	No.	%	No.	%
Male	83	93.26	6	6.74	89	100.00
Female	51	83.61	10	16.39	61	100.00
Total	134	89.33	16	10.67	150	100.00

Chi-square = 2.598 with 1 degree of freedom; P = 0.107

Table 5: Distribution of Nonfermenters Among Patients of ICU/ IPD / OPD

ICU/IPD / OPD	Pseudomonas aeruginosa		Acinetobacter baumannii		Total	
	No.	%	No.	%	No.	%
ICU	28	73.68	10	26.32	38	100.00
IPD	79	92.94	6	7.06	85	100.00
OPD	27	100.00	0	0.00	27	100.00
Total	134	89.33	16	10.67	150	100.00

Chi-square = 14.151 with 2 degrees of freedom; P = 0.000

Table 6: Types of Clinical Samples From which Nonfermenters were isolated

Specimens	Pseudomonas aeruginosa		Acinetobacter baumannii		Total	
	No.	%	No.	%	No.	%
Pus and Wound discharge	52	96.30	2	3.70	54	100.00
Sputum	33	84.62	6	15.38	39	100.00
Ear swab	28	96.55	1	3.45	29	100.00
Urine	16	84.21	3	15.79	19	100.00
Blood	4	57.14	3	42.86	7	100.00
CSF	0	0.00	1	100.00	1	100.00
Pleural fluid	1	100.00	0	0.00	1	100.00
Total	134	89.33	16	10.67	150	100.00

Chi-square = 21.874 with 6 degrees of freedom; P = 0.001

Table 7: Biochemical Characteristics of Nonfermenters

Tests		Acinetobacter baumannii (n=16)		Pseudomonas aeruginosa (n=134)		Total (n=150)	
		No.	%	No.	%	No.	%
Motility	+ ve	0	0.00	134	100.00	134	89.33
Catalase	+ ve	16	100.00	134	100.00	150	100.00
Oxidase	+ ve	0	0.00	134	100.00	134	89.33
Indole	-ve	16	100.00	134	100.00	150	100.00
Methyl red	-ve	16	100.00	134	100.00	150	100.00
Voges Proskauer	-ve	16	100.00	134	100.00	150	100.00
Urease producers	+ve	0	0.00	5	3.73	5	3.33
Citrate Utililzer	+ve	16	100.00	134	100.00	150	100.00
TSI	K/K	16	100.00	134	100.00	150	100.00
OF Glucose	A	16	100.00	134	100.00	150	100.00
OF Lactose	A	16	100.00	00	00	16	10.67
OF Xylose	A	16	100.00	134	100.00	150	100.00
OF Mannitol	A	0	0.00	116	86.56	116	77.33
OF Maltose	-ve	16	100.00	134	100	150	100.00
Lysine	-ve	16	100.00	134	100.00	150	100.00
Ornithine	-ve	16	100.00	134	100.00	150	100.00
Arginine	+ve	16	100.00	134	100.00	150	100.00
DNase Test	-ve	16	100.00	134	100.00	150	100.00
ONPG Test	-ve	16	100.00	134	100.00	150	100.00
Esculin test	-ve	16	100.00	134	100.00	150	100.00

Discussion

Pseudomonas aeruginosa and Acinetobacter baumannii are known to be common nosocomial pathogens. Risk factors for acquisition of NFGNB infection include: Immunosuppression, Surgery, Instrumentation, Trauma, Wounds, Pneumonia, Steroid therapy, Burns, Excessive and indiscriminate use of Broad spectrum antibiotics.

Because of increasing frequency of human infections by Nonfermentative gram negative bacteria the diagnostic laboratory must be able to identify these organisms accurately.

A total 150 of strains of Nonfermenters were isolated from 1200 various non repetitive clinical samples. The samples were processed and NFGNB identified as far as possible up to species level as per standard protocols.

In the present study, 12.50% of incidence / isolation rate of Nonfermenters in various clinical samples was found.

Studies carried out by different researchers have reported varied isolation rates of NFGNB. In the present study, 12.50% of incidence of Nonfermenters in various clinical samples was parallel to the results of a study from Samanta et al. (2011)[3], they isolated NFGNB in 10% of clinical samples, a study Eltahawy AT et al. (2001)[4] NFGNB accounted for 16% of all the gram negative bacilli isolates and another study reported 21.80% from Vijya et al (2000)[5].

In the present study we isolated only Pseudomonas aeruginosa and Acinetobacter baumannii. Pseudomonas aeruginosa was most commonly isolated NFGNB about 89.33%. This result has correlated well with the study conducted Gokale et al (2012)[6] who isolated from 95.28% of cases, while Vijya et al.(2000)[5] reported 78.94%, Arora et al. (2010)[7] reported 72.83%, Veenu et al. (1999)[8] reported 72.6%, Zhang et al. (2011)[9] 71.7%, Juyal D et al. (2013)[10] 38.21%, while Samanta et al. (2011)[3] reported a low isolation rate of 26% of Pseudomonas aeruginosa. The variation is likely due to number and type of clinical samples and geographical area of the study.

In the present study the second commonest isolate was Acinetobacter baumannii 10.67%. The result correlated well with the study conducted by Arora et al. (2010)[7] who reported 8.4%. Gokale SK et al. (2012)[6] 15.4%, Zhang et al. (2011)[9] 14%. While Juyal D et al. (2013)[10] 29.27%, Samanta et al. (2011)[3] 66% and Vijya et al. (2000)[5] 6.1%. The variation is likely due to number and type of clinical samples and geographical area of the study.

Out of 150 non fermenters, 89 (59.33%) were from males and 61(40.67%) were from females. Similar observation of male prepondance was made by Ranjan et al.(2001)[11], Benachinmardi K K et al. (2014)[12], and Wispinghoff H et al. (1999) [13]. Most of the our isolates belonged to the age group 21-30Years 37 (24.67%) followed by age group 51-60 years 26 (17.33%) and least isolates were from age group <10 years 14 (9.33%). Which correlates with study done by Benachinmardi K K et al (2014)[12] they reported maximum number of cases in the age group of 21-30 years 20% (20).

In our study, out of 150 NFGNB, isolates were 85 (56.67%) from IPD, 38 (25.33%) ICU and 27(18%) from OPD which

was correlate with study done by Benachinmardi K K et al. (2014)[12] NFGNB isolated from IPD 39 (39%), ICU 37 (37%), and OPD 24 (24%) indicative of the fact that NFGNB association as a cause of nosocomial infection.

In our study maximum number of NFGNB were isolated from Pus & Wound discharge 54 (36%) followed by Sputum 39 (26%), Ear swab 29 (19.33%), Urine 19 (12.67%), Blood 7(4.67%), CSF 1 (0.67%) and Pleural fluid 1 (0.67%).This was similar to other studies done by Mishra E et al. (1986),[14] Yashodhara P et al. (1977)[15], Mindoli P B et al. (2010)[2] who reported maximum number of NFGNB from pus samples. It indicates that they are a common cause of suppurative pyogenic localized infections.

In our study, out of 134 Pseudomonas aeruginosa, most of the patients 30 (22.38 %) were aged between 21 - 30 years which was correlate with study done by Kireçc E et al. (2014)[16] found that 30.66% patients were aged between 20-40 years In our study, out of 16 Acinetobacter baumannii, most of the patients 7 (43.75%) were aged between 21 - 30 years.

In our study, sex wise distribution of NFGNB caused by P. aeruginosa was more common in males 83/134 (61.94%) compared to females 51/134 (38.06%).which was correlate with study done by Kireçc E et al. (2014)[16] showed that cases caused by P. aeruginosa are more common in males (57%) compared to females (43%). In our study, sex wise distribution of NFGNB caused by Acinetobacter baumannii shows that infection was more in female (62.5%) than in males (37.5%).

In our study among the total 134 Pseudomonas aeruginosa isolated it was most common in IPD 79/134 (58.9%) followed by ICU 28/134 (20.8%) and OPD 27/134 (20.1%). which correlates well with study of Benachinmardi K K et al. (2014)[12] that showed that highest percentage of Pseudomonas aeruginosa from IPD i.e. 64.10%.

In our study among 16 Acinetobacter baumannii isolated it was most common in ICU 10/16 (62.5%) followed by IPD 6/16 (37.5%) and none was isolated from OPD which correlates with the study done by Benachinmardi K K et al. (2014)[12] that showed that 37.83% percentage of Acinetobacter baumannii isolation from ICU and 26.09% respectively.

Out of 134 Pseudomonas aeruginosa isolated from various clinical samples, maximum number were isolated from pus and wound discharge 54 (36%) followed by sputum 33 (24.62%),while Benachinmardi K K et al. (2014)[12] isolated 15 (25%) Pseudomonas aeruginosa from the total of 60 Pseudomonas isolates from 100 clinical samples from pus samples. In present study total of 16 Acinetobacter baumannii were isolated from 150 clinical samples. The maximum number of Acinetobacter baumannii were isolated from sputum 6 (37.5%), urine and blood 3 each (18.75%).

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 non-fermenters and the acids produced are often too weak to convert the pH indicator. Hugh and Leifson's OF medium that accommodated the metabolic properties of nonfermenters is used for the identification and speciation. Most common isolate in our study Pseudomonas aeruginosa oxidized Glucose, Xylose, Mannitol to produce acid, Acinetobacter baumannii oxidized Glucose, xylose, Lactose sugars in Hugh and Leifson's OF sugar media. All the isolates of Pseudomonas aeruginosa and Acinetobacter baumannii dehydrolysed Arginine. Commonly urea hydrolysis is not seen in Pseudomonas aeruginosa isolates. In our study urea hydrolysis was seen in 3.73% of P. aeruginosa.

Conclusion

The study identified the incriminating NFGNB i.e. Pseudomonas aeruginosa, Acinetobacter species as pathogens from the various clinical samples from IPD, OPD and ICU.. NFGNB should not be ignored as mere contaminant but correlate clinically for its pathogenic potential and identified using standard protocol so as to institute appropriate and timely antibiotic coverage.

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