

Original Research Article

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Isolation and Identification of Non-Fermentative Gram Negative Bacilli

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Nonfermentative group of bacteria have been incriminated as emerging opportunistic pathogens, especially from hospital settings due to their increasing frequency of isolation from clinical specimens. Nonfermenters show natural resistance to a wide range of commonly used antibiotics. This study includes 50 isolates of nonfermenters from different clinical specimens like Pus, Sputum, blood and urine obtained from patients attending SVS Medical college & Hospital from Jan 2007 to March 2008. Out of 50 nonfermenters, *Pseudomonas* sp. (82%), *Acinetobacter* sp. (14%), *Alcaligenes* sp. (4%) were isolated. Sensitivity pattern was Piperacillin (60%), Amikacin (52%), Ciprofloxacin (46%), Ceftazidime (46%), Cefotaxime (44%), Cefi-pime (36%), Gentamycin (24%). This study showed that non-fermenters were frequently isolated from clinical specimens and that they are becoming resistant to the routinely used antibiotics and hence should be taken seriously rather than regarding them as contaminants or commensals.

Introduction

In medical microbiology, non fermentative group of bacteria have been incriminated as emerging opportunistic pathogens, especially from hospital settings. Current interest in non fermenters is fully justified by their increasing frequency of isolation from clinical specimens and their natural resistance to a wide range of commonly used antibiotics.

A number of these organisms are associated with serious disease in whom it is difficult to distinguish between clinical infection and colonization.

The nonfermenters comprise a heterogenous group of gram negative aerobic bacilli / coccobacilli, that are either incapable of utilizing carbohydrates as a source of energy or degrade them via oxidative rather than fermentative metabolic pathways.

The majority of non fermenters are found in nature as inhabitants of soil and water, as harmless parasites on mucous membranes of humans and animals, causing opportunistic infections in immunocompromized individuals. These organisms owe their invasiveness or infectivity to an altered immune status or an already debilitated host.

The pathogenic potential of non fermenters has been proved beyond doubt by their frequent isolation from clinical samples and their association with the disease. Recent literature shows that these organisms cause generalized infections in man like urinary tract infections, septicemias, subacute bacterial endocarditis, and meningitis etc (Mackie and Mc Cartney, 1996).

About 15% of all the isolates encountered in clinical microbiological laboratories are non fermenters, out of which two thirds belong to *Pseudomonas* species (Bailey and Scott's, 10th ed.).

Most laboratories include in their daily identification schemes, tests for the detection of *Pseudomonas aeruginosa* as it accounts for the majority of pathogenic nonfermenters.

Their identification requires more efforts as many members of this group are slow growing and requires the use of special culture media and biochemical tests (Koneman, 1988).

Since many of them are resistant to common antibiotics, early identification seems imperative to institute an appropriate treatment which may reduce the mortality due to these organisms in hospitalized patients.

Despite widespread use of antibiotics, the nosocomial infections still pose a great problem. Several factors influence the changing spectrum of etiological agents like increased level of drug resistance, bacterial synergism, some environmental risk factors and a higher number of compromised hosts.

For these reasons a lot of opportunists such as the non-fermenting gram negative bacilli emerge creating a significant therapeutic challenge.

Materials and Methods

Clinical Samples like pus', sputum, urine, blood and csf were collected from in and out patients attending S.V.S Hospital Mahabubnagar. During the period of 01.04.2007 to 30.01.2008.

The Specimens were inoculated on nutrient, blood and Mac conkey agar and incubated aerobically over night at 37° for 24hrs and then examined next day.

Out of which 50 samples were showing growth of non – lactose fermenting colonies which were gram negative bacilli. They were then inoculated into TSI agar to see for acid production. An organism is considered non – fermenter if it fails to produce any acid on TSI agar and is then subjected to a battery of tests as follows.

Gram stain for morphology
Hanging drop for motility
Study of cultural characters

BA } 37°
MA } 37°
NA } Both 37°C and 42°C

For production of enzymes:

Catalase
Oxidase
Urease
Arginine dihydrolase
Nitrate reduction

For substrate utilization

Citrate test

For metabolism of proteins and aminoacids
Gelatin liquefaction, Indole production

Utilization of carbohydrates of media containing

Glucose Lactose Xylose Sucrose Maltose
Mannitol

Antibiotic sensitivity testing by standard disc diffusion method

Results and Discussion

Non fermentative bacilli in clinical Specimens

Non – fermentative bacilli	No. of isolates	Percentage
<i>Pseudomonas</i> spp.	41	82%
<i>Acinetobacter baumannii</i>	5	10%
<i>Acinetobacter lwoffii</i>	2	4%
<i>Alcaligenes faecalis</i>	2	4%

Table above show the incidence of different non fermentative bacilli in 50 clinical specimens. 41 of the isolates were *Pseudomonas* spp forming the majority. 7 isolates were *Acinetobacter* spp; 4 isolates were *Alkaligenes* spp.

Sensitivity pattern of non – fermenters to some of the antibiotics

26 isolates found to be sensitive to AK (Amikacin), 23 isolates to CF (Ciprofloxacin),

23 isolates to CA (Ceftazidime), 22 isolates to CE (Cefotaxime), 18 isolates to CPM (Cefipime),

12 isolates G (Gentamicin), 7 isolates to NF (Norfloxacin).

Percentage of non fermenters sensitive and resistant to antibacterial agents

Table 4 shows the sensitivity pattern of nonfermentative bacilli to various antibiotics.

69% *P. aeruginosa* were sensitive to Amikacin

58% to Ceftazidime, 69% Cefipime
61% to Ciprofloxacin

Acinetobacter baumannii sensitive to Cefperazone.

P. fluorescens was sensitive to Ciprofloxacin

Acinetobacter lwoffii – sensitive to Amikacin – 100%.

Alkaligenes faecalis – sensitive to Ciprofloxacin and Cefotaxime.

Percentage of nonfermenters resistant to antibacterial agents

AMP	-	100
S	-	100
NF	-	86
NF	-	86
G	-	76
CPM	-	64
CE	-	56
CA	-	54
CF	-	54
AK	-	48

The isolation of non- fermenters frequently from clinical materials has proved their pathogenicity (Topley and Wilson's 8th ed, 1999). One-fifth of all the gram negative bacilli isolated from various clinical specimens in a laboratory have been found to be non-fermenters (Bailey and Scott's, 10th ed).

About two-thirds of these are *Pseudomonas* spp. The remaining one-third include named and unnamed groups which are important because of their role in hospital acquired and frequent antimicrobial resistance (Homes *et al.*, 1977) (Fig. 1-4 and Table 1-4).

Table.1 Non fermentative bacilli in relation to the various clinical specimens i.e. pus, blood, urine; sputum, CSF and pleural fluid

Isolate	Pus	Urine	Sputum	Blood	CSF	Pleural Fluid
1. <i>P. aeruginosa</i>	10	7	4	3	1	1
2. <i>P. flourecens</i>	2	1	1	-	-	-
3. <i>P. cepacica</i>	1	1	1	-	-	-
4. <i>P. putida</i>	1	1	1	-	-	-
5. <i>P. stutzerl</i>	1	1	-	1	-	-
6. <i>P. maltophilia</i>	1	-	1	-	-	-
7. <i>P. baumannii</i>	2	3	-	-	-	-
8. <i>A. lwoffii</i>	-	2	-	-	-	-
9. <i>Alk. faecalis</i>	1	1	-	-	-	-
Total	19	17	8	4	1	1

Table.2 Incidence of nonfermenters in relation to age

CLINICAL SPECIMENS	NUMBER	0-20YRS	21-40YRS	41-60YRS	>60YRS
PUS	19	2	4	4	9
URINE	17	-	2	12	3
BLOOD	4	-	3	-	1
SPUTUM	8	-	3	4	1
PLEURAL FLUID &CSF	2	-	1	-	1

Table.3 Sex wise distribution

TOTAL NUMBER	MALE		FEMALE	
	NUMBER	PERCENTAGE	NUMBER	PERCENTAGE
50	35	70%	15	30%

Table.4

Isolate	AK		CF		CA		CPM		CE		G		NF		A		S	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
<i>P. aeruginosa</i> (26)	69	31	61.5	38.5	58	42	69	31	50	50	38.5	61.5	11.5	88.5	-	-	-	-
<i>P. flourecens</i> (4)	25	75	50	50	25	75	-	100	25	75	-	100	-	100	-	100	-	100
<i>P. cepacia</i> (3)	33.3	66.7	-	100	-	100	-	100	33.3	66.7	-	100	33.3	66.7	-	100	-	100
<i>P. putida</i> (3)	33.3	66.7	-	100	-	100	-	100	33.3	66.7	-	100	33.3	66.7	-	100	-	100
<i>P. stutzeri</i> (3)	66.7	33.3	-	100	100	-	-	100	33.3	66.7	-	100	-	100	-	100	-	100
<i>P. maltophilia</i> (2)	50	50	-	100	50	50	-	100	50	50	-	100	-	100	-	100	-	100
<i>A. baumannii</i> (5)	20	80	40	60	20	80	-	100	60	40	20	80	-	100	-	100	-	100
<i>A. lwoffii</i> (2)	100	-	50	50	-	100	50	50	50	50	-	100	-	100	-	100	-	100
<i>Alk. faecalis</i>	50	50	100	-	50	50	-	100	100	-	-	100	100	-	-	100	-	100

Fig.1

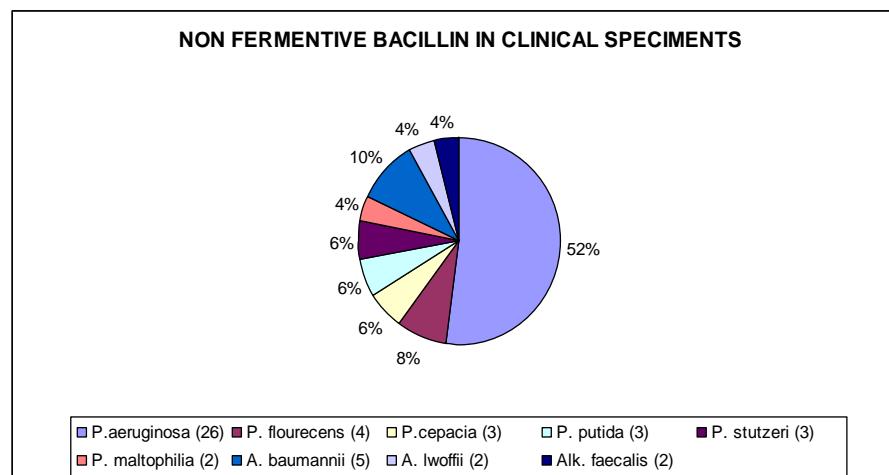


Fig.2 Incidence of nonfermenters in clinical specimens

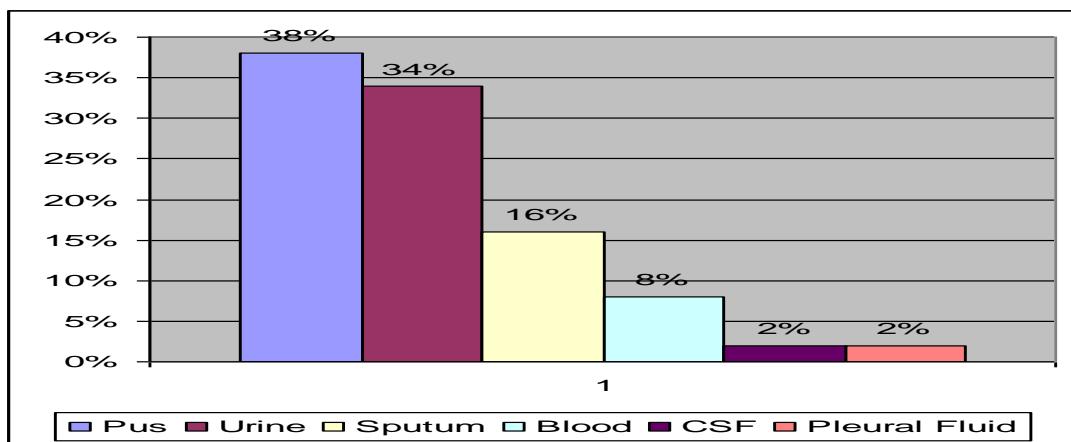


Fig.3

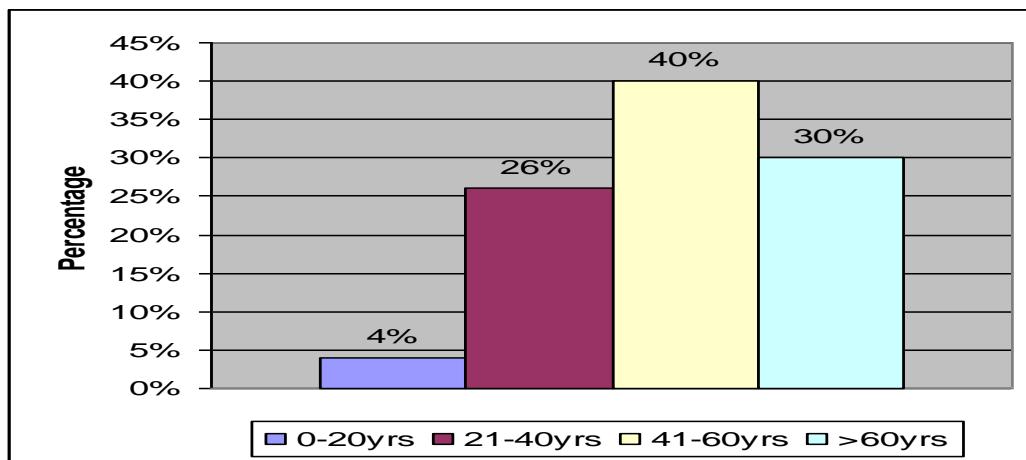
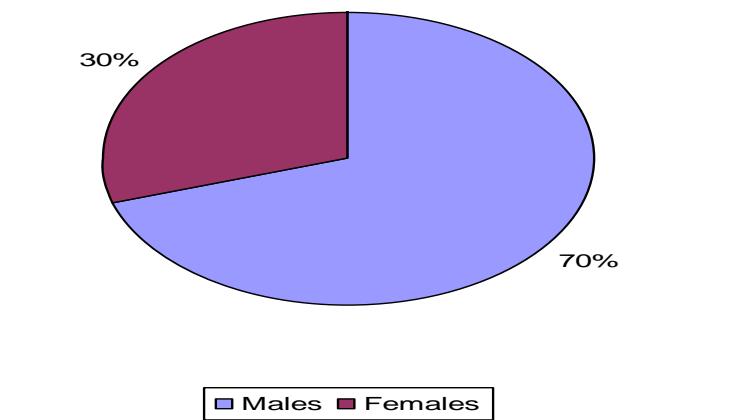


Fig.4



Pseudomonas species were found to be the predominant organisms isolated amongst the non – lactose fermenters in our study (82%) which is in agreement with earlier studies (Picket *et al.*, 1970; Gilardi *et al.*, 1985).

The *Pseudomonas* species isolated were *Ps aeruginosa* (26) *Ps. flourescens* (4), *Ps. cepacia* (3), *Ps. putida* (3) *Ps. stutzeri* (3) *Ps. maltophilia* (2), other non – fermenters isolated were *Acinetobacter baumannii* (5), *A. lowffii* (2) and *Alcaligenes faecalis* (2)

Pseudomonas aeruginosa is resistant to a large number of commonly used drugs. They are usually susceptible to Aminoglycosides which are the drugs of choice.

Out of 26 clinical isolates of *Pseudomonas aeruginosa* (69%) were sensitive to Amikacin and 58% to Ceftazidime these two being highly effective against *Pseudomonas*. Antimicrobial susceptibility of all the isolates being resistant two or more drugs. Only 11.5% were sensitive to all drugs (Coyle *et al.*, 1976; Venu *et al.*, 1999).

Majority of the isolates were from males (70%) which is akin with the studies of others (Coyle *et al.*, 1976; Neena *et al.*, 2001).

Most of the isolates were from patients between 41 and 60 (20) more than 60 years (15) and between 21 and 40 (13). This is in coincidence with earlier reports only two isolates were from patients between 0 – 20 years.

Our studies showed that out of 50 – non fermenters, *A. baumannii* (3) and *A. lwoffii* (2) were isolated from urine. Only 2 Isolates of *A. baumannii* are from pus.

Alcaligenes faecalis (1) each from urine and pus have been isolated which is in agreement with other studies (Michell *et al.*, 1966).

Non fermenters are normal inhabitants of the mucous membrane of humans and animals causing opportunistic infections. The pathogenic role of them has been proved beyond doubt by their frequent isolation from clinical samples and their association with this case. Since many of them are resistant to common antibiotics, early identification seems imperative.

As the organisms colonise in the tissues they should not be discarded as contaminants.

As non fermenters exhibit resistance to commonly used antibiotics combination of drugs may be useful in treating infections.

Conclusions are as follows:

- 1) Clinical Samples like Pus, Sputum, Urine, Blood and CSF were collected from in and out patients attending S.V.S Hospital Mahabubnagar. During the period of 01.04.2007 to 30.01.2008.
- 2) 50 isolates of non-fermenters characterized and identified.
- 3) Out of 50, 41 were *Pseudomonas* spp, 7 *Acinetobacter* Sp, 2 *Alkaligenes*.
- 4) Majority of the isolates were from Pus (19), Urine (17), Sputum (8), Blood (4), Pleural fluid (1) and CSF (1).
- 5) Maximum number of isolates was from patients between the ages of 41and60 years (40%).
- 6) The non-fermentative bacilli were resistant to the commonly used antibiotics like Ampicillin and streptomycin
- 7) They were sensitive to Amikacin (52%), Ciprofloxacin (46%), Ceftazidine (46%), Cefotaxime (44%), Cefipime (36%), Gentamicin (24%), Norfloxacin (14%)
- 8) Study shows that non-fermenters are frequently isolated from clinical specimens and that they are becoming resistant to the routinely used antibiotics and hence should be taken seriously rather

than regarding them as contaminants or commensals.

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