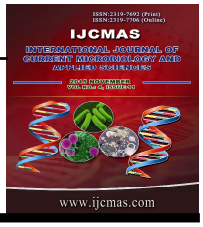
Int.J.Curr.Microbiol.App.Sci (2015) 4(11): 623-629

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 11 (2015) pp. 623-629**
http://www.ijcmas.com



Original Research Article

Characterization and Antibiotic Susceptibility Pattern of Gram Negative Non Fermenters in Various Clinical Samples in Tertiary Care Hospital of Navi Mumbai

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Keywords

Non-fermenting Gram-negative bacilli, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*. Nosocomial pathogen

A B S T R A C T

Gram negative non fermentative bacteria (GNNFs) are a group of aerobic, non spore forming, gram negative bacilli or coccobacilli that either do not use carbohydrate as a source of energy or degrade them through oxidative pathway rather than fermentation (Malini, 2009). Once considered as contaminants GNNFs now associated with life-threatening infections and emerging as multi drug resistant nosocomial pathogens. Aim of the work is to study the incidence and the antibiotic susceptibility patterns of GNNF organisms in various clinical samples. This study was conducted in the Department of Microbiology at a tertiary care teaching hospital over a period of 1 year. GNNFs were isolated and identified from clinical specimens by standard procedure and antibiotic sensitivity test was performed. A total 4146 samples were received for bacteriological isolation. Maximum samples received were urine, stool followed by respiratory samples. Out of 4146 samples 150 GNNF's were isolated (3.61%). Maximum isolates were *Ps. aeruginosa* 94 (62.66%) followed by *Acinetobacter baumannii* 35 (23.33%) and *Ps. fluorescens* 06 (4%). out of 150 GNNFs 56 (37.33%) were isolated from respiratory samples, 33(22%) were from pus, 23 (15.33%) were from urine samples. Nonfermenters have been attracting the attention of Clinicians and Microbiologist as emerging etiological agents of various kinds of infection, especially in the hospital environment. The role of non fermenters should not be underestimated as causative agent of various kinds of infections, especially in the hospital environment.

Introduction

Gram negative non fermentative bacteria are a group of aerobic, non spore forming, gram negative bacilli or coccobacilli that either do not use carbohydrate as a source of energy or degrade them through oxidative pathway rather than fermentation (Koneman, 2006). GNNF are ubiquitous in nature and widely distributed in soil, water, sewage and plants or as harmless bacteria on mucous membrane of humans or animals. They also grow in faucets, aerators, respirators, sinks and water baths. They contaminate medications or sterile solutions intended for intravenous therapy. These bacteria can cause disease by colonizing or subsequently infecting immunocompromised patient or

gaining access to normally sterile body site through trauma (Finegold Sm and Martin, 1982).

Infections by this microorganism are often difficult to treat because of its virulence, intrinsic and acquired antibiotic resistance, and the relatively limited choice for effective antimicrobial agents. It is particularly resistant to biocides (disinfectants, antiseptics, and preservatives) and thrives well in the hospital environment (Kaushal *et al.,* 1996).

Materials and Methods

The present study was carried out over a period of one year from January 2009 to January 2010 at microbiology department, MGM Medical College & Hospital, Kamothe, Navi Mumbai.

A total of 150 GNNF's (Gram negative non fermentative bacteria) were isolated from 4146 different clinical samples such as urine (1100), stool (900), respiratory samples (766), pus (670), blood (550), miscellaneous samples (160). Clinical details were recorded in a proforma.

Samples were collected by using standard methods. All these specimens were processed by standard techniques.

Preliminary identification of GNNF's was done by observing

TSI reaction: only those producing no acid in TSI medium will be termed as non fermenters and subjected to a battery of tests like:

1. Oxidase test
2. Motility test (Hanging drop preparation)
3. Indole test (by Kovac's method)
4. Citrate utilization test (Simmon's citrate medium)
5. Urease test (using Christensen's urea slant)
6. Methyl red test (by using MR reagent)
7. Sugar reaction in Hugh Leifson's O-F media
8. Pigment production
9. Nitrate reduction test
10. Decarboxylation test
11. Esculin hydrolysis
12. Sensitivity to polymyxin disc.

For identification of other non fermenters flow chart were prepared according to various biochemical tests helpful to identify organism upto species level, the flow chart was prepared by combining the principles of various schemes (Weaver-Hollis, Gilardi & Pickett) & Koneman's Color Atlas and Textbook of Diagnostic Microbiology (Koneman, 2006).

Antimicrobial sensitivity was determined using Kirby Bauer disc diffusion method.

MacFarland standard (0.5) was used to compare the inocula while testing the sensitivity (Pederson *et al.,* 1970).

Ps. aeruginosa (ATCC- 27853) was used as standard sensitive strain.

Primary line of drugs used for isolates other than those from urine.

Amikacin (30mcg), ciprofloxacin (5), cephotaxime (30), cefuroxime (30), augmentin (30) lomefloxacin (30), ceftazidime (30), cefaperazone (30), gentamycin (75), netilmicin (30), pefloxacin (5) and oflaxacin (5).

For isolates from urine sample:

Ampicillin (20mcg), cotrimoxazole (25), ceftizoxime (30), chloramphenicol (30), cephalexin (30), tetracyclin (30), ciprofloxacin (5), sparfloxacin (300), gatifloxacin (10), norfloxacin (10) and ofloxacin (5)

Second line antibiotics:

Imipenem (10), cefepime(30), cefoperazone/sulbactem (75/30), ceftazidime (30), piperacillin/ tazobactem (100/10), meropenem (10), cefuroxime (30), ticarcillin/clavulanic acid (75/10)

Results and Discussion

Out of 4146 clinical samples examined 150 (3.61%) yielded nonfermenters, of which 23 (2.09%), 07 (0.77%), 56 (7.31%), 33 (4.92 %,), 12 (2.18%) and 19 (11.87%) were from urine, stool, respiratory samples, pus, blood and miscellaneous samples respectively.

Table 1 shows that total 4146 samples were received for bacteriological isolation. Maximum samples received were urine, stool followed by respiratory samples. Total GNNF's isolated were 3.61%.

Pie chart showing spilt of total No. of non-fermenters isolated from various clinical samples (n= 150).

Out of total 150 non fermenters isolated in the present study, 56 (37.33%) were isolated from respiratory samples, 33(22%) were from pus, 23 (15.33%) were from urine, 19 (12.66%) were from miscellaneous samples, 12 (8%) were from blood and 7 (4.66%) were from stool samples.

The nonfermantative gram negative bacilli (NFGNB) are distributed widely in nature and have been isolated from soil, water and medical devices as well as from clinical specimens (Finegold Sm and Martin, 1982). The widespread use of antibiotics and other chemotherapeutic agents in the treatment of diseases had played a major role in the frequency of infection by these organisms. Another factor is the increase in the number of debilitated people with chronic diseases that may impair the immunological defense mechanisms.

In our study, out of 4146 samples, 150 were non fermenters. Thus the incidence of non fermenters is 3.16%. Kaushal *et al.* (1998) they also reported 4.4% incidence of GNNF's from cases of urinary tract infections. Gardner and Griffith (1970) reported incidence of 4.4%. Thus the finding of this study shows comparable results with other workers.

Maximum non fermenters were isolated from respiratory sample (sputum, endotracheal secretion and tracheotomy tube) 56 (37.33%) followed by pus samples 33 (22%), urine 23 (15.33%), miscellaneous samples 19 (12.66%), blood samples 12 (8%) and stool samples 7 (4.66%). Comparable isolation rates of non fermenters from Yashodhara and Shyamala *et al.* (1997) reported 34.88%, 21% and 27.90% from urine, pus and blood respectively.

Ps. aeruginosa was the most common isolate found (62.66%) in the present study followed by *Acinetobacter baumannii* 23.33% of the total non fermenters isolated. Similar results were obtained by Malini et al (2009) and Arora Usha *et al.* (2006)

Ps. fluorescens was found to be 4% in the present study. Similar results were obtained by Rao and Shivnanda (1993) 3.89%

In the present study majority of GNNF's were resistant to commonly used antibiotics.

There was no single antibiotic to which all the non fermenters were sensitive. This was also reported by Rao and Shivnanda (1993) and Yashodhara and Shyamala (1996).

Most of the GNNF's isolated in the present study were sensitive to amikacin (60.66%), cefotaxime (59.33%), ofloxacin (58.66%), ceftazidime (55.33%), ciprofloxacin and gentamycin (53.33%).

Vijaya *et al.* (2000) reported sensitivity to amikacin 85 (63.90%), fluroquinolone, ciprofloxacin and norfloxacin 48.12 and 27.80% respectively.

In the present study, higher resistance was observed to augmentin (84%) and cefuroxime (79.33%).

In the present study *Ps. aeruginosa* were found to be sensitive to imipenem (76.66%), amikacin (69.14%), cefotaxime (64%), ceftazidime (63.82%) and ofloxacin (60.63%) and cefaperazone (58.6%). Similar results were seen with the studies of Malini *et al.* (2009).

Acinetobacter baumannii were tested for antibiotic sensitivity and were found to be sensitive to ofloxacin, pefloxacin (48.57%) each, and cefotaxime (37.14%) and resistant to cefaperazone (14.28%), gentamycin (20%) and amikacin (28.57%).

Similar results were reported by Malini (2009) with 100% sensitivity to Imipenem, 53.5% to amikacin, 27% to ciprofloxacin, and14% to cefaperazone. All strains of *Alcaligenes denitrificans* were 100% sensitive to ciprofloxacin. In pus sample most isolates were resistant to augmentin, amikacin, cefuroxime. Most of the isolates from respiratory sample were sensitive to gentamycin, ofloxacin and amikacin.

Summary and Conclusion

Main purpose of the present study was isolation and identification of non fermenters from various clinical specimens.

A total of total 4146 samples were received over a period of January 2009 to January 2010 for bacteriological isolation. Maximum samples received were urine and stool followed by respiratory samples. The incidence of GNNF's in our study was 3.61%.
In the present study, out of total 150 non fermenters isolated, most isolates were from respiratory samples (37.33%), followed by pus (22%) and (15.33%) were from urine sample.

Out of total 150 GNNF's isolated, maximum isolates were *Ps. aeruginosa* 94 (62.66%) followed by *Acinetobacter baumannii* 35 (23.33%) and *Ps. fluorescens* 06 (4%).

Most of the GNNF's isolated in the present study were sensitive to amikacin (60.66%), cefotaxime (59.33%), ofloxacin (58.66%), ceftazidime (55.33%), ciprofloxacin and gentamycin (53.33%).

Nonfermenters have been attracting the attention of clinicians and microbiologist as emerging etiological agents of various kinds of infection, especially in the hospital environment.

The role of non fermenters should not be underestimated as causative agent of various kinds of infections, especially in the hospital environment.

Table.1 showing data of number of samples received and total non fermenters isolated

Sample	No. of samples received	Total non fermenters isolated	% of isolation
Urine	1100	23	2.09%
Stool	900	07	0.77%
Respiratory samples	766	56	7.31%
Pus	670	33	4.92%
Blood	550	12	2.18%
Miscellaneous samples	160	19	11.87%
Total	4146	150	3.61%

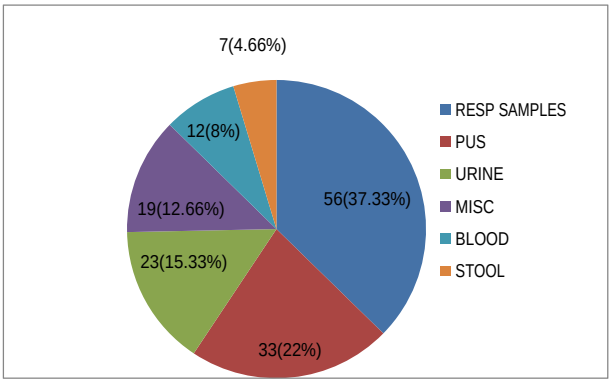
Table.2 Showing isolation of different species of GNNF's from clinical samples (n= 150)

Name of organism	Total No. isolated	% of isolation
Ps.aeruginosa	94	62.66%
Acinetobacter baumannii	35	23.33%
Ps. Fluorescens	06	4%
Ps.stutzeri	03	2%
CDC group – 03	02	1.33%
Shewanella	02	1.33%
Alkaligenes faecalis	02	1.33%
Alcaligenes denitrificans	02	1.33%
Sphingobacterium multivorum	01	0.66%
Stenotrophomonas maltophila	01	0.66%
Moraxella catarrhalis	01	0.66%
Sphingomonas paucimobilis	01	0.66%

Table.3 Sample wise isolation of GNNFs

SAMPLES.																		
Sr. No	**Organism Isolated**	**Respiratory**			Pus	U	Bld	St	**Miscellaneous**									**Total**
		SP	ET	TT					CT	ES	CSF	CL tip	Vg Swab	BS	Ts	PF	Pl F	
1	*Ps. aeruginosa*	18	10	3	18	21	5	7	4	4	1	1	1	1	-	-	-	94
2	*A. baumanni*	5	9	1	6	1	7	-	1	-	1	1	1	-	-	1	1	35
3	*Ps. fluroscence*	2	-	-	3	1	-	-	-	-	-	-	-	-	-	-	-	06
4	*Ps. stutzeri*	1	-	-	2	-	-	-		-	-	-	-	-		-	-	03
5	*CDC-03*	1	-		1	-	-	-	-	-	-	-	-	-	-	-	-	02
6	*Shewanella*	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	02
7	*Alcaligenes faecalis*	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	02
8	*Alcaligenes denitrificans*	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	02
9	*Sphingobacterium Multivorum*	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	01
10	*Stenotrophomonas maltophila*	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	01
11	*Moraxella catarralis*	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	01
12	*Sphingomonas paucimobilis*	1	-	-	-	-	-	-	-	-	-	-	-	-	-		-	01
	Total	31	21	04														
		56			33	23	12	07	05	04	02	02	02	01	01	01	01	150

Fig.1 Pie chart showing spilt of total number of non-fermenters isolated from various clinical samples (n= 150)



Reference

Arora Usha *et al.* 2006. *Acinetobacter* spp. - An emerging pathogen in neonatal septicemia in Amritsar. *Indian J. Med. Microbiol.*, 24(1): 81.

Finegold, S.M., Martin, W.J. 1982. Non-fermentative Gram negative bacilli and coccobacilli. In: Baly and Scotts diagnostic microbiology, 6th edn, CV Mosby CO., St. Louis Toronto, London. Pp. 249–265.

Gardner, P., Griffith, W.B. 1970. Non fermemtative gram negative bacilli of nosocomial interest. *Am. J. Med.*, 48: 735–748.

Kaushal, M.L., Grover, P.S., Gupta M.L. 1996. Non fermentative Gram negative bacteria assosciated with pyogenic infection. *Indian J. Med. Microbiol.*, 14(3): 163–165.

Kaushal, M.L., Grover, P.S., Gupta, M.L. 1998. Non-fermenters in urinary tract infection. *J. Assoc. Physicians India*, 46(9): 798–800.

Koneman, E.W. 2006. The non fermentative Gram negative bacilli. In: Koneman, E.W., Atlan, S.D., Dowell, Sommers, H.B. (Eds), Color atlas and text book of diagnostic microbiology, 6th edn. JB Lipincot Co, Philadelphia. Pp. 309–375.

Malini, A, Deepa, E.K. 2009. Non fermenting Gram negative bacilli infections in tertiary care hospitals in Kolar, Karnataka. *J. Lab. Phy.*, I(2): 62–66.

Pederson, M.M., Marso, E., Picklet, M.J. III. 1970. Non fermeters bacteria associated with man. *Am. J. Clin. Pathol.*, 54: 178–192.

Rao, Shivananda, 1993. Bacteraemia due to non-fermenting gram negative bacilli in immunecompromised patients. *Indian J. Med. Microbiol.*, 11(2): 95–99.

Vijaya, D., Kamala, *et al.* 2000. Prevalence of nonfermenters in clinical specimens. *Indian J. Med. Microbiol.*, 54(3): 87–91.

Yashodhara, P., Shyamala, P.S. 1997. Identification and characterization of non fermentors from clinical specimens. *Ind. J. Med. Microbiol.*, 15(4): 195–197.