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Isolation and Identification of Non-Fermenting Gram Negative Bacteria

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Abstract

Background: In medical microbiology, Non-Fermentative group of bacteria have been incriminated as emerging opportunistic pathogens, especially from hospital settings. Non fermenting gram negative bacilli are a group of heterogeneous, aerobic (grow in presence of oxygen), non sporing bacteria.

Materials & Methods: 150 isolates from various age groups of both male and female patients were included in the study. A detailed history was elicited and the clinical specimens were collected under aseptic precautions and subjected to preliminary biochemical test and further speciation was done.

Results: In the present study *Pseudomonas aeruginosa, Acinetobacter baumanii, Pseudomonas fluorescens* were isolated of which *Pseudomonas aeruginosa* Was the most commonest. The non fermenting gram negative bacilli were Isolated 28. 68% from local wound infection, 22. 3% from post operative wound infection, 19. 9% from respiratory tract infections, 8. 37% from gastrointestinal tract infections, 6. 22% from urinary tract infections and 4. 68% from septicemia cases.

Conclusion: The non fermenting gram negative bacilli infection is mainly seen in patients with serious underlying risk factors like prolonged stay in hospital, catheterization, underlying diseases like diabetes, malignancies and chronic pulmonary disease. Ciprofloxacin, Ofloxacin, Amikacin, Imipenem appeared to be effective drugs in treating non fermenting gram negative bacilli infections.

Keywords: Non Fermenting Gram Negative Bacilli; Antimicrobial Susceptibility.

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

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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 immunocompromised 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.

MATERIAL AND METHODS

Study place: The study was carried out in the Department of Microbiology, L. N Medical College Bhopal M.P.

Study design: Prospective study.

Study duration: One year

A total of 250 clinical samples like pus, sputum, urine, blood and csf were collected from in and out patients attending LN Medical College & Hospital. The Specimens were inoculated on Nutrient agar, Blood agar and MacCconkey agar and incubated aerobically over night at 37° for 24hrs and then examined next day. Out of which 150 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 containing of carbohydrates of media Glucose Lactose Xylose Sucrose Maltose Mannitol Antibiotic sensitivity testing by standard disc diffusion method.

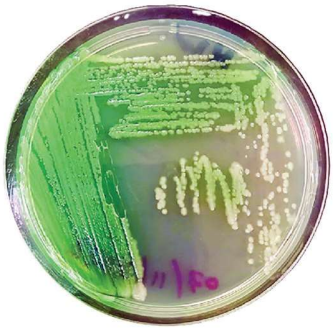


Fig. 1: ***Pseudomonas aeruginosa*** **growth on Nutrient agar**

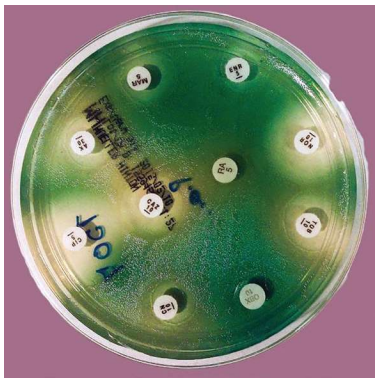


Fig. 2: Antimicrobial Susceptibility of Pseudomonas on Muller Hinton Agar

RESULT

Table 1 shows that the Pus samples constituted majority of specimens accounting 45. 3%. Urine and sputum samples accounted for 15. 3% & 14% of specimens respectively. Stool, Blood, Pleural fluid, and CSF samples accounted for remaining 26%.

Table 2 shows that Pseudomonas aeruginosa was isolated from 15 cases of local infection, 9 cases each of respiratory tract and post operative infection, 4 cases of each of post traumatic and infection related to gastrointestinal tract, 3 cases of urinary tract infection and 2 cases of septicaemia. *Pseudomonas fluorescens* was isolated from 4 cases of post op infection and 2 cases of respiratory tract infections.

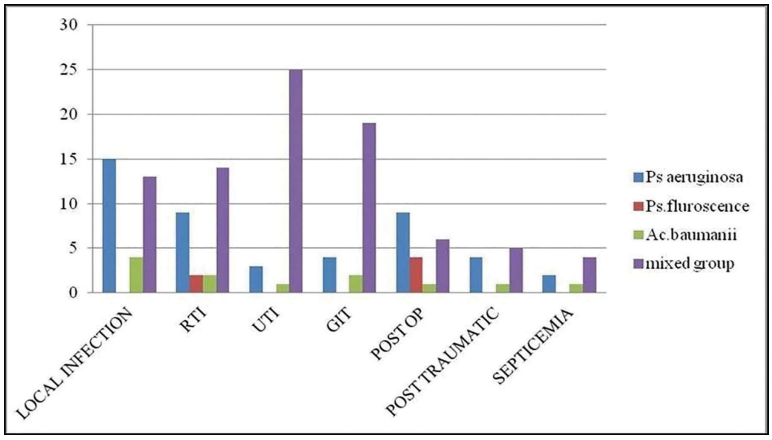
Acinetobacter baumanii was isolated from 4 cases of local infection, 2 cases each of respiratory tract infection and infection related to gastro intestinal tract, 1 case each of septicemia, post op infection, post traumatic and urinary tract infection. Mixed growth *(Proteus mirabilis, Proteus vulgaris, Citrobacter, MRSA, E coli, Klebsiella, Enterococci, Enterobacter species, Group A beta haemolytic streptococci, Salmonella spp, Shigella spp)* were mostly seen in local infection – 13 cases, 6 cases of post-op infections, 19 cases of infection related to gastro intestinal tract, 25 cases of urinary tract infection, 14 cases of respiratory tract infection, 5 cases of post traumatic infection and 4 cases of septicemia (Table 3).

Table 1: Number of Cases reported from various Clinical Specimen

S. No	Sample	No. of cases	Percentage (%)
1	Pus	68	45. 33
2	Sputum	21	14
3	Ascitic fluid	5	3. 33
4	Blood	7	4. 66
5	Urine	23	15. 33
6	Stool	11	7. 33
7	Cervical Discharge	4	2. 66
8	Pleural Fluid	6	4
9	CSF Fluid	5	3. 33

Table 2: Bacterial species isolated under each clinical infections

Species	Local infection	RTI	UTI	GIT	Post op infection	Post traumatic infection	Septicemia	Total
Ps. *aeruginosa*	15	9	3	4	9	4	2	46
Ps. *fluorescens*	-	2	-	-	4	-	-	6
Ac. *baumanii*	4	2	1	2	1	1	1	12
Mixed Group	13	14	25	19	6	5	4	86



Graph 1: Bacterial species isolated under each clinical infections

Table 3: Antibiotic susceptibility pattern of NFGNB for Penicillin group of drugs

Antibiotic	Sensitive		Resistant	
	No of Cases	%	No of Cases	%
Penicillin	3	4. 68%	61	95. 32%
Amoxyclav	14	21. 87%	50	78. 13%
Carbenicillin	35	54. 68%	29	45. 32%
Piperacillin+ Tazobactam	29	45. 31%	35	54. 69%
Netilmicin	9	14. 06%	55	85. 94%
Ticarcillin	16	25%	48	75%
Piperacillin	18	28. 12%	46	71. 98%

Table 4: Antibiotic susceptibility pattern of NFGNB for Carbapenem group of drugs

Antibiotic	Sensitive		Resistant	
	No. of cases	%	No. of cases	%
IMIPENEM	54	84. 3%	10	15. 6%
MEROPENEM	38	59. 4%	26	40. 6%

Table 4 showed a sensitivity of 84. 3% to Imipenem and 59. 4% sensitivity were seen with Meropenem.

DISCUSSION

The Non fermenting bacilli are widely distributed in nature as saprophytes or as commensals and pathogen to man. NFGNB earlier considered as a contaminant is now gaining importance as a Nosocomial pathogen. During the study period, 150 samples from various clinical conditions like local infection, post-operative infection, post traumatic infection, respiratory tract infections, urinary tract infection, septicaemia, gastrointestinal tract infection and genital tract infection were collected and subjected for further processing. NFGNB were isolated from 64 samples which included infections caused by Ps. *aeruginosa*, Ps. *fluorescens*, Ac. *baumani* were isolated similar to other studies by Yashodhara P*et al.*[9]

In our study NFGNB's were most commonly isolated from pus sample. This is similar to earlier studies done by Mishra E *et al.*[2] and Yashodhara Petal[9].

Ps. *aeruginosa*, Ac. *baumanii* were the most common isolates fromlocal infectionlike cellulitis, diabetic foot, ear discharge and burns in our study which was similar to other studies by Rajan R *et al.*[10] Ps. *aeruginosa* was the main etiological agent responsible for 52.7% local infections in our study. However, it was higher in studies by Yashodhara P *et al.*[9] 66. 95%, in Mishra *et al.* study[2] 66%, in Resmi Rajan *et al.*[10] 89.9% and in Cristane *et al.* study 72. 5%.

The differences in the percentages of various parameters may be due to the variation in the sample size. In our study *Pseudomonas aeruginosa* caused 58% of Post-operative wound infection and *Pseudomonas fluorescents* caused 23.57%. In a study by Resmi Rajan *et al. Pseudomonas aeruginosa*. caused 34.09% of post-operative wound infection. In a study by Yashodhara *et al. Pseudomonas fluorescens* caused 5.8% of post-operative infection. Infections related to abdomen included peritonitis cases in our study Ac. Baumanii was the most common NFGNB isolate. In our study patients who had been catheterized for >72 hours, urinary tract infection was common with *Pseudomonas aeruginosa* and *Acinetobacter baumanii*. Not much inference could be obtained as the number isolated was very small 2%. Ps. *aeruginosa* and Ac. *baumanii* both are known to cause recurrent and chronic urinary tract infection and often multi drug resistant.

NFGNB's showed an overall 15.66% resistance to Imipenem in our study. In a study by Taneja *et al.* it showed 36%. *Pseudomonas aeruginosa* showed 13.04% resistance to Imipenemin our study, in other studies done by Taneja *et al.*[6], Rajan Retal[10], Gupta E *et al.*, Troillet N *et al.*, Smitha S *et al.*, Wong fu *et al.*[56] showed a range of 11. 8% - 81. 5%.

CONCLUSION

Large number of NF isolated from different patients has an etiological role to play in infections and is reflected by the fact that, in repeated cultures same organisms were re-isolated. Most of the patients had high risk factors like prolonged stay in hospital especially in ICUs, catheterisation (both urinary and intravenous), diabetes, burns and malignancy. The most common isolates were Ps. *aeruginosa* 30. 66% followed by Ac. *baumanii* 8 % Ps. *fluorescens* 4%. Most common clinical conditions were ulcers, post-operative wounds, COPD, peritonitis and burns cases.

The sensitivity pattern changes from hospital to hospital and population to population. Treating NFGNB systemic infection is usually by broad spectrum intensive treatment and specific therapy is based on laboratory data after identifying the causative agent and antibiotic susceptibility results. Minimized use of available antimicrobial, regular antimicrobial susceptibility surveillance and strict infection control measures are required to contain this emerging antibiotic resistance among NFGNBs.

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