International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Significance of Multi Drug Resistant Non Fermenting Gram Negative Bacilli Isolated From Multiple Clinical Samples in a Tertiary Care Hospital, Nellore, Andhra Pradesh

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Abstract

Objective:

Gram negative bacilli of Enterobacteriaceae & non Enterobacteriaceae form a major group of isolates from multiple clinical samples in a tertiary care hospital and also in diagnostic laboratories. Among them Non fermenting Gram negative bacilli are posing a major clinical challenge owing to their multi drug resistance & form a major group of health care associated infections (HAI). The current study was designed to isolate & characterize bio-chemically various non fermenting gram negative bacilli & to establish their antibiotic susceptibility pattern from multiple clinical samples.

Results:

A total of 1000 clinical samples were collected from the hospitalized patients. Of these, 560(56%) were culture positive. 166 Gram negative non fermenting bacilli(29.64%) were isolated from these culture positive samples.

Of these 166 non fermenting gram negative bacilli, majority of isolates were from sputum, urine specimens followed by pus, blood & ET secretions, Catheter tips. Common non fermenters isolated in our study include Pseudomonas aeruginosa, Acinetobacter, Burkholderia cepacia .

Majority of these isolates were resistant to Aminoglycosides, fluoroquinolones and also to some extent for Carbapenems. All the isolates were found to be susceptible to Colistin , followed by Polymyxin-B. Maximum rate of Multi drug resistance was exhibited by Acinetobacter species .

Keywords: Multi drug resistance, Non fermenting Gram negative bacilli, Pseudomonas aeruginosa, Acinetobacter, Health care associated infections.

Introduction:

Gram negative bacilli either Enterobacteriaceae or non Enterobacteriaceae form major part of health care associated & community acquired infections like UTI, Respiratory, Wound infections & blood stream infections. Among these Non fermenting Gram negative bacilli have emerged as major nosocomial pathogens and posing a clinical & diagnostic challenge owing to their multi drug resistance. Non fermenting Gram negative bacilli are widely distributed & can be isolated from commonly used

instruments in hospitals, laboratories and from the body surface of health care workers. More than 15% of Gram negative bacterial infections are caused by Non fermenting Gram negative bacilli. NFGNB cause wide range of infections in Immuno compromised , hospitalized individuals and patients with associated co morbidities.

Greater antibiotic resistance exhibited by NFGNB is due to their immense rate of anti microbial treatment than the Pathogenicity of a particular strain. Because of their multi drug resistance nature the study performed aims to enlighten the significance of commonly isolated Non fermenting Gram negative bacilli and implementation of specific antibiotic policy there by reducing the rate of infections and resistance to newer generation antibiotics.

Methodology:

The study was conducted in the department of clinical Microbiology, Narayana Medical College & Hospital from March 2022 to February 2023. Samples were collected from hospitalized patients under aseptic conditions which include Urine, Pus, Sputum, Blood, ET secretions, Catheter tips, BAL .Collected samples were processed according to standard protocols and inoculated on to Mac Conkey agar, Blood agar, Chocolate agar. Blood cultures were performed and bottles were incubated in BAC T ALERT automated system. Positive cultures were further processed on Mac Conkey, Blood agar & Nutrient agar media. Identification of NFGNB was carried out according to regular protocol which includes microscopic methods such as Gram's staining, motility and major bio-chemical tests to determine the Phenotypic characters of the isolate. Antibiotic susceptibility was performed by using Kirby Bauer Disk diffusion method & interpretation was performed according to CLSI guidelines.

SPSS version was employed for data analysis in the form of frequency distribution and percentages.

Results:

A total of 1000 different clinical samples were collected from hospitalized patients of which 560(56%) were culture positive with significant growth . Of these 560 culture positive cases, Non fermenting Gram negative bacilli were isolated from 166 specimens (29.64%)which include Sputum, Urine, Pus, Blood, Catheter tips, BAL .Of the 1000 clinical samples 570 samples were from males & 430 were from females . Among 166 Non Fermenting Gram Negative Bacilli, 112 isolates were from males (60.5%)& 54 from females (39.45%)

Table-1: %of isolation of Non Fermenting Gram negative Bacilli

Total No: of Samples (N)	Positive cultures with significant growth	Total No: of NFGNB	% of NFGNB
1000	560(56%)	166	29.64

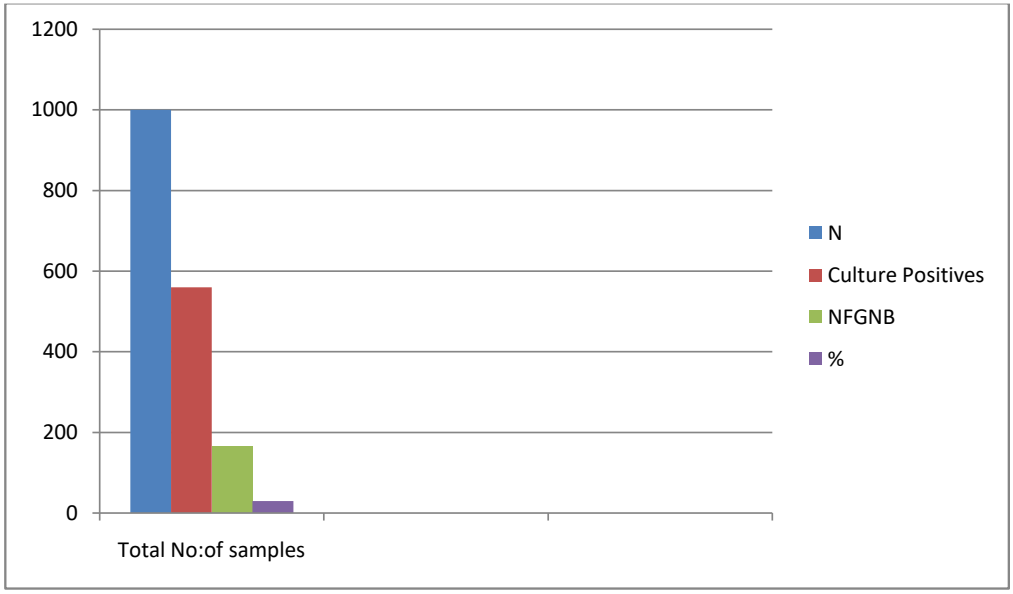
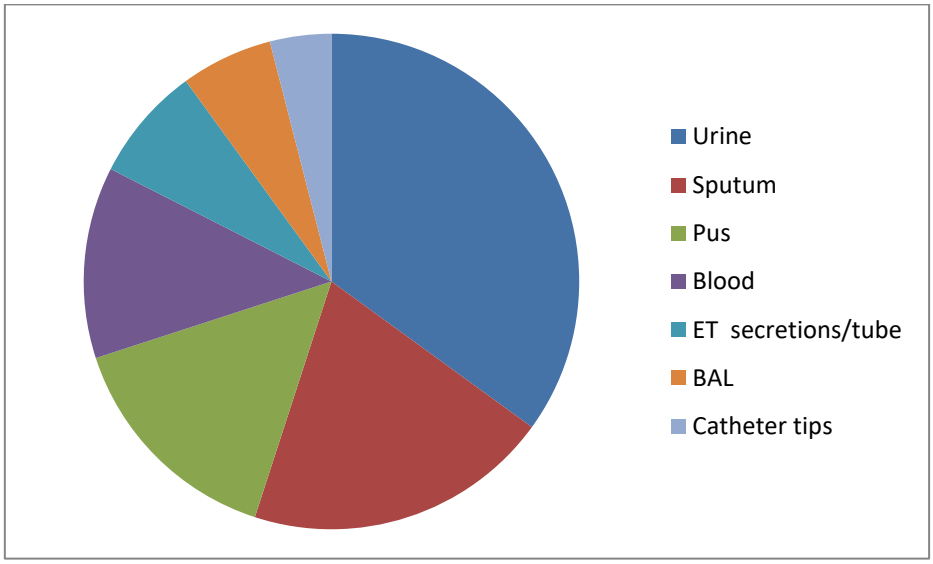


Table-2: Sample wise distribution (N=1000)

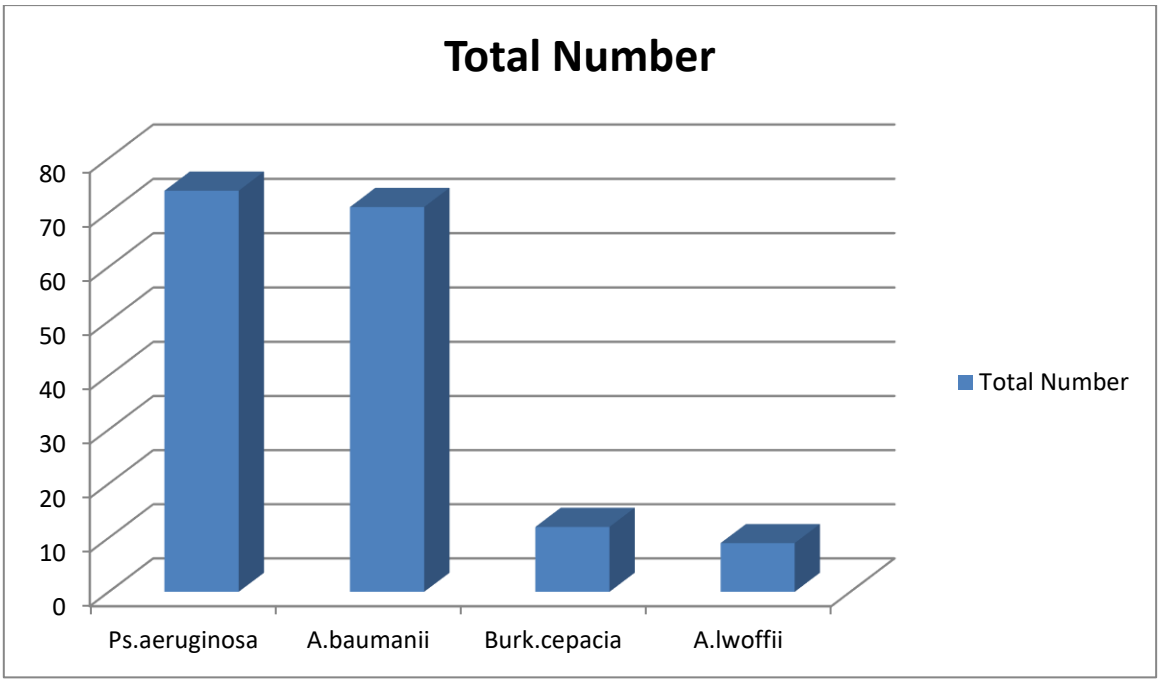
Urine	**350**
Sputum	**200**
Pus	**150**
Blood	**125**
ET secretions/ ET tube	**75**
Catheter tips	**40**
Broncho Alveolar Lavage(BAL)	**60**



Non fermenting Gram Negative Bacilli isolated in the current study include:

Pseudomonas aeruginosa, Acinetobacter baumannii, Burkholderia cepacia, Acinetobacter lwoffii and their distribution is tabulated as given below.

Name of the isolate	Total Number isolated(N_t=166)	% of isolation
Pseudomonas aeruginosa	74	44.57
Acinetobacter baumanii	71	42.77
Burkholderia cepacia	12	7.22
Acinetobacter lwoffii	09	5.42



Majority of Pseudomonas aeruginosa isolates were recovered from urine followed by sputum, pus, broncho alveolar lavage specimens. Maximum number of Acinetobacter baumanii isolates were from Urine, Sputum, Broncho alveolar lavage & from Catheter tips.

Isolates of Pseudomonas aeruginosa were identified based on Pigment production , Catalase positive, Oxidase positive & active motility.

Table showing rate of isolation from collected clinical specimens:

Bacterial isolate	No: of isolates from Urine	No: of isolates from Sputum	No: of isolates from Pus	No:of isolates from BAL
Ps.aeruginosa(N=74)	31	20	12	4
A.baumanii(N=71)	26	25	10	3
Burk.cepacia(N=12)	5	1	3	1
A.lwoffii(N=09)	3	2	1	1

A total of 4 isolates of Pseudomonas aeruginosa were recovered from blood (Blood cultures) & 3 from Catheter tips.

Rate of isolation of A.baumanii , B.cepacia & A.lwoffii from other clinical specimens

Name of the isolate	**Blood**	**Catheter tips**
A.baumanii	4	2

B.cepacia	1	1
A.lwoffii	1	1

Pseudomonas aeruginosa & Acinetobacter baumanii exhibited minimal range of sensitivity to majority of commonly tested antibiotics in clinical practice . All the isolates of Acinetobacter baumanii & Pseudomonas aeruginosa were sensitive for Colistin & Polymyxin-B. Maximum rate of MDR was identified among the isolates of Pseudomonas aeruginosa , followed by Acinetobacter baumanii, Burkholderia cepacia. Both Pseudomonas aeruginosa & Acinetobacter baumanii exhibited equal range of resistance towards the commonly used antibiotics which are tabulated as below:

Name Of The Antibiotic	**Sensitive/ Resistance**
Amoxycillin + Clavulanic acid	Resistance
Ceftazidime, Cefipime	Resistance
Ciprofloxacin	Resistance
Amikacin	Resistance
Gentamicin	Resistance
Co-trimoxazole	Resistance
Piperacillin +Tazobactam	Resistance

Sensitivity & Resistance range for Carbapenems tested in the study:

Name of the isolate	**Carbapenem group of drug**	**Sensitive**	**Resistance**
Pseu.aeruginosa(N=74)	Imipenem Meropenem	52(70.27%) 58(78.3%)	22(29.72%) 16(21.62%)
Ac.baumanii(N=71)	Imipenem Meropenem	49(69.01%) 38(53.52%)	22(30.98%) 33(46.47%)
Burkh cepacia(N=12)	Imipenem Meropenem	07(58.33%) 08(66.66%)	05(41.66%) 04(33.33%)
Ac.lwoffii(N=09)	Imipenem Meropenem	05(55.55%) 03(33.33%)	04(44.44%) 06(66.66%)

Data analysed suggests that carbapenamase producing Non fermenting gram negative bacilli were incresing alarmingly and posing a threat in patient recovery especially among ICU cases.

Bio-chemical characterization of the isolates in the current study:

Name of the isolate& Motility nature	**Citrate utilization test**	**Urease production test**	**TSI reaction**
Ps.aeruginosa-Motile	Positive (Citrate utilized)	Negative	Alkaline slant/Alkaline butt(K/K)
Ac.baumanii-Non motile	Positive	Variable	K/K
Burk.cepacia- Motile	Positive (Utilized)	Negative	K/K

Ac.lwoffii- Non motile	Negative	Negative	K/K

Discussion:

Health care associated infections (HAI) are always a major concern because of emergence of Multi drug resistant bacterial strains which are a real menace for the clinicians posing a challenge in patient recovery. Both Enterobacteriaceae & non Enterobacteriaceae Gram negative bacilli exhibit multi drug resistance . Non fermenting gram negative bacilli have emerged as major threat in health care sector because of their MDR nature.

In this study a total of 166(29.64%) Non fermenting Gram negative bacilli were isolated from 560 culture positive cases which is slightly more, but nearly corelates with the study performed by Santhosh yadav etal (27.1%). Studies conducted by Rahbar etal documented 15% isolation rate for NFGNB exhibiting multi drug resistance . The difference in the rate of isolation of NFGNB in various health care settings is probably due to poor infection control practices, indiscriminate us of antibiotics leading to emergence of MDR strains and lack of standard antibiotic policy in the respective hospitals . In our study majority of the NFGNB isolates were from Urine sample followed by Sputum, Pus . Pseudomonas aeruginosa is the most common isolate in our study (44.57%), followed by Acinetobacter baumanii(42.77%) followed by Burkholderia cepacia(7.22%) which is closely in accordance with studies done by Santhosh yadav etal which revealed Acinetobacter baumanii(44%) as most common isolate with slightly higher isolation rate than Pseudomonas aeruginosa(41%). These bacteria are ubiquitous in presence & can be isolated from multiple places in hospitals like Ots, Ward surfaces, beds, cots , skin of health care personnel, instruments used in Ots, surgeries etc..NFGNB isolated in our study are highly potent in causing wide disseminated type of infections ranging from simple wound infections, UTI to severe Sepsis . Strains of Acinetobacter baumanii, Pseudomonas aeruginosa exhibit wide range of antibiotic resistance either acquired or intrinsic drug resistance. Few isolates in our study exhibited resistance even to Carbapenems and are potent causes for nosocomial & community acquired infections. Infections especially because of Carbapenem resistant Acinetobacter baumanii, Pseudomonas aeruginosa are associated with alarming rise in morbidity & mortality in the infected / hospitalized patients. All the isolates in our study were susceptible to “last resort” drugs i.e; Colistin, Polymyxin-B which is in accordance with the study performed by Santhosh yadav etal, Anil C etal, Rit K, Nag F etal quoting the susceptibility patterns of NFGNB.

Strict, vigilant & meticulous antibiotic policy should be implemented in health care settings, tertiary care hospitals to minimize the emergence of multi drug resistant Gram negative bacilli . Indiscriminate use of antibiotics should be curtailed especially in ICUs, Post operative units thus minimizing the out break of SSIs. Regular Surveillance to trace out the emergence of new MDR Strains should be conducted which is vital for reducing the out break of SSIs also.

Ethics approval:

The study was performed after attaining clearance from Instituitional ethics board . No special invasive procedures were done during the study and all the samples collected were regularly drawn from the infected clients.

Acknowledgements:

Our sincere thanks to all the faculty & technical experts of the microbiology laboratory.

Constraints/limitations of the study:

Because of lack of complete data we couldn't extend the study for estimating the rate of SSIs, % of Carbapenemases & Carbapenem resistant enterobacteriaceae & non enterobacteriaceae. We are keen to extend our study in future if feasible even at molecular level also.

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