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Bacteriological study of non-fermenters and their antibiogram in tertiary care hospital

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Abstract--Non –fermenting Gram-negative bacilli are a group of heterogeneous, aerobic, nonsporing bacteria. They are saprophytes in nature and are also found as commensals in man and other animals. This study aims to isolate and identification of NFGNB from various clinical specimens and to find out their clinical significance among the in-patients admitted at Vijayanagar Institute of Medical Sciences, Bellary. 110 isolates from various age groups and sexes were included in the study. A detailed history was elicited and the clinical specimens were collected under aseptic precaution and subjected to preliminary biochemical tests and further speciation was done. In the present study commonest isolates were *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Acinetobacter haemolyticus*, *Pseudomonas fluorescense*, *Acinetobacter lwoffii* *Acinetobacter junii*, *Acinetobacter radioresistant* and from urinary tract infection which accounted for (34.5%), followed by respiratory tract infection (21.2%), local infection (20.9%), Post operative infection (8.18%), infection related to the abdomen (6.36%), Septicemia (5.45%), and post-traumatic infection (2.7%) in decreasing order of frequency. They showed variability in their antibiotic susceptibility results, Most of them were sensitive to Carbapenem groups of drugs. The NFGNB infection is mainly seen in patients with serious underlying risk factors like prolonged stay in Hospital, catheterization, underlying diseases like COPD, Diabetes, and Malignancy. Amikacin, Imipenem, and Ciprofloxacin appear to be effective drugs in treating NFGNB infections.

Keywords--NFGNB-non-fermenting gram-negative bacilli, UTI-urinary tract infection, RTI-respiratory tract infection.

Introduction

The Non-fermentative Gram-negative bacilli are a group of aerobic, non-spore-forming bacilli that either don't use carbohydrates as a source of energy or degrade them through metabolic pathways other than the oxidative method. They

comprise about 1/5th of all Gram-Negative bacilli (GNB)¹. They are common inhabitants of soil and water. They also exist as harmless parasites on the mucous membranes of humans and animals. Though primarily regarded as contaminants or incidental organisms, they are emerging as an important nosocomial pathogen causing opportunistic infections in immunocompromised hosts and can also cause infection by gaining access to normally sterile body sites through trauma^{2,3}.

Among the species that are opportunistic pathogens in immunologically compromised hosts either by disease or treatment, *Pseudomonas aeruginosa* is predominant followed by *Acinetobacter baumannii* (*A.baumannii*), *P.flourescens*, *Stenotrophomonas maltophilia*, *Alkaligenes*, *Flavobacteria* and *Flavimonas*. Recently *Acinetobacter* species played a significant role in the colonization and infection of hospitalized patients^{4,2}. These organisms have been implicated in causing septicemia, meningitis, Osteomyelitis, Pneumonia, ventilator-associated pneumonia, Urinary tract infection, and surgical wound infection^{5,2}. Antimicrobial treatment of the infection caused by these agents is difficult due to its multi-drug resistance (MDR) and rapid selection of high-level MDR to various groups of antibiotics like β -lactam, Aminoglycosides, and Fluoroquinolones posing a problem for both treatment and infection control⁶. The isolation rate of NFGNB was increasing in our lab, hence this study was undertaken to identify and study the sensitivity pattern of NFGNB.

Materials and Methods

The study comprises 110 samples of clinical specimens collected from inpatients admitted to various departments of Vijayanagar Institute of Medical Sciences, Bellary, during a period of 1-year study from January 1st, 2012, to 31st December 2013. All clinical samples received from various departments under aseptic precaution are transported to the microbiology laboratory. The study was conducted as follows:

- Gram stain
- Culture
- Blood agar
- Mac Conkey agar

Incubate aerobically at 37^oc For 24 Hours. Isolates are inoculated on Triple Sugar Iron agar (TSI) medium. Organisms that failed to acidify the butt of TSI (that is produce an alkaline reaction). Were provisionally considered as Nonfermenters and were identified further as per standard protocols by using appropriate biochemical tests. Characters were assessed by:

- Morphology on Gram's stain
- Motility
- Pigment production
- Oxidase test
- Catalase test
- Indole production

- Urease test
- Nitrate reduction test
- Citrate utilization
- OF reactions of Glucose, Sucrose, Lactose, Maltose, Mannitol, xylose.
- Growth on 10% lactose agar
- Lysine and arginine decarboxylase tests
- Gelatin liquefaction test.

Identification Of SPP^{1,8}

A biochemical test performed for NFGNB

- OF medium (Hugh and Leifson)
- Nitrate-reducing broth
- Citrate utilization test
- Growth at room temperature 25^o-30^o, 37^o, 44 °c
- Hemolysis on BA
- Gelatin liquefaction test.
- Malonate test.

Antibiotic susceptibility testing was done by the Kirby-Bauer disc diffusion method. The various antibiotics with their concentration that will be used are listed below:

Amikacin(AK)-30mcg	Ceftazidime(Ca)-30mcg	Meropenem(Mr)-10mcg
Amoxiclav (AMC)30mcg	Ciprofloxacin(Cf)-5mcg	Piperacillin+tazobactam(Pt) 10/100mcg
Cefepime(Cpm)-30mcg	Gentamycin(G)-10mcg	
Cefotaxime(Ce)-30mcg	Imipenem(I)-10mcg	

Result

The present study included 110 samples from patients with various clinical conditions like RTI, UTI, Infection related to the abdomen, Septicemia, and Local infections, satisfying the inclusion criteria and exclusion criteria attending Vijayanagar Institute of Medical Sciences, Bellary. Pus was the commonest sample from which NFGNB were isolated the most 38.4% followed by Urine 34.5%, Sputum 22.8%, and Blood 5.3%. Out of 110 cases, the youngest patient was a 6-month-old baby, and the oldest patient was 79 years old. The age group 21 to 70 years accounted for 80% of patients highest between 41-50yrs were as least below 1-10yr 2.7% and 71-80yr 6.5% of age group. Total no of the representative sample =110, In that 72(66%) were Males and 38(34%) were females. Out of 110 isolates, 31.8% were isolated from the surgical ward followed by 22.7% from the orthopedics ward, 13.6% from both ICU and Medical ward, and 9.1% from Pediatrics and Burns unit. Out of 110 NFGNB isolates showed Resistance of 75.5% to Amoxyclav, 70.9%, to Piperacillin+tazobactum, 61.8% to cefotaxime, 53.6% to ceftazidime, 58.2% to cefepime. Amikacin showed resistance to 40% followed by 48.2% to Gentamicin, 36% to Ciprofloxacin, 43.6% to Imipenem, and 55.5% to Meropenem.

Antibiotic	Resistant	
	N	%
Amikacin	44	40
Gentamicin	53	48.2
Ciprofloxacin	66	36
Imipenem	48	43.6
Amoxiclav	83	75.5
Meropenem	61	55.5
Piperacillin+tazobactam	78	70.9
Cefotaxime	68	61.8
Ceftazidime	59	53.6
Cefepime	64	58.2

Discussion

The non-fermenting bacilli are widely distributed in nature as saprophytes or as commensals and pathogens to man⁸. NFGNB earlier considered a contaminant is now gaining importance as a nosocomial pathogen. During the study period from January 2012 to December 2013 at Vijayanagar Institute of Medical Sciences, 110 isolates from various clinical conditions like Septicemia, local infection, post-operative infection, post-traumatic infection, and Respiratory tract infection. All these clinical conditions caused by *Ps.aeruginosa*, *Ps.flourescence*, and *Ac.baumannii*, were isolated, Similar to studies by Prashanth K.Parendakar et al⁷ and Taneja et al. NFGNB are known to cause infection at extreme age, which was also seen in our study, 14.5% in the age group of 61 to 70 years and 4.5% in <1 year of age similar to the study by Gardener et al⁸ which could be due to subnormal immune system. There was a preponderance of the nosocomial infection in males in our study as shown in Similar observation was made in other studies^{9,10}.

The mean duration of stay in the hospital was 16 days. In our study, 12.7% were admitted to ICUs whereas in Algun et al¹¹ study it was 47.2%. other patients were admitted to the surgical ward, Orthopedics, and medical wards including Pediatrics wards. Earlier studies have shown that NFGNBs has been mostly isolated from the pus sample⁸. In our study also NFGNBs were isolated from the pus sample. *Pseudomonas aeruginosa*, *Ac.baumannii* was the most common isolate, Local infections like cellulitis, diabetic foot, and burns in our study which was similar to other studies^{9,10}. *Ps.aeruginosa* as the main etiological agent responsible for 31.1% of urinary tract infections, and 27.5% of local infections in our study, however, it was higher in studies by Resmi Rajan et al 89.9%,72.5% in Cristiane et al study^{12,9}. The differences in the percentages of various parameters may be due to the variation in the sample size.

Infection at burns site is because by injury associated with the breakdown of normal skin, immune defects, and selection of antibiotics with inadequate coverage for this pathogen¹³. Polymicrobial infection was commonly seen in diabetic foot and burn wound⁷. Postoperative wound infection was caused by *Pseudomonas aeruginosa* (8.62%) were in Resmi Rajan et al isolated it was (34.09)

% . Infections related to the abdomen included peritonitis cases in our study. *Ac.baumannii* and *Pseudomonas aeruginosa* was the most common organism isolated in our study. In our study, in patients who had been catheterized for >72 hrs, post-operative urinary tract infection was common with *Pseudomonas aeruginosa* and *Ac.baumannii*. *Ps.aeruginosa* and *Ac.baumannii*, both are known to cause chronic & recurrent Urinary tract infections and are often Multi-drug resistant¹³ The most common organism causing respiratory tract infection was *Ps.aeruginosa* 18.9% followed by *Ac.baumannii* 17.8%.In study by Mark et al¹⁴. *Pseudomonas aeruginosa* isolated was 6%. Most patients had underlying pathologies like COPD, tuberculosis, and pneumonic consolidation and were exposed to repeated nebulization.

NFGNB displays a wide and variable spectrum of antibiotic sensitivity. There is no antibiotic to which all strains are susceptible¹⁵. *Ac.baumannii* showed a resistance of 78.6% resistance to Piperacillin tazobactam combination in our study, and the study by Jawad et al. (58.2%)¹⁶. *Ps.aeruginosa* showed a resistance of 68.9% to Piperacillin tazobactam in our study as compared to 2 % resistance in study by¹⁷. The low sensitivity in our study could be due to excessive use of Piperacillin tazobactam combination in our hospital. Cefepime which is commonly used by the clinicians in our hospital showed 71.4% resistance to *Ac.baumannii* whereas a study by Mohammad et al showed resistance of 64.8%¹⁸*Ps.aeruginosa* shows 51.7% resistance to ciprofloxacin in our study, in various other studies it ranged from 12.5% to 83% by Smitha et al,Troilet et al,Algun et al^{19,11,20,6,21}. *Pseudomonas aeruginosa* showed 25.8% resistance to Imipenem in our study, studies in India and Abroad showed a range of 11.8%-81.5%^{6,19,18,9,20} Imipenem showed resistance of 25.8% for *Ps.aeruginosa* in our study whereas in a study by Jayanthi et al resistance to imipenem was 6.6%²³.In a study Ekta Gupta et al.¹⁹Resistance to Meropenem was 22.16% . *Ps. aeruginosa* showed 55.2% resistance to Meropenem and it ranged from 4.2% to 37.3% in different studies^{24,19}. *Ac.baumannii* showed resistance of 50% for Imipenem and 64.3% for Meropenem. It is known that Meropenem develops resistance earlier than Imipenem¹⁵. The various risk factors observed in our study in decreasing order of frequency are: ^{1,8,10, 24}

- Catheterization (urinary/i.v)-49
- Duration of Hospital stay 16 days
- Stay in ICU- 11 patients stayed in ICU in our study.
- Surgery- 17 underwent various surgery
- Diabetic – 15 patients had an underlying metabolic disorder
- Preexisting respiratory illness – 15 patients
- Trauma – patients had a history of trauma
- Neonate – 5 in number
- Immunosuppression due to malignancy – 6 patients
- On broad spectrum antibiotic-5 patients

Conclusions

But the rate of isolation of NF 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, catheterization (both urinary and intravenous) diabetes, burns, and malignancy. The most common isolates were *Pseudomonas aeruginosa* followed by *Acinetobacter baumannii*. Common clinical conditions were ulcers, postoperative wounds, COPD, burns cases, and peritonitis. In the present study, the most effective antibiotics were Amikacin, Imipenem, and Ciprofloxacin. Most of the NFs isolated were resistant to the Penicillin group of drugs. Repeated exposures of organisms to antimicrobial agents is thought to enhance the development and maintenance of resistance. Also, the presence of an antimicrobial agent in sub-lethal concentrations makes an environment suitable for the development of resistance.

Organisms that are resistant to drugs commonly employed in therapy emphasize that NFs need to be taken more seriously and should not be discarded as mere contaminants or nonpathogens. Identification of these organisms can throw more light on their prevalence and pathogenic role. 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 NFGNB.

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