"Isolation of Non-Fermenting Gram-Negative Bacilli (NFGNB) in Patients Attending a Tertiary Care Hospital in Kanpur"

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Abstract:

Introduction: Due to the liberal and empirical use of antibiotics, NFGNB have emerged as important healthcare-associated pathogens. Very few laboratories in India identify these NFGNB other than Pseudomonas routinely, hence this study was undertaken to identify, isolate and study the NFGNB in patients attending tertiary care hospital. **Materials and Methods:** Present prospective research was carried out in the Department of Microbiology, in patients with NFGNB infection, attending Rama Medical College Hospital and Research Centre Kanpur, from January 2017 to December 2017. NFGNB were isolated from blood, CSF, pus, urine, drain tip, cerebrospinal fluid and other body fluids. **Result:** A total of 100 samples attending hospital were isolated. The prevalence rate of NFGNB was found to be 60% from various clinical samples. Maximum number of the clinical samples infected by NFGNB were from Surgery wards (55.5%) followed by Obstetrics & gynecology (36.6%) & Urology (28.3%). Higher incidence of NFGNB infection was observed in the age group of 21-40 years (44.0%), whereas, the number of infected male patient (65%) was much higher than female (35%).The bacilli were mostly observed in pus samples (41.0%) followed by urine (30.0%) and tracheal aspirates (17%). Pseudomonas *aeruginosa* (65%) was the predominant isolate among the non-fermenters, followed by Acinetobacter *baumanii* (30.10%), *S. maltophilia* (3.33%) & *B.cepacia* (1.67%). **Conclusion:** The aerobic NFGNB, which were usually considered as contaminants are now emerging as important nosocomial pathogens. Care in detection, evaluation of effective antibiotic options and the judicious use of antibiotics by instituting antibiotic policy of combination therapy and rigorous infection control measures will help to fight against these multidrug resistant NFGNB in the effective management of patients.

Keywords: NFGNB; non-fermenters; nosocomial pathogens; S. maltophilia; P. aeruginosa.

Introduction

Non-fermenting gram-negative bacilli (NFGNB) are a taxonomically diverse group of aerobic, non-sporing, bacilli that either do not utilize glucose as a source of energy or utilize it oxidatively [1]. They are physiologically versatile group of bacteria that flourish as saprophytes in warm moist situation in the humid environment. Recently they are recognized as opportunistic pathogens that cause infection mainly in debilitated and immune-compromised individuals [2, 3]. Some of them are found as commensal in the human gut as well [4]. These Non- fermenters (NF) are emerging with increasing frequency as agents of often serious infection as well as nosocomial infection [5, 6]. They are frequently isolated from cases such as septicemia, meningitis, pneumonia, urinary tract infection and surgical wound infection. The identification of these non-fermenters is important because of the fact that most of them are resistant to many antibiotics [7]. In recent years, due to the liberal and empirical use of antibiotics [5]. Hence this study was undertaken to identify, isolation and study the NFGNB in patients attending Rama Medical College Hospital & Research Centre, Kanpur.

Material and Methods

This prospective study was carried out in the Department of Microbiology, Rama Medical College Hospital and Research Centre for a period of one year from January 2017 to December 2017. Ethical clearance was obtained from Institute. In-patients with Non Fermenting gram Negative bacilli (NFGNB) infection, admitted during the period of study were included. A total of 100 non-fermenting bacteria isolated from various clinical specimens like pus, urine, blood, bronchoalveolar lavage, endotracheal aspirations, drain tip and cerebrospinal fluid were collected from both OPD and IPD of Rama Medical College Hospital and Research Centre.

Sample Processing: The samples were processed according to standard procedures available. The collected samples were subjected to direct Gram stain and all specimens were inoculated onto 5% sheep blood agar and MacConkey's agar medium. Urine samples were inoculated onto Cystine Lactose Electrolyte Deficient agar (CLED). NFGNB isolates were isolated and identified by standard protocol.[8] Antibiotic susceptibility pattern was done on Mueller Hinton Agar by Kirby- Bauer disc diffusion method as recommended by Clinical and Laboratory Standards Institute(CLSI-2016). Following antibiotic discs were used for disc diffusion testing: Amikacin(AK), Gentamicin(Gen), Cephotaxime(CTX), Ceftazidime(CAZ), Cefepime(CPM), Ciprofloxacin(CIP), Ofloxacin(OF), Piperacillin (Pi), Piperacilline tazobactam(PIT), Imipenem(IMP), meropenem(MRP), Aztrionem(AT). The control strains used were *E.coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 Overnight broth culture compared to 0.5 McFarland's was used as inoculum. After incubation at 37oC for 16-18 hrs, zone of inhibition was noted. Results were interpreted according to CLSI standard.

Results

The present study was conducted on a total of 100 clinical samples were collected of these 60 NFGNB was isolated. [**Fig 1**] The prevalence rate of NFGNB was found to be 60% from different clinical samples. Age wise distribution of patients was mentioned in [**Table 1**]. Maximum number of isolates were in the age

group of 21 – 40 years (44%) followed by 41 – 60 years (26%). Out of total subjects, Male and female were 60% & 40% respectively. The Male: Female ratio = 3**:** 2. Distribution of NFGNB isolates from various types of clinical samples mentioned in [**Table 2**]. Among different types of clinical samples; Pus samples 41%, the predominating clinical sample harboring the non-fermenters followed by urine (30%). Maximum number of the clinical samples infected by NFGNB were from Surgery wards (55.5%) followed by Obs. & gynae (36.6%) & Urology (28.3%). [**Table 3**] Species wise distribution of NFGNB isolates was mentioned in [**Table 4**]. Pseudomonas *aeruginosa* (65.00%) was the predominant isolate among the non-fermenters, followed by Acinetobacter *baumanii* (30.10%), *S. maltophilia* (3.33%) & *B.cepacia* (1.66%). [**Table 4**] Antibiotic Sensitivity pattern of isolates was mentioned in **Fig 4** and **Fig 5**.

Fig 1: Rate of NFGNB

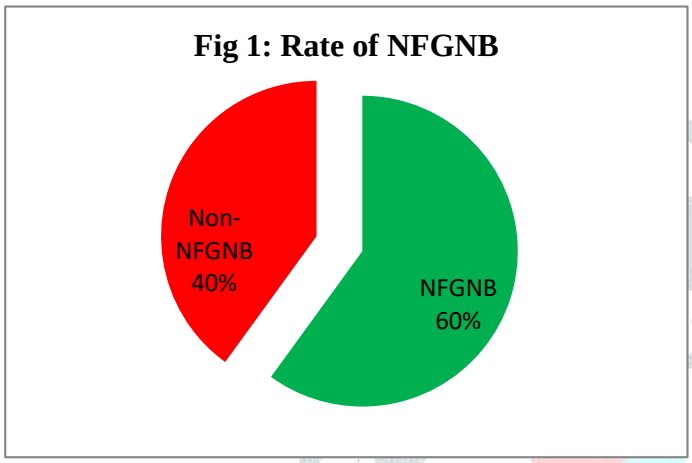


Table - 1: Age wise distribution of patients (n=100)

	Age	No. of patients
1	0-20	13
2	21 – 40	44
3	41 – 60	26
4	>60	17

Fig 2: Gender wise distribution of patients (n = 100)

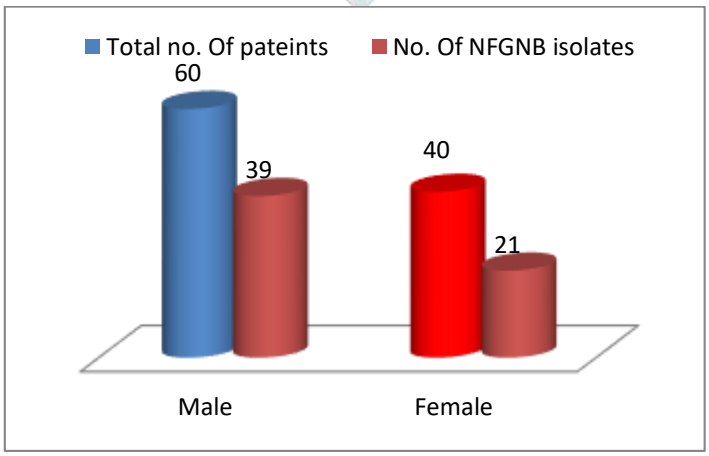


Table – 2: NFGNB isolates from various types of clinical samples:

Sample	Total no. of samples (n=100)	Total no. of isolates (n=60)
Pus	41	24 (40%)
Urine	30	12 (20%)
E. Tube	17	17 (28.34%)
Sputum	5	04 (6.67%)
Swab throat	3	01 (1.67%)
Foley's tip	2	01 (1.67%)
Central line tip	1	01 (1.67%)
CSF	1	Nil

Table -3: Source of samples (n=100)

Specialty	No. of cases	Percentage
Surgery	34	34
Obs. & gynae	22	22
Urology	17	17
Medicine	10	10
Casuality	8	8
Thoracic medicine	6	6
Cardiac surgery	3	3

Table – 4: Species wise distribution of NFGNB isolated (n=60)

Clinical isolates	Number	Percentage
Pseudomonas aeruginosa	39	65.%
Acinetobacter.baumanii	18	30.10%
Burkholderia cepacia	1	1.66%
Stenotrophomonas.maltophilia	2	3.33%

Figure- 3: Antibiotic Sensitivity pattern of Pseudomonas aeruginosa and Acinetobacter baumannii

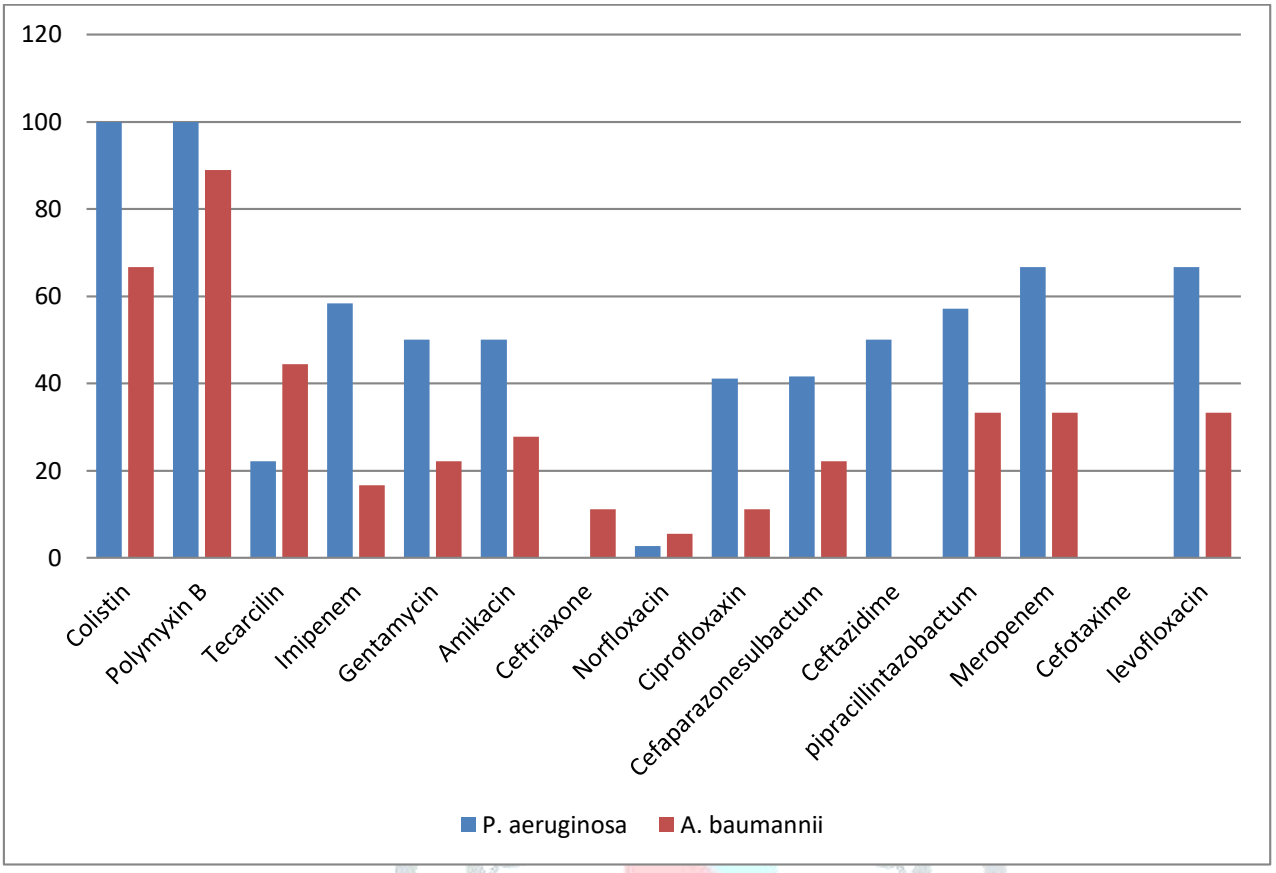
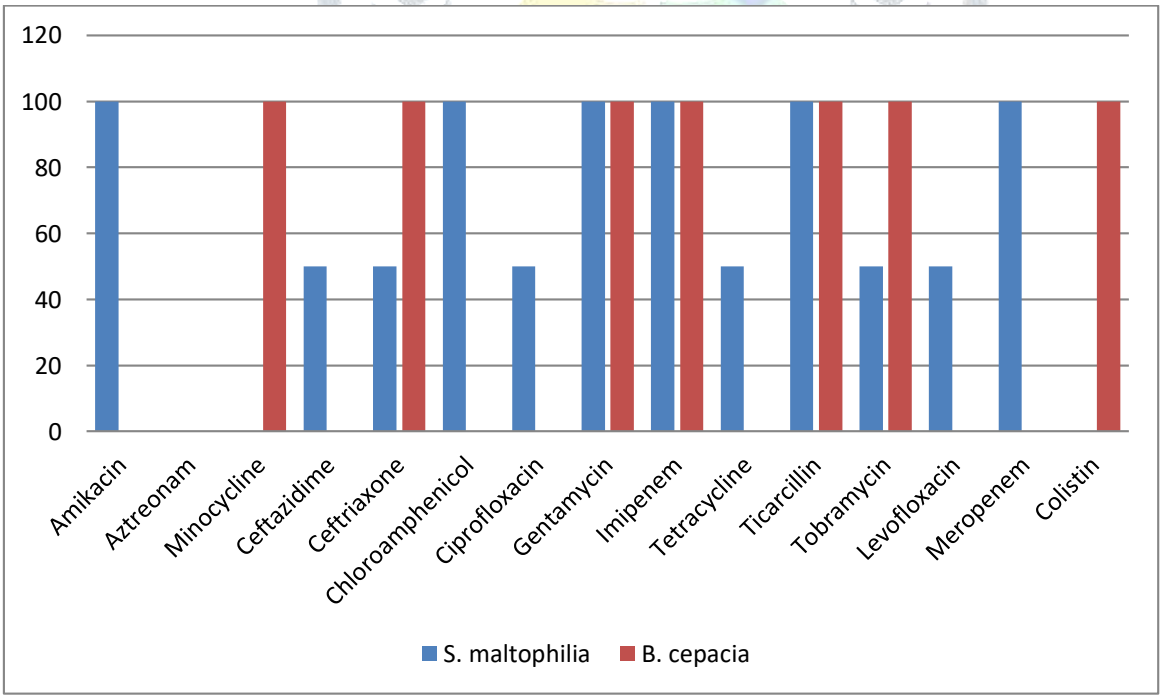


Figure-4: Antibiotic sensitivity patterns of B. *cepacia* and S. *maltophilia*



Discussion

Aerobic Non-Fermenting Gram Negative Bacilli (NFGNB) usually considered as contaminants are emerging as important nosocomial pathogens. Non-fermenting Gram Negative bacilli (NFGNB) are being isolated with increasing frequency from clinical specimens. During the study period Out of 100 clinical

samples 60 Non-fermenter gram negative were isolated. Analyzing the age group, higher incidence of infection by NFGNB was seen in the age group of 21-40 years (44.0%). This was comparable with the study by Meharwal et al from Chandigarh (53%). It can be attributed to the increased activity of the age group and higher chance of exposure to infections. The incidence of infections by non-fermenters is very high in males (65%) as compared to females (35%).[9,10]. This correlated with the study by Chacko B et al who observed 66.6% of males to be affected as compared to (33.3%) of females. The increase in male to female ratio (1.86:1) could be due to the involvement of outdoor activities by males and therefore increased chance of exposure to infections.

Among the clinical samples, non-fermenters were predominantly observed in pus samples (41.0%) followed by urine (30.0%) and tracheal aspirates (17%) in the present study. Kharangate et al from India reported 50% incidence in pus samples [11]. Vijaya et al from India reported a lower incidence of 32.4% in pus samples.[12] Veenu et al reported 29% incidence in urinary tract infections.[13] Gladstone et al from CMC Vellore showed a higher incidence in endotracheal aspirates (42.4%) due to large number of samples[14].

Pseudomonas *aeruginosa* was the predominant isolate (65%) among the Non-fermenting gram negative bacilli observed in the present study followed by Acinetobacte *baumanii* (30%), Stenotrophomonas *maltophilia* (3.34%), Burkholderia *cepacia* (1.67%). Kharangate et and Ezeltahawy et al. and reported 56% isolation of Ps *aeruginosa and*[11,15] Taneja et al, from Chandigarh, India and Wang H et al from China reported found Ps *aeruginosa* 51% and 46.9% respectively as the predominant isolate followed by A *baumanii and* S. *maltophilia*[16,17].

In the present study, 66% of *Ps. aeruginosa* isolates were susceptible to Imepenum & Meropenam, which was similar to Indian studies by Prakesh et al 86% & Anupurba et al 84% [18], An Italian study also reported 81% susceptibility similar to present study. Susceptibility to Piperacillin – Tazobactum in the present study was 57.14% comparable to the study in Canada conducted in the year 2008 (80.5%) [19].

A. *baumanii* is an organism which exhibits high resistance to antibiotics in hospital environment. In the present study 58.33% of isolates were sensitive to Imipenam & Meropenam, 57.14% to Piperacillin-Tazobactum, 50% to Amikacin, 50% to ceftazidime, while Cefotaxime & gentamicin showed maximum resistance at 67% and 75%. *S. maltophilia* is an emerging nococomial pathogen in hospital settings. The significance of this organism lies in its intrinsic multidrug resistance [20]. S. *maltophilia* is highly resistant to many of the antibiotics commonly used in hospital settings. In the present study these isolates were sensitive to quinolones (50%), and cotrimoxatole (100%), Resistance was observed with beta-lactam agents – ceftazidime (50%), and cefotaxime (100%) as observed by Robert et al [21].

Burkholderia *cepacia* has emerged as a significant respiratory pathogen. Study done in Chandigarh show majority of these isolates from blood. In the present study organism were isolated from Endotrachial tube. These organisms were sensitive to carbapenams (100%), quinolones (50%) and ceftazidime (100%). A report by V.Gautam et al found a similar susceptibility profile with resistance to aminoglycosides as observed in the current study [22].

Conclusion

P. *aeruginosa* and Ac. *baumanii* are the two common NFGNB which are isolated from wound infections, post-operative wound infection and post traumatic wound infections. Carbapenems(Imipenem) to be kept as reserve drug for use in drug resistant organisms. Awareness of their entry into a hospital environment is the first step that clinical microbiologists can take to address this problem. Care in detection, evaluation of effective antibiotic options, and judicious use of antibiotics by instituting antibiotic policy of combination therapy and rigorous infection control measures will help to fight against these multidrug resistant non-fermenters in the effective management of patients.

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