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RESEARCH ARTICLE

IDENTIFICATION OF NON-FERMENTING GRAM NEGATIVE BACILLI FROM CLINICAL SAMPLES AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN A TERTIARY CARE HOSPITAL, MANIPUR

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ABSTRACT

Background: Non-fermenting gram negative bacilli are a group of aerobic, non-spore forming bacilli. They are ubiquitous in nature, inhabiting soil, water and also present in the hospital environment. NFGNB accounts for nearly 12-16% of all bacterial isolates in a clinical microbiology laboratory. The important members are *Pseudomonas aeruginosa*, *Acinetobacter baumannii*. They cause various infections such as septicaemia, pneumonia, urinary tract infection, surgical site infections and meningitis. **Aims and Objectives:** To estimate the prevalence and antimicrobial susceptibility pattern of non-fermenters in various isolated clinical samples in a tertiary care hospital**. Materials and Methods:** A hospital based cross sectional study was done between August 2018 to July 2019 to isolate NFGNB from various clinical samples received from OPD and IPD for culture and sensitivity in the department of Microbiology, JNIMS, Imphal. The non-fermenters were identified using standard methods and antimicrobial susceptibility test was performed to determine the sensitivity pattern of the isolates. **Results:** A total of 162 isolates of non-fermenters were identified from various clinical samples processed during a period of 1 year. Pus (51.3%) was the commonest sample from which NFGNB were isolated, followed by sputum (20.9%), urine (16.60%), catheter tip (5.5%), blood (4.9%), ear swab (1.6%). Out of 162 isolates, the most common organism isolated was Pseudomonas aeruginosa (57%) followed by Acinetobacterbaumannii (25%). The most effective drug in this study was found to be Imipenem which showed sensitivity percentage of 86%. **Conclusion:** The present study highlights the importance of speciating NFGNB and to know the resistance pattern of carbapenems so as to establish strict antibiotic policies.

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INTRODUCTION

Non-fermenting gram negative bacilli (NFGNB) are a group of aerobic, non-spore forming bacilli that either do not utilize glucose as a source of energy or utilize it oxidatively (Winn, 2006). They are ubiquitous in nature (Malini, 2009). In the hospital environment, they have been isolated from instruments such as ventilator machine, mattresses, and other equipments as well as from the skin of healthcare workers (Chawla *et al*., 2013). They can cause device associated infections, and are often resistant to disinfectants and they also have the potential to spread infection from patient-to-patient via fomites or the hands of health care professionals (Rit,m 2013). NFGNB accounts for nearly 12-16% of all bacterial isolates in a clinical microbiology laboratory.

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The important members of the group in non-fermenters include *Pseudomonas aeruginosa, Acinetobacter baumannii, Stenotrophomonas maltophilia, Burkholderi acepacia, Burkholderia pseudomallei* (Rahbar *et al*., 2010). They cause life-threatening infections such as septicemia, meningitis, pneumonia, urinary tract infections, and surgical site infections (SSI), ventilator associated pneumonia, wound infection, osteomyelitis[2]. Risk factors include immunosuppression, trauma, foreign body, indwelling catheters, invasive diagnostic as well as therapeutic procedures, prolonged hospital stay and using broad spectrum antibiotics (Kukkamalla, 2011; Juyal, 2013). *P.aeruginosa* has greater potential to become resistant to most antibiotics as it is clear from the fact that its genome contains the largest resistance island with more than 50 resistance genes. Mechanism for this antibiotic resistance is due to the production of antibiotic-degrading or antibiotic-inactivating enzymes, outer membrane proteins to expel the antibiotics and mutations that change the antibiotic targets (Hancock, 1998). Carbapenem group of antibiotics are essential in the treatment of nosocomial gram negative infections (Hemalatha, 2005; Karthika, 2009; Marra, 2006).

Hence this study was undertaken to isolate and identify the various non-fermenters from patients admitted in our tertiary care hospital and to study their antimicrobial susceptibility pattern at a tertiary care hospital in Manipur.

MATERIALS AND METHODS

This is a hospital-based, cross-sectional study from August 2018 to July 2019 conducted in Dept. of Microbiology, JNIMS. Isolates from all clinical samples received from Indoor Patient Department (IPD) which were identified as Non-fermenting Gram negative bacilli were included in the study. Mixed growth of > 3 types (probably contaminated sample) were excluded from the study. Processing of clinical samples were done conventionally by plating on Nutrient agar, Blood agar and Mac Conkey Agar and incubated at 37 ° C for 18-24hrs.Gram staining from culture smear was performed which has shown gram negative bacilli. Hanging drop preparation showed motile/non-motile bacilli. Further identification was done by oxidase test, Hugh Leifson's oxidative-fermentative test, indole test, citrate utilization test, urea hydrolysis test, triple sugar iron agar, 10% lactose test, growth at 42°C.Antibiotic susceptibility testing was performed by using Kirby Bauer disc diffusion technique and diameter of zone of inhibition were measured according to CLSI 2019 guideline. The following antibiotics were used for susceptibility testing such as Ceftazidime, Cefepime-tazobactum, Ciprofloxacin, Levofloxacin, Pipercillin, Imipenem, Gentamicin which were procured commercially from Himedialaboratories limited.*P. aeruginosa* ATCC 27853 strain was used as quality control. Data analysis was done by using descriptive statistics.

RESULTS

A total of 162 NFGNB isolates were identified from 16163 clinical samples processed during a period of 1year. The age group above 60 years accounted for 22.2% which is the highest percentage followed by 31-40(18.6%),41-50(17.4%) and 51-60(16.3%) and the least percentage was seen below 10 years of age as depicted in Tab 1. Out of 162 NFGNB isolated 102(62.96%) were from males and 60(37.03%) were from females.

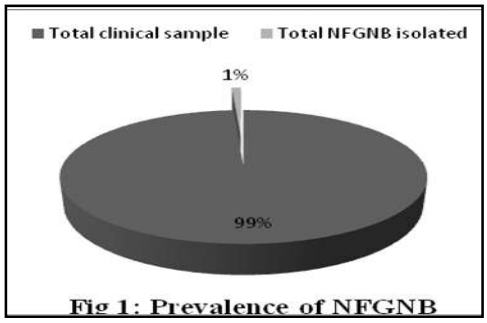

Fig 1: Prevalence of NFGNB

Table 1: Age-wise distribution of NFGNB

Age in Years	No. of isolates	Percentage
1-10	7	4.32
11-20	12	7.40
21-30	19	11.72
31-40	32	19.75
41-50	30	18.51
51-60	26	16.04
> 60	36	22.22
Total	162	100

The above figure 2 shows the sample wise distribution of NFGNB. Pus was the commonest sample from which NFGNB were isolated the most 51.3% followed by sputum (20.9%), urine (16.60%), catheter tip (5.50%), blood (4.9%) and ear swab (1.6%). The above table 3 depicts the number of different isolates from various clinical samples. *P.aeruginosa* was mostly isolated from pus sample (52), followed by sputum (20), urine (12), catheter tip(4), blood (4) and ear swab(1). *P.fluorescens* was mostly isolated from pus(4), followed by sputum (4), urine (2) and catheter tip(1). *A.baumannii* was isolated most commonly from pus (21), followed by urine (10), sputum (5), blood (3), catheter tip (2). *A.lwoffii* wereisolated from pus (8), sputum (3), urine (3) and Catheter tip (1). Among various wards, Surgery (66) has the highest number of non-fermenters followed by ICU (43), Medicine (21), Orthopaedics (15), Obstetrics and Gynaecology (9), Paediatrics (7), Otorhinolaryngology (1) shown in Fig 5. For Pseudomonas sp. Ceftazidime has shown highest resistance pattern at 29% followed by Levofloxacin at 27% and Ciprofloxacin at 22%. Meropenem and Imipenem showed sensitivity of 87% and 86% followed by Piperacillin at 78% respectively. For Acinetobacter sp. sensitivity of the drugs gentamycin, levofloxacin, ciprofloxacin and meropenem were 54%, 55%, 47% and 45% respectively. Resistance was observed more for the drugs ceftazidime (60%), cefipime+tazobactum (62%) and imipenem (57%), ciprofloxacin (50%) and meropenem (51%).

DISCUSSION

In our study the highest percentage was seen in the age group above 60 years which is 22.2% which is similar to other studies conducted by Kalidas Rit *et al*. (2013) in Kolkata in2013 where 72% of the patients were adults over 45 years of age and a study by Meenakumari *et al*. (2011) where 90% were adults. This could be due to sub normal immune system.In our study males (62.96%) are more commonly affected than the females (37.03%). Similar observation was made in other studies conducted by Kalidas *et al*. (2013) where 55% were males and 45% were females and in a study in 2012 by Piyush *et al*. (2011), 71.5% were males and 28.5% were females. In the present study, NFGNB were most commonly isolated from pus samples (51.3%) followed by sputum (20.9%), urine (16.6%), catheter tip (5.5%), blood (4.9%) and ear swab (1.6%). This is in concordance with a study conducted in 2009 by Malini *et al*. (2009) wherein NFGNBs were most commonly isolated from pus samples. Out of 193 samples, 120(62.2%) isolates of NFGNB were from pus samples. Another study conducted by Gokale *et al*. (2012) also showed that maximum number of NFGNB was from pus samples (58.4%).

In our study *P. aeruginosa* (57%) was the most common organism isolated from the clinical samples, followed by *A.baumannii* (25%), *A.lwoffii* (10%), *P. fluorescens* (7%), similar results include study by Chawla *et al*. (2013) in 2013,where 56.7% isolates were *P.aeruginosa* and 39.3% were Acinetobactersp and a study by Gokale (2012) in 2012 106 where *P.aeruginosa* constituted 82.3% and *A.baumannii* 15.4%. In this study, *P.aeruginosa* (55.5%) and *A.baumannii* (20%) were the most commonisolates from pus samples. This is similar to the study by Rashid *et al*. (2007) Among various wards, Surgery (66) has the highest number of non-fermenters followed by ICU (Intensive Care Unit).Other studies done by Eltahawy *et al*. (2001) and Rahbar *et al*. (2010) has shown highest number in ICU.For Pseudomonas sp.

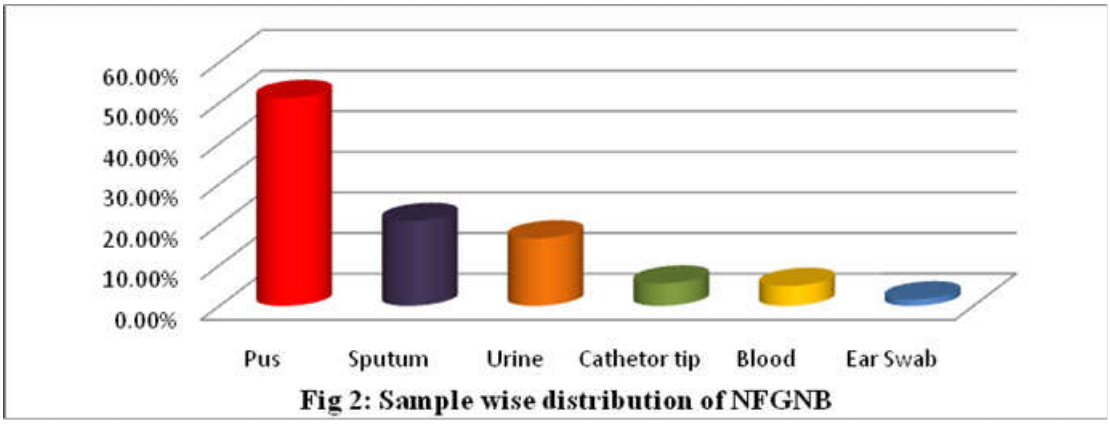

Fig 2: Sample wise distribution of NFGNB

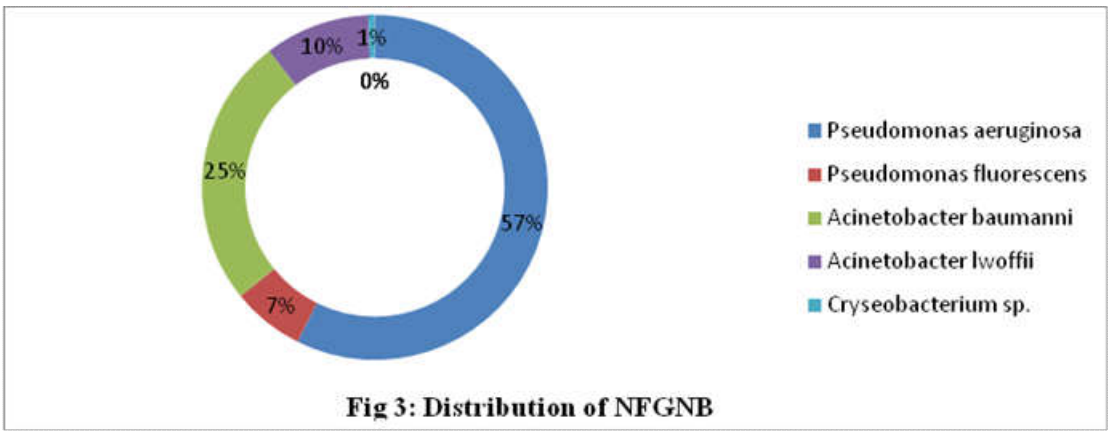

Fig 3: Distribution of NFGNB

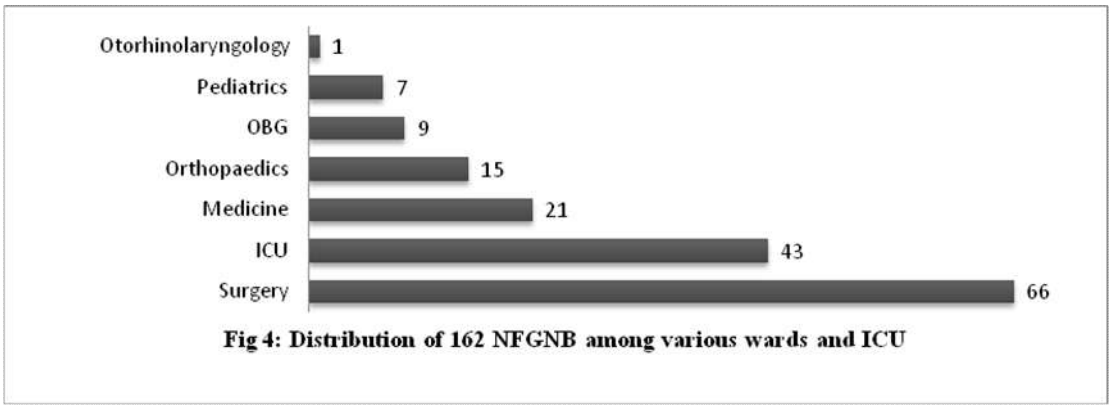

Fig 4: Distribution of 162 NFGNB among various wards and ICU

Table 3. Sample wise analysis of NFGNB

Samples	*P. aeruginosa*	*P.fluorescens*	*A.baumannii*	*A.lwoffi*	Chyseobacterium sp.
Pus	52	4	21	8	0
Sputum	20	4	5	3	0
Urine	12	2	10	3	0
Catheter tip	4	1	2	1	1
Blood	4	0	3	1	0
Ear swab	1	0	0	0	0
Total	93	11	41	16	1

Table 4. Antibiotic susceptibility pattern

Antibiotics	Pseudomonas sp.			Acinetobacter sp.		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Ceftazidime	65	6	29	37	3	60
Cefepime+Tazobactum	74	10	16	32	6	62
Piperacillin	78	8	14	42	4	54
Ciprofloxacin	70	8	22	47	3	50
Levofloxacin	68	5	27	55	5	40
Imipenem	86	4	10	39	6	57
Meropenem	87	2	11	45	4	51
Gentamicin	68	12	20	54	4	42

Ceftazidimehas shown highest resistance pattern at 29% followed by Levofloxacin at 27% and Ciprofloxacin at 22%. Meropenem and Imipenem showed sensitivity of 87% and 86% followed by Piperacillin at 78% respectively.The resistance pattern to ceftazidime was similar to study done by Tripathi *et al*. (2011) and sensitivity to imipenem is similar to study conducted by Mohammad Rahbarin 2010 where it was 78.9%.

Conclusion

Identification of non-fermenters and their antibiotic susceptibility pattern should be regarded with all seriousness in clinical practice because by being resistance to multiple antibiotics, their prevalence not only limits the treatment options but also act as areservoir of drug resistance genes. More importantly these organisms have great potential to survive in hospital environment, so effective methods of sterilization and infection control measures like hand hygiene, using personal protective equipments, environmental cleaning and disinfection, sterilization and disinfection of various instruments and devices used in patient care and biomedical waste management should be implemented.

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