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Research Article

A Study of Prevalence and Antimicrobial Susceptibility Pattern of Non Fermenter Gram Negative Bacilli Isolated From Various Clinical Samples at a Tertiary Care Center, Jaipur

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Abstract
Background: *The present study was done to examine the prevalence and antimicrobial susceptibility pattern of NFGNB.*
Introduction: *Non Fermenter Gram Negative Bacilli were previously considered as Non-pathogenic or contaminants but in past few years they have become a serious threat to the society as the frequency of their isolation and resistance towards antimicrobial agents is increasing rapidly. They have developed resistance towards commonly used antimicrobial agents as well as towards higher class also.*
Material & Methods: *This study was done on all clinical samples received for culture and sensitivity over a period of 6 months in department of Microbiology, NIMS Medical College, Jaipur. Samples were received and processed according to standard procedures and Antimicrobial susceptibility testing was done by using Kirby Bauer's disc diffusion method.*
Result: *From 674 bacterial isolates, 122 isolates (18.10%) were identified as NFGNB. Male population was highly affected by NFGNB as compared to female population. Pseudomonas species was commonly isolated followed by Acinetobacter species and Proteus species. Meropenem was most sensitive drug followed by Imipenem. Cephalosporins showed high resistance.*
Conclusion: *Higher isolation rate of NFGNB seen in our study with high resistance towards first line antibiotics. Resistance towards Cephalosporins and Carbapenems indicates Beta-lactamases production by these organisms. So this study will be helpful in initiating proper empirical therapy of such patients, thus reducing the morbidity rate.*
Keywords: *NFGNB, Pseudomonas species, Acinetobacter species, Proteus species, Kirby-Bauer's disc diffusion method, Carbapenem, Cephalosporins.*

Introduction

Organisms which are aerobic, non-spore forming, Gram Negative rod and either do not take carbohydrates as their energy source or utilize them by various metabolic pathways except fermentation are known as Non fermenter Gram Negative bacilli. These organisms show growth on Surface of TSI (triple sugar iron) medium but not in the butt part. Also these organisms never acidify the butt of the test media[1]. Infections caused by this group are 15% of the total infection caused by the Gram Negative Bacilli[2]. Pseudomonas species, Acinetobacter species, Proteus species, Alkaligenes, Burkholderia, Moraxella, Strenotrophomonas, Flavobacter, Oligella, Flavinomas etc. are some of the organisms present in this group[3].These organisms shows great resistance towards routinely used disinfectants and they have the ability to colonize on different surfaces and that's why they are also important nosocomial pathogens. According to some recent literatures, these organisms are also associated with many life-threatening conditions like Septicemia, Urinary tract infection, Ventilator associated pneumonia, wound infection, meningitis etc[4]. Normally most of the infections caused by these organisms are secondary infections because their infections are mainly seen in patients already suffering from any other primary conditions like burns, prolonged antimicrobial therapy, patient on any immunosuppressive agents, old age etc[5]. Recent studies have shown that Pseudomonas aeruginosa is the second most common cause of nosocomial pneumonia and ventilator associated pneumonia[6,7]. Infections of Acinetobacter species are normally seen in patients with endotracheal intubation, central venous catheterization or peritoneal dialysis[8]. Resistance to antimicrobial agent developed in NFGNB can be due to mutation in genes encoding porins, efflux pump mechanisms, due to chromosomal beta lactamases or due to penicillin binding proteins[9]. Improper empirical therapy and excessive use of broad spectrum antimicrobial agents is one of the main factor responsible for the antimicrobial resistance[10]. Because of the great antimicrobial resistance and increasing frequency of isolation of NFGNB, current study was done to know the prevalence of NFGNB and their antimicrobial susceptibility pattern in our hospital setting.

Material & Methods

This study was done in Bacteriology lab of Department of Microbiology in National Institute of Medical Sciences & Research, Jaipur over a period of 6 months from August 2018 to January 2019. Samples were received and processed as soon as possible. Samples like Urine, Sputum, Wound swab, Blood, Endotracheal tube, Body fluids, Pus, CSF etc. were received from patients admitted in different wards, ICU and from OPD patients.

Samples were cultured on 5% Sheep Blood agar, Mac-Conkey agar and growth was observed and processed by series of test like Gram staining, Growth at 25°C ,37°C ,42°C, Motility (Hanging Drop Method), catalase test, Oxidase test, Citrate test, urease test, Pigment production, in dole production, Methyl Red test, Voges Proskauer test, Triple sugar Iron test, Oxidation/ Fermentation test for Glucose, Lactose, Xylose, Mannitol and Maltose (Hugh and Leifson's media), Lysine and Ornithine decarboxylase and Arginine dihydrolase activity test etc. were done for isolation of the Non-Fermentative Gram Negative Bacilli[11].

Antimicrobial susceptibility testing was done by using Kirby Bauer's disc diffusion method as per CLSI guidelines 2017 using commercially available Antimicrobial disc. Escherichia coli ATCC 25922 & Pseudomonas aeruginosa ATCC 27853 were used as control organisms during the study[12].

Result

Total 674 bacterial isolates were obtained from various clinical samples during the study period. Among these, 122 isolates were identified as NFGNB at an isolation rate of 18.10%.

Out of these 122 NFGNB, 86 (70.49%) strains were isolated from male patients and 36 (29.51%) strains were isolated from female patients. Among the 122 NFGNB, 100 isolates (81.96%) were identified as Pseudomonas species, 18 isolates (14.75%) were identified as Acinetobacter species and 4 isolates (3.2%) were identified as Proteus species.

Most of the NFGNB were isolated from sputum sample (27.04%) followed by ear swab (21.3%), pus (16.39%), Endotracheal tube (14.75%) etc. Pseudomonas species is mainly isolated from sputum (29%) sample. Acinetobacter species is mainly isolated from endotracheal tube (33.33%) and Proteus species is mainly isolated from urine (50%) sample.

Among total Pseudomonas species isolates, 71 isolates (71%) were obtained from male patients and 29 isolates (29%) were obtained from female patients. Among Acinetobacter species, 12 isolates (66.67%) were obtained from male patients and 6 isolates (33.33%) were obtained from female patients and among the Proteus species, 3 isolates (75%) were obtained from male patients and 1 isolate (25%) was obtained from female patient.

Antimicrobial sensitivity testing showed that among the 122 NFGNB, 80.3% organisms were sensitive to Meropenem, 60.6% organisms were sensitive to Imipenem, 56.5% organisms were sensitive to Piperacillin-Tazobactum.

83% Pseudomonas isolates were sensitive to Meropenem followed by 61% isolates sensitive to Imipenem and 59% isolates sensitive to Piperacillin-Tazobactum. 61.11% isolates of Acinetobacter spp. were sensitive to Meropenem followed by 55.5% isolates sensitive to Imipenem and Aztreonam. Meropenem showed 100% sensitivity rate against Proteus spp. followed by Imipenem, Gentamycin, Piperacilin-Tazobactum with a sensitivity rate of 75%. Ceftazidime was least sensitive drug among the Pseudomonas spp. with a sensitivity rate of 43%. Ciprofloxacin was the least sensitive drug among the Acinetobacter spp. and Proteus spp. with a sensitivity rate of 33.3% and 25% respectively.

Chart 1 – Distribution of isolated organisms.

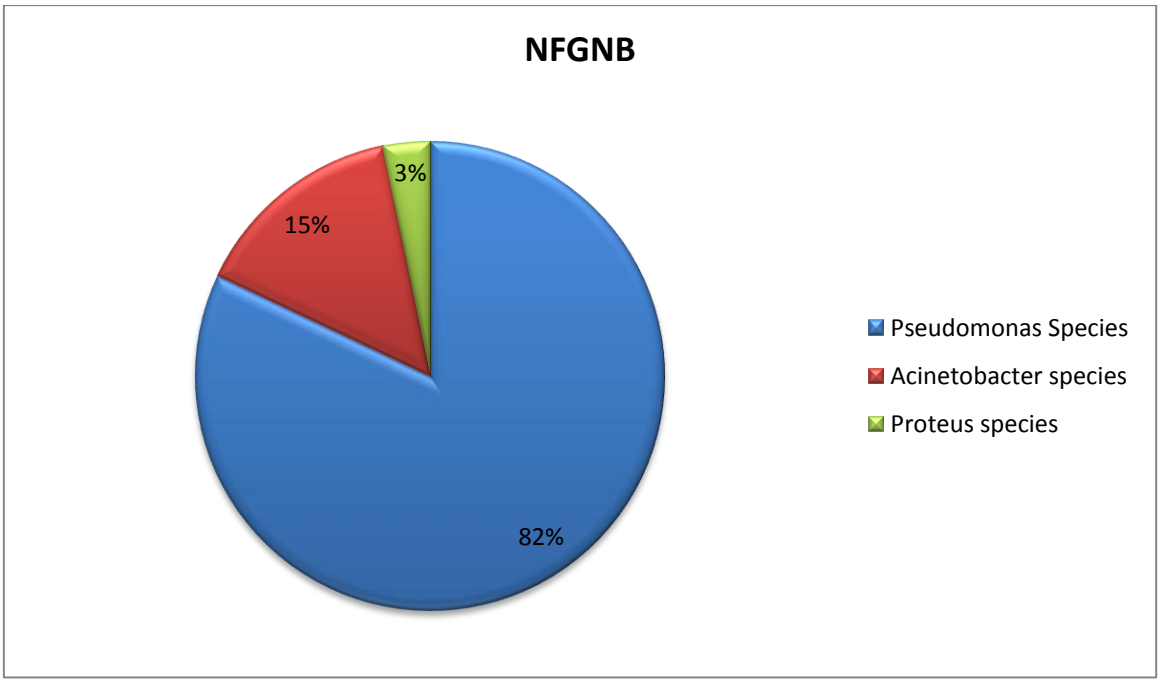
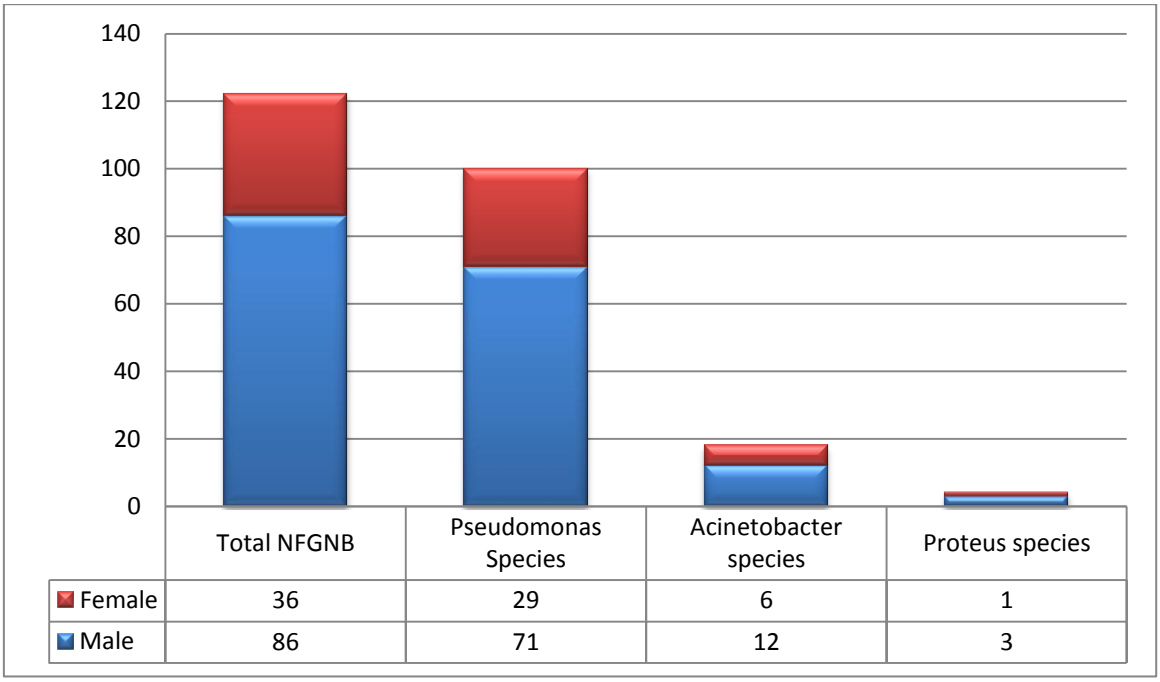
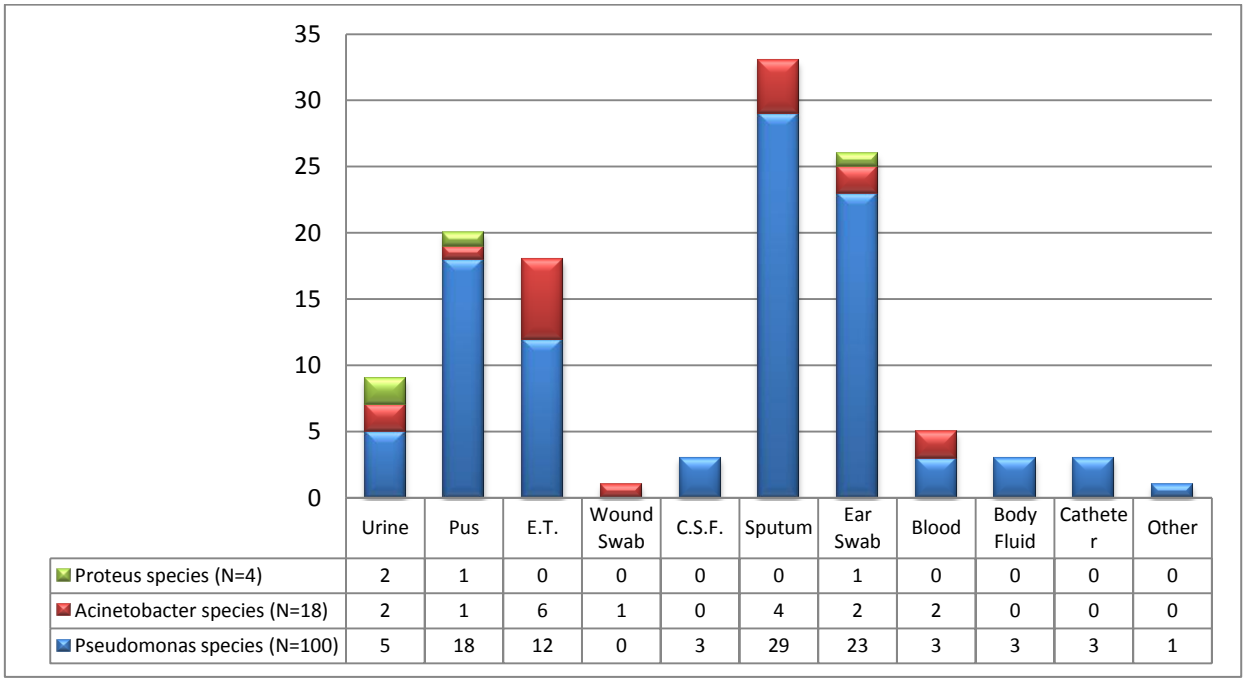


Chart 2 – Gender wise distribution of isolates.



	Total NFGNB	Pseudomonas Species	Acinetobacter species	Proteus species
Female	36	29	6	1
Male	86	71	12	3

Chart 3 – Sample wise isolation of Different NFGNB



	Urine	Pus	E.T.	Wound Swab	C.S.F.	Sputum	Ear Swab	Blood	Body Fluid	Catheter	Other
Proteus species (N=4)	2	1	0	0	0	0	1	0	0	0	0
Acinetobacter species (N=18)	2	1	6	1	0	4	2	2	0	0	0
Pseudomonas species (N=100)	5	18	12	0	3	29	23	3	3	3	1

Chart 4 – Antimicrobial Susceptibility pattern of NFGNB.

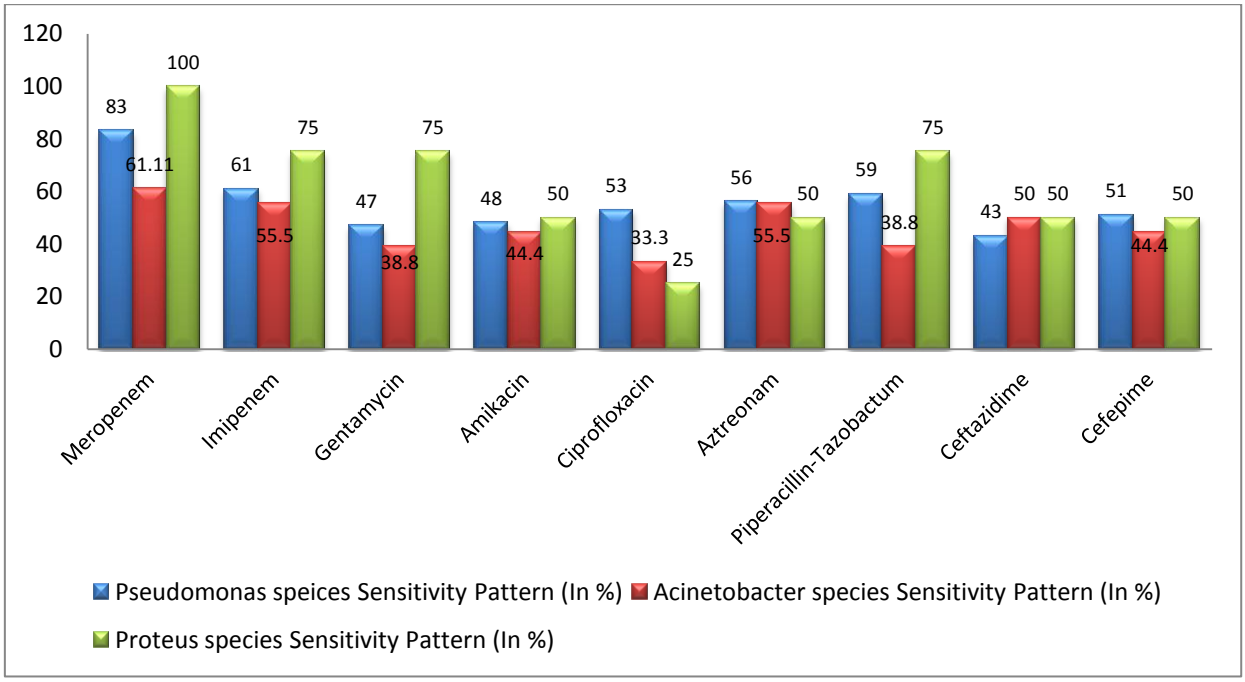


Table 1 Sample wise isolation of Different NFGNB

Sample	Pseudomonas species (100)	Acinetobacter species (18)	Proteus species (4)
Urine	5	2	2
Pus	18	1	1
E.T.	12	6	0
Wound Swab	0	1	0
C.S.F.	3	0	0
Sputum	29	4	0
Ear Swab	23	2	1
Blood	3	2	0
Body Fluid	3	0	0
Catheter	3	0	0
Other	1	0	0

Table 2 Antimicrobial Susceptibility pattern of NFGNB. (S = Sensitive isolates)

Anti-Microbial Drugs	Pseudomonas speices (100)		Acinetobacter species (18)		Proteus species (4)		Total (122)	
	S	%	S	%	S	%	S	%
Meropenem	83	**83**	11	**61.11**	4	**100**	98	**80.3**
Imipenem	61	**61**	10	**55.5**	3	**75**	74	**60.6**
Gentamycin	47	**47**	7	**38.8**	3	**75**	57	**46.7**
Amikacin	48	**48**	8	**44.4**	2	**50**	58	**47.5**
Ciprofloxacin	53	**53**	6	**33.3**	1	**25**	60	**49.1**
Aztreonam	56	**56**	10	**55.5**	2	**50**	68	**55.7**
Piperacillin-Tazobactum	59	**59**	7	**38.8**	3	**75**	69	**56.5**
Ceftazidime	43	**43**	9	**50**	2	**50**	54	**44.2**
Cefepime	51	**51**	8	**44.4**	2	**50**	61	**50.0**

Discussion

Non fermenter Gram Negative Bacilli are ubiquitous in nature. Although these organisms were considered as non-pathogenic or commensals or contaminants but in recent time the pathogenic potential of NFGNB has been known[1]. But recent studies have shown that these organisms have emerged as important nosocomial

pathogens and mainly attacking the immunocompromised patients. Antimicrobial resistance is very common and is increasing rapidly. And now a days they are resistant to routinely used antimicrobial agents[13].

In our study the prevalence of NFGNB is 18.10% which is in accordance with the study done by Amandeepkaur et al (2018), in which the prevalence of NFGNB was 16.1%[14]. Vijaya et al (2000) in her study got the prevalence rate of 21.80% which slightly higher than our study[15]. A great variation in prevalence rate is seen in many studies like Sidhu et al (2010)[16] got very high prevalence rate 45.9% whereas Malini et al (2009)[17] got prevalence rate 4.5% which is very low.

In current study the isolation rate of NFGNB from sputum sample is 27.04%. Many authors have reported variable isolation rate of NFGNB from sputum like the isolation rate of NFGNB from sputum was 22.5% in study of Savita Singh et al (2017)[18]. In study of Malini et al (2009)[17] and Patel et al (2013)[19] isolation rate of NFGNB from sputum was 6.7% and 7% respectively.

Isolation of NFGNB from urine sample is 7.3% in our study which correlates with the result of study done by Gokale et al (2012)[20]. Isolation rate was 8.2% in his study. Benanchinmardi et al (2014)[21], Malini et al (2009)[17] and Patel et al (2013)[19] have reported NFGNB isolates obtained from urine as 11%, 11.9% and 11.8& respectively.

In the present study the isolation rate of NFGNB from blood sample is 4.09% which is showing similarity with the result of Benanchinmardi et al (2014)[21]. In this the isolation rate was 6%.

Shilpa K. Gokale et al. (2012)[20] in her study got isolation rate of Pseudomonas species and Acinetobacter species as 82.3% and 16% respectively. We also got similar results. Vijaya et al. (2000)[15] in her study identified 78.1% isolates as Pseudomonas species and it also correlates with result of our study. Great variation in isolation of Acinetobacter species is seen in different studies.

In our study, most sensitive drug among NFGNB was Meropenem. This was in accordance with the results of Shilpa K. Gokale et al. (2012)[20] and Jitendra Nath et al. (2016)[22]. In our study Piperacillin-Tazobactum was 59% sensitive and Ceftazidime was 43% sensitive for Pseudomonas spp. This correlates well with the findings of Savitasingh et al (2017)[18]. Sensitivity rate of Amikacin and Ciprofloxacin for Pseudomonas spp. was 49.5% and 50.4% respectively in our study and this result is in concordance with the study done by Shilpa K. Gokale et al (2012)[20]. For Ciprofloxacin, Piperacillin-Tazobactum and Ceftazidime we got sensitivity rate of 53%, 59% and 43% respectively. These result correlates with the findings of Kaur A et al. (2018)[14]. Among the Acinetobacter isolates, we got sensitivity rate of Meropenem, Amikacin and Ceftazdime as 61.11%, 44.4% and 50.0% respectively and this result are quite similar to the study of Kaur A et al. (2018)[14] and Jitendra Nath et al. (2016)[22].In our study we got 44.4% and 55.5% sensitivity rate of Amikacin and Imipenem respectively and Nabamita Chaudhary et al. (2019) reported almost similar sensitivity rate of Amikacin and Imipenem (47.78% and 60.0% respectively)[23].

Conclusion

Higher prevalence of NFGNB is seen in our study and in other studies also. It should be noted that there is great resistance among the NFGNB isolates against the routinely used first line antimicrobial agents. Higher isolation and higher antimicrobial resistance is an alarming sign for healthcare professionals. These organisms can survive in hospital environment that's why proper housekeeping, equipment decontamination and strict guidelines for sterilization need to be implemented. Further studies will definitely help in better understanding of changes in its antimicrobial resistance pattern. This study can be very helpful in initiating the empirical treatment of such patients thereby reducing the morbidity rate and also reducing the emergence of multidrug resistant non fermenter Gram Negative Bacilli.

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