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Prevalence and *in vitro* antibiogram of non-fermenting gram negative bacilli in a tertiary care hospital of Karnataka

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ABSTRACT

Background and Objectives: Nonfermenting gram-negative bacilli (NFGNB), are a taxonomically diverse group of aerobic, nonsporing, bacilli that are saprophytic in nature. They have emerged as an important healthcare-associated pathogens. They have exhibited resistance clinically to a variety of antibiotic groups. Thus, the present study was conducted to isolate, identify and carry out in vitro susceptibility antibiogram of NFGNB from various clinical specimens and also to highlight their clinical significance among the in-patients admitted at Basaveshwar Teaching and General Hospital, Gulbarga, Karnataka.
Materials and Methods: 120 isolates from various age groups of both male and female patients were included in the study. A detailed history was elicited and the clinical specimens were collected under aseptic precautions and subjected to preliminary biochemical test and further speciation was done.
Results: NFGNB were most frequently isolated from local infections, with Pseudomonas aeruginosa, Acinetobacter baumanii and Pseudomonas fluorescens, being the more common. These species showed variability in their antibiotic susceptibility results, most of them exhibiting resistance to Penicillin group of drugs and high sensitivity to Carbapenem group of drugs.
Conclusion: *P.aeruginosa* had shown good sensitivity to imipenem, carbenicillin and of loxacin. *A. baumannii* showed good sensitivity to imipenem, amikacin and gentamicin. As, the sensitivity pattern varies from hospital to hospital and population to population, it is essential to establish the clinical relevance of the isolated NFGNB. This would avoid unnecessary usage of antibiotics and emergence of drug-resistant strains.

1. Introduction

Non fermenting gram negative bacilli (NFGNB) are taxonomically diverse heterogenous group of aerobic, nonsporing, bacilli that either do not utilize carbohydrates as source of energy or degrade them by various metabolic pathways other than fermentation. NFGNB are known to account for about 15% of all bacterial isolates from a clinical microbiology laboratory.[1,2] They are saprophytes which may be found inhabiting the soil and water or as commensals in humans and animals. Their emergence as important nosocomial pathogens can be attributed to the liberal use of antibiotics, their inherent resistance to common disinfecting agents and their ability to colonize a variety of surfaces.[3]

These bacteria can be frequently isolated from samples of patients suffering from septicemia, meningitis, pneumonia, urinary tract infection and surgical wound infection. Some of the risk factors that can contribute to NFGNB infections include immunosuppression (oncology patients on cytotoxic therapy/radiotherapy, organ transplant patients and even patients with AIDS), neutropenia, mechanical ventilation, cystic fibrosis, indwelling catheters, invasive diagnostic and therapeutic procedures. Currently *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are the most commonly isolated nonfermenters pathogenic for humans. Other species that can be isolated include opportunist pathogens like

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P. fluorescence, *P.stutzeri*, Stenotrophomonas *maltophilia*, P.*putida* and P.*cepacia*.[4,5]

A difficulty has been encountered recently in treating NFGNB infections as they exihibit a multidrug resistance (MDR). MDR is common and increasing among these bacteria. A number of strains have been identified to be resistance towards various groups of drugs including Beta-lactam, Aminoglycosides and fluroquinolones. This in turn is posing a barrier to treatment and hospital infection control.[6,7]

There have been very few studies to report on the prevalence of NFGNB and their antibiogram from this part of the country. The present study was hence taken up to help bridge this gap of knowledge. This study was aimed to isolate, identify and carry out in vitro susceptibility antibiogram of NFGNB from various clinical specimens and also to highlight their clinical significance among the in-patients admitted at Basaveshwar Teaching and General Hospital, Gulbarga, Karnataka.

2. Materials and Methods

This study was carried out on 120 samples of clinical specimens collected from in- patients admitted to various departments of Basaveshwar teaching and general hospital, Gulbarga during a period of 1 year study from January 2012 to December 2012. A detailed history of the patients was recorded including the current underlying disease like Diabetes or malignancy.

The samples for the study were collected from patients with NFGNB infection, admitted during the period of study. NFGNB was isolated from blood, CSF and other body fluids. Patients with colonisation of NFGNB with no apparent clinical infection and isolates from improperly collected samples were excluded from the study.

The samples were collected from respiratory tract (RT), pus, wound, urine and blood. A preliminary (gram stain) examination was carried out for RT, pus, wound and urine samples, following which they were incubated in appropriate media. The RT samples were inoculated in 5% sheep blood agar (BA), Macconkey agar (MA), and chocolate agar (CA) which were incubated overnight at 37°c and were observed for growth for 48 hrs.

The pus and wound swab sample were plated on 5% sheep BA, MA, Thioglycollate which were incubated overnight at 37°c and were observed for growth for 48 hrs. For the urine samples, gram stain smear were made by placing a loopful urine sample on a clean slide and allowed to air dry. These samples were then plated by a 4mm loop onto 5% sheep BA and MA for semi-quantitative analysis. Isolates which were significant in semi quantitative culture of urine were included in the study.

A Brain Heart Infusion broth was used for blood culture. The bottle was examined duly for turbidity and subculture was made at regular intervals on to BA, MA and any growth was processed further for identification. Cultures that showed growth in the first three days were included in the study.

For identification of species, biochemical tests were performed which include OF medium(Hugh and Leifson), Nitrate reducing broth, Citrate utilization test, Growth at room temperature 25°c-30°c, 37°c, 44°c, Hemolysis on a 5% sheep BA, Gelatin liquefaction and Hanging drop preparation for motility testing. Antibiogram was done by KIRBY-BAUER disc diffusion method. The various antibiotics with their concentration that were used are shown in Table 1.

Only those NFGNB which grew either in pure culture or as predominant growth were identified in the study. All the tests were performed with positive and negative control.

3. Results

In the present study, 120 samples were collected from clinical specimens with local infections, Septicemia, Respiratory tract infections, Urinary tract infections, Ear infections, meningitis and cervicitis samples from patients admitted to Basaveshwar Teaching and General Hospital, Gulbarga.

The various samples from which NFGNB were isolated are shown in Table 2. Pus samples constituted majority of specimens accounting for 50.83%. Urine and Sputum samples accounted for 14.17% & 15% of specimens respectively. Stool, Blood, Pleural fluid, Ascitic fluid and CSF samples accounted for remaining 20%.

Various bacterial species isolated from each clinical diagnosis have been shown in Table 3. Out of all the cases, Pseudomonas *aeruginosa* was isolated in 48 cases which included 18 cases of local infection, 7 cases of respiratory tract infection (RTI), 4 cases each of Gastro intestinal tract (GIT) and post traumatic, 3 cases each of urinary tract infection (UTI) and septicaemia and 9 cases of post-operative (post OP) infection.

Pseudomonas *fluorescens* was isolated from 5 cases including 2 cases of RTI and 3 of post OP infection. Acinetobacter *baumanii* was isolated from 15 cases which included 5 cases of local infection, 3 cases of RTI, 2 cases each of GIT and post OP and 1 case each of UTI, post traumatic and septicaemia.

Mixed growth (Proteus mirabilis, Proteus vulgaris, Citrobacter, MRSA, E coli, Klebsiella, Enterococci, Enterobacter species, Group A beta haemolytic streptococci, Salmonella spp, Shigella spp) were seen a total of 52 cases which included 16 cases of local infection, 13 cases of UTI, 9 cases of RTI, 5 cases of GIT, 4 case of septicaemia, 3 cases of post OP infection and 2 cases of post traumatic infection.

The antibiotic susceptibility pattern for various NFGNBs for penicillin group of drugs is shown in Table 4. In the decreasing order of frequency, the NFGNB showed 54.41% sensitivity to Carbenicillin, 45.58% to Piperacillin

Table 1: Various antibiotics and their concentrations used for the study

Amikacin(AK)-30mcg	Ceftizoxime(Ck)	Lomifloxacin(Lo)-10mcg
Ampicillin(A)-30mcg	Ciprofloxacin(Cf)-5mcg	Ofloxacin(Of)-5mcg
Carbenicillin(Cb)-100mcg	Cotrimoxazole(Co)-1.25mcg	Penicillin(P)-10u
Cefaperazone(Cs)-75mcg	Gentamycin(G)-10mcg	Piperacillin(Pc)-100mcg
Cefepime(Cpm)-30mcg	Imepenem(I)-10mcg	Polymyxin(Pb)-300u
Cefotaxime(Ce)-30mcg	Kanamycin(K)-30mcg	Tetracyclin(T)-30mcg
Ceftriaxone(Ci)-30mcg	Netilimicin(Nt)-30mcg	Ticarcillin(Ti)-75mcg
Ceftazidime(Ca)-30mcg	Norfloxacin(Nx)-10mcg	Tobramycin(Tb)-10mcg

Table 2: Various samples from which NFGNB were isolated

S. No	Sample	No of cases	Percent
1	Pus	61	50.83%
2	Sputum	18	15%
3	Ascitic fluid	3	2.5%
4	Blood	5	4.17%
5	Urine	17	14.17%
6	Stool	8	6.67%
7	Cervical discharge	2	1.67%
8	Pleural fluid	3	2.5%
9	CSF Fluid	3	2.5%

Table 3: Bacterial species isolated under each clinical diagnosis

Species	Local	RTI	UTI	GIT	Post op	Post Traumatic	Septicemia	Total
Ps.aeruginosa	18	7	3	4	9	4	3	48
Ps.fluorescens	-	2	-	-	3	-	-	5
Ac.baumanii	5	3	1	2	2	1	1	15
Mixed Group	16	9	13	5	3	2	4	52

Table 4: Antibiotic susceptibility pattern of NFGNB for penicillin group of drugs

Antibiotic	Sensitive		Resistant	
	No of Cases	%	No of Cases	%
Penicillin	5	7.36%	63	92.64%
Amoxyclav	16	23.53%	52	76.47%
Carbenicillin	37	54.41%	31	45.58%
Piperacillin tazobactam	31	45.58%	37	54.41%
Netilmicin	11	16.18%	57	83.82%
Ticarcillin	18	26.48%	50	73.52%
Piperacillin	20	29.42%	48	70.58%

and Tazobactam, 29.42% to Piperacillin, 26.48% sensitivity to Ticarcillin, 23.53% sensitivity to Amoxyclav, 16.18% sensitivity to Netilmicin and 7.36% sensitivity to Penicillin. Maximum resistance was exhibited against penicillin (92.64% cases) and minimum against carbenicillin (45.58% cases).

Ps.*aeruginosa* showed a sensitivity of 68.75% to Carbenicillin followed by 41.66% sensitivity to Piperacillin+Tazobactam. Ps.fluroscens showed a uniform sensitivity of 60% to Carbenicillin and Piperacillin+Tazobactam. Acinetobacter *baumanii* showed a sensitivity of 40% to Piperacillin+Tazobactam, 26.66% sensitivity to Carbenicillin and Amoxyclav.

The antibiotic susceptibility pattern of NFGNB for cephalosporin group of drugs is shown in Table 5. NFGNB showed a sensitivity of 52.95% to Ceftazidime, 48.53% to Cefaperazonea and 42.65% to Ceftriaxone. Maximum resistance was observed for Cefuroxime and minimum for Ceftazidime.

Ps.*aeruginosa* showed a sensitivity of 54.16% for Cefoperazone, 60.42% sensitivity for Ceftazidime, 49.84% sensitivity for Cefepime. Ps. *Fluorescens* showed uniform sensitivity of 60% to Ceftriaxone, Ceftazidime, and Cefaperazone. Ac. *baumanii* showed a susceptibility of

Table 5: Antibiotic susceptibility pattern of NFGNB for cephalosporin group of drugs

Antibiotic	Sensitive		Resistant	
	No of cases	%	No of cases	%
Cefuroxime	8	11.77%	60	88.23%
Cefotaxime	17	25%	51	75%
Ceftriaxone	29	42.65%	39	57.35%
Cefaperazone	33	48.53%	35	51.47%
Ceftazidime	36	52.95%	32	47.05%
Cefipime	29	42.65%	39	57.35%

46.66% to Ceftazidime, 53.34% to Ceftriaxone and 33.33% to Cefepime and Cefaperazone.

The antibiotic susceptibility pattern of NFGNB for aminoglycosides and quinolone group of drugs is shown in Table 6. NFGNB showed a sensitivity of 69.11% to Amikacin, 55.18% to Gentamicin, 61.76% to Ciprofloxacin and 55.88% to Ofloxacin.

Ps.*aeruginosa* showed a sensitivity of 75% to Amikacin, 60.42% sensitivity to Gentamicin, 72.92 % sensitivity to Ciprofloxacin and 64.58 % sensitivity to Ofloxacin. Ps.*fluorescens* showed a sensitivity of 40% to Amikacin and Ofloxacin and 20% sensitivity to Gentamicin and Ciprofloxacin. Ac.*baumanii* showed a sensitivity of 60% to Amikacin and Gentamicin, 40% sensitivity to Ciprofloxacin and 33.33% sensitivity to Ofloxacin.

The antibiotic susceptibility pattern of NFGNB for carbapenems group of drugs is shown in Table 7. NFGNB's showed a sensitivity of 82.35% to Imipenem and 58.82% sensitivity to Meropenem.

Ps.*aeruginosa* showed a sensitivity of 85.42% to Imipenem and 62.5% sensitivity were seen with Meropenem. Ps.*fluorescens* showed a sensitivity of 80% to Imipenem and 60% sensitivity were seen with Meropenem. Ac.*baumanii* showed a sensitivity of 73.34% to Imipenem and 40% sensitivity were seen with Meropenem.

The antibiotic susceptibility pattern of NFGNB for other group of drugs is shown in Table 8. The isolated organisms showed a sensitivity of 30.88% to Tetracycline, 25% to Co-trimoxazole, 19.12% to Chloramphenicol and a susceptibility of 35.29% to Polymyxin B.

Ps. *aeruginosa* showed a sensitivity of 39.58% to Polymyxin B, 31.25% to Tetracycline, 22.92% to Cotrimoxazole and 12.5% to Chloramphenicol. Ps. *fluorescens* showed a uniform sensitivity of 40% to Tetracycline, Cotrimoxazole and Chloramphenicol. Ac. *baumanii* showed a uniform sensitivity of 26.66% to Tetracycline, Cotrimoxazole and Chloramphenicol.

The individual sensitivity and resistance of P. aeruginosa, P. fluorescens and A. baumannii to various antibiotics has been shown in Table **??**.

4. Discussion

The non-fermenters are present everywhere in the environment. Usually they are considered as commensals or Contaminants but their pathogenic potential has been established owing to their frequent isolation from clinical materials.[4,8] Their association with nosocomial infections has also been observed frequently. The facts of an epidemiological complexity, efficacy to cause outbreaks of infection and antimicrobial resistance has brought the focus of attention on these NFGNBs.[9–12] Resistance to antimicrobials has increased over the years as a result of which many strains exhibit resistance to all commonly used antibiotics. This multi-drug resistance in turn increases the difficulty and cost of treatment for these infections.[13–16]

During the study period from January 2012 to December 2012 at Basaveshwar Teaching and General Hospital, Gulbarga, 120 samples from various clinical conditions like septicaemia, local infection, post-operative infection, post traumatic infection, RTI, UTI and GIT were collected and subjected for further processing. NFGNB were isolated from 68 samples.

The most common NFGNB isolated in our study was *P. aeruginosa* in 48/120 isolates (40%) followed by A. baumannii in 15/120 cases (12.5%) which is similar to the results obtained by Malini *et al.* who reported *P. aeruginosa* as the most common isolate accounting for 104/189 (53.8%) isolates, followed by *A. baumannii* (43/189, 22.2%).[3]

In the present study, the highest number of isolates were isolated from pus swabs (50.83%), which is in accordance with the observations made by Rit *et al.*[17] and Gokale and Metgud[18] who also reported pus swabs as the source of maximum percentage of the isolates i.e., 27.86% and 58.4%, respectively. P. aeruginosa and A. baumannii were mostly associated with local infections (37.5% and 33.33% respectively) while P. fluorescens was mostly associated with RTI (40%) and post OP infections (60%) as is evident from Table 3.

NFGNB displays a wide and variable spectrum of antibiotic sensitivity. There is no antibiotic to which all strains are susceptible. NFGNB are uniformly resistant to Penicillin group of drugs. NFGNB showed sensitivity of 29.42% to Piperacillin in our study and the sensitivity ranged from 40% to 85% in other studies by Rajan R et

Table 6: Antibiotic susceptibility pattern of NFGNB for aminoglycosides and quinolone groups of drugs

Antibiotic	Sensitive		Resistance	
	No. of cases %		No of cases %	
Amikacin	47	69.11%	21	30.88%
Gentamicin	38	55.18%	30	44.82%
Ciprofloxacin	42	61.76%	26	38.23%
Ofloxacin	38	55.88%	30	44.11%

Table 7: Antibiotic susceptibility pattern for carbapenems group of drugs

Antibiotic	Sensitive		Resistant	
	No. of cases	%	No. of cases	%
Imipenem	56	82.35%	12	17.65%
Meropenem	40	58.82%	28	41.18%

Table 8: Antibiotic susceptibility pattern of NFGNB for other group of drugs

Antibiotic	Sensitive		Resistant	
	No. of cases	%	No. of cases	%
Polymixin B	24	35.29%	44	64.71%
Chloramphenicol	13	19.12%	55	80.88%
Tetracycline	21	30.88%	47	69.12%
Co-trimoxazole	17	25%	51	75%

al. and Prakash K S et al. Piperacillin+Tazobactam is a preferred drug for treating NFGNB infections and showed a sensitivity of 45.58% in our study.[19–23]

NFGNB's showed an overall 17.16 % resistance to Imipenem in our study. In a study by Taneja et al. it showed 36%.[24] NFGNB's showed a resistance of 41.18% to Meropenem which was higher, compared to Imipenem in our study. It is known that Meropenem develops resistance earlier than Imipenem. In study by Gupta E et al resistance to Meropenem was 22.16%.[25]

Maximum sensitivity by Ps.*aeruginosa* was shown to Imipenem (85.42%) followed by carbenicillin (68.75%) and ofloxacin (64.58%) which are similar to results of studies conducted by Malini A. et al. and Rit et al. where the susceptibility to imipenem shown was 94.2% and 91.08%, respectively.[3,17] In agreement with the studies done by Benachinmardi *et al*. and Naqvi *et al*. that showed higher susceptibility to quinolones, *P. aeruginosa* isolates in the present study showed susceptibility of 75.92% and 64.58% to the quinolones such as ciprofloxacin and ofloxacin, respectively.[11,12]

In our study, *P. aeruginosa* showed lesser susceptibility to amoxicillin + clavulanic acid (18.75%) and least to chloramphenicol (12.5%). Ps.*aeruginosa* showed a resistance of 54.42 % to Piperacillin+Tazobactam in our study. The low sensitivity in our study could be due to excessive use of Piperacillin+Tazobactam combination in our hospital.

Ac.*baumanii* showed maximum sensitivity towards imipenem (73.34%) followed by amikacin and gentamicin (each 60%). The results obtained in our study are similar to the studies conducted by Rit et al. and Tunyapanit et al. where the susceptibility to imipenem was 90% and 100% respectively.[13,25] Ac.*baumanii* showed a sensitivity of 30% to Piperacillin in our study. In studies conducted by Wong fu et al. it showed a sensitivity of 30% and in a study by Taneja et al it showed 40% sensitivity. Ac. *baumanii* showed a resistance of 60% to Piperacillin+Tazobactam in our study, similar to other studies.[9,24] Ac .*baumanii* showed a sensitivity of 26.66 % to Amoxyclav in our study. In studies conducted by Jawad et al. it showed a sensitivity of 57% and in study by Wong fu et al., it was 25%.[9,10]

NFGNBs showed resistance of 47.05 % to Ceftazidime, 51.47 % to Cefaperazone, 57.35% to Cefepime which are commonly used by the clinicians in our hospital. Ps.*aeruginosa* showed 27.08% resistance to Ciprofloxacin in our study. In various other studies by Taneja et al., Algun U et al., Prakash KS et al., Wong fu et al. and Smitha S et al., it ranged from 12.5% to 83%.[9,23,24,26,27]

NFGNBs showed a good sensitivity to Amikacin 69.11% in our study which is similar to other studies by Prakash K S et al., Wong Fu et al. and Taneja et al. Gentamicin showed a sensitivity of 55.18 % in our study. However Ps.*fluorescens* showed least sensitivity of 20% to Gentamicin which was comparable to study by Yashodhara et al. where it was 25%.[9,24,27,28]

5. Conclusion

NFGNB though regarded as contaminants are important bacteria causing wide range of nosocomial infections. A variability in their sensitivity pattern emphasizes the need

Table 9: Antibiotic susceptibility pattern for various group of drugs against each organism

Antibiotic		Ps.aeruginosa		Ps.fluorescens		Ac.baumanii	
			%		%		%
Amoxyclav	R	39	81.25%	4	80%	11	73.34%
		9	18.75%	1	20%	4	26.66%
Carbenicillin	R	15	31.25%	2	40%	11	73.34%
		33	68.75%	3	60%	4	26.66%
Piperacillin+ Tazobactam	R	28	58.34%	2	40%	9	60%
		20	41.66%	3	60%	6	40%
Cefaperazone	R	22	45.84%	2	40%	10	66.67%
		26	54.16%	3	60%	5	33.33%
Ceftazidime	R	19	39.58%	2	40%	8	53.34%
		29	60.42%	3	60%	7	46.66%
Ceftriaxone	R	29	60.42%	2	40%	7	46.66%
		19	39.58%	3	60%	8	53.34%
Cefepime	R	26	54.16%	4	80%	1	66.67%
		22	49.84%	1	20%	5	33.33%
Amikacin	R	12	25%	3	60%	6	40%
		36	75%	2	40%	9	60%
Gentamicin	R	19	39.58%	4	80%	6	40%
		29	60.42%	1	20%	9	60%
Ciprofloxacin	R	13	27.08%	4	80%	9	60%
		35	72.92%	1	20%	6	40%
Ofloxacin	R	17	35.42%	3	60%	10	66.67%
		31	64.58%	2	40%	5	33.33%
Imipenem	R	7	14.58%	1	20%	4	26.66%
		41	85.42%	4	80%	11	73.34%
Meropenem	R	18	37.5%	2	40%	9	60%
		30	62.5%	3	60%	6	40%
Polymixin B	R	29	60.42%	4	80%	12	80%
		19	39.58%	1	20%	3	20%
Chloramphenicol	R	42	87.5%	3	60%	11	73.34%
		6	12.5%	2	40%	4	26.66%
Tetracycline	R	33	68.75%	3	60%	11	73.34%
		15	31.25%	2	40%	4	26.66%
Co trimoxazole	R	37	77.08%	3	60%	11	73.34%
		11	22.92%	2	40%	4	26.66%

for identification and isolation of NFGNB and to perform antibiogram to help in decide proper line of treatment and antibiotic protocol.

P. aeruginosa and *A. baumannii* were the most common NFGNB isolated in our study. *P. aeruginosa* had shown good sensitivity to imipenem, carbenicillin and ofloxacin. *A. baumannii* showed good sensitivity to imipenem, amikacin and gentamicin. The different species of NFGNB have shown a varied sensitivity pattern in our study.

The multidrug resistance of these organisms is thought to be enhanced and maintained through repeated exposure of organisms to antibiotics. Presence of a sub lethal concentration of antibiotics creates a suitable environment for development of resistance. Also, the sensitivity pattern varies from hospital to hospital and population to population depending upon the frequently prescribed antibiotics to the infected patients.

Thus, it is essential to establish the clinical relevance of the isolated NFGNB, before they are considered as pathogens. This would avoid unnecessary usage of antibiotics and emergence of drug-resistant strains.

6. Source of Funding

None.

7. Conflict of Interest

None.

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