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Original Research Article

Study of Non-Fermentative Gram Negative Bacilli

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Abstract

Aim and Objectives: To isolate and identify Non-fermenting Gram-negative bacilli (NFGNB) from clinical specimens and to study the antimicrobial susceptibility pattern of the isolates.
Methods: This study was conducted in the Department of Microbiology in a Tertiary Care Hospital during the period of two years. NFGNB were isolated and identified from a variety of clinical specimens by standard procedure and antibiotic sensitivity test was performed.
Results: NFGNB isolation rate was found to be 5.19%. *Acinetobacter spp.* was the most common isolate (56.82%) followed by *Pseudomonas spp.* (40.92%), *Stenotrophomonas maltophilia* (1.36%) and *Burkholderia cepacia complex* (0.90%). Maximum sensitivity of *Acinetobacter* was seen to Imipenem (82.40%), followed by Amikacin (62%), Piperacillin-tazobactamin combination (52.40%). *Pseudomonas* isolates showed 100 % sensitivity to Colistin and Polymyxin B followed by Imipenem (86.67%), Amikacin (71.67%) and Piperacillin-tazobactam (70.56%). *Stenotrophomonas maltophilia* isolates were sensitive to Trimethoprim-Sulfamethoxazole (83.33%) and 66.67% were sensitive to Levofloxacin. Isolates of *Burkholderia cepacia complex* (BCC) were 100% sensitive to Meropenem and Ceftazidime followed by Trimethoprim-Sulfamethoxazole (75%).
Conclusion: Early detection and Identification of NFGNB and monitoring their susceptibility pattern are necessary to guide the clinician for better care and management of patients. NFGNB are now emerging as organisms of nosocomial infections. Hence, regular antimicrobial susceptibility surveillance and strict infection control measures are needed to prevent the emergence and spread of multi drug resistant NFGNB in health care settings.
Keywords: Non-fermenting Gram-negative bacilli (NFGNB), *Acinetobacter, Pseudomonas, Stenotrophomonas, Burkholderia, Antimicrobial susceptibility pattern*.

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1. Introduction

Non-fermentative gram negative bacilli (NFGNB) have emerged as important healthcare-associated pathogens [1]. Although, frequently considered to be commensals or contaminants, their pathogenic potential has been established without doubt because of their frequent isolation from clinical specimens [2]. The most commonly occurring NFGNB are *Pseudomonas aeruginosa, Acinetobacter spp., Stenotrophomonas maltophilia, Burkholderia spp., Alcaligenes spp.* etc [3, 4]. Prevalence of these pathogens often varies dramatically between communities, hospitals in the same community and among different patient populations in the same hospital [5].

Antimicrobial treatment of the infection caused by NFGNB 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 problem for both treatment and infection control [6]. These organisms are potentially dangerous in the ICU setting and can lead to increased financial burden for the patients. They may also spread resistance to other susceptible bacteria by horizontal gene transfer [7].

NFGNB are not routinely identified up to species level in microbiology laboratories, as their identification requires a number of biochemical tests.

Since there has been an increase in incidence of infection and drug resistance by these organisms, it is required to identify them up to species level [8]. With this background the present study was undertaken to isolate and identify NFGNB from clinical specimens and to study the antimicrobial susceptibility pattern of the isolates.

2. Materials and Methods

The present observational study was carried out in the Department of Microbiology, at Tertiary Care Institute during the period of two years. The study participants were patients who were referred to our department for culture and antibiotic susceptibility testing and were found to be culture positive for non-fermenters. No specific exclusion criteria envisaged.

Depending on sites of infections various samples like urine, pus, wound swabs, sputum, ET aspirate, blood and ascitic fluid, were collected from inpatient department (IPD), outpatient department (OPD) and intensive care units (ICU) and processed as per the standard guidelines [13]. NFGNB were isolated from a variety of clinical specimens, plated on blood agar and MacConkey agar and incubated at 37°C for 18–24 h under aerobic conditions.

NFGNB were identified by standard microbiological techniques [9,10] by studying their morphology [11], colony characteristics [9] and biochemical reactions [12]. Each isolate was subjected to antimicrobial susceptibility test as per CLSI 2014 guidelines [13] by Kirby-Bauer disk diffusion technique [14].

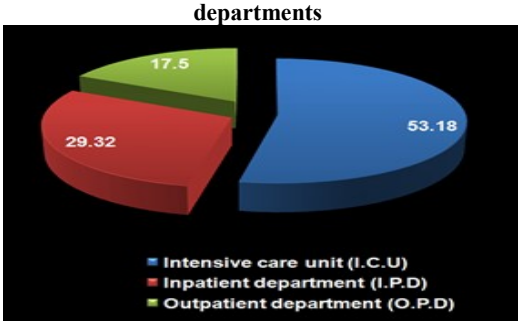
2.1 Statistical Analysis

Chi square test was used with appropriate correction to see the significance of difference between the sensitivity of various drugs using SPSS software. $p \leq 0.05$ was considered significant.

3. Observations and Results

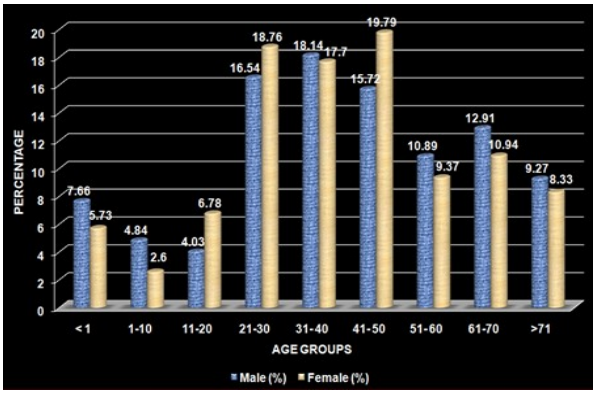
During two years of study period, total 440 NFGNB were isolated from 8,468 clinical specimens with isolation rate of 5.19%. The distribution of specimens and NFGNB isolates from various departments is shown in figure 1. Significantly higher percentage of NFGNB strains were found in ICU (20.24%) compared to IPD (2.40%) and OPD (3.94%).

Figure 1: Distribution of specimens and Non-fermentative gram negative bacilli (NFGNB) isolates from various departments



NFGNB isolates were more common in males (56.36%) as compared to females (43.64%) with Male to Female ratio of 1.29:1. Also from the figure 2, it is observed that the NFGNB infection was more common in age group of 21 to 50 years (52.97%).

Figure 2: Age and gender-wise distribution (n=440)



Majority of NFGNB were isolated from ET aspirates 136 (28.57%), followed by pus 124 (6.37%), sputum 44 (3.85%) and blood 40 (2.84%).Out of 440 isolates, most frequently isolated NFGNB were *Acinetobacter spp.*(250; 56.82%) followed by *Pseudomonas spp*. (180; 40.92%). Other NFGNB isolated were *Stenotrophomonas maltopilia* (6; 1.36%) and *Burkholderia cepacia complex* (BCC) (4; 0.90%).

A. baumannii was the most common isolate (58.82%) in ET aspirate followed by *P. aeruginosa* (20.58%) whereas *P. aeruginosa* was the most common isolate (54.83%) in Pus followed by *A. baumannii* (25.80%). *P. aeruginosa* was also the most common isolate in urine (44.56%) and sputum (40.90%), (Table 1).

Table 1: Distribution of various NFGNB species in different clinical specimens

Specimens	NFGNB species							
	Acinetobacterbaumannii	*Pseudomonas aeruginosa*	*Acinetobacter lwoffii*	*Pseudomonas putida*	*Stenotrophomonasmaltophilia*	*Pseudomonas fluorescens*	*Burkholderiacepacia complex*	Total
ET aspirate	80 (58.82)	28 (20.58)	26 (19.12)	01 (0.74)	-	01 (0.74)	-	136 (30.91)
Pus	32 (25.80)	68 (54.83)	16 (12.90)	04 (3.23)	02 (1.62)	02 (1.62)	-	12 (28.18)
Urine	34 (36.96)	41 (44.56)	14 (15.22)	01 (1.08)	02 (2.18)	-	-	92 (20.91)
Sputum	16 (36.36)	18 (40.90)	04 (9.09)	02 (4.55)	-	02 (4.55)	02 (4.55)	44 (10.00)
Blood	18 (45.00)	10 (25.00)	08 (20.00)	-	02 (05.00)	-	02 (05.00)	40 (09.09)
Body Fluids	02 (50.00)	02 (50.00)	-	-	-	-	-	4 (0.91)
Total	182 (41.36)	167 (37.96)	68 (15.46)	08 (01.82)	06 (01.36)	05 (01.14)	04 (0.90)	440

Maximum sensitivity of *Acinetobacter* was seen to Imipenem (82.40%), followed by Amikacin (62%), Piperacillin-tazobactamin combination (52.40%) whereas maximum resistance was observed to Piperacillin alone (86.40%), Trimethoprim-sulfamethoxazole (83.17%) and Cephalosporines, (Table 2).

Table 2: Antimicrobial susceptibility pattern of *Acinetobacter* isolates (n=250)

Test/ Report Group	Antibiotic	Sensitive (%)	Resistant (%)
Group A	Ceftazidime (CAZ)	52 (20.80)	198 (79.20)
	Ciprofloxacin (CIP)	46(18.40)	204 (81.60)
	Imipenem (IPM)	206 (82.40)	44 (17.60)
	Gentamicin (GEN)	67 (26.80)	183 (73.20)
	Tobramycin (TOB)	125 (50.00)	125 (50.00)
Group B	Amikacin (AK)	155 (62.00)	95 (38.00)
	Piperacillin (PIP)	34 (13.60)	216 (86.40)
	Piperacillin-tazobactam (P/T)	131 (52.40)	119 (47.60)
	Cefotaxime (CTX)	52 (20.80)	198 (79.20)
	Cefepime (CPM)	58 (23.20)	192 (76.80)
	Tetracycline (TET)	121 (48.40)	129 (51.60)
	Trimethoprim-Sulfamethoxazole (COT)	34 (16.83)	168 (83.17)

Pseudomonas isolates showed 100 % sensitivity to Colistin and Polymyxin B followed by Imipenem (86.67%), Amikacin (71.67%), Piperacillin-tazobactam (70.56%) and Tobramycin (69.44%) whereas maximum resistance was observed to Ceftazidime (66.61%), Cefepime (61.67%), Piperacillin (56.67%) and Ciprofloxacin (54.44%). NFGNB isolates from urine showed 66.67% resistance to Norfloxacin, (Table 3).

Table 3: Antimicrobial susceptibility pattern of *Pseudomonas* isolates (n=180)

Test/ Report Group	Antibiotic	Sensitive (%)	Resistant (%)
Group A	Piperacillin (PIP)	78 (43.33)	102 (56.67)
	Ceftazidime (CAZ)	61 (33.39)	119 (66.61)
	Gentamicin (GEN)	90 (50.00)	90 (50.00)
	Tobramycin (TOB)	125 (69.44)	55 (30.56)
Group B	Amikacin (AK)	129 (71.67)	51 (28.33)
	Piperacillin-tazobactam (P/T)	127 (70.56)	53 (29.44)
	Cefepime (CPM)	69 (38.33)	111 (61.67)
	Aztreonam (AT)	102 (56.67)	78 (43.33)
	Imipenem (IPM)	156 (86.67)	24 (13.33)
	Ciprofloxacin (CIP)	82 (45.56)	98 (54.44)
Group O	Colistin (CL)	180 (100.00)	0 (00)
	Polymyxin B	180 (100.00)	0 (00)
	Netilmicin	136 (75.56)	44 (24.44)
Group U	Norfloxacin (NX) (n=42)	14 (33.33)	28 66.67)

Stenotrophomonas maltophilia isolates were sensitive to Trimethoprim-Sulfamethoxazole (83.33%) and 66.67% were sensitive to Levofloxacin whereas isolates of *Burkholderia cepacia complex* (BCC) were100% sensitive to Meropenem and Ceftazidime followed by Trimethoprim-Sulfamethoxazole (75%), (Table 4).

Table 4: Antimicrobial susceptibility pattern of *Stenotrophomonas maltophilia* and *Burkholderia cepacia complex* (BCC) isolates

Antimicrobial susceptibility pattern of *Stenotrophomonas maltophilia*			
Test/ Report Group	**Antibiotic**	**Sensitive (%)**	**Resistant (%)**
Group A	Trimethoprim-Sulfamethoxazole (COT)	05 (83.33)	01 (16.67)
Group B	Minocycline (MIN)	03 (50.00)	03 (50.00)
	Levofloxacin (LEVO)	04 (66.67)	02 (33.33)
Antimicrobial susceptibility pattern of BCCisolates			
Group A	Trimethoprim-Sulfamethoxazole (COT)	03 (75.00)	01 (25.00)
Group B	Ceftazidime (CAZ)	04 (100.00)	00 (00)
	Minocycline (MIN)	02 (50.00)	02(50.00)
	Meropenem (MP)	04 (100.00)	00 00)

4. Discussion

The Non fermentative gram negative bacilli (NFGNB) are widely distributed in nature as saprophytes or as commensals and pathogen to man [1]. NFGNB earlier considered as a contaminant is now gaining importance as a nosocomial pathogen. Also, over the last decade, NFGNBs have emerged as important opportunistic pathogens in the increasing population of patients who are immunocompromised by their disease or medical/surgical treatments. During routine clinical microbiology work in

most laboratories, these are generally not pursued for identification due to the general notion of physicians that NFGNBs other than *Pseudomonas aeruginosa* are generally commensals or non-pathogens and if required, can be treated easily with any of the broad spectrum antibiotics and also due to the tedious and time taking process of their species identification [15].

In the present study, a total number of 440 (5.19%) NFGNB were isolated from 8,468 clinical specimens this is correlated with study done by Karjigi *et al* [16] which reported isolation rate of 5.8%. The maximum numbers of specimens collected were more from IPD than from ICU and OPD but maximum number of NFGNB isolates were from ICU, which was statistically significant and comparable with the study done by Algun *et al* [17]. Most of the cases were found in the age group of 21-50 years followed by > 61 years with male preponderance. Similar observations have been found in the other studies [18, 19].

The majority of NFGNB were isolated from ET aspirates followed by pus and sputum; this result is comparable with study done by Nautiyal *et al* [20]. The most frequently isolated NFGNB were *Acinetobacter spp.* followed by *Pseudomonas spp*. The other organisms isolated were *Stenotrophomonas maltophilia* and *Burkholderia cepacia complex*; our results are in concordance with studies of other researchers [21, 22]. *A. baumannii* infections have become increasingly common among critically-ill patients in intensive care units (ICUs) worldwide [21]. In current study also, we have found *A. baumanii* to be the most common NFGNB isolated from Endotracheal Tube-aspirate from ICU patients.

Acinetobacter baumannii was the most common NFGNB isolated from ET aspirate followed by *Pseudomonas aeruginosa* and *Acinetobacterl woffii* which was correlated with the prior studies [23, 24]. *Pseudomonas aeruginosa* (54.83%) was the most common NFGNB isolated from pus (which also included wound swabs, ear swabs from OM, etc.) followed by *Acinetobacter baumannii* (25.80%). Similar findings were seen in study done by Sarkar *et al* [25]. Also, *Pseudomonas aeruginosa* (44.56%) was the most common NFGNB isolated from urine followed by *Acinetobacter baumannii* (36.96%) and *Acinetobacterl woffii* (15.22%). NFGNB isolated from sputum were *P. aeruginosa* (40.90%), *Acinetobacter baumannii* (36.36%), *Acinetobacterl woffii* (9.09%), P. putida (4.55%), *P. fluorescens* (4.55%) and *Burkholderia cepacia complex* (4.55%). These results are correlated with the other studies [5, 19]. *Acinetobacter species* (45%) were the most common NFGNB isolated in blood, which is comparable with study done by Gautam *et al* [26].

In the present study, it was observed that, *Acinetobacter species* showed the maximum sensitivity to Imipenem followed by Amikacin, Piperacillin-tazobactam and Tobramycin while maximum resistance was observed to Piperacillin, Trimethoprim-sulfamethoxazole, Ciprofloxacin, Ceftazidime, Cefotaxime and Cefepime which is similar to the study done by Rit *et al* [19]. *Pseudomonas* possesses intrinsic resistance to many antibiotics classes and has an ability to develop resistance by mutations in different chromosomal loci or by horizontal acquisition of resistant genes carried on plasmids, transposons or integrons. Polymyxin B and Colistin were the most efficient drugs for *Pseudomonas* isolates with 100% sensitivity followed by Imipenem, Amikacin, Piperacillin-tazobactam and Tobramycin whereas maximum resistance was observed to Ceftazidime, Cefepime and Ciprofloxacin. Similar findings were reported by Malini *et al* [1]. This study shows 66.67% *Pseudomonas* isolates from urine were resistance to Norfloxacin which is comparable with other studies [17, 27]. *Stenotrophomonas maltophilia* isolates were sensitive to Trimethoprim - Sulfamethoxazole, Levofloxacin and Minocycline whereas *Burkholderia cepacia complex* (BCC) isolates showed 100% sensitivity to Meropenem and Ceftazidime followed by Trimethoprim-Sulfamethoxazole (75%) and Minocycline (50%). These results are correlated with the study done by Samanta *et al* [28].

5. Conclusion

Considering the wide spread variability of sensitivity profiles of common NFGNB isolates, it is imperative that every hospital should monitor the antibiogram profile of these isolates from time to time to serve as a basis for empirical therapy in emergency situation. NFGNB need to be taken more seriously and should not be discarded as mere contaminants or non-pathogens. Microbiology report is important for the clinicians in deciding the selection of antibiotics to be used in infections caused by NFGNB. Early detection and Identification of these resistant organisms is crucial which can throw more light on their prevalence and pathogenic role. Judicious use of available antimicrobial agents, regular antimicrobial susceptibility surveillance and strict infection control measures are required to control this emerging antibiotic resistance among non-fermentative gram negative bacilli.

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