A study on non-fermenting Gram-negative bacilli: their isolation and *in vitro* susceptibility in a tertiary care hospital in Central India

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Summary

Background: aerobic, non-spore forming Non-Fermenting Gram-Negative Bacilli (NFGNB) are significant nosocomial agents. They can cause infections such as bacteraemia, meningitis, pneumonia, urinary tract infection and osteomyelitis especially in immunocompromised hosts. Identification and monitoring of susceptibility pattern thus become of utmost importance in the management of these Multidrug Resistant (MDR) pathogens.

The aim of this study was to determine the isolation rate of NFGNB along with its susceptibility pattern in all the clinical samples.

Materials and Methods: the study was conducted in the Microbiology Department of a tertiary care hospital. The NFGNB were identified using a standard protocol that included tests for motility, oxidase production, oxidation-fermentation test, gelatin liquefaction, and utilization of 10% lactose. Antibiotic susceptibility testing was performed with Kirby-Bauer disc diffusion method.

Results: from a total of 15847 samples 935 (5.90%) NFGNB were isolated. *Acinetobacter spp.* (50.37%) and *Pseudomonas spp.* (47.05%) were the most common NFGNB isolated followed by *Burkholderia cepacia complex* (2.4%) and *Stenotrophomonas maltophilia* (0.2%). High resistance was observed for cephalosporins, monobactams and quinolones in *Pseudomonas aeruginosa*. In *Acinetobacter spp.* high resistance was observed for cephalosporins, cotrimoxazole and Beta Lactam and Beta Lactamase Inhibitor (BL-BLI) combination and quinolones.

Conclusions: NFGNB have emerged as an important nosocomial agent, therefore early detection in routine laboratory, monitoring susceptibility pattern, immediate infection control and antimicrobial stewardship programs should be implemented in order to limit the spread of MDR organisms.

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Introduction

Non-Fermenting Gram-Negative Bacilli (NFGNB) are aerobic and non-spore forming. It consists of a taxonomically diverse group of organisms that do not use glucose as an energy source or utilize glucose oxidatively [26].

Previously regarded as commensals and contaminants, these NFGNB now make up 15% of all bacterial isolates from a clinical microbiology laboratory [15]. Saprophytic in nature, they are capable of causing infections especially in hospitalized patients, immunocompromised individuals and patients with haematological malignancies [20]. NFGNB are known to cause infections such as bacteraemia, meningitis, pneumonia, urinary tract infection, surgical site infection, wound infection, osteomyelitis, *etc*. [18].

Their innate resistance to widely used disinfectants and injudicious use of antimicrobials are the reasons behind their rise to prominence as nosocomial agents [4,10,21,22]. Multidrug resistance is due to the mobile genetic elements such as plasmids and transposons that are transferred between, and across different bacterial species [13]. They are also known to produce Extended Spectrum Beta Lactamases (ESBL's) and Metallo Beta-Lactamases (MBL's) giving rise to Multi Drug Resistant (MDR) isolates [6].

This emphasizes the importance of inclusion of tests for the isolation, identification and susceptibility testing of NFGNB routinely, which can show the prevalence and pathogenic role of these organ-

isms [27]. Thus, this study was done to identify non fermenting gram-negative bacilli their speciation, distribution in various clinical samples and antibiotic susceptibility pattern.

Materials and Methods

Study setting

The prospective study was carried out in the department of Microbiology in Government Medical College, Nagpur for a period of 1 year from January 2023 to January 2024. Clinical specimens like blood, pus, sputum, tracheal aspirate, broncho alveolar lavage, pleural fluid, Cerebro-Spinal Fluid (CSF), urine from patients were collected from inpatient departments by standard collection procedures.

Sample processing

All the samples received were inoculated on blood agar and MacConkey agar, incubated at 37°C for 48 h, before being reported as sterile. The isolates which were non-lactose fermenting (Figure 1) and showed alkaline/no change (K/K) reaction on triple sugar iron agar (Figure 2) were provisionally considered as NFGNB and they were further identified using standard protocol for identification [26]. The battery of tests included were Gram stain, hanging drop for motility, catalase test, oxidase test, oxidative-fermentative test (Hugh-Leifson medium) for glucose, citrate utilization, utilization of 10% lactose, gelatin liquefaction, lysine and ornithine decarboxylation, arginine dihydrolase test and growth at 42°C.

The significance of the isolated non-fermenter isolate was assessed by the presence of pus cells along with the gram negative bacilli/coccobacilli in the stained smear from the sample, Monomicrobial infection and isolation of the same organism from repeat sample [8].

Antimicrobial susceptibility testing

Antimicrobial sensitivity was determined by Kirby-Bauer disc diffusion method on Mueller Hinton agar using commercially available antimicrobial discs (Hi-media) following CLSI guidelines (2022) for all the antimicrobials against *Pseudomonas aeruginosa* and *Acinetobacter spp*. Disc diffusion was also performed for testing levofloxacin, minocycline and cotrimoxazole in *Stenotrophomonas maltophilia* isolates. Isolates of *Burkholderia cepacia complex* were tested against ceftazidime, meropenem, minocycline and trimethoprim- sulfamethoxazole via disc diffusion [12]. Controls used were *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853.

The minimum inhibitory concentration was determined by VITEK 2 Compact Instrument for testing resistance for all the drugs of *Pseudomonas putida*, *Pseudomonas fluorescens* and *Pseudomonas stutzeri* isolates. MIC testing was also done for testing resistance of Chloramphenicol and ceftazidime for *S. maltophilia*. In *B. cepacia* MIC testing was done for ticarcillin clavulanate, chloramphenicol and levofloxacin as per CLSI 2022 guidelines [12]. Controls used were *S. maltophilia* ATCC 17666 and *P. aeruginosa* ATCC 27853.

Results

A total of 15847 samples were received, out of which 935 non-fermenting Gram-negative bacilli were isolated accounting an isolation rate of 5.90%. Among these 517 isolates were from male patients and 418 isolates were from female patients. Male to female ratio was 1.23:1. Age of our cases ranged from day 10 to 71 years. The maximum 210 isolates were observed in age group 41-50 years followed by 165 isolates in age group 51-60 years. The age and gender distribution are shown in Figure 3.

From various clinical specimens, maximum number of non-fermenter organisms were isolated from pus and wound swab specimens (42.9%). It was followed by blood (19.1%), urine (11.2%), ET secretions (9.8%), Bronchoalveolar Lavage (BAL) (7.3%), sputum (2.6%), ascitic fluid (2.5%), tissue (1.6%), catheter tips (1.5%), pleural fluid (1.3%) and CSF (0.2%). Among the NFGNB isolated, the most common isolate being *P. aeruginosa* (46.3%) followed by *Acinetobacter baumannii* (32%) and *Acinetobacter lwoffii* (18.4%). Other species isolated includes *B. cepacia* (2.4%),

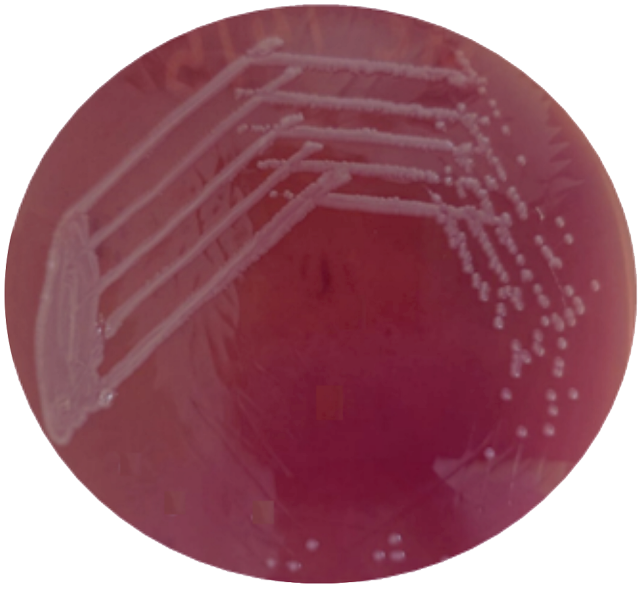

Figure 1. Non-lactose fermenting colony on MacConkey agar.

Figure 2. Triple sugar iron agar showing K/K reaction.

P. stutzeri (0.4%), *P. putida* (0.2%), *S. maltophilia* (0.2%) and *P. fluorescens* (0.1%). NFGNB from various clinical samples is depicted in Table 1.

Antibiotic susceptibility pattern is shown in Table 2, Table 3 and Table 4.

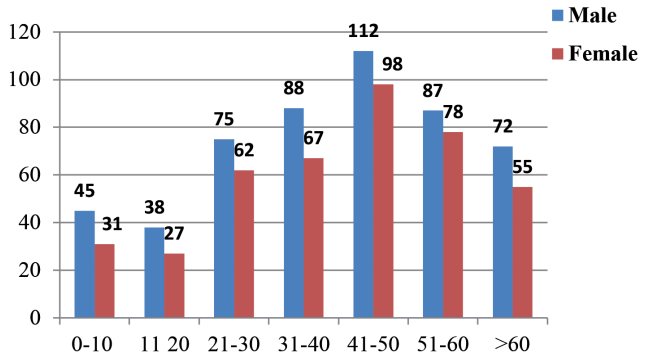

Figure 3. Distribution of non-fermenter isolates according to the age and gender.

Discussion

In the present study, a total of 15847 clinical specimens were processed, from which 935 (5.90%) non-fermenter strains were isolated. In studies of Benachinmardi *et al*., Chawla *et al*., Malini *et al*. similar isolation rate of non-fermenters was found which was 3.58%, 4%, and 4.5% respectively [2,5,14]. Isolation rates of 16.18% and 12.18% were seen in studies by Nautiyal *et al*. and Rit *et al.* which is in contrast with the present study [16,23].

These variations in the prevalence of NFGNB in different healthcare settings might be due to the different infection control practices and circulation of these bacterial pathogens in respective hospitals.

In the current study males were most commonly affected. In studies by Meherwal *et al*., Benachinmardi K *et al.* and Rit K *et al*., similar male preponderance was found [15,2,23]. Where as in a study conducted by Prasanna S *et al.* female preponderance was found [19].

Maximum (22.5%) isolates belonged to the 41-50 years age group followed by 51-60 years. The findings are in concordance with Rit K *et al*. [23]. However in a study conducted by Benachinmardi K *et al*. maximum non fermenter isolate belonged to 21-50 years [2].

Table 1. Non-fermenter isolated from various clinical specimens.

Clinical sample	*P. aeruginosa* (%)	*A. baumannii* (%)	*A. lwoffii* (%)	*B. cepacian* (%)	*P. stutzeri* (%)	*P. putida* (%)	*S. maltophilia* (%)	*P. fluorescens* (%)
Pus/WS	191 (44.1)	127 (42.5)	75 (43.6)	-	4(100)	2(100)	2 (100)	-
Blood	82 (18.9)	42 (14.2)	34 (19.8)	20(90.1)	-	-	-	1 (100)
Urine	42 (9.7)	34 (11.4)	29 (16.9)	-	-	-	-	-
ETT	42 (9.7)	41 (13.4)	9 (5.2)	-	-	-	-	-
BAL	29 (6.7)	30 (10.1)	9 (5.2)	-	-	-	-	-
Sputum	13 (3.1)	8 (2.7)	3 (1.7)	-	-	-	-	-
Ascitic fluid	9 (2.1)	8 (2.7)	6 (3.5)	-	-	-	-	-
Tissue	8 (1.8)	4 (1.3)	3 (1.7)	-	-	-	-	-
CVC tips	8 (1.8)	2 (0.6)	2 (1.2)	2 (9.1)	-	-	-	-
Pl. fluid	7 (1.6)	3 (1.1)	2 (1.2)	-	-	-	-	-
CSF	2 (0.5)	-	-	-	-	-	-	-
Total	433	299	172	22	4	2	2	1

ETT, Endotracheal Tube; BAL, Bronchoalveolar Lavage; CVC Tips, Central Venous Catheter Tips; Pl. fluid, Pleural fluid; CSF, Cerebrospinal Fluid

Table 2. Antibiotic sensitivity pattern of Pseudomonas spp.

Antibiotic drug	Resistant isolates %			
	P. aeruginosa (n=433)	*P. stutzeri* (n=4)	*P. putida* (n=2)	*P. fluorescens* (n=1)
Ceftazidime	310 (71.5)	2 (50)	1 (50)	1 (100)
Gentamicin	234 (54.1)	1 (25)	1 (50)	0
Tobramycin	238 (54.9)	1 (25)	1 (50)	0
Piperacillin tazobactam	252 (58.1)	2 (50)	1 (50)	0
Cefepime	281 (64.8)	2 (50)	1 (50)	1 (100)
Aztreonam	304 (70.2)	2 (50)	1 (50)	0
Meropenem	210 (48.4)	2 (50)	1 (50)	0
Amikacin	188 (43.5)	1 (25)	1 (50)	0
Levofloxacin	350 (80.8)	2 (50)	1 (50)	0
Trimethoprim sulfamethoxazole	-	2 (50)	1 (50)	0
Netilmicin	291 (67.1)	1 (25)	0)	0
Norfloxacin(n=42)	22 (52.4)	-	-	-

Table 3. Antibiotic resistance pattern of *Acinetobacter* spp.

Antibiotic drug	Resistant isolates (%)			
	A. baumannii (n=299)		*A. lwoffii* (n=172)	
Ampicillin sulbactam	181 (60.6)		99 (57.6)	
Ceftazidime	238 (79.4)		135 (78.4)	
Meropenem	153 (51.2)		83 (48.1)	
Gentamicin	172 (57.6)		95 (55.3)	
Tobramycin	177 (59.1)		101 (58.7)	
Levofloxacin	247 (82.6)		142 (82.4)	
Piperacillin tazobactam	187 (62.6)		106 (61.3)	
Cefepime	225 (75.2)		123 (71.5)	
Amikacin	159 (53.3)		85 (49.4)	
Minocycline	172 (57.6)		91 (52.7)	
Co trimoxazole	186 (62.3)		99 (57.6)	
Tetracycline	n=34	16 (47.1)	n=29	12 (41.2)

Table 4. Antibiotic resistance pattern of *Burkholderia cepacia* (n=22) and *Stenotrophomonas maltophilia* (n=2).

Antibiotic drug	Resistant isolates %	
	Burkholderia cepacia (n=22)	*Stenotrophomonas maltophilia* (n=2)
Meropenem	10 (45.4)	-
Trimethoprim-sulfamethoxazole	8 (36.3)	0
Levofloxacin	16 (72.7)	0
Ceftazidime	10 (45.4)	2 (100)
Minocycline	9 (40.9)	0
Ticarcillin-clavulanate	18 (81.8)	0
Chloramphenicol	-	2 (100)

The detailed categorization helps in identifying the gender and age groups that are at higher risk. In our study, the higher age groups were more affected as the infections caused by NFGNB have a predilection for severely ill and immune-compromised people [11].

The current study reports *P. aeruginosa* as the most common non-fermenter isolated accounting 46.3% isolation rate. Study conducted by Chawla K *et al.* reported similar observations where *P. aeruginosa* was the most common non-fermenter isolated with an isolation rate of 56.7% followed by *Acinetobacter spp*. (39.3%). But isolation rate of *S maltophilia* was 45.5% which is in contrast with the present study [5].

The resistance patterns observed in hospital-acquired bacterial pathogens can differ significantly; thus, it is essential to monitor these pathogens for their antibiotic susceptibility profiles in specific settings to guide empirical treatment choices. Each hospital should develop its own antibiogram, as the typical antibiotic sensitivity patterns may not be applicable in every location [25]. In the present study resistance pattern of *P. aeruginosa* against cephalosporins like ceftazidime and cefepime was 71.5 % and 64.8% respectively. Rit K *et al.* and Biswal I *et al*. also obtained similar results (71.29% and 70.66% respectively) [23,3]. For meropenem, the resistance was 48.4% in the present study. Baruah P *et al.* reported 33.33% resistance against carbapenem [1]. It is known that *P. aeruginosa* carries plasmids which codes for multidrug resistance and this has led to the organism being resistant to commonly used antibiotics [26].

The resistance of *A. baumannii* to carbapenems was 51.2% in the current study. Benachinmardi K *et al*. reports 59.1% resistance of *A baumannii* to Carbapenems which is in concordance with the current study [2]. In *A. lwoffii* resistance to meropenem was 48.1%. *A. baumannii* is receiving greater attention because of its possible biofilm-forming capacity, which may also account for its exceptional antibiotic resistance, enhanced virulence, and survival traits [9].

In the present study *B. cepacia* were isolated from blood culture and catheter tips. The resistance to ticarcillin clavulanate and levofloxacin was 81.8% and 72.7% respectively. Resistance to meropenem was 45.4%. Sidhu *et al.* and Rit K *et al.* reported susceptibility of *B. cepacia* to imipenem to be 100% and 92.85% respectively [23,24]. Thus it can be concluded that carbapenems offer an excellent therapeutic effect in infections due to *B. cepacia*, which has shown resistance to many first line antibiotics like beta lactams, polymyxin B and aminoglycosides [7].

In the current study, the isolates of *S. maltophilia* were susceptible to minocycline, cotrimoxazole, levofloxacin and ticarcillin-clavulanate and resistant to ceftazidime and chloramphenicol. In a study conducted by Patel P *et al. S. maltophilia* was susceptible to cotrimoxazole but resistant to ceftazidime and chloramphenicol [17].

Conclusions

Non Fermenting Gram-Negative Bacilli (NFGNB) are an important nosocomial pathogen, primarily causing infection in immunocompromised and hospitalized patients. Accurate identification of non-fermenters to species level is vital for appropriate patient management and care.

P. aeruginosa and *A. baumannii* were amongst the most commonly isolated non-fermenter spp. Resistance for imipenem and piperacillin-tazobactam was less in *P aeruginosa*. So, for *P aeruginosa* infections they should be the reserve drugs. *A baumannii*

showed higher degree of resistance to various classes of antibiotics suggesting multidrug resistance.

Judicious and cautious use of older and newer antimicrobial agents is essential to prevent the emergence of multi drug resistant bacteria.

Multi drug resistant isolates in a hospital environment poses therapeutic problem and is also a serious concern for infection control management. Therefore, early detection in routine laboratory, immediate infection control and antimicrobial stewardship programs should be implemented in order to limit the spread of multi drug resistant organisms.

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