

Characterization and Antimicrobial Susceptibility Profile of Non-Fermenting Gram Negative Bacilli with Special Reference to *Pseudomonas spp.* And *Acinetobacter spp.*: A Tertiary Care Center's Perspective

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ABSTRACT

Background: The non-fermenting gram-negative bacilli (NFGNB) are widely distributed in the environment and have become increasingly common isolates in the clinical microbiology laboratory. Although, they are often disregarded as contaminants, but their pathogenic potential has been proved beyond doubt and over the period of time NFGNBs have emerged as opportunistic pathogens, particularly in immunocompromised hosts, and are difficult to treat because of widespread antibiotic resistance. **Objective:** The objective of this study was to determine the prevalence of NFGNB isolated from various clinical specimens with special reference to *Pseudomonas spp.* and *Acinetobacter spp.*, and to evaluate their antimicrobial susceptibility profiles. **Methods:** Study was conducted in the Department of Microbiology at a tertiary care teaching hospital of Uttarakhand State over a period of one year (January 2014 to December 2014). A total of 4483 clinical specimens were received in laboratory and were processed. NFGNB were isolated and identified using a standard protocol. Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method. **Results:** NFGNB isolation rate in the present study was 11.4%. Pus/wound swab was the most common specimen (21%) followed by sputum/endo-tracheal aspirate (17%) and ear swab. *Pseudomonas aeruginosa* was the most common isolate (34.5%) followed by *Acinetobacter baumannii* (25.5%) and *Acinetobacter lwoffii* (16.7%). A high level of antibiotic resistance was recorded for most of the first and second line drugs. Imipenem and amikacin were the drugs with maximum activity. **Conclusion:** Identification of NFGNB and monitoring their susceptibility patterns will not only help in effective and efficient management of infections caused by them but also in better care and management of patients. Improved antibiotic stewardship and infection-control measures will be needed to prevent or slow the emergence and spread of multidrug-resistant NFGNB in the healthcare setting.

Keywords: Amikacin, Carbapenemase, Imipenem, Multi drug resistant, Nosocomial infections *Pseudomonas aeruginosa*.

INTRODUCTION

Aerobic non-fermenting gram negative bacilli (NFGNB) are a taxonomically diverse group of organisms that either do not utilize glucose as a source of energy or utilize it oxidatively.^[1] They do not require many nutrients for their development, can tolerate harsh environmental conditions, show remarkable resistance to antimicrobials and are frequently described as hospital acquired opportunistic pathogens.^[2-4] All these organisms have the potential to spread horizontally on fomites or the hands of healthcare workers.^[5] Moreover, inherent resistance of these bacterial agents to

commonly used disinfectants and their tendency to colonize various surfaces has been pivotal in their emergence as important nosocomial pathogens.^[6] NFGNB is a heterogeneous group and includes organisms like *Pseudomonas spp.*, *Acinetobacter spp.*, *Alkaligenes spp.*, *Stenotrophomonas maltophilia*, and *Burkholderia cepacia* complex (BCC). Due to the rampant use of antimicrobials in recent years, most of these organisms are now resistant to majority of the routinely used antimicrobials and have emerged as important health care associated pathogens. They have been incriminated in variety of infections such as bacteremia, meningitis, pneumonia, urinary tract infections, surgical site infections, wound infections, osteomyelitis etc.^[7] Major risk factors include prolonged hospitalization, broad-spectrum antibiotic use, mechanical ventilation, cystic fibrosis, indwelling catheters, neutropenia, invasive diagnostic/ therapeutic procedures and immunocompromised state.^[8,9]

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Currently *Pseudomonas aeruginosa* and *Acinetobacter spp.* are the most commonly isolated NFGNB pathogenic to humans and infections caused by other species are relatively infrequent.^[10,11] Organisms belonging to both these genus are known for their multidrug resistant (MDR) or pan drug resistant (PDR) strains. The MDR potential of these organisms stems from different factors, such as up-regulated production of enzymes metabolizing the drugs, target site modifications, overexpression of efflux pumps, and porin deficiency.^[12,13] Carbapenems are considered as potent antimicrobial weapon against such MDR strains, particularly against *Pseudomonas*, however, both *Pseudomonas* and *Acinetobacter* have developed resistance even against this group of drug.^[9,14] As organisms belonging to both these genus are among the most predominantly isolated NFGNB so carbapenem resistance among them is of major concern. The prevalence, phenotypic characteristics, and antimicrobial susceptibility profile of NFGNB strains may show regional variation. Therefore, periodic epidemiological studies are needed to establish appropriate therapeutic management strategies to prevent the infections caused by NFGNB. With this background, the current study was undertaken to isolate, identify and characterize NFGNB along with their antimicrobial susceptibility profile from various clinical samples of the patients attending our hospital a tertiary care center.

MATERIALS AND METHODS

This study was conducted for a period of one year (January 2014 to December 2014) in a tertiary care hospital of Uttarakhand state, India. A total of 4483 clinical specimens were received in laboratory, which included urine (1277), blood (1186), pus (692), sputum and other respiratory samples (634), ear swab (431) and other body fluids (263). Samples were plated on blood agar (BA) and MacConkey's agar (MA) and incubated at 37°C for 48 hours before being reported as sterile. The isolates that showed non lactose fermenting (NLF) colonies

on MA and failed to acidify the butts of triple sugar iron (TSI) agar were provisionally considered as NFGNB and they were further identified by using a standard protocol for identification.^[1] The characters assessed were gram staining morphology, motility (by hanging drop), catalase test, oxidase test, citrate utilization, urea hydrolysis, hemolysis on 5% sheep blood agar, growth on 6.5% NaCl, nitrate reduction, pigment production, indole production, lysine and ornithine decarboxylation, arginine dihydrolase test, growth at 40°C and 42°C, oxidation of 1% glucose, lactose, sucrose, maltose, mannitol, xylose (Hugh and Leifson's medium), growth on 10% lactose agar and gelatin liquefaction test.

Antimicrobial sensitivity testing was done by Kirby-Bauer disc diffusion method on Muller Hinton agar (MHA). Briefly a suspension of each isolate was made so that the turbidity was equal to 0.5 McFarland standards and then plated as a lawn culture onto MHA. Antibiotic discs were placed and plates were incubated at 37°C for 18- 24 hrs. Results were interpreted in accordance with Central Laboratory Standards Institute (CLSI) guidelines.^[15] *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains. All dehydrated media, reagents and antibiotic discs were procured from Hi-Media Laboratories Pvt. Ltd, Mumbai, India.

RESULTS

Out of the total 4483 clinical samples, NFGNB were isolated from 513 (11.4%) samples. A total of 522 NFGNB were isolated from 513 clinical samples as nine samples yielded two types on NFGNB. Monomicrobial growth was seen in 319 (62.2%) samples whereas 194 (37.8%) samples showed polymicrobial growth where along with non-fermenters some other organisms were also isolated, among which the most predominant were *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter spp.*, *Klebsiella pneumonia* and *Proteus spp.*

Table 1: Non fermenting gram negative bacilli isolated from various clinical samples

Organism	Pus/ Wound swab	Sputum/ Endo-tracheal aspirate	Ear Swab	Urine	Blood	Other Body fluids	Total no. (%)
<i>Pseudomonas aeruginosa</i>	67	29	31	27	19	07	180 (34.5)
<i>Acinetobacter baumannii</i>	31	46	27	17	08	04	133 (25.5)
<i>Acinetobacter lwoffii</i>	19	33	15	13	05	02	87 (16.7)
<i>Pseudomonas fluorescens</i>	15	12	17	09	07	00	60 (11.5)
<i>Alcaligenes fecalis</i>	07	03	01	05	01	02	19 (3.6)
<i>Stenotrophomonas maltophilia</i>	08	05	02	02	00	00	17 (3.3)
<i>Achromobacter xylosoxidans</i>	04	01	02	00	00	00	07 (1.3)
<i>Chryseobacterium indologens</i>	04	01	00	02	00	00	07 (1.3)
Others	02	01	03	01	03	02	12 (2.3)
Total no. of isolates (%)	157 (30.1)	131 (25.1)	98 (18.8)	76 (14.6)	43 (8.2)	17 (3.2)	522

Table 2: Antimicrobial susceptibility profile of the isolated non-fermenting gram negative bacilli

Antimicrobials	<i>Pseudomonas aeruginosa</i> (n=180)	<i>Acinetobacter baumannii</i> (n=133)	<i>Acinetobacter lwoffii</i> (n=87)	<i>Pseudomonas fluorescens</i> (n=60)	<i>Alcaligenes fecalis</i> (n=19)	<i>Stenotrophomonas maltophilia</i> (n=17)	<i>Achromobacter xylosoxidans</i> (n=07)	<i>Chryseobacterium indologens</i> (n=07)
AMK	76.11	76.69	81.69	73.33	89.47	23.52	0	85.70
IMI	74.44	68.42	74.71	78.33	89.47	0	100	NT
TCV	72.22	40.60	48.27	70.00	100	23.52	100	NT
PTZ	70.56	38.34	44.82	71.66	100	23.52	100	85.70
CFS	52.22	36.09	39.08	58.33	89.47	17.64	100	100
GEN	51.67	50.37	52.87	48.33	78.94	17.64	28.57	NT
CEF	46.11	33.08	39.08	53.33	89.47	0	71.42	NT
PIP	59.44	19.54	24.13	53.33	78.94	0	71.42	NT
CFT	36.11	17.29	21.83	48.33	63.15	0	71.42	NT
CPZ	33.89	17.29	21.83	35.00	63.15	0	71.42	85.70
CIP	28.89	14.28	19.54	31.66	52.63	94.11	28.57	100
CTX	11.67	23.30	29.88	20.00	63.15	94.11	0	0
AZT	21.67	14.28	19.54	23.33	63.15	0	57.14	85.7

AMK: amikacin; AZT: aztreonam; CEF: cefepime; CFS: cefoperazone-sulbactam; CFT: ceftazidime; CIP: ciprofloxacin; CPZ: cefoperazone; CTX: cotrimoxazole; GEN: gentamicin; IMI: imipenem; PTZ: piperacillin-tazobactam; PIP: piperacillin; TCV: ticarcillin-clavulanic acid

Susceptibility pattern shown in the table is the percentage of isolates found sensitive to the particular antimicrobial agent. Intermediately sensitive isolates were considered as resistant.

Table 3: Isolation rate of non-fermenting gram negative bacilli, *Pseudomonas spp.* and *Acinetobacter spp.* among the various studies conducted in the past two decades.

Authors	Year & Place	Isolation Rate		
		NFGNB	<i>Pseudomonas spp.</i> %	<i>Acinetobacter spp.</i> %
Vijya et al. ^[28]	2000, Bengaluru, Karnataka (India)	21.8	78.9	6.1
Eltahawy et al. ^[23]	2001, Jeddah, Saudi Arabia	16.0	56.0	34.0
Malini et al. ^[26]	2009, Kolar, Karnataka (India)	4.5	64.6	25.3
Sidhu et al. ^[27]	2010, Amritsar, Punjab, (India)	36.5	42.5	23.3
Samanta et al. ^[22]	2011, Chandigarh (India)	10.0	26.0	66.0
Bruno et al. ^[24]	2011, Brazil	2.2	65.0	16.6
Juyal et al. ^[10]	2013, Pauri Garhwal, Uttarakhand (India)	9.3	49.6	43.1
Benachinmardi et al. ^[25]	2014, Bengaluru, Karnataka (India)	3.6	61.0	34.0
Baruah et al. ^[21]	2015, New Delhi (India)	4.9	15.0	20.0
Argenta et al. ^[29]	2017, Brazil	4.8	69.3	35.6
Grewal et al. ^[11]	2017, Patiala, Punjab (India)	11.6	87.9	7.9
Kaur et al. ^[30]	2018, Bhatinda, Punjab (India)	16.1	55.1	35.9
Sarkar et al. ^[31]	2018, Bhubaneswar, Odisha (India)	13.9	42.1	53.5
Deb et al. ^[32]	2019, Kolkata, (India)	29.6	53.9	41.5

NFGNB: non-fermenting gram negative bacilli

NFGNB were isolated from variety of clinical specimens. Majorities were isolated from pus/wound swab, sputum/endo-tracheal aspirate and ear swab. Table 1 depicts the distribution and percentage of NFGNB isolated from various clinical samples. *P.aeruginosa* was the most common isolate, accounting for 180 (34.5%) followed by *A.baumannii*; 133 (25.5%), *A.lwoffii*; 87 (16.7%), *P.fluorescens*; 60 (11.5%) *Al.fecalis*; 19 (3.6%) and *S.maltophilia*; 17 (3.3%). *Achromobacter xylosoxidans* and *Cryseobacterium indologens* were rarely isolated together accounting for 14 (2.6%) of the isolates. Six isolates of *Shewanella putrefaciens* and three isolates each of *Sphingomonas paucimobilis* and *Pseudomonas stutzeri* were found to be contaminants. *Pseudomonas spp.* and *Acinetobacter spp.* were the most commonly isolated NFGNB and together both of them accounted for 87.2% of the isolates.

The antibiotic susceptibility test results of the isolated NFGNB are depicted in Table 2 and shows the percentage of susceptible isolates. High level of resistance was recorded among most of the isolates. Except for *S.maltophilia*, amikacin and imipenem

were found to be the most effective antibiotics. High level of resistance for most of the first line drugs was noted among majority of the organisms.

DISCUSSION

NFGNB are widely distributed in the environment and have become increasingly common isolates in the clinical laboratory. They account for 15% of all bacterial isolates from clinical microbiological laboratory.^[16] Being ubiquitous in nature NFGNB are often disregarded as contaminants, however their pathogenic potential has been undoubtedly proved by their frequent isolation from various clinical specimens and their association with disease.^[17] NFGNB have emerged as opportunistic pathogens particularly in immunocompromised hosts more so in hospital settings. The available data suggests that NFGNB are remarkable microorganisms because of their epidemiological complexity, propensity to cause outbreaks of infection and antimicrobial resistance.^[18-20] Resistance to antimicrobials is common and has increased over the years among

NFGNB and number of strains are now resistant nearly to all commonly used antibiotics. Multi drug resistance among these organisms makes the treatment of infections caused by them difficult and expensive.

Studies carried out by various researchers have reported varied isolation rates, ranging from 2.2% to 45.9%.^[21] In the present study NFGNB were isolated in 11.4% of clinical samples, the finding which was in parallel to the studies from Uttarakhand, Chandigarh and Saudi Arabia, where NFGNB were isolated in 9.3%, 10% and 16% of the clinical samples, respectively.^[10,22,23] In contrast to our results, studies from Brazil, Bengaluru-Karnataka, Kolar-Karnataka and New Delhi reported a very low isolation rate of 2.2%, 3.6%, 4.5% and 4.9% respectively, whereas a study from Amritsar reported very high isolation rate of 45.9% while another study from Bengaluru-Karnataka reported it to be 21.80%.^[21,24-28] Among most of the aforesaid studies, *Pseudomonas spp.* and *Acinetobacter spp.* were found to be commonest NFGNB and this was in concordance to our findings. Table 3 depicts comparative analysis of the isolation rate of NFGNB, *Pseudomonas spp.* and *Acinetobacter spp.* among the various studies conducted in the past two decades.

NFGNB are known for causing a wide range of nosocomial infections and drug resistance pattern among such nosocomial bacterial pathogens may vary widely from country to country at any given time and within the same country over a period of time.^[33,34] Considering these variations and in order to guide appropriate selection of empiric therapy, periodic surveillance of the nosocomial pathogens and their antibiogram in a given set up is imperative. Since the standard antibiotic sensitivity pattern may not hold true for every area, various international authorities also emphasize that every hospital should have its individual antibiotic sensitivity pattern, based on which not only the local antibiotic policy should be formulated but should also be updated time to time.^[28]

Both *Pseudomonas* and *Acinetobacter* isolates showed high degree of resistance against the commonly administered antibiotics, however amikacin, imipenem and ticarcillin-clavulanic acid were found to be the most effective. *Pseudomonas aeruginosa* was found to be more sensitive to most of the antibiotics tested than *P.fluorescens* whereas *A.baumannii* showed a higher rate of resistance than *A.lwoffii*. Though imipenem showed good activity to all the NFGNB except for *S.maltophilia*, but emerging resistance to this group of drug is of major concern. Previous studies by other authors also have reported carbapenem resistance among NFGNB.^[35-37] In the present study 29.09% of *Acinetobacter spp.* and 24.58% of *Pseudomonas spp.* were imipenem resistant. Carbapenemase activity in *Acinetobacter spp.* is mainly due to

carbapenem hydrolyzing class D β -lactamases (CHDLs) that is mostly specific for this genus. These enzymes belong to 3 unrelated groups of clavulanic acid resistant β -lactamases represented by OXA-23, OXA-24, and OXA-58 that can be either plasmid or chromosomally encoded.^[38] In case of *P.aeruginosa* the dominant mechanism of carbapenem resistance is loss of carbapenem specific porin OprD2.^[10,39] Number of strains have now been identified that exhibit resistance to essentially all commonly used antibiotics. We believe that documenting resistance among NFGNB is very important especially the carbapenem resistance, as these strains may often cause outbreaks in the ICU setting and can limit therapeutic option due to the high degree of multi drug resistance. These organisms can also spread resistance to other susceptible bacteria by horizontal gene transfer.

CONCLUSION

NFGNB though regarded as contaminants are important bacteria causing wide range of nosocomial infections. Regional and chronological changes in the antimicrobial susceptibility pattern of the microorganisms highlight the importance of periodic epidemiological surveys in the hospitals. The data extrapolated from such surveys will be helpful for development of therapeutic protocols as well as for tuning existing antibiotic policies in a given healthcare set up. Effective and efficient implementation of such policies will restrict/control haphazard use of antimicrobials and will also be helpful in limiting the spread of resistant nosocomial pathogens. Prevalence of pathogens often varies dramatically between communities, hospitals in the same community and among different patient populations in the same hospital. Thus it is important for clinicians to remain updated with prevalence and antimicrobial susceptibility pattern of the circulating pathogens in their practice setting and the antimicrobials to be used for empiric therapy should be selected accordingly. More importantly these organisms have great potential to survive in hospital environment so improved antibiotic stewardship and infection control measures will be needed to prevent or slow the emergence and spread of multidrug resistant NFGNB in the healthcare setting.

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