

Original Research Article

Isolation and Identification of Non Fermenting Gram Negative Bacilli in A Tertiary Care Hospital

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Abstract: Non-Fermenting Gram-Negative Bacilli (NFGNB) group includes numerous organisms but the ones which are known to cause nosocomial infections are *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Burkholderia cepacia* complex (BCC) and *Stenotrophomonas maltophilia*. This study was undertaken to identify the various nonfermenters isolated from patients admitted to our hospital, a tertiary care center, at Jammu. A total of 4585 clinical specimens were received in the laboratory, which included 1234 urine, 742 pus, and 1864 blood cultures, collected in a brain-heart infusion broth, 110 endotracheal catheter tips, 529 CSF samples and 115 body fluids. These samples were placed on blood agar, chocolate agar, and MacConkey's agar, and incubated at 37°C for 18-24 hours. The clinical isolates were identified using the conventional biochemical tests as per the standardized protocols. Non-fermenting Gram-negative bacilli were isolated from 572 out of 4585 clinical specimens accounting for an isolation rate of 12.40%. *Pseudomonas aeruginosa* was the most common isolate, accounting for 312 (54.54%), followed by *Acinetobacter baumannii* 235 (41.08%), *Stenotrophomonas maltophilia* 15 (2.62%), and *Burkholderia cepacia* complex 10 (1.70%). In our study, a total of 13 (86.66%) of the isolates were sensitive to Colistin and a total of 4 (26.60%) were sensitive to Imipenem. Thus NFGNB are emerging as important opportunistic pathogens and are resistant to commonly used antimicrobials. Therefore early diagnosis and institution of empirical therapy based on local antibiogram data of the institute would reduce mortality and improve patient management.

Keywords: Non-Fermenting Gram-Negative Bacilli (NFGNB), *Pseudomonas*.

INTRODUCTION:

Non-Fermenting Gram-Negative Bacilli (NFGNB) are a group of aerobic, non-spore-forming, bacilli/coccobacilli that are either incapable of utilizing carbohydrates as a source of energy or degrade them via oxidative, rather than fermentative pathway. This group includes numerous organisms but the ones which are known to cause nosocomial infections are *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Burkholderia cepacia* complex (BCC) and *Stenotrophomonas maltophilia*.

NFGNB were earlier considered to be commensals or contaminants. But, the pathogenic potential of these organisms has been established beyond doubt because of their frequent isolation from clinical specimens and their association with the disease. These apparently heterogeneous microorganisms have common traits of clinical importance that justify their inclusion and study in a

single group. They can be recovered from hospital environment, commonly cause device related infections, are often resistant to disinfectants and have the potential to spread from patient to patient via fomites or the hands of medical personnel.

Also the antimicrobial resistance exhibited by the NFGNB creates an epidemiologic niche for these pathogens that facilitates colonization and superinfection in antibiotic-treated patients [1-4].

NFGNB are known to account for about 15% of all bacterial isolates from a clinical microbiology laboratory. In recent years, due to the liberal and empirical use of antibiotics, NFGNB have emerged as important healthcare-associated pathogens [5].

It has always been a tedious task for a routine microbiological laboratory to identify the NFGNBs and poor laboratory proficiency in identification of these

NFGNBs prevails worldwide, including in our own country. For this reason, reports of disease due to these organisms are rare from India. Identification through commercial kits and automated systems is not fool-proof as many non-Burkholderia betaproteobacteria (*Ralstonia picketti* and *Pandoraea* species) are labeled as BCC and some BCC strains as *Pseudomonas aeruginosa* [6].

Hence, this study was undertaken to identify the various nonfermenters isolated from patients admitted to our hospital, a tertiary care center, at Jammu. The study was also done to assess their clinical significance and antimicrobial susceptibility pattern, and to identify the various healthcare-related infection they cause.

AIMS AND OBJECTIVES

- To isolate, identify and characterize non-fermenting Gram-negative bacilli from clinical isolates received from indoor hospital patients for a period of one year.
- To analyze the antibiotic sensitivity patterns of non-fermenting Gram-negative bacilli.

MATERIAL & METHODS

This prospective study was conducted in the Department of Microbiology, Government Medical College and Hospital, Jammu which is an 850 bedded tertiary care hospital, for a period of one year.

A total of 4585 clinical specimens were received in the laboratory, which included 1234 urine, 742 pus, and 1864 blood cultures, collected in a brain-heart infusion broth, 110 endotracheal catheter tips, 529 CSF samples and 115 body fluids. These samples were placed on blood agar, chocolate agar, and MacConkey's agar, and incubated at 37°C for 18-24 hours. The organisms isolated were identified using the appropriate biochemical tests [1]. All the organisms giving non-lactose fermenting (NLF) colonies on MacConkey agar medium. And producing an alkaline reaction (K/K) on triple sugar iron agar grew on Triple Sugar Iron agar were provisionally considered to be NFGNB.

ISOLATE SPECIATION / IDENTIFICATION:

The clinical isolates were identified using the conventional biochemical tests as per the standardized protocols [19]. The characters assessed included morphology on Gram-stain, motility, cytochrome oxidase activity, OF test (Hugh-Leifson medium) for glucose and mannitol, growth on 10% lactose agar, decarboxylase test, growth on aerobic low peptone medium, Triple sugar iron fermentation with hydrogen

sulphide production on lead acetate paper and gelatin liquefaction.

ANTIMICROBIAL SUSCEPTIBILITY TESTING:

The sensitivity test was performed with the help of the Kirby-Bauer disc diffusion method using commercially available discs (Hi-media). The different antibiotics tested were Ceftazidime (30mcg), Ceftriaxone (30mcg), Cefepime (30mcg), Imepenem (10µg), Piperacillin-Tazobactam (100/10mcg), Ticarcillin-Clavulanic acid (75/10mcg), Ciprofloxacin (5mcg), Ofloxacin (5mcg), Amikacin (30mcg), Gentamycin (10mcg), Tobramycin (10mcg), Trimethoprim-Sulfamethoxazole (1.25/23.75mcg), and Colistin (10mcg). The results were interpreted as per the Clinical and Laboratory Standards Institute (CLSI) guidelines⁷

Following ATCC strains were used as Quality control strains:

ATCC-25922 *E. coli* – Gram-negative bacilli
ATCC-25923 *S. aureus* – Gram-positive cocci
ATCC 27853 *P. aeruginosa*- Non-Lactose Fermenters

RESULTS

A total of 4585 clinical samples were received from the indoor patients in the microbiology laboratory. Non-fermenting Gram-negative bacilli were isolated from 572 out of 4585 clinical specimens accounting for an isolation rate of 12.40%. Two ninety seven specimen (50.16%) showed polymicrobial infection where non-fermenters were isolated along with other organisms, of which *E. coli* and *S. aureus* were commonly associated. The remaining two ninety five specimens (49.83%) showed monomicrobial infection.

The majority of patients (35.13%) belonged to the age group 40-60 years. 342 NFGNB(59.79%) were isolated from males and 230 (40.20%) were from females. Regarding invasive devices, 292 (51.04%) patients had peripheral venous and 229 (40.03%) had urinary catheter placed. Central venous line and endotracheal tube were found in 119 (19.93%) and 82 (14.33%) cases respectively. This association was highly statistically significant. (P value: 0.000001)

History of previous antibiotic therapy was received from 188 (32.86%) patients, recent hospitalisation from 275 (48.07%) and recent surgery in the preceding year from 182 (31.81%). Out of the 572 NFGNB 343 (59.96%) were isolated from pus, 36 (6.29%) from urine, 33 (5.76%) from body fluids, 70 (12.23%) from blood, 48 (8.39%) from CSF, 42 (7.34%) from endotracheal secretions

Table-1: Microorganisms obtained from different specimens

Organisms	Pus	Urine	Blood	Endotracheal aspirates	CSF	Fluid Aspirates
Pseudomonas aeruginosa	232	25	16	21	3	15
	74.35%	8.01%	5.12%	6.73%	0.96%	4.80%
Acinetobacter baumannii	108	10	42	12	45	18
	45.95%	4.25%	17.87%	5.10%	19.14%	7.65%
Stenotrophomonas maltophilia	2	1	9	3	0	0
	13.33%	6.66%	60.00%	20.00%	-	-
Burkholderia cepacia	1	0	3	6	0	0
	10.00%	-	30.00%	60.00%	-	-

Pseudomonas aeruginosa was the most common isolate, accounting for 312 (54.54%), followed by Acinetobacter baumannii 235 (41.08%),

Stenotrophomonas maltophilia 15 (2.62%), and Burkholderia cepacia complex 10 (1.70%)

Table-2: NFGNB Isolated

Organisms isolated	NFGNB Isolated (n=572)	Percentage
Pseudomonas aeruginosa	312	54.54%
Acinetobacter baumannii	235	41.08%
Stenotrophomonas maltophilia	15	2.62%
Burkholderia cepacia	10	1.70%

The table-1 depicts numbers and percentages of Pseudomonas aeruginosa, Acinetobacter baumannii, Stenotrophomonas maltophilia and Burkholderia cepacia complex obtained from different specimens.

Pseudomonas aeruginosa isolates in our study were highly susceptible to Colistin (96.79%),

Amikacin, Tobramycin (75%), Piperacillin/Tazobactam (62.85%) and imipenem (59.61%) Like Pseudomonas aeruginosa, Acinetobacter baumannii was also highly susceptible to Colistin (85.10%).The drug Imipenem in our institute was more sensitive to Acinetobacter (75.31%) as compared to Pseudomonas (59.61%).

Table-3: Antimicrobial sensitivity study

Name of Antibiotic	Number and percentage of sensitive Pseudomonas isolates	Number and percentage of Sensitive Burkholderia isolates	Number and percentage of Sensitive Acinetobacter isolates	Number and percentage of Sensitive Stenotrophomonas isolates	Number and percentage of Sensitive Stenotrophomonas isolates
Ceftazidime	119(38.14%)	6(60%)	29(12.34%)	4(26.60%)	4(26.60%)
Ceftriaxone		2(20%)	52(22.12%)	3(20.00%)	3(20.00%)
Cefepime	100(32.05%)	1(10%)	31(13.19%)	2(13.30%)	2(13.30%)
Imipenem	186(59.61%)	2(20%)	177(75.31%)	4(26.60%)	4(26.60%)
Piperacillin/Tazobactam	193(62.85%)	6(60%)	141(60.00%)	6(40.00%)	6(40.00%)
Ticarcillin/Clavulanic Acid	106(33.97%)	4(40%)	137(58.29%)	6(40.00%)	6(40.00%)
Ciprofloxacin	175(56.08%)	5(50%)	52(22.12%)	7(46.60%)	7(46.60%)
Ofloxacin	156(50.00%)	1(10%)	92(39.14%)	5(33.33%)	5(33.33%)
Amikacin	234(75.00%)	4(40%)	97(41.27%)	4(26.60%)	4(26.60%)
Gentamycin	116(37.17%)	2(20%)	43(18.29%)	2(13.30%)	2(13.30%)
Tobramycin	234(75.00%)	2(20%)	104(44.25%)	2(13.30%)	2(13.30%)
Trimethoprim/Sulfamethoxazole	-----	8(80%)	59(25.10%)	12(80.%)	12(80.%)
Colistin	302(96.79%)	8(80%)	200(85.10%)	13(86.66%)	13(86.66%)

Strains of *Burkholderia cepacia* were 80% sensitive to Trimethoprim/Sulfamethoxazole and Colistin and 60% to Piperacillin/Tazobactam and Ceftazidime.

DISCUSSION

In our study, a total of 572 (12.4%) isolates of NFGNB were isolated from 4585 clinical specimens received from indoor admitted patients of our hospital.

Various workers have reported variable results in their studies. Mohammad Rahbar *et al.*; [8] and A. Malini *et al.*; [5] reported a positivity rate of 15% where as Shailpreet Sidhu *et al.*; [9] reported 45.91% of non-fermenters. These variations might be because of the hospital infection control practices of respective institutes.

In our study 297 specimens (50.16%) showed polymicrobial infection which is corroborated with the study done in tertiary care hospital in Kolar, Karnataka by A Malini *et al.*; [5]. As with other studies [10, 11] most of the isolates 343 (59.96%) of NFGNB were from pus specimens.

The frequent use of invasive devices in the form of peripheral venous catheter (51.04%), urinary catheter (40.03%), central venous catheter (19.93%) and Endotracheal Tube (14.33%) were found in most of the patients. These devices probably could have acted as a source of infection in these patients. Costerton JW *et al.*; [12] documented that Biofilm formation by *Pseudomonas aeruginosa* plays an important role in the pathogenesis of central venous catheter-related infection, urinary catheter cystitis, contact lens-associated corneal infection, lung infection in cystic fibrosis, and ventilator-associated pneumonia.

Pseudomonas aeruginosa and *Acinetobacter* species were found to be the common nosocomial pathogens.. NFGNB belonging to *Pseudomonas* species along with the *Acinetobacter* species accounted for 95.62% of the isolates. Other workers [13] also found these two organisms as predominant pathogen.

Pseudomonas aeruginosa isolates were highly susceptible to were highly susceptible to Colistin (96.79%), Amikacin, Tobramycin (75%), Piperacillin/Tazobactam (62.85%) and Imipenem (59.61%). This contrasts with the antibiotic sensitivity pattern of isolates from a Bangalore study in which *Pseudomonas aeruginosa* showed 60-70% resistance to Amikacin, Ceftazidime, and Ciprofloxacin [14]. These differences in rate of drug resistance might be because of the variations in type of antimicrobials being prescribed by the clinicians. In a study from Chandigarh [15] 42% of *Pseudomonas aeruginosa* isolates were

found to be resistant to imipenem. This is in concordance with our study where 40.39% of the isolates were resistant to Imipenem.

Siegman Igra Y *et al.*; [16] reported Nosocomial *Acinetobacter* meningitis secondary to invasive procedures and this is in concordance with our study where out of the total NFGNB isolated from CSF, *Acinetobacter* tops the list. In our study, *Acinetobacter* strains percentage sensitivity for Colistin and Imipenem was 85.10% and 75.31% respectively. Imipenem monotherapy have also been proved effective in many studies [17].

In our study, a total of 13 (86.66%) of the isolates were sensitive to Colistin and a total of 4 (26.60%) were sensitive to Imipenem. This is in contrast with the study done by Mohamad Rahbar [8] where nearly 98% of *Stenotrophomonas maltophilia* isolates were resistant to Imipenem.

Administration of Cotrimoxazole immediately brought a cure in these patients. This is in concordance with the study done by A. Malini *et al.*; in Kolar [5]. Our study also showed that Fluoroquinolones such as Ciprofloxacin and Ofloxacin are also effective antibiotics against *Stenotrophomonas maltophilia*

Gilligan PH *et al.*; reported that the risk factors associated with acquisition of *Burkholderia cepacia* include older age, more advanced pulmonary disease, and exposure to *Burkholderia cepacia* from previous hospitalization or a sibling with *Burkholderia cepacia* colonization In our study 30% of the isolates of *Burkholderia cepacia* were from old age patients and 40% of the patients gave the history of previous hospitalization [18].

As concluded by the studies done by Fass RJ *et al.*; Quinn JP and Speert DP, *Burkholderia cepacia* complex strains are resistant to most antibiotics commonly used for treatment of Gram-negative bacterial infections, including the extended-spectrum Penicillins and Amino glycosides [19-21]. This is in concordance with our study where percentage resistance of amino glycosides was 80% in *Burkholderia cepacia*. Thus in our study Colistin was the most effective antibiotic to be active against non fermenting Gram-negative bacilli.

CONCLUSION

NFGNB are emerging as important opportunistic pathogens and are resistant to commonly used antimicrobials. For each of these organisms, underlying host factors were strongly associated with outcome. The interplay between these multidrug resistant pathogens and the increasing number of

immune-compromised patients poses a challenge for the microbiologists and clinicians likewise. Early diagnosis and institution of empirical therapy based on local antibiogram data of the institute would reduce mortality and improve patient management.

The present study has sufficient power to identify factors, organisms and antibiotics influencing the outcome of infection which will help us to understand the role of these organisms in human disease process better. However, use of additional features and large study sample would have enhanced the value of study and may have provided greater insight into a possible link.

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