

Antibiotic Susceptibility Pattern of Non-Fermenting Gram-Negative Rods Isolated from Blood Culture of Patients: an Observational StudyArchana¹, Aradhana Bharati², Samir Alam³, Vijay Kumar⁴¹Tutor, Department of Microbiology, Patna Medical College and Hospital, Patna, Bihar India²Tutor, Department of Microbiology, Patna Medical College and Hospital, Patna, Bihar, India³Tutor, Department of Microbiology, Patna Medical College Hospital, Patna, Bihar, India⁴Professor and HOD, Department of Microbiology, Patna Medical College and Hospital, Patna, Bihar, India

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Conflict of interest: Nil

Abstract**Aim:** The aim of the present study was to assess frequency and antibiotic susceptibility pattern of non-fermenting gram-negative rods isolated from blood culture of patients.**Material & methods:** The prospective study was conducted in the Department of Microbiology, Patna Medical College and Hospital, Patna, Bihar, India in between the duration of 1 year. Blood Stream Infection (BSI) was defined as the isolation of a pathogen microorganism from >1 blood culture bottle. BSIs were classified as community- and hospital-acquired infections if detected within the first 48 h of hospitalization, or after 48 h of hospitalization, respectively.**Results:** Total 200 NFGNB were isolated from 1425 culture positive clinical samples accounting for an isolation rate of 14.03%. Urine was the most common specimen (30%) followed by pus (26%), blood (16%), sputum (12%), tracheal aspirate (8%) and remaining 8% included other samples. *Acinetobacter baumannii* was the predominant isolate, 104 (52%) followed by *Pseudomonas aeruginosa* 80 (40%) and *Burkholderia cepacia* complex (BCC) 10 (5%). *Burkholderia pseudomallei*, *Acinetobacter lwoffii* and *Stenotrophomonas maltophilia* altogether accounted for 3%. Among the NFGNB isolated, *A. baumannii* showed highest sensitivity to gentamicin and lowest sensitivity to ceftriaxone. *P. aeruginosa* was mostly sensitive to amikacin but least sensitive to ceftriaxone. *B. cepacia* complex, *B. pseudomallei* and *S. maltophilia* showed 100% susceptibility to cotrimoxazole. *A. lwoffii* showed sensitivity to most of the antibiotics. *A. baumannii* and *P. aeruginosa* were mostly sensitive to gentamicin and amikacin and least sensitive to ceftriaxone.**Conclusion:** This study underlines the need to identify NFGNB in tertiary care hospitals and to monitor their susceptibility pattern to guide the clinician for better care and management of patients. Improved antibiotic stewardship and strict infection control measures especially hand washing need to be implemented to prevent emergence and spread of multidrug resistant NFGNB in health care settings.**Keywords:** *Acinetobacter Sp.*, Antibiotic susceptibility, Nosocomial pathogens, non-fermentersThis is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided original work is properly credited.**Introduction**

The increasing emergence of multidrug-resistant organisms is a public health threat that is recognised worldwide. [1] The World Health Organization (WHO) has published the Priority Pathogens List as part of its efforts to address the increase in the global resistance to antimicrobial agents. In the list, the threat of Gram-negative bacilli (GNB) that are resistant to multiple antibiotics is emphasised. Two non-fermenting Gram-negative bacilli (NFGNB), namely *Acinetobacter baumannii* (carbapenem resistant) and *Pseudomonas aeruginosa* (carbapenem resistant) are among the organisms in this list. [2]

Non-fermenting Gram-negative bacilli often colonise the hospital environment, [3] hospitalised patients and the hands of healthcare workers and pose a challenge as they are resistant to a variety of disinfectants commonly used in the hospital environment. [4-6] Non-fermenting Gram-negative bacilli are increasingly being cultured from normally sterile sites such as cerebrospinal fluid (CSF), blood, tissue, pus, fluid and catheter tips. [7] The role of NFGNB in causing disease is well described especially in patients who are or have been recently hospitalised. [8]

NFGNBs are known to colonize initially and then subsequently invade the otherwise normally sterile site through trauma. It has been noted that disruption of natural barriers is an important route of entry of infections. [9,10] Rates of colonization increase in hospitalized patients particularly in those who have been hospitalized for extended periods or / and have received broad spectrum antimicrobial therapy/chemotherapy. [11] Most of the non-fermenters cause nosocomial blood stream infections particularly in debilitated and immunocompromised hosts and are usually multidrug resistant.

There are very few studies from India wherein the various NFGNB, isolated from patients' samples, have been identified and their clinical significance assessed. Hence, the present study was therefore taken to identify the non-fermenters from blood specimens and to determine their antimicrobial susceptibility pattern.

Material & Methods

The prospective study was conducted in the Department of Microbiology, Patna Medical College and Hospital, Patna, Bihar, India in between the duration of 1 year. Blood Stream Infection (BSI) was defined as the isolation of a pathogen microorganism from >1 blood culture bottle. BSIs were classified as community and hospital-acquired infections if detected within the first 48 h of hospitalization, or after 48 h of hospitalization, respectively. The recovery of different species 72 h after the previous positive blood culture in a single patient was considered to be a distinct episode.

Isolation of the same microorganism from a single patient was considered to be a single episode even if the culture was obtained after 72 h. Multiple bacteremic episodes in a single patient were considered to be distinct episodes, if separated by at least 7 days.

Sample Collection and Processing

Blood samples were collected from the patients before the administration of any antibiotic. For adults, after aseptic precautions, 5-10 mL of blood subsequently incubated in BacTAlert3D (Biomérieux, France), a fully automated blood culture system for detection of growth in blood culture. On getting a positive alarm, Gram stain were carried out on positive bottles, followed by sub culture on 5% sheep blood agar and MacConkey agar plates which were incubated aerobically at 37°C overnight for bacterial isolation. Isolates were identified by Vitek 2 Compact (Biomérieux).

Antimicrobial susceptibility testing was done with an automated microbiology system, Vitek 2 compact 60 system BioMérieux India®) and interpreted according to CLSI criteria. [12]

The patient data that were collected included age, sex, underlying diseases and risk factors. Quality control was performed by testing these same antimicrobials against reference strains of bacteria.

Statistical Analysis

Descriptive statistics were used to express overall results. Data were analysed using SPSS 22.0.

Results

Table 1: NFGNB isolates obtained from various clinical specimens

NFGNB isolates	200 (14.03)
Others	1425 (85.53)

Total 200 NFGNB were isolated from 1425 culture positive clinical samples accounting for an isolation rate of 14.03%.

Table 2: Sample-wise distribution of NFGNB isolates

Samples	No. of NFGNB(n=200)	Percentage
Urine	60	30
Pus	52	26
Blood	32	16
Sputum	24	12
E.T. tube	16	8
Catheter Tip	4	2
CVP tip	4	2
Drain tip	3	1.5
Throat swab	3	1.5
Wound swab	1	0.5
Other body fluids	1	0.5

Urine was the most common specimen (30%) followed by pus (26%), blood (16%), sputum (12%), tracheal aspirate (8%) and remaining 8% included other samples.

Table 3: Prevalence of NFGNB isolates

Isolates	Number (n=200)	Percentage
<i>A. baumannii</i>	104	52
<i>P. aeruginosa</i>	80	40
<i>B. cepacia complex</i>	10	5
<i>B. pseudomallei</i>	3	1.5
<i>A. lwoffii</i>	2	1
<i>S. maltophilia</i>	1	0.5

Acinetobacter baumannii was the predominant isolate, 104 (52%) followed by Pseudomonas aeruginosa 80 (40%) and Burkholderia cepacia complex (BCC) 10 (5%). Burkholderia pseudomallei, Acinetobacter lwoffii and Stenotrophomonas maltophilia altogether accounted for 3%.

Table 4: Sensitivity pattern of non-fermenters to antimicrobial agents

Antimicrobials	<i>A. Baumannii</i>	<i>P. Aeruginosa</i>	<i>B. Cepacia complex</i>	<i>B. Pseudomallei</i>	<i>A. Lwoffii (%)</i>	<i>S. Maltophilia</i>
Piperacillin/tazobactam 100/10 mcg	30	28	0	0	3	0
Ceftazidime 30 mcg	28	26	0	0	3	0
Ceftriaxone 30 mcg	24	22	0	0	3	0
Cefepime 30 mcg	40	32	0	0	3	0
Amikacin 30 mcg	62	145	0	0	3	0
Gentamicin 10 mcg	80	70	0	0	3	0
Ciprofloxacin 5 mcg	70	65	0	0	1	0
Cotrimoxazole 25 mcg	80	72	15	4	3	1
Meropenem 10 mcg	72	64	5	3	3	0

Among the NFGNB isolated, *A. baumannii* showed highest sensitivity to gentamicin and lowest sensitivity to ceftriaxone. *P. aeruginosa* was mostly sensitive to amikacin but least sensitive to ceftriaxone. *B. cepacia complex*, *B. pseudomallei* and *S. maltophilia* showed 100% susceptibility to cotrimoxazole. *A. lwoffii* showed sensitivity to most of the antibiotics. *A. baumannii* and *P. aeruginosa* were mostly sensitive to gentamicin and amikacin and least sensitive to ceftriaxone.

Discussion

Nonfermenting gram-negative bacilli (NFGNB) are a taxonomically diverse group of aerobic, nonsporing, bacilli that either do not utilize glucose as a source of energy or utilize it oxidatively. [6] They occur as saprophytes in the environment and some are also found as commensals in the human gut. [13,14]

Total 200 NFGNB were isolated from 1425 culture positive clinical samples accounting for an isolation rate of 14.03%. Urine was the most common specimen (30%) followed by pus (26%), blood (16%), sputum (12%), tracheal aspirate (8%) and remaining 8% included other samples. Nevertheless, in many studies, NFGNB were most commonly isolated from pus. [2,15] *Acinetobacter baumannii* was the predominant isolate, 104 (52%) followed by *Pseudomonas aeruginosa* 80 (40%) and

Burkholderia cepacia complex (BCC) 10 (5%). *Burkholderia pseudomallei*, *Acinetobacter lwoffii* and *Stenotrophomonas maltophilia* altogether accounted for 3%. These results corroborated well with the studies of Goel V et al, where, *A. baumannii* (48.78%) was the most commonly isolated pathogen followed by *P. aeruginosa* (37.71%). [16] According to Samanta P et al, the isolation rate of *Acinetobacter* species was 66%, and *Pseudomonas* species was 26%. However, in other studies, the most common isolate was *P. aeruginosa*, followed by *A. baumannii*. [15,17-19] In our study, prevalence of *A. baumannii* was more in high risk areas, possibly due to increased colonisation of *A. baumannii* in hospital environment, including humidifiers, nebulizers, anaesthetic equipment's, ventilators, healthcare workers etc. causing nosocomial opportunistic infections in patients with severe underlying illnesses. [16,18]

Among the NFGNB isolated, *A. baumannii* showed highest sensitivity to gentamicin and lowest sensitivity to ceftriaxone. *P. aeruginosa* was mostly sensitive to amikacin but least sensitive to ceftriaxone. *B. cepacia complex*, *B. pseudomallei* and *S. maltophilia* showed 100% susceptibility to cotrimoxazole. *A. lwoffii* showed sensitivity to most of the antibiotics. *A. baumannii* and *P. aeruginosa* were mostly sensitive to gentamicin and amikacin and least sensitive to ceftriaxone. In the study of

Jayanthi S et al, isolation rate for *P. aeruginosa* was 41.2%, followed by *Acinetobacter* species (26.29%). [17] Upgade A et al, reported 43% *Pseudomonas* spp. followed by *Acinetobacter* spp. 21%. [20] *P. aeruginosa* was mostly sensitive to amikacin (84%) but least sensitive to ceftriaxone (28%). *B. cepacia* complex, *B. pseudomallei* and *S. maltophilia* showed 100% susceptibility to cotrimoxazole. *A. lwoffii* showed sensitivity to most of the antibiotics. *A. baumannii* and *P. aeruginosa* were mostly sensitive to gentamicin and amikacin and least sensitive to ceftriaxone. Gokale S et al, showed highest susceptibility to meropenem (96.2%) and 45% susceptibility to ciprofloxacin for *A. baumannii*. In the study of Gokale S et al, *P. aeruginosa* showed good sensitivity to meropenem (96.2%), followed by ciprofloxacin (50%) and amikacin (49.5%). [2]

In recent years, due to the liberal and empirical use of antibiotics, NFGNB have emerged as important healthcare-associated pathogens. They have been incriminated in infections, such as, septicemia, meningitis, pneumonia, urinary tract infections (UTI), and surgical site infections (SSI). [14] NFGNB are innately resistant to many antibiotics and are known to produce extended spectrum β -lactamases and metallo β -lactamases. [14,21]

Conclusion

This study underlines the need to identify NFGNB in tertiary care hospitals and to monitor their susceptibility pattern to guide the clinician for better care and management of patients. Improved antibiotic stewardship and strict infection control measures especially hand washing need to be implemented to prevent emergence and spread of multidrug resistant NFGNB in health care settings.

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