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Original Research Article

A Comprehensive Investigation of Non-Fermenting Gram Negative Bacilli Focusing on both Clinical and Microbiological Aspects

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Abstract

Aim: A comprehensive investigation of non-fermenting gram negative bacilli at a tertiary care hospital, focusing on both clinical and microbiological aspects.

Material and Methods: This study had a retrospective design and was conducted at Department of Microbiology, Darbhanga Medical College and Hospital, Laheriasarai, Darbhanga, Bihar, India from January 2021 to December 2021. A total of 4025 clinical samples including urine, pus, blood, wound swab and body fluids were received in the laboratory and inoculated on blood and MacConkey agar or CLED agar and incubated aerobically at 37°C for 18 to 24 hours. The isolates which were non-lactose fermenting and showed alkaline change (K/NC) reaction in triple sugar iron agar media were provisionally considered as NFGNB.

Results: Acinetobacter baumannii was the predominant isolate, 211 (51.34%) followed by Pseudomonas aeruginosa 173 (42.09%) and Burkholderia cepacia complex (BCC) 18 (4.38%). Burkholderia pseudomallei, Acinetobacter lwoffii and Stenotrophomonas maltophilia altogether accounted for 2.19%. Among the NFGNB isolated from high-risk areas including intensive care units and dialysis units, A. baumannii (60.36%) was the most prevalent pathogen, followed by P. aeruginosa (28.40%). Chi-squared ($\chi 2$) value is 9.341 and p-value <0.05. In other clinical areas P. aeruginosa accounted for 51.65% followed by A. baumannii (45.04%). A. baumannii was more prevalent in high-risk areas (ICUs and Dialysis Units) in comparison to other clinical areas. Chi-squared ($\chi 2$) value is 9.341 and p-value < 0.05. Similarly, P. aeruginosa is more prevalent in other clinical areas, than in high-risk areas. Chi-squared ($\chi 2$) value is 22.069 and p-value < 0.05.

Conclusion: To conclude, despite earlier being regarded as contaminants, NFGNB are now emerging as important pathogens causing a wide range of nosocomial infections. Identification of NFGNB and monitoring of their susceptibility profiles are essential due to their variable sensitivity patterns and to help in proper management of the infections caused by them.

Keywords: Acinetobacter baumannii, Antibiotic stewardship, Nonfermenters, Nosocomial infection, Pseudomonas aeruginosa.

Introduction

Non fermenters are a group of aerobic, non-spore forming gram negative bacilli that are either incapable of utilizing carbohydrates as a source of energy, or degrade them via oxidative rather than a fermentative pathway. [1] non-fermenters can cause a vast variety of infections and accounts for approximately 15% of all Gram negative bacilli cultured from clinical specimen. [2] Less than 1/5th of all Gram negative bacilli isolated from clinical specimens received in the routine laboratories are likely to be nonfermentive bacilli. Although non-fermenters are commonly considered as commensals or contaminants; they have emerged as important nosocomial pathogens with frequent outbreaks. [3-5] Spectrum of disease by Pseudomonas aeruginosa and Acinetobacter baumannii, the most common NFGNB are well established as nosocomial pathogens. Other NFGNBs like Burkholderia cepacia, Stenotrophomonas maltophilia, Sphingomonas paucimobilis, Ralstonia pickettiior, Achromobacter spp. have been increasing since the early 1970s. [6]

These pathogens primarily affect patients with co-morbidities such as cystic fibrosis (CF), immunosuppression, organ transplantation, and malignancy. Higher rate of hospitalized patients with serious underlying diseases, large environmental distribution as potential reservoirs for human infections and intrinsic high-level of antibiotic and biocide resistance in NFGNB are contributing factors for this emergence. [7] In-spite of being important as human pathogens, very few clinical microbiology laboratories are able to identify these organisms as a routine because of their complicated taxonomy, slow growth, need for use of special culture media and large spectrum of complex biochemical test required for their identification by conventional techniques. [8] To overcome this problem a number of semi or fully automated systems like Phoenix, Microscan, Vitek 2 etc. have been introduced which are expected to give faster and better results that can be very critical in-patient care but they are not available in a routine microbiology laboratory for use.

Material and Methods

This study had a retrospective design and was conducted at Department of Microbiology, Darbhanga Medical College and Hospital, Laheriasarai , Darbhanga, Bihar, India from January 2021 to December 2021. A total of 4025 clinical samples including urine, pus, blood, wound swab and body fluids were received in the laboratory and inoculated on blood and MacConkey agar or CLED agar and incubated aerobically at 37°C for 18 to 24 hours. The isolates which were non-lactose fermenting and showed alkaline change (K/NC) reaction in triple sugar iron agar media were provisionally considered as NFGNB. They were further identified using standard protocols for identification, like gram staining for morphology, hanging drop for motility, pigment production, oxidase test, catalase test, Hugh-Leifson oxidative fermentative test for glucose, lactose, sucrose, maltose and mannitol, nitrate reduction test, indole test, citrate utilization test, urease test, utilization of 10% lactose, lysine and ornithine decarboxylation, arginine dehydration, growth at 42°C and 44°C.[1] The clinical significance of isolated NFGNB was assessed retrospectively by analyzing the case sheets for relevant laboratory and clinical criteria. Laboratory criteria included the presence of pus cells along with gram-negative bacilli in the stained smear from the sample, isolation of the same organism from a repeat sample, leukocytosis, and relevant radiological evidence. The clinical criteria included the presence of risk factors such as underlying diseases (diabetes mellitus, chronic renal failure, malignancy, cystic fibrosis, pneumonia and other immunosuppressive conditions), presence of intravenous or urinary catheters, duration of stay in intensive care unit (ICU), mechanical ventilation and recent surgery.[7,8] Antimicrobial susceptibility test was performed by Kirby- Bauer disc diffusion method using commercially available disc (Hi-Media). The different antimicrobials used were gentamicin (10µg), amikacin (30 µg), ceftazidime (30µg), ceftriaxone (30µg), piperacillin/ Tazobactum (100µg/10µg), imipenem (10µg), meropenem (10µg), ciprofloxacin (5µg), and cotrimoxazole (25µg). The results were interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 were used as control strains. [9]

Statistical Analysis: Statistical analysis was done by using Excel and SPSS V21. The result of this analysis was used for comparison of data and to finalize the study results. p-value was determined to evaluate the levels of significance using Excel and SPSS V21, p-value of < 0.05 was considered to be significant.

Results

Total 411 NFGNB were isolated from 3116 culture positive clinical samples accounting for an isolation rate of 13.19%. Urine was the most common specimen (29.44%) followed by pus (27.49%), blood (15.57%), sputum (12.90%), tracheal aspirate (8.27%) and remaining 6.33% included other samples (Table 1).

Table 1: Sample-wise distribution of NFGNB isolates.

Samples	No. of NFGNB (n=411)	Percentage
Urine	121	29.44
Pus	113	27.49
Blood	64	15.57
Sputum	53	12.90
E.T. tube	34	8.27
Catheter Tip	6	1.46
CVP tip	6	1.46
Drain tip	4	0.97
Throat swab	4	0.97
Wound swab	4	0.97
Other body fluids	2	0.49

Acinetobacter baumannii was the predominant isolate, 211 (51.34%) followed by Pseudomonas aeruginosa 173 (42.09%) and Burkholderia cepacia complex (BCC) 18 (4.38%). Burkholderia pseudomallei, Acinetobacter lwoffii and Stenotrophomonas maltophilia altogether accounted for 2.19% (Table 2).

Table 2: Prevalence of NFGNB isolates.

Isolates	Number (n=411)	Percentage
A. baumannii	211	51.34
P. aeruginosa	173	42.09
B. cepacia complex	18	4.38
B. pseudomallei	4	
A. lwoffii	3	2.19
S. maltophilia	2	

Among the NFGNB isolated from high-risk areas including intensive care units and dialysis units, A. baumannii (60.36%) was the most prevalent pathogen, followed by P. aeruginosa (28.40%). Chi-squared ($\chi 2$) value is 9.341 and p-value <0.05. In other clinical areas P. aeruginosa accounted for 51.65% followed by A. baumannii (45.04%). Chi-squared ($\chi 2$) value is 22.069 and p-value <0.05 (Table 3). Majority of the patients were adults aged above 45 years and isolation rate in males (60.10%) was higher than that in females (39.90%). Isolation of NFGNB was maximum from urine sample (29.44%) followed by, pus (27.49%), blood (15.57%), sputum (12.90%) and then ET tube (8.27%). A. baumannii was the most common species, accounting for 51.34% of the isolates, followed by P. aeruginosa 49.09% and B. cepacia complex (4.38%).

Table 3: Species-wise distribution in different clinical areas.

Ward	Total no.	A. baumannii	P. aeruginosa	BCC	B. pseudomallei	A. lwoffii	S. maltophilia
High risk areas	169	102 (60.36%)	48 (28.40%)	14 (8.28%)	2 (1.18%)	2 (1.18%)	1 (0.59%)
Other areas	242	109 (45.04%)	125 (51.65%)	4 (1.65%)	2 (0.83%)	1 (0.41%)	1 (0.41%

A. baumannii was more prevalent in high-risk areas (ICUs and Dialysis Units) in comparison to other clinical areas. Chi-squared ($\chi 2$) value is 9.341 and p-value < 0.05. Similarly, P. aeruginosa is more prevalent in other clinical areas, than in high-risk areas. Chi-squared ($\chi 2$) value is 22.069 and p-value < 0.05.

Table 4: Sensitivity pattern of no fermenters to antimicrobial agents.

Antimicrobials	A. Baumannii (%)	P. Aeruginosa (%)	B.Cepacia complex (%)	B.Pseudomallei (%)	A. Lwoffii (%)	S. Maltophilia (%)
Piperacillin/tazobactam 100/10 mcg	64 (30.33)	66 (38.15)	0	0	3 (100)	0
Ceftazidine 30 mcg	50 (23.70)	53 (30.64)	0	0	3 (100)	0
Ceftriaxone 30 mcg	49 (23.22)	51 (29.48)	0	0	3 (100)	0
Cefepime 30 mcg	68 (32.23)	60 (34.68)	0	0	3 (100)	0
Amikacin 30 mcg	107 (50.71)	144 (83.24)	0	0	3 (100)	0
Gentamicin 10 mcg	125 (59.24)	131 (75.72)	0	0	3 (100)	0
Ciprofloxacin 5 mcg	122 (57.82)	125 (72.25)	0	0	1 (33.33)	0
Cotrimoxazole 25 mcg	119 (56.40)		18 (100)	4 (100)	3 (100)	2 (100)
Meropenem 10 mcg	119 (56.40)	113 (65.32)	8 (44.44)	3 (75)	3 (100)	0

Among the NFGNB isolated, A. baumannii showed highest sensitivity to gentamicin (59.24%) and lowest sensitivity to ceftriaxone (23.22%). P. aeruginosa was mostly sensitive to amikacin (83.24%) but least sensitive to ceftriaxone (29.48%). B. cepacian complex, B. pseudomallei and S. maltophilia showed 100% susceptibility to cotrimoxazole. A. lwoffii showed sensitivity to most of the antibiotics (Table 4). A. baumannii and P. aeruginosa were mostly sensitive to gentamicin and amikacin and least sensitive to ceftriaxone.

Discussion

Nonfermentive gram-negative bacilli are ubiquitous in environment. They used to be considered as contaminants or commensals in the past. They have now emerged as important healthcare-associated and opportunistic pathogens due to their frequent isolation from clinical materials and their association with various diseases. In the present study, the isolation rate of NFGNB from clinical samples was 13.19%. [10] This was parallel to the results of a study from Kolkata by Rit K et al, where NFGNB were isolated in 12.18% of clinical samples.10 However, the prevalence of no fermenters varies greatly from time to time and place to place. A study from Amritsar reported a very high isolation rate of 45.9% whereas, it was 3.58% in a study from Bangalore and 5.2% in another study from Chennai. In a study from Saudi Arabia NFGNB isolation rate was 16%. [11-14] In the present study, NFGNB were most frequently isolated from urine samples (29.44%), followed by pus (27.49%). Nevertheless, in many studies, NFGNB were most commonly isolated from pus. [4,12] According to a study by Shobha KL et al, no fermenters were emerging as an important cause of urinary tract infections (9.44%). [15] Frequent isolation of NFGNB from urine and pus samples in this study, could be attributed to the increase in number of critically ill, hospitalized patients requiring urinary tract catheterization and other instrumentations. Prolonged hospital stay, bed sores, burns, open wounds, surgical site infections, diabetes, malignancies and several underlying illnesses made these patients more vulnerable to NFGNB infections. In this study, A. baumannii was the most common species isolated, accounting for 51.34%, followed by P. aeruginosa (49.09%) and B. cepacia complex (4.38%). A. lwoffii, B. pseudomallei and S. maltophilia together accounted for (2.19%). 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.[12,13,17,18] In the present study, in high-risk areas, A. baumannii was the most common isolate (60.36%), followed by P. aeruginosa (28.40%) which was statistically significant ($\chi2 = 9.341$; p-value < 0.05). This study corroborated well with the result of the study by Goel V et al, showing A. baumannii being the commonest isolate followed by P. aeruginosa from high risk areas. [16] In our study, prevalence of A. baumannii was more in high risk areas, possibly due to increased colonization of A. baumannii in hospital environment, including humidifiers, nebulizers, anesthetic equipment, ventilators, healthcare workers etc. causing nosocomial opportunistic infections in patients with severe underlying illnesses. [16,17] In other clinical areas, P. aeruginosa was the commonest isolate (51.65%), followed by A. baumannii (45.04%). This was statistically significant ($\chi2 = 22.069$; p-value <0.05). Most of the isolates were from surgery and orthopedic wards, where patients with road traffic accidents, burn, open wounds, abscesses, and surgical site infections were frequently admitted. In the study of Jayanthi S et al, isolation rate for P. aeruginosa was 41.2%, followed by Acinetobacter species (26.29%). [13] Upgade A et al, reported 43% Pseudomonas spp. followed by Acinetobacter spp. 21%. [19]

A. baumannii showed highest susceptibility to gentamicin (59.24%) and lowest susceptibility to ceftriaxone (23.22%). This organism exhibited 56.40% susceptibility to both meropenem and cotrimoxazole and 57.82% susceptibility to ciprofloxacin. However, Gokale S et al, showed highest susceptibility to meropenem (96.2%) and 45% susceptibility to ciprofloxacin for A. baumannii.[4] P. aeruginosa showed highest susceptibility to amikacin (83.24%), but least susceptibility to ceftriaxone (29.48%). Susceptibility to piperacillin/tazobactam combination was 38.15% and to cefepime 34.68%. 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%).[4]

Conclusion

To conclude, despite earlier being regarded as contaminants, NFGNB are now emerging as important pathogens causing a wide range of nosocomial infections. Identification of NFGNB and monitoring of their susceptibility profiles are essential due to their variable sensitivity patterns and to help in proper management of the infections caused by them.

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