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Original Research Article

Prevalence of nonfermentative gram-negative bacilli and their antimicrobial susceptibility profiles in a tertiary care hospital of Eastern India

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ABSTRACT

Background: Nonfermentative gram-negative bacilli (nonfermenters) have emerged as a major concern for nosocomial infections. They exhibit resistance not only to the beta-lactam and other group of antibiotics but also to carbapenems. This study was undertaken to know the prevalence of nonfermenters from clinical samples along with their antimicrobial susceptibility profile.
Methods: A cross-sectional study over a period of 21 months in the microbiology laboratory of a tertiary care hospital was done. Clinical samples were processed by conventional bacteriological methods for isolation and identification. Susceptibility testing was done by Kirby-Bauer disc diffusion method as recommended by Clinical and Laboratory Standard Institute.
Results: 411 nonfermenters (13.18%) were isolated from 3116 culture positive clinical samples. Out of these nonfermenters, most were *Acinetobacter baumannii* (51.34%) followed by *Pseudomonas aeruginosa* (42.09%), *Burkholderia cepacia* complex (4.38%) and others (2.19%). Others included *Burkholderia pseudomellei*, *Acinetobacter lwoffii* and *Stenotrophomonas maltophilia*. Highest sensitivity to gentamicin and amikacin were shown by *A. baumannii* and *P. aeruginosa* respectively while both were mostly resistant to ceftriaxone. Burkholderia and Stenotrophomonas species showed 100% sensitivity to cotrimoxazole. *A. baumannii* was the most prevalent nonfermenter in intensive care units.
Conclusions: Timely identification of nonfermenters and monitoring their susceptibility patterns will help in proper management of infections caused by them. Improved antibiotic stewardship and infection control measures should be implemented to prevent nosocomial infections and spread of drug resistant nonfermenters.

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

INTRODUCTION

The nonfermentative gram-negative bacilli (NFGNB) are a group of aerobic, non-spore-forming bacilli that either do not use carbohydrates as a source of energy or degrade them through metabolic pathways other than fermentation.[1] They are widely distributed in nature as saprophytes or as commensals and act as opportunistic pathogens for man.[2] Review of recent literatures show that nonfermenters are being recovered with increasing frequency from clinical specimens in a higher proportion of hospitalized patients suffering from illnesses like urinary tract infection, ventilator associated pneumonia, surgical site infection and septicaemia.[3,4]

Nonfermenters are now resistant to many routinely used antibiotics and even to cephalosporins and carbapenems. Resistance compromises treatment, prolongs hospital stay, increases mortality and healthcare costs.[5,6] The aim of the present study was to isolate and identify NFGNB from clinical samples and to assess prevalence and antimicrobial susceptibility profiles in a tertiary care hospital of Eastern India.

METHODS

This study had a cross sectional design and was conducted between January 2015 to October 2016 in the Department of Microbiology, Kalinga Institute of Medical Sciences, Bhubaneswar.

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 dehydrolation, 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% (Figure 1).

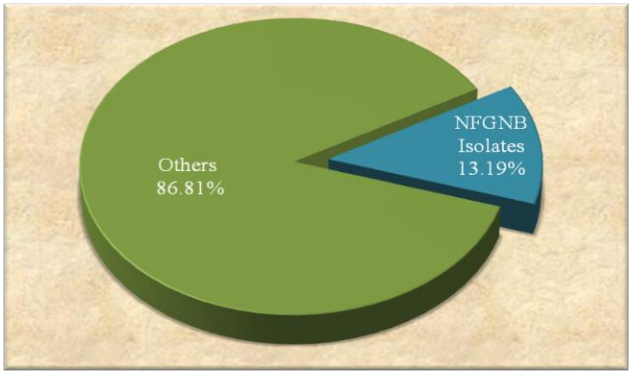

Figure 1: NFGNB isolates obtained from various clinical specimens.

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	2.19
A. lwoffii	3	
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 nonfermenters 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. cepacia* 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

Nonfermentative 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%. 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 nonfermenters 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, nonfermenters 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, hospitalised 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 ($\chi 2 = 9.341$; 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 colonisation of *A. baumannii* in hospital environment, including humidifiers, nebulizers, anaesthetic equipments, 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 ($\chi 2 = 22.069$; p-value <0.05). Most of the isolates were from surgery and orthopaedic 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/tazobactum 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.

Prevalence of pathogens often varies dramatically between communities, hospitals in the same community and among different patient populations in the same hospital. Therefore, clinicians must be updated with the prevalence and antimicrobial susceptibility pattern of the circulating pathogens in their healthcare settings. Appropriate antimicrobials should be used for empiric therapy. Since, these organisms have great potential to survive in hospital environment, improved antibiotic stewardship and infection control measures will be needed to prevent the emergence and spread of drug resistant NFGNB in healthcare settings.

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