Prevalence and antibiogram of nonfermenting gram negative bacilli isolates obtained from various clinical samples in a tertiary care hospital, Bathinda, Punjab, India

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ABSTRACT

Background: Non-fermenting gram-negative bacilli (NFGNB) have emerged as important healthcare associated pathogens in recent years. Infections caused by these bacteria are almost always secondary to some predisposing factors in patients such as burns, prolonged antimicrobial therapy, immunosuppression etc. The Objectives of the study was to be carried out with an objective to identify NFGNB upto genus and species level and study their antimicrobial sensitivity/resistance pattern so that empiric therapy could be selected accordingly.

Methods: A total of 2261 clinical samples were collected from patients admitted in ICU and different wards of the hospital. All samples were processed according to standard microbiological procedures. Identification of NFGNB upto genus and species level was done by various biochemical tests. Antimicrobial susceptibility testing was done by Kirby Bauer disc diffusion method results were interpreted in accordance with clinical laboratory standards institute guidelines.

Results: In this study, 365 NFGNB were obtained accounting for their prevalence of 16.1%. P. aeruginosa was the commonest NFGNB isolated in this study accounting for 52.6%, A. baumannii was the second common NFGNB isolated (31.7%). Other NFGNB isolates were obtained with a lesser frequency. P. aeruginosa isolates were highly sensitive to polymyxin B and colistin followed by imipenem. Most of the A. baumannii isolates were multidrug resistant.

Conclusions: This study gives an alarming sign towards high prevalence of multi drug resistant NFGNB in our hospital. Therefore, improved antibiotic stewardship and strict protocols for hand washing need to be implemented to prevent emergence and spread of multidrug resistant NFGNB in health care settings.

Keywords: A. baumannii, Antibiotic resistance, Non-fermenting gram-negative bacilli, P. aeruginosa

INTRODUCTION

The non-fermentative gram negative bacilli (NFGNB) consists of a diverse group of non-spore forming, aerobic bacilli that either do not use carbohydrates as the source of energy or degrade them through metabolic pathways other than fermentation.¹ Certain conditions or diseases predispose the patients to infection with non-fermenters like malignancies particularly of reticuloendothelial system, instrumentation, surgery, catheterizations particularly of urinary tract, intravascular catheterisation, lumbar puncture, tracheostomy, dialysis, lavages, placement of shunts, prosthesis and prolonged antibiotic usage and chronic infections. Burns, open wounds and
Exudative lesions are other predisposing factors.² NFGNB account for about 15% of all gram-negative bacilli isolated from clinical specimens. Infections caused by these bacteria are almost always secondary to some predisposing factors in patients such as burns, prolonged antimicrobial therapy, introduction of immnosuppressive agents, extremes of age and prolongation of life of a patient with many severe diseases, by advanced surgical and medical treatment.³ In routine, they are identified only in few laboratories in India as they are slow growing and require special culture media and biochemical tests for their identification.¹⁴ Resistance to antimicrobials is common in these organisms and has increased over the years among NFGNB and number of strains are now resistant nearly to all commonly used antibiotics. Development of resistance in non-fermenters is multifactorial. Factors involved are mutations in genes encoding porins, efflux pump mechanisms, penicillin binding proteins, chromosomal beta lactamasers.³⁶ Success of antimicrobial therapy depends on the appropriateness of the choice of antibiotics that should be used on the basis of prior knowledge of the susceptibility pattern of the agent; therefore this study was conducted with an objective to identify non-fermenting Gram negative bacilli isolated from various clinical samples up to genus and species level along with study of their antimicrobial sensitivity/resistance pattern.

**METHODS**

This present study was conducted in Bacteriology Section of Microbiology Department, Adesh Institute of Medical Sciences and Research (AIMSR), Bathinda after getting approval from the Thesis Research Degree Committee and Ethical Committee of the Institute.

A total of 2261 clinical samples were collected from patients admitted in ICU and various wards of the hospital of depending upon the clinical diagnosis of respective patients. These included: urine, pus, blood, ear swabs, high vaginal swabs, sputum, endotracheal secretions, tracheal aspirate and various body fluids. Out of total samples, 858 samples were collected from ICU patients and 1403 samples were collected from patients admitted in various wards of the hospital.

All samples were collected and processed as per standard microbiological guidelines. Samples were inoculated on to Blood Agar (BA) and MacConkey Agar (MA) plates under strict aseptic conditions and plates were incubated at 37°C for 24-48 hours under aerobic conditions. All isolates that showed non-lactose fermenting colonies on MA and those which grew only on BA and not on MA were subjected to Gram staining and all gram-negative bacilli/cocci/coccobacilli obtained were then subjected to triple sugar iron test. The bacterial isolates which produced alkaline/acid (K/A) reaction and acid/acid (A/A) reaction were excluded. Isolates which produced an alkaline/alkaline (K/K) reaction were provisionally identified as non-fermenters and were included in this study and subjected to identification up to genus/species level by a battery of biochemical tests.⁸ Oxidative/Fermentative (O/F) test for glucose, lactose, sucrose, mannitol and xylose, oxidase test, motility test, nitrate reduction test, lysine and ornithine decarboxylase test, arginine dihydrolase test, gelatin liquefaction test, urease test, indole production test, citrate utilization test, growth at 42°C and 44°C. Antimicrobial susceptibility testing was performed by Kirby Bauer disc diffusion method on Mueller Hinton agar as per CLSI guidelines using commercially available discs.¹⁰ Following antimicrobial discs were used: ceftazidime (30µg), cefepime (30µg), piperacillin-tazobactum (100µg/10 µg), aztreonam (30µg), imipenem (10µg), meropenem (10 µg), gentamicin (10µg), amikacin (30µg) netilmicin (30 µg), ciprofloxacin (5µg), norfloxacin (30µg; for urinary isolates), polymyxin B (300 units) and colistin (10µg). Plates were incubated at 37°C for 18-24 hours and results were interpreted according to zone sizes mentioned in the CLSI guidelines.¹⁰

**RESULTS**

Out of total samples processed, 1136 (50.3%) samples showed growth and in 1125 (49.7%) samples, no growth was obtained after appropriate incubation period and a total of 365 NFGNB were isolated thus accounting for their isolation rate as 16.1%. 270 (76%) isolates were obtained from male patients and 95 (24%) were isolated from female patients.

![Figure 1: Distribution of various NFGNB isolates obtained in the study.](image-url)
Maximum NFGNB (37.4%) were obtained from age group of 41 to 60 years followed by age- group of 21 to 40 years; 61-80 years; 0 to 20 years and minimum isolates were obtained from age group of more than 80 years. 208 NFGNB isolates were obtained from ICU patients and 157 were isolated from patients admitted in different wards. P. aeruginosa was the commonest NFGNB isolated in this study with the prevalence of 52.6% among total NFGNB isolates. A. baumannii was the second common NFGNB isolated (31.7%) and others accounted for a total of 15.6%. These included P. fluorescens, P. stutzeri, P. putida, A. lwaffii, S. maltophilia, A. faecalis, S. paucimobilis, B. cepacia, A. xylosoxidans and B. diminuta (Figure 1).

Maximum NFGNB isolates were obtained from pus samples followed by urine, endotracheal secretions and tracheal aspirate. Sputum and blood, each accounted for 4.9% and 3.6% of total isolates were obtained from ear swabs. Very few isolates were obtained from CSF, intercostal fluid, pleural fluid and central line tip culture as shown in Figure 2. Sample-wise distribution of all NFGNB isolates obtained is shown in Table 1.

**Figure 2: Distribution of various NFGNB isolates obtained from various samples.**

| Type of sample       | Total Isolates | P. aeruginosa | P. fluorescens | P. stutzeri | P. putida | A. baumannii | A. lwaffii | S. maltophilia | B. cepacia | A. faecalis | B. diminuta | A. xylosoxidans | S. paucimobilis |
|----------------------|----------------|---------------|----------------|-------------|-----------|--------------|------------|----------------|------------|-------------|-----------|---------------|----------------|
| Urine                | 85             | 54            | 5              | 1           | 1         | 9            | 5          | 1              | 2          | 4           | 1         | 2             | 1              |
| Pus                  | 91             | 54            | -              | 1           | -         | 29           | 5          | 1              | -          | -           | -         | -             | -              |
| Blood                | 18             | 41            | -              | -           | -         | 6            | 1          | 3              | 4          | -           | -         | -             | -              |
| Tracheal aspirate    | 62             | 29            | -              | -           | -         | 28           | 1          | 3              | 1          | -           | -         | -             | -              |
| Ear secretions       | 65             | 20            | -              | -           | -         | 34           | 3          | 6              | 2          | -           | -         | -             | -              |
| Sputum               | 18             | 10            | -              | -           | -         | 7            | -          | 1              | -          | -           | -         | -             | -              |
| HVS                  | 5              | 4             | -              | -           | -         | -            | -          | 1              | -          | -           | -         | -             | -              |
| Ear swabs            | 14             | 13            | 1              | -           | -         | -            | -          | -              | -          | -           | -         | -             | -              |
| Pleural fluid        | 2              | 2             | -              | -           | -         | -            | -          | -              | -          | -           | -         | -             | -              |
| Intercostal fluid    | 3              | 1             | -              | -           | -         | 2            | -          | -              | -          | -           | -         | -             | -              |
| CSF                  | 1              | 1             | -              | -           | -         | 1            | -          | -              | -          | -           | -         | -             | -              |
| Central line tips    | 1              | -             | -              | -           | -         | -            | -          | -              | -          | -           | -         | -             | -              |
| Total                | 365            | 192           | 6              | 2           | 1         | 116          | 15         | 15             | 9          | 4           | 1         | 2             | 2              |

P. aeruginosa isolates were 100% sensitive to polymyxin B and colistin, 71.4% sensitivity was reported towards imipenem and very less sensitivity was reported towards cephalosporins. A. baumannii isolates showed high level of resistance to most of the antibiotics tested. However, 96.5% sensitivity was recorded for polymyxin B and 97.4% for colistin indicating them as the drugs to be used for treatment of A. baumannii infections. Antibiogram obtained for A. lwaffii was observed as much more susceptible to antibiotics tested. B. cepacia showed very good sensitivity towards ceftazidime (77.7%), imipenem (66.6%), meropenem (77.7%) and all isolates were sensitive to piperacillin-tazobactam and cotrimoxazole. S. maltophilia was also found to be multidrug resistant pathogen showing resistance to various groups of antibiotics. All isolates of S. maltophilia were resistant towards ceftazidime, cefepime, imipenem and meropenem. All isolates were sensitive to cotrimoxazole.
Antibiotic susceptibility pattern obtained for most commonly isolated NFGNB is shown in Table 2.

Table 2: Antibiotic susceptibility profile of commonly isolated NFGNB in present study.

| Antibiotic Tested | P. aeruginosa (n=192) | A. baumannii (n=116) | A. lwoffii (n=15) | B. cepacia (n=9) | S. maltophilia (n=15) |
|-------------------|-----------------------|----------------------|-------------------|-----------------|----------------------|
| Cefazidime        | 75 (39.1%)            | 4 (3.4%)             | 8 (53.3%)         | 5 (55.5%)       | 0 (0%)               |
| Cefepime          | 81 (42.2%)            | 6 (5.2%)             | 9 (60%)           | 7 (77.7%)       | 0 (0%)               |
| Piperacillin-     | 124 (64.6%)           | 15 (12.9%)           | 13 (86.6%)        | 9 (100%)        | 4 (26.6%)            |
| tazobactum        |                       |                      |                   |                 |                      |
| Cotrimoxazole     | Not tested            | 9(7.8%)              | 10 (66.6%)        | 9 (100%)        | 15 (100%)            |
| Aztreonam         | 86 (44.8%)            | Not-tested           | Not tested        | Not tested      | Not tested           |
| Gentamicin        | 107 (55.7%)           | 11 (9.5%)            | 14 (93.3%)        | 1 (11.1%)       | 2 (13.3%)            |
| Amikacin          | 117 (60.9%)           | 12 (10.3%)           | 12 (80%)          | 0 (0%)          | 2 (13.3%)            |
| Netilmicin        | 120 (62.5%)           | Not tested           | Not tested        | Not tested      | Not tested           |
| Ampicillin-       | Not tested            | 30 (25.8%)           | 14 (93.3%)        | Not tested      | Not tested           |
| sulbactum         |                       |                      |                   |                 |                      |
| Norfloxacin       | 26 (48.1%)            | 2 (22.2%)            | 3 (60%)           | 0 (0%)          | Not tested           |
| (for urinary      |                       |                      |                   |                 |                      |
| isolates)         |                       |                      |                   |                 |                      |
| Ciprofloxacin     | 79 (57.2%)            | 12 (11.3%)           | 7 (70%)           | 2 (28.5%)       | 8 (53.3%)            |
| (for non-urinary   |                       |                      |                   |                 |                      |
| isolates)         |                       |                      |                   |                 |                      |
| Imipenem          | 137 (71.4%)           | 46 (39.6%)           | 15 (93.3%)        | 6 (66.6%)       | 0 (0%)               |
| Meropenem         | 124 (64.5%)           | 37 (31.9%)           | 15 (93.3%)        | 7 (77.7%)       | 0 (0%)               |
| Polymyxin b       | 192 (100%)            | 112 (96.5%)          | 15 (100%)         | 0 (0%)          | 15 (100%)            |
| Colistin          | 192 (100%)            | 113 (97.4%)          | 15 (100%)         | 0 (0%)          | 15 (100%)            |

DISCUSSION

Non-fermenters were usually considered as commensals or contaminants in the past but have now emerged as important health care pathogens. These organisms are associated with life threatening infections such as septicemia, pneumonia, UTI, meningitis, surgical site infections, ventilator associated pneumonia, osteomyelitis etc. and resistance to antimicrobials have resulted in difficulty in treatment of infections caused by these bacteria. NFGNB are intrinsically resistant to various antimicrobials and are known to produce extended spectrum beta-lactamases (ESBL’s) and metallo-beta-lactamases (MBL’s). In the present study, isolation rate of NFGNB out of total samples processed was 16.1%. Similar isolation rate has been reported by other authors: 16%, 19%, 12.2%, 7,13,14 Very less isolation rate has been reported by Benachinmardi et al and Malini et al i.e. 3.5% and 4.5% respectively.6,15 However, some authors have also reported higher isolation rates of NFGNB in their studies: 75.9%, 66.8% and 36.5%.16-18 76% isolates of NFGNB were obtained from male patients and 24% from female patients. These results are similar to Jayapriya et al who has reported NFGNB isolates from males as 71% and females as 29%.19 In another study by Ridhima et al 69.7% isolates were obtained from males and 30.3% from females whereas in a study by Kalidas et al and Aamal et al NFGNB isolates obtained from males was 55% and 52% respectively whereas from females was 45% and 42% respectively.14,20,21

In our study, maximum NFGNB (37.4%) were isolated from age group of 41-60 years and minimum (1.7%) were isolated from patients above 80 years. Ridhima et al reported that the age group which was maximum infected with NFGNB was 45-60 yrs which is similar to this study.20 According to Aamal et al group in which NFGNB were more frequently isolated was 15-62 yrs.21 In a study by Kalidas et al 72% isolates of NFGNB were from the age above 45 yrs and Benachinmardi et al, reported maximum NFGNB isolates were obtained from 21-50 yrs age group whereas in a study by Jayapriya et al, most of the NFGNB isolates were obtained from 21-40 yrs age group.6,14,19

In this study, 56.9% NFGNB isolates were obtained from ICU patients and 43.1% from IPD patients admitted in surgery, medicine, gynaecology and paediatric wards. The results are in concordance with studies by Juyal et al, Jayapriya et al, and Patel et al, who had reported isolation rate of NFGNB isolates from ICU samples to be 67%, 58% and 52% respectively and isolation rate from IPD as 33%, 48% and 42% respectively.11,19,22

In the present study, maximum NFGNB isolates (24.9%) were obtained from pus samples but variable isolation rates of NFGNB from pus samples have reported by other
In this study, 23.2% NFGNB were obtained from urine samples. A study by Rajendra et al shows similar results with the isolation rate of NFGNB from urine to be 25.4%. Jayapriya et al reported the NFGNB isolates obtained from urine to be 30.8%. Many authors have reported very less isolation rates of NFGNB from urine samples. Benanchinmardi et al, Malini et al and Patel et al have reported NFGNB isolates obtained from urine as 11%, 11.9% and 11.8% respectively. A study by Gokale and Metgud showed that only 8.2% NFGNB isolates were obtained from urine samples. In the present study, 17.8% NFGNB were isolated from endotracheal secretions, 16.9% from tracheal secretions and 4.9% from sputum samples. In studies by Malidas et al, Malini et al, Gokale and Metgud the NFGNB reported from endotracheal secretions were 18.4%, 16.4%, 6.8% and 7.8% respectively. In a study by Malini et al, and Patel et al, NFGNB obtained from sputum samples were 6.7% and 7% respectively which also correlates with results of this study. In this study, NFGNB isolated from blood samples were 4.9%. Benanchinmardi et al, and Aamal et al, have reported NFGNB isolates obtained from blood as 6% and 8% respectively thus showing similarity with the results of this study. On the other hand, Sidhu et al, and Rajendra et al, have reported higher isolation rate of NFGNB from blood samples i.e.-36.3% and 24.5% respectively. Very few NFGNB isolates (1.5%) were obtained from body fluids. Similar results have been reported by other studies in which NFGNB isolated from body fluids were 1.5%, 2.4% and 2.3% respectively.

In the present study, P. aeruginosa was the most frequently isolated NFGNB as 52.6% of total NFGNB isolates were of P. aeruginosa. Various authors have reported similar prevalence of P. aeruginosa in their studies: 53.8%, 53%, 50.2%, 58.9% and 56.9%. Higher prevalence of P. aeruginosa has also been reported by some authors: 72.6% 78.9%, 82.3% and 76.9%. Lesser prevalence of P. aeruginosa has been reported by Samanta et al and Juyal et al i.e. -26% and 38.2% respectively. A. baumannii was the second most frequently isolated NFGNB as 31.7% of total NFGNB isolates were of A. baumannii. Different authors have reported similar prevalence of A. baumannii in their studies: 39%, 30.3%, 30.5%, 26.19

In the present study, S. maltophilia showed the prevalence of 4.1% which is very close to the results reported by Jayapriya et al who had reported it to be 4.5%. Kasidas et al and Rajendra et al have reported the prevalence of S. maltophilia as 3% and 3.6% respectively. In this study, A. lvofii showed the prevalence of 4.1% which is very close to the results reported by Kalidas et al who reported it to be 5.4%. However, Juyal et al and Rajendra et al have reported the prevalence of A. lvofii as 13.8% and 9.1% respectively which is slightly higher as compared to this study. B. cepacia showed the prevalence of 2.5% in this study which is very near to the results reported by Jayapriya et al who reported it to be 3.2% whereas Kalidas et al had reported the prevalence of B. cepacia to be 6.9% which is slightly higher as compared to this study. P. fluorescens, P. putida, P. stutzeri, A. faecalis, A. xylosoxidans, S. paucimobilis, B. diminuta were less frequently isolated NFGNB accounting to the total prevalence of 4.7%. Various studies around the globe also reported very less prevalence of these NFGNB isolates.

P. aeruginosa was found resistant to most of the commonly used antimicrobial agents. It was found highly resistant to ceftazidime (60.9%) and cefepime (57.8%) which is similar to other studies. Juyal et al, Kalidas et al and Patel et al, have reported resistance of P. aeruginosa for ceftazidime to be 68.8%, 71.3% and 75.4% respectively. Juyal et al reported resistance towards cefepime as 61.4% whereas very low level of resistance was recorded by Sadhna et al, 28% and Bimla and Rekha -25.3%. Higher resistance to cephalosporins might be due to production of ESBL’s by this bacteria. In this study, pipercillin-tazobactam was effective antibiotic as only 35.4% resistance was recorded for this antibiotic. This is almost similar to studies by Kalidas et al and Patel et al who have recorded it as 46.5% and 24.1% respectively. Sensitivity of P. aeruginosa to imipenem in this study was 71.3%. Various other studies have also reported imipenem as effective antibiotic to treat infections caused by P. aeruginosa with sensitivity of 94%, 91% and 89% respectively. In our study, resistance towards meropenem was higher as compared to imipenem. Overexpression of the MexAB-Opq M efflux system is known to affect meropenem efficacy but not that of imipenem. In addition, the MexCD- OprJ and Mex XY-Opq M efflux systems may be involved in reduced susceptibility to meropenem. Polymyxin B and colistin were the most effective antimicrobial agents against P. aeruginosa as 100% sensitivity was recorded for them in this study and various other studies.

A. baumannii isolates were found to be extremely resistant to ceftazidime (96.6%) and cefepime (94.8%) and pipercillin-tazobactam (94.8%). Higher level of resistance was also recorded for cotrimoxazole (92.2%), ampicillin-sulbactam (74.2%) amikacin (89.7%) and ciprofloxacin (88.7%). Resistance towards imipenem and meropenem was recorded as 60.4% and 68.1% respectively. This correlates with the studies by Kalidas et al and Jaggi et al and who have reported resistance towards imipenem and meropenem to be 65.4% and 62.6% respectively. Lower resistance was seen in polymyxin B and colistin in this study. Kalidas et al, Taneja et al and Nahar et al and also recorded 10.5%, 3.5% and 5% resistance of A. baumannii towards...
colistin.\textsuperscript{14-26} \textit{A. baumannii} was found much more susceptible to antibiotics as compared to \textit{A. baumannii}. Many authors have reported \textit{A. baumannii} as a drug susceptible organism as compared to \textit{A. baumannii}.\textsuperscript{36-38} All isolates of \textit{S. maltophilia} were resistant towards cefazidime, cefepime, imipenem and meropenem. However, it was found that only 26.6\% isolates were resistant to piperacillin-tazobactum and 13.3\% towards gentamicin and amikacin. All isolates were sensitive to cotrimoxazole. These results are in correlation with the results reported by Kalidas et al Nonika et al and Paez et al.\textsuperscript{14,39,40} In the present study, \textit{B. cepacia} showed very good sensitivity towards cefepime (77.7\%), imipenem (66.6\%), meropenem (77.7\%). All isolates were sensitive to piperacillin-tazobactum and cotrimoxazole. All isolates were resistant to polymixin B and colistin as \textit{B. cepacia} shows intrinsic resistant towards these drugs.\textsuperscript{3,3} Kalidas et al and Sidhu et al have also reported similar antibiogram of \textit{B. cepacia} in their studies.\textsuperscript{14,18}

**CONCLUSION**

It may be concluded that growth of NFGNB cannot be overlooked and should be confronted with high index of suspicion. Precise identification of these bacteria upto genus and species level, imperative clinic-microbiological correlation and careful antibiotic prescription shall go a long way in improving clinical outcomes of patients. This study also gives an alarming sign towards high prevalence of multi drug resistant NFGNB. It is noteworthy that as these bacteria also have a great potential to survive in hospital environment therefore, improved antibiotic stewardship, good housekeeping, equipment decontamination, strict protocols for hand washing, isolation procedures need to be implemented to prevent emergence and spread of multidrug resistant NFGNB in health care settings.

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