**Characterization and Antibiotic Sensitivity Pattern of Nonfermenting Gram Negative Bacilli from various Clinical Samples at a Tertiary Care Hospital**

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**A B S T R A C T**

Non-fermenting Gram negative bacilli (NFGNB) are a group of bacteria which are aerobic, non sporing and most commonly they are saprophytes. They are also found as commensals in both man and animals. NFGNB are previously considered as a contaminant but now it is emerged as a major cause for life threatening nosocomial infections and also multidrug resistant organisms. Materials and methods: One year prospective study was conducted and our aim is to characterise the non fermenters from various clinical samples and their antibiogram. 110 isolates from various clinical samples with different age groups were included. The clinical samples like pus, urine, endotracheal aspirates, blood, sputum and body fluids were collected under aseptic precaution and identified by using standard protocol. It includes Gram’s staining, Motility Testing, Catalase test, Oxidase test, OF test and various biochemical reactions. The susceptibility testing was done by Kirby bauer disc diffusion method. A total of 110 NFGNB were isolated and among that nonfermenters, *Pseudomonas aeruginosa* (49%) was the predominant, followed by *Acinetobacter baumanii* (35%), *Acinetobacter lwoffi* (7.3%), *Stenotrophomonas maltophilia* (5.4%), and *Burkholderia cepacia* (2.8%). *Pseudomonas aeruginosa* showed good sensitivity to Polymyxin B (100%), Meropenem and Imipenem (79.6%) followed by Piperacillin tazobactam (72%) and Amikacin (59.3%). *Acinetobacter baumanii* showed 92% sensitivity to Polymyxin B followed by 69% sensitivity to Meropenem and Imipenam. *P.aeruginosa* and *A.baumanii* were the commonest nonfermenters that are isolated in our study. They are associated with various infections like urinary tract infection, blood stream infections, surgical site infections and ventilator associated pneumonia. *P.aeruginosa* showed better sensitivity to following antibiotics: Polymyxin B, Meropenem, Imipenem and Amikacin. Therefore it is essential to identify the nonfermenters and to know their antibiotic sensitivity pattern.

**Keywords**

Non-Fermenting Gram negative Bacilli, Antibiotic sensitivity, *Pseudomonas aeruginosa*, Polymyxin B, Meropenem.

**Article Info**

Accepted: 15 August 2019
Available Online: 10 September 2019

**Introduction**

Non fermenting Gram Negative Bacilli (NFGNB) are a group of organisms which are aerobic and non-sporing that either do not use carbohydrates as a source of energy or degrade them through metabolic pathways other than fermentation. (1)

Most commonly these bacteria occur as
saprophytes in the environment and also found as commensals in the human gut. These are ubiquitous in nature particularly in soil and water. Although frequently considered as contaminants, most of them have emerged as important nosocomial pathogens causing opportunistic infections in immunocompromised hosts. NFGNB accounts for about 15% of all bacterial isolates from a clinical microbiology laboratory.

Non fermenting Gram Negative Bacilli can cause various infections including wound infections, urinary tract infections, meningitis, pneumonia, septicemia, osteomyelitis, etc., Associated with risk factors like immunosuppression, neutropenia, mechanical ventilation, cystic fibrosis, indwelling catheters, invasive diagnostics and therapeutic procedures. Outcome of the disease mainly depends on prolonged hospital stay, broad spectrum antibiotic use and underlying host factors.

In the National Nosocomial Infection Surveillance (NNIS) survey from the Centre for Disease Control and Prevention (CDC), Infections caused by nonfermenters is the fourth most common cause of hospital acquired infections.

This group of non fermenters includes Pseudomonas, Acinetobacter, Stenotrophomonas, Burkholderia, Alcaligenes and Weeksella spp, etc. Among these non fermenters, Pseudomonas aeruginosa is the commonly isolated non fermenter followed by Acinetobacter baumanii. Infection caused by these two nonfermenters is pathogenic for humans whereas infections caused by other species are less frequent.

NFGNB show resistance to antibiotics due to production of extended spectrum β lactamases and metallo β lactamases. Nonfermenters can cause opportunistic as well as nosocomial infections and this nosocomial infections caused by nonfermenters are most commonly observed in debilitated and immune compromised patients.

This study was undertaken to identify the nonfermenters isolated from various clinical samples and their antimicrobial susceptibility pattern.

Materials and Methods

The present study was undertaken at the Department of Microbiology, Vijayanagara Institute of Medical Sciences, Ballari for a period of one year. A total of 110 non fermenters were isolated from various clinical samples such as pus, sputum, urine, blood, ET tube and body fluids were included.

Identification is mainly based on the Gram staining, Motility testing, and growth on Nutrient Agar, Mac Conkey Agar and Blood Agar. The isolates which are catalase positive, oxidase positive or negative, non lactose fermenting colonies on Mac Conkey agar were identified by colony morphology and pigment production. They were inoculated in Triple sugar iron (TSI) agar slope. The colonies which failed to acidify the TSI agar were considered as non fermenters and subjected to the following tests such as Indole, Citrate, Urease, Nitrate reduction, growth at 42°C and sensitivity to Polymyxin B.

The sensitivity test was performed by Kirby-bauer disc diffusion method using commercially available discs (Himedia). The results were interpreted as per the CLSI guidelines. Pseudomonas aeruginosa ATCC 27853 was used as control strain.

Results and Discussion

A total of 110 non fermenters were isolated from various clinical samples. Among the 110
non fermenters, 43 (39%) were isolated from pus, 20 (18.1%) were from urine, 19 (17.2%) from wound swab, 11 (10%) from blood, 9 (8.1%) from sputum, 5 (4.5%) from endotracheal aspirate and 3 (2.7%) from body fluids. (Table: 1)

Majority of isolates of non fermenters were from Surgical ward (40%) followed by ICU (20%), Medicine (14.6%), OBG (10%), Urology (8%), Burns (5.5%), Ortho (4.6%), Paediatrics (3.6%), Otorhinolaryngology (2.7%), TB ward (0.9%), Dermatology (0.9%). (Table: 2)

Among the nonfermenters, *Pseudomonas aeruginosa* (49%) was the predominant isolate followed by *Acinetobacter baumanii* (35%), *Acinetobacter lwoffii* (7.3%), *Stenotrophomonas maltophilia* (5.4%), and *Burkholderia cepacia* (2.8%). (Table: 3)

*Pseudomonas aeruginosa* showed good sensitivity to Polymyxin B (100%), Meropenem and Imipenam (79.6%) followed by Piperacillin tazobactam (72%) and Amikacin (59.3%). *Acinetobacter baumanii* showed 92% sensitivity to Polymyxin B followed by 69% sensitivity to Meropenem and Imipenam.

Among all the isolates maximum resistance was recorded for Gentamycin (61.8%), Cotrimoxazole (60%), followed by Ciprofloxacin (50.9%) and Cefotaxime (47.3%). (Table: 4)

Non fermenting Gram Negative bacilli (NFGNB) are being isolated with increasing frequency from various clinical samples. In recent years, the failure to treatment due to their multidrug resistance has led to the interest to carry out this study. In the present study out of 110 non fermenters, 43(39%) were isolated from pus, 20 (18.1%) from urine, 19(17.2%) wound swab, 11(10%) blood, 9(8.1%) sputum, 5 (4.5%) from endotracheal aspirate and 3(2.7%) from body fluids. In a study conducted by Gokale et al they reported that 58.4% non fermenters were isolated from pus/wound discharge followed by 23% from blood, 8.2% from urine, 4.5% from sputum and 2.3% from pleural fluid. (2)

A study conducted by Kirtilaxmi et al also stated that the isolation rate of pus was 21%, 11% from urine, 7% from blood and 17% from tracheal aspirate. (3)

In another study conducted by Kalidas et al they observed that the isolation rate was 27.9% from pus sample, 18.4% from tracheal aspirate, 16.4% from sputum and16.4% from bloodand15.9% from urine. (12)

In the present study maximum number of non fermenters were isolated from Surgical wards (28.2%) followed by Intensive care unit (20%) and Medicine ward (14.6%). Similar findings were also reported by Anupurba et al., that higher prevalence rate of non fermenters was observed in surgery wards (29.9%).

The second highest prevalence observed in this study was intensive care units (20%) followed by Medical ward (14.6%).

In another study conducted by Keertilaxmi B etal they also reported that the isolation of NFGNB from intensive care units was 37%. (3)

Among the non fermenters in recent years there are outbreaks of *Burkholderia cepacia* complex septicaemia have been documented worldwide in intensive care units (ICUs), oncology units and renal failure patients. (13)

In this present study, the commonest NFGNB isolated were *Pseudomonas aeruginosa* 54(49%) followed by *Acinetobacter baumanii* 39(35%), *Acinetobacter lwoffii* 8(7.3%), *S.maltophilia* 6 (5.4%), and *Burkholderia cepacia* 3(2.8%).
**Table 1** Nonfermenting gram negative bacilli from various clinical samples

| Clinical Samples | No. of Isolates | % of Isolates |
|------------------|----------------|--------------|
| Pus              | 43             | 39%          |
| Urine            | 20             | 18%          |
| Wound swab       | 19             | 17%          |
| Blood            | 11             | 10%          |
| Sputum           | 9              | 8.5%         |
| ET tube          | 5              | 4.6%         |
| Body fluids      | 3              | 2.8%         |
| **Total**        | **110**        | **100%**     |

**Table 2** Distribution of clinical isolates

| Speciality              | Clinical Isolates | Percentage (%) |
|-------------------------|-------------------|----------------|
| Surgery                 | 32                | 29.1           |
| Intensive care unit     | 22                | 20             |
| Medicine                | 16                | 14.6           |
| OBG                     | 11                | 10             |
| Urology                 | 9                 | 8.2            |
| Burns                   | 6                 | 5.4            |
| Ortho                   | 5                 | 4.6            |
| Paediatrics             | 4                 | 3.6            |
| Otorhinolaryngology     | 3                 | 2.7            |
| TB ward                 | 1                 | 0.9            |
| Dermatology             | 1                 | 0.9            |
| **Total**               | **110**           | **100%**       |

**Table 3** Speciation of nonfermenting gram negative bacilli

| Organisms               | No. of Isolates | Percentage (%) |
|-------------------------|-----------------|----------------|
| *Pseudomonas aeruginosa*| 54              | 49             |
| *Acinetobacter baumanii*| 39              | 35             |
| *Acinetobacter lwaffi*  | 8               | 7.3            |
| *Stenotrophomonas maltophilia* | 6 | 5.4 |
| *Burkholderia cepacia*  | 3               | 2.8            |
| **Total**               | **110**         | **100%**       |
Table 4: Antimicrobial susceptibility pattern of nonfermenting gram negative bacilli

| Antibiotics       | P. aeruginosa (n=54) | B. cepecia (n=3) | A. baumannii (n=39) | A. Iwofi (n=8) | S. maltophilia (n=6) |
|-------------------|----------------------|------------------|---------------------|---------------|----------------------|
|                   | S   %      | S   %      | S   %      | S   %      | S   %      |
| Gentamicin        | 22  40.7   | -       | 18   46   | 4   50    | -       |
| Amikacin          | 32  59.3   | -       | 26   66   | 6   80    | 2   33.3  |
| Ciprofloxacin     | 23  42.6   | 1   33.3  | 18   46   | 4   50    | 6   100   |
| Ofloxacin         | 23  42.6   | 1   33.3  | 18   46   | 4   50    | 6   100   |
| Ceftazidime       | 30  56    | 1     33.3 | 20   51   | 6   80    | -       |
| Cefotaxime        | -     -    | 1     33.3 | 20   51   | 6   80    | -       |
| Piperacillin-      | 39  72     | 1     33.3 | 26   66   | 8   100   | 1   16.7  |
| tazobactam        |          |        |        |          |          |
| Cotrimoxazole     | -     -    | 3     100 | 20   51   | 4   50    | 6   100   |
| Imipenem          | 43  79.6   | 2     66.7 | 27   69   | 8   100   | -       |
| Meropenem         | 43  79.6   | 2     66.7 | 27   69   | 8   100   | -       |
| Polymyxin B       | 54  100    | -     -    | 36   92   | 8   100   | 6   100   |

*S-Sensitive and *- Not tested

A study conducted by Kalidas et al., also reported that *Pseudomonas* aeruginosa (50.2%) was the predominant isolate followed by *A. baumannii* (24.9%), *A. Iwofi* (5.5%), *S. maltophilia* (3%) and *Burkholderia cepacia* (7%). In the present study *Pseudomonas spp* and Acinetobacter spp were the commonest NFGNB isolated which correlates with other studies. (3,16,17)

Because of the prevalence of high intrinsic resistance of different NFGNB to different antimicrobial agents in recent years, the absolute identification of non fermenters and their resistance pattern should be performed at microbiology laboratory. As this can be an important guide for the clinicians for appropriate selection of empiric therapy. In this study, the antimicrobial susceptibility pattern of *P. aeruginosa* showed 54 (100%) sensitivity to Polymyxin B, 43 (79.6%) sensitivity to Imipenem and Meropenem followed by Piperacillin tazobactum 39 (72%), Amikacin 32 (59.3%), Ceftazidime 30 (56%), Ciprofloxacin and Ofloxacin 23 (42.6%) and Gentamycin 22 (40.7%) which correlates with

the study conducted by Grewal et al., (18) and Kaur et al., (19).

A Study from Gokale et al., also reported that most of the isolates of *Pseudomonas aeruginosa* were sensitive to Meropenem (96.2%), followed by Ciprofloxacin (50.4%) and Amikacin (49.5%).

A study conducted by Nautiyal et al. reported that all the isolated *Pseudomonas aeruginosa* were sensitive to Polymyxin B (14) which correlates with our study as well. Kirtilaxmi et al. reported the sensitivity of *Pseudomonas aeruginosa* were ceftazidime (60%), Gentamycin (65%), Piperacillin-tazobactum (73.3%), Imipenem (80%), Amikacin (83.3%), Ciprofloxacin (58.3%) (3) It is similar to our study.

In the present study, the isolates of *A. baumannii* showed 27 (69%) sensitivity to Meropenem and Imipenem followed by Amikacin and Piperacillin tazobactum 26 (66%), Ceftazidime and Cotrimoxazole 20 (51%), Gentamycin and Ciprofloxacin and
Ofloxacin 18 (46%) each respectively. Most of the isolates were sensitive to Polymyxin B 36 (92%). These results are similar to the study conducted by Nautiyal et al., (14)

In the present study, all the isolates of A.lwoffi were sensitive to Imipenem, Meropenem and Piperacillin tazobactum 8(100%) followed by Cefotaxime, Ceftazidime and Amikacin 6 (80%), Gentamycin, Cotrimoxazole and Ciprofloxacin and Ofloxacin 4 (50%), Polymyxin B (100%). Similar to the present study, another study conducted by Nautiyal etal reported that all the isolated A.lwoffi were 100% sensitive to Polymyxin B. (14)

In our study, among the isolated S.maltophilia majority were sensitive to Cotrimoxazole, Ciprofloxacin and Ofloxacin and Polymyxin B 6 (100%), followed by Amikacin 2(33.3%) and Piperacillin tazobactum1 (16.7%). These results are similar to another study conducted by Nautiyal etal They reported that all the isolated S.maltophilia were 100% sensitive to polymyxin B. Similar results were also shown in another study conducted by Deepak etal They reported that all the isolated S.maltophilia showed 100% sensitivity to Ciprofloxacin and Cotrimoxazole and 33.3% to piperacillin tazobactum and 16.67% to Gentamycin (15). S. maltophilia is intrinsically resistant to most β lactams, including carbapenems. (13) In our study, among the isolates of Burkholderia cepacia (3), all were sensitive to Cotrimoxazole 3(100%), followed by Imipenem and Meropenem 2(66.7%), Ciprofloxacin, Ofloxacin, Cefotaxime and Ceftazidime 1(33.3%). In contrast to the present study, Kalidas et al. showed the 92.8% sensitivity to Imipenem and Cotrimoxazole, 85% to Ceftazidime and Ciprofloxacin and 57% to Piperacillin tazobactum. (12)

Burkholderia cepacia show intrinsic resistance to aminoglycosides and polymyxins due to the presence of inducible chromosomal β-lactamases and altered penicillin-binding proteins leads to resistance to β-lactams.

They also mediate resistance to chloramphenicol, trimethoprim and fluoroquinolones through its antibiotic efflux pumps. (13)

Observations from the present study showed that aerobic NFGNB which are usually considered as contaminants are now emerging as important nosocomial pathogens. Different antimicrobial susceptibility pattern and multidrug resistance by nonfermenters cause difficulty in treating the infections. ESBL and MBL production by these organisms lead to high morbidity and mortality and we have only option of treating them by potentially toxic drugs like Colistin and Polymyxin B. All the health care institutions should have their own antimicrobial policy, regular surveillance and infection control protocols to avoid high incidence of resistant non fermenters.

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**How to cite this article:**

Narayan Shrihari and Iswarya M 2019. Characterization and Antibiotic Sensitivity Pattern of Nonfermenting Gram Negative Bacilli from various Clinical Samples at a Tertiary Care Hospital. *Int.J.Curr.Microbiol.App.Sci.* 8(09): 1502-1508.

doi: [https://doi.org/10.20546/ijcmas.2019.809.172](https://doi.org/10.20546/ijcmas.2019.809.172)