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**Original Research Article**

# Current Challenges in Antibiotic resistance: Non-fermenting Gram-negative bacilli with Special References to Pseudomonas species and Acinetobacter species

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**Abstract**
**Background:** *Non-fermenting Gram-negative bacilli (NFGNB) have emerged as a major cause of health care-associated infections and are innately resistant to many antibiotics. The aim of this study was to determine the prevalence of NFGNB isolated from various clinical specimens and evaluate their antimicrobial resistance profiles.*
**Materials and Methods:** *A prospective, cross-sectional, laboratory-based study was conducted from January 2017 to January 2020 at the Department of Microbiology, Chitwan Medical College and Teaching Hospital, Nepal. NFGNB were isolated from a different clinical specimens, plated on Nutrient agar, MacConkey agar and Blood agar and incubated aerobically at 37°C for 18-24 h. Identification was done on the basis of colony morphology, Gram stain, catalase test, oxidase test and standard biochemical tests. Antimicrobial susceptibility test was performed using the Kirby-Bauer disc diffusion method using commercially available discs on Mueller-Hinton agar. Data was analyzed using SPSS IBM version 20.*
**Result:** *A total 24550 samples were studied; where 4910 (20.00%) show bacterial growth and 982 (4.00%) were NFGNB. Majority 736 (74.95%) of NFGNB were isolated from hospitalized patients. Most of them were recovered from sputum 392 (39.22%) and 245 (24.95%) urine sample. Pseudomonas species 540 (54.99%) was the leading pathogens followed by Acinetobacter species 403 (41.04%). NFGNB showed resistance to several antibiotics tested. Overall, Colistin (100%) and Polymyxin B (91-93%) was the most effective antimicrobial agent.*
**Conclusion:** *Pseudomonas species and Acinetobacter species are the leading NFGNB; mostly recovered from respiratory specimens and are resistance to several antibiotics. Timely identification of NFGNB and monitoring their susceptibility patterns will help in proper management of infections.*
**Keywords:** *Non-fermenting Gram-negative bacilli (NFGNB), Pseudomonas, Acinetobacter, Antimicrobial susceptibility test, Piperacillin-Tazobactum, Carbapenems, Polymyxin B, Colistin.*

## Introduction

Non-fermenting gram-negative bacilli (NFGNB) are a taxonomically diverse group of aerobic, non-sporing, bacilli that either do not utilize glucose as a source of energy or utilize it oxidatively.[1] They are saprophytes and previously considered as contaminants or commensal of little significance.[2] Commonly NFGNB like, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia* and *Burkholderia cepacia* complex are responsible for human infection.[3] However, recent literature shows that these organisms are now associated with life-threatening infections such as pneumonia, ventilator associated pneumonia (VAP), meningitis, urinary tract infection, surgical site infection, septicemia, wound infection, osteomyelitis etc.[2] Predisposing factors for such infection are abusive use of wide spectrum antimicrobial agents, use of immunosuppressant substances, diabetes mellitus, chronic renal failure, malignancy, hepatitis, cystic fibrosis, pneumonia, presence of intravenous or urinary catheters, mechanical ventilation, surgical procedures, stay in intensive care unit (ICU), history of recent hospitalization.[4] They can be recovered from hospital environment, commonly cause device related infections, are often resistant to disinfectants and have the potential to spread from patient-to-patient via fomites or the hands of health care personnel.[5,6] The isolation rates of NFGNB from clinical specimens vary from 2.18 to 45.9%.[7] Recently there has been a tremendous interest in these organisms as they are being isolated from clinical specimens with increasing frequency.[8] Worldwide the public health risk of NFGNB is growing rapidly due to their role in nosocomial infections. [9]

NFGNB are innately resistant to many antibiotics and are known to produce extended spectrum ß-lactamases, Amp C β-lactamases, and metallo β-lactamases.[10,11] In recent years due to the liberal and empirical use of antibiotics, NFGNB have emerged as important health care associated pathogens.[12-14] The emergence in causing infection is of great concern to clinicians, spreading of resistance to commonly used antibiotics in humans has posed adverse impact on morbidity and mortality due to diseases caused by resistant bacteria.[15] Also the antimicrobial resistance exhibited by the NFGNB creates an epidemiologic niche for these pathogens that facilitates colonization and super infection in antibiotic-treated patients.[16-19] Antibiotic resistance among NFGNB are mostly due to antimicrobial inactivating enzymes, reduced access to bacterial targets and point mutations that change targets or cellular functions.[20]

*Pseudomonas* species and *Acinetobacter* species are the most common NFGNB isolated. Carbapenems are the last resort, but Carbapenems resistance is on rise among both these species.[15] Hence, present study was done to find the prevalence of NFGNB in various clinical specimens and evaluate their antimicrobial resistance profiles.

## Method

A prospective, cross-sectional, laboratory-based study was conducted from January 2017 to January 2020. The study conducted in the Microbiology Department of Chitwan Medical College and Teaching Hospital, Nepal. A total of 24550 specimens of Sputum, Urine, ET tube, Pus, Blood and Body fluid were collected from patients and plated on Nutrient agar, MacConkey agar and Blood agar and incubated aerobically at 37°C for 18-24 h. Identification was done on the basis of colony morphology, Gram stain, catalase test, oxidase test and standard biochemical tests. Fermentation test for Glucose, Lactose, Xylose, Mannitol and Maltose (Hugh and Leifson's media), Lysine and Ornithine decarboxylase and Arginine dihydrolase activity test etc. were done for isolation of the Non-Fermentative Gram Negative Bacilli. [21]

Antimicrobial susceptibility test was performed by way of Kirby-Bauer disc diffusion method; all isolates were swabbed on Muller-Hinton agar and commercially available discs were placed and

incubated aerobically at 37°C for 18-24 h. All isolates were tested against the following antibiotics: Amikacin (30µg), Gentamycin (10µg), Ciprofloxacin (5µg), Cefotaxime (30µg), Piperacillin-Tazobactum (100/10µg) Cotrimoxazole (25µg), Imipenem (10µg), Meropenem (10µg), Polymyxin-B (300 units), Colistin (10µg). Results were interpreted according to the Clinical Laboratory Standards Institute (CLSI) guidelines.[22] Quality control strains *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were utilized in biochemical tests and antimicrobial susceptibility testing. The study was approved by Institutional Review Committee of Chitwan Medical College (CMC-IRC), under reference number CMC-IRC/2073/074-78. Data was analyzed using SPSS IBM version 20.

## Result

### Demographic Distribution and Identification of Bacterial Isolates

A total of 24550 specimens were studied for culture and sensitivity among that, 4910 (20.00%) were positive for bacterial growth and 982 (4.00%) were NFGNB. Out of 4910 positive samples 2946 (60.00%) were from males and 1964 (40.00%) from females. Majority 736 (74.95%) of NFGNB were isolated from inpatient department. Most of them were recovered from sputum 392 (39.92%) and 245 (24.95%) urine sample (Table-1). The isolate *Pseudomonas* species 540 (54.99%) was most common, followed by *Acinetobacter* species 403 (41.04%), remaining 39 (3.97%) were other NFGNB (Table-2).

**Table1:** Distribution of NFGNB in different clinical specimen

| Specimen | No. of specimen | Bacterial growth | NFGNB | |
|---|---|---|---|---|
| | | | Frequency | Percent (%) |
| Urine | 14825 | 2980 | 245 | 24.95 |
| Blood | 4050 | 350 | 30 | 8.15 |
| Sputum | 2450 | 650 | 392 | 39.92 |
| Pus | 1945 | 602 | 105 | 12.22 |
| Body fluid | 810 | 40 | 10 | 0.50 |
| ET tube | 470 | 288 | 200 | 14.26 |
| **Total** | **24550** | **4910** | **982** | **100** |

**Table 2:** Individual NFGNB organism isolated from different clinical specimen

| Specimen | Organism isolated | | | Total |
|---|---|---|---|---|
| | Pseudomonas species No. (%) | Acinetobacter species No. (%) | Other NFGNB No. (%) | No. (%) |
| Sputum | 275 (28.00) | 100 (10.19) | 17 (1.73) | 392 (39.92) |
| Urine | 134 (13.64) | 100 (10.19) | 11 (1.12) | 245 (24.95) |
| ET tube | 80 (8.15) | 116 (11.81) | 4 (0.40) | 200 (20.37) |
| Pus | 42 (4.27) | 60 (6.11) | 3 (0.31) | 105 (10.69) |
| Blood | 6 (0.61) | 20 (2.04) | 4 (0.40) | 30 (3.05) |
| Body fluid | 3 (0.31) | 7 (0.71) | 0 (0.00) | 10 (1.02) |
| **Total** | **540 (54.99)** | **403 (41.04)** | **39 (3.97)** | **982 (100.00)** |

### Antimicrobial Resistance Pattern

The isolates *Pseudomonas* species showed highest percentage of resistance to be 74.19% against Cotrimoxazole, followed by 55.74% Gentamicin, 55.56% Cefotaxime, 39.26% Ciprofloxacin and Piperacillin-Tazobactum. Imipenem, Meropenem, Amikacin, Polymyxin-B and Colistin were the most efficient antibiotics tried, with resistance rate of 30.00%, 35.18%, 13.89%, 8.89% and 0.00%, respectively. The isolates *Acinetobacter* species showed highest percentage of resistance to be 91.56% against Cefotaxime, 91.32% Cotrimoxazole, 80.40% Ciprofloxacin, 76.18% Piperacillin-Tazobactum, 62.03% Gentamicin, 55.08% Imipenem, 50.12% Meropenem, 47.89% Amikacin, and 6.95% Polymyxin-B; whereas all

the isolates were sensitive to Colistin. The examination of the antimicrobial susceptibility pattern of these isolates demonstrated that NFGNB showed high rates of multidrug resistance, being resistance to most third-generation cephalosporins and other antibiotics as well. NFGNB isolates with antimicrobial resistance pattern are shown in Table-3.

**Table 3:** Antimicrobial resistance profiles

| Antimicrobial agent | Bacterial isolates | |
|---|---|---|
| | *Pseudomonas* species (n=540) | *Acinetobacter* species (n=403) |
| | Resistance No (%) | Resistance No (%) |
| Amikacin | 75 (13.89) | 193 (47.89) |
| Gentamicin | 301(55.74) | 250 (62.03) |
| Ciprofloxacin | 212 (39.26) | 324 (80.40) |
| Cefotaxime | 300 (55.56) | 369 (91.56) |
| Piperacillin-Tazobactum | 212 (39.26) | 307 (76.18) |
| Cotrimoxazole | 406 (74.19) | 368 (91.32) |
| Imipenem | 162 (30.00) | 222 (55.08) |
| Meropenem | 190 (35.18) | 202 (50.12) |
| Polymyxin-B | 48 (8.89) | 28 (6.95) |
| Colistin | 0 (0.00) | 0 (0.00) |

**Discussion**

NFGNB were previously considered to be contaminants but have now emerged as significant hospital-associated pathogens. Pseudomonas species and Acinetobacter species are known to be the regular nosocomial pathogens. In the present study isolation rate of NFGNB was 4.00%, which is similar to the results obtained by Benachinmardi *et al.*[23] (3.58%) and is lower than the report of Rit *et al.*[24] (12.18%) and Mahajan *et al.*[25] (12.40%) where the isolation rate is calculated from the total specimen. In this study, the isolation rate out of positive culture was 20.00% which is higher than the study of Grewal *et al.*[26] (11.6%) and lower than the study of Nautiyal *et al.*[27] (25.6%), Sharma *et al.*[28] (25.6%) and Bhargava *et al.*[29] (29.62%). These variations in the prevalence of NFGNB in different hospital might be due to variations in infection control practice as well as circulation of these pathogens in respective health care setting. The present study showed that, the majority of NFGNB were isolated from male patients (60.00%), which is lower than the results of Maniyan *et al.*[30] (65.4%) and Benachinmardi *et al.*[23] (68%). Current study showed that, the majority of the non-fermenters were isolated from sputum 39.92% followed by urine 24.95% and ET tube 20.37%. This result slightly complies with the study of Nautiyal S *et al.*[27] where majority of the non-fermenters were isolated from respiratory specimen 42.3% but differs from the study of Maniyan *et al.*[30], Rit *et al.*[24], Bhatnagar *et al.*[31], Benachinmardi *et al.*[23] and Bhargava *et al.*[29] where the majority of the non-fermenters were isolated from pus.

In the present study, out of 982 NFGNB, 54.99% of the isolates were *Pseudomonas* species and 41.04% isolates were *Acinetobacter* species which was similar to the study of Maniyan *et al.*[30] where *Pseudomonas aeruginosa* isolates were 51.8% and *Acinetobacter baumanii* were 40.00%. Similarly, Rit *et al.*[24] showed that, *Pseudomonas aeruginosa* were 53.7% and *Acinetobacter baumanii* 30.3% which was close to our study. Likewise, Mahajan *et al.*[25] isolated 54.54% *Pseudomonas aeruginosa* and 41.8% *Acinetobacter baumanii*. Bhargava *et al.*[29] showed that isolates of *Pseudomonas aeruginosa* were 56.94% which was similar to our study and 20.83% were *Acinetobacter baumanii* which contrast with our study. Similarly, Bhatnagar *et al.*[31] isolated 83.33% *Pseudomonas* species and 16.67% *Acinetobacter* species which contrast with our study. The present study showed that, the majority (74.95%) of the NFGNB were isolated from IPD which is similar to the report of Benachinmardi *et al.*[23] Similarly, the study by

Maniyan *et al.* [30] showed majority of the isolates were isolated from surgery and intensive care units but overall maximum number the NFGNB were from isolated from IPDs which comply with our study.

Resistance patterns among bacterial pathogens may vary from country to country and also within the same country, over time. *Pseudomonas* species isolates in our study exhibited higher sensitivity to Colistin 100% and Polymyxin-B 91.11%, which was similar to the report of Maniyan *et al.*[31] where sensitivity to Polymyxin-B was 100% and Rit *et al.*[24] where sensitivity to Colistin was 100%. In addition the sensitivity of the *Pseudomonas* species to Amikacin was 86.11%, which contrast with the study of Grewal *et al.*[26] 46.8%. As well the sensitivity of the *Pseudomonas* species to Gentamycin was 44.26%; slightly lower 38.4% has been reported by Grewal *et al.*[26]. Furthermore the sensitivity pattern of Imipenem, Meropenam, Piperacillin-Tazobactum, Ciprofloxacin in present study resembles with the other study Mahajan *et al.*[25] and Simgamsetty *et al.*[32] *Pseudomonas* species was least sensitive towards Cotrimoxazole (25.32%); whereas higher sensitivity 41.27%, 44.73% and 100% have been reported by Mahajan *et al.*[25], Nautiyal *et al.*[27]and Maniyan *et al.*[30] respectively. The finding of present study showed that, *Acinetobacter* species exhibited higher sensitivity to Colistin (100%) and Polymyxin- B (93.05%), which is similar to the report of Maniyan *et al.*[30] and Rit *et al.*[24] Moreover the sensitivity of the organism to Amikacin and Gentamycin in current study was 52.11% and 37.97% which is in between the finding of Rit *et al.*[24] In addition the sensitivity of the organism to Imipenam and Meropenam in current study was 44.92% and 49.88%. In present study *Acinetobacter* species exhibited minimum sensitivity towards Cotrimoxazole 8.68% which comply with the study of Nautiyal *et al.*[27] 9.8% and contrast with Maniyan *et al.*[30] 55.6%.

## Conclusion

NFGNB though regarded as contaminants are important bacteria causing wide range of nosocomial infections. This study showed 4.00% prevalence of NFGNB. *Pseudomonas* species 540 (54.99%) and *Acinetobacter* species 403 (41.04%) were the most commonly isolated NFGNB from the clinical specimens. The isolation of MDR *Pseudomonas* species and MDR *Acinetobacter* species in current study raises the concern of rapidly emerging antibiotic resistance in this group of bacteria in Chitwan, Nepal. On in vitro susceptibility testing Colistin (100%) and Polymyxin B (92-93%) was the most effective antimicrobial agents against NFGNB. Timely identification of non-fermenters and monitoring their antibiotic susceptibility patterns are suggested for effective management of infections and limitation of the emergence of MDR.

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## Authors' Contributions

Chaudhary N K contributed to the concept and design of the study, reporting of test result, collection of data, data analysis and interpretation of data, drafting the final version of manuscript. Dhakal S contributed to the collection and processing of specimen as well as helped in preparation of manuscript.

## Conflicts of Interest

There are no conflicts of interest regarding the publication of this article.

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