

Original Research Article

## Identification and Antimicrobial Susceptibility Patterns of Non-Fermentative Gram Negative Bacilli in a Tertiary Care Hospital: Our Experience

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Received: 12/05/2016

Revised: 16/07/2016

Accepted: 18/07/2016

### ABSTRACT

**Background:** Non-Fermentative Gram Negative Bacilli (NFGNB) are a group of aerobic non-spore forming gram negative bacilli that are either incapable of utilizing carbohydrates as a source of energy or are able to degrade them via oxidative rather than fermentative pathway. Being saprophytic in nature, they have been increasingly reported from the cases of nosocomial infections. The current study was taken up to characterize NFGNB and also to establish their antibacterial susceptibility pattern isolated from clinical samples of patients who were admitted in Intensive Care Unit (ICU).

**Materials and methods:** A total of 260 strains were included in the study that grew from various clinical specimens like pus, urine, sputum, blood, Bronchoalveolar Lavage (BAL), Endotracheal aspiration, Pleural fluid, Central line tip, Wound swab, etc. in culture collected from patients who were admitted in the ICU's at NRI General Hospital, Chinakakani, Guntur District, A.P. The study was carried out from July 2015 to December 2015. Characterization and Antimicrobial Susceptibility testing was carried out using the VITEK-2 Compact system (Biomérieux, France).

**Results:** Out of the 260 strains, 74 strains were isolated from Blood, 71 from tracheal aspirate, 50 from pus/wound infections, 36 from urine, 25 from sputum, 2 from Bronchoalveolar lavage (BAL), one from central line tip and one from pleural fluid. *Pseudomonas aeruginosa* was found to be the most common isolate accounting for 151 (58 %) followed by *Acinetobacter baumannii* 94 (36.1%), *Burkholderia cepacia* (1.92%), *Acinetobacter lwoffii* (1.15%), *Pseudomonas stutzeri* (1.15%) and one each of *Achromobacter xylosoxidans* (0.38%), *Sphingomonas paucimobilis* (0.38%) were isolated. Most of the isolates of *Pseudomonas aeruginosa* were found to be sensitive to Cefoperazone/Sulbactam (90%), Imipenem (70.2%), Meropenem (68.1%), Aztreonam (66.6%), Amikacin (66.6%), Netillin (66.6%), Ciprofloxacin (65.4%), Cefepime (61.9%), Gentamicin (57.5%) and Levofloxacin (55.5%).

**Conclusion:** Prevalence of NFGNB varies between communities, hospitals in the same community and among different patient populations in the same hospital. The pathogens have a great potential to survive in a hospital environment so implementation of stringent antibiotic stewardship programs and strict infection control practices will be required to prevent or slow down their emergence and spread.

**Key words:** NFGNB, Characterization, Nosocomial infections, VITEK-2 Compact.

### INTRODUCTION

Nonfermenters are being isolated from various clinical specimens. Although frequently considered as contaminants, the pathogenic potential has been proved

beyond doubt by their frequent isolation from clinical material and their association with disease. [1-3] 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 medical personnel. [4-6] Non-Fermenting Gram Negative Bacteria (NFGNB) associated with different nosocomial infections are becoming increasingly resistant to commonly used antimicrobial agents. [6,7] NFGNB are known to account for about 15% of all bacterial isolates from a clinical microbiology laboratory. [1,3,6,8,9]

In the recent years due to the liberal and empirical use of antibiotics, NFGNB have emerged as important health care associated pathogens. [1,3,10] Several automated systems are available for the identification and susceptibility of the clinically important bacteria. [3,11,12] More recently the new VITEK-2 Compact system (Biomérieux, France) has been introduced. The VITEK-2 compact system detects metabolic changes by fluorescence based methods which facilitate the identification of gram negative bacteria within 6 hours. This system monitors the kinetics of bacterial growth and calculates Minimum Inhibitory Concentrations (MIC) using a unique algorithm. [3,9,13]

The current study was taken up to characterize NFGNB and also to establish their antibacterial susceptibility pattern isolated from clinical samples of patients in Intensive Care Unit (ICU).

## MATERIALS AND METHODS

A total of 260 strains were included in the study that grew in culture from various clinical specimens like pus, urine, sputum, blood, Bronchoalveolar Lavage (BAL), Endotracheal aspiration, Pleural fluid, Central line tip and Wound swab that came for culture and Sensitivity testing to the Clinical Microbiology Laboratory of NRI General Hospital, Chinakakani. Only the clinical samples that were collected from the patients who were admitted in various ICU's of NRI General Hospital, Chinakakani, Guntur District, A.P. during the period from July 2015 to December 2015 were included in the study. Only Non-

Lactose Fermenting (NLF) Gram Negative bacteria that grew well in Macconkey's agar were considered for further characterization.

### Identification of NFGNB

**A. Inoculum Preparation:** From the isolated colonies grown on the media, a bacterial suspension was prepared in 3 mL of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) in a 12x75 mm clear plastic (polystyrene) test tube. The turbidity of the suspension was adjusted to a McFarland standard of 0.5 with the help of a VITEK-2 Densi Check instrument. [3,12] The time between the preparation of inoculum and filling of the card was always less than 30 min. Identification with the VITEK-2 compact system was performed using a Gram Negative (GN) card according to the Manufacturer's instructions. The 64 well plastic GN card contains 41 tests including 18 tests for sugar assimilation, 18 tests for sugar fermentation, 2 decarboxylase tests and 3 miscellaneous tests (for urease, utilization of malonate and tryptophan deaminase).

**B. Quality control:** The Vitek-2 Compact machine was validated using the standard strains as per the manufacturer's instructions. *Acinetobacter baumannii* ATCC BAA-747, *Aeromonas hydrophila* ATCC 35654, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used. During the study period, the control strains were checked at regular intervals.

### Antimicrobial Susceptibility Testing

Antimicrobial Susceptibility testing with the VITEK-2 compact system was performed using an AST N281 card according to the Manufacturer's instructions. The VITEK-2 AST N281 susceptibility card is intended for use with the VITEK-2 systems in clinical laboratories as an in-vitro test to determine the susceptibility of clinically significant aerobic gram negative bacilli to

antimicrobial agents. [14,15] Antibiotics tested in AST N281 card included Levofloxacin, Gentamicin, Cefepime, Meropenem, Imipenem, Ticarcillin/Clavulanic acid, Doripenem, Ceftazidime, Cefoperazone/Sulbactam, Amikacin, Ciprofloxacin, Minocycline, Tigecycline, Colistin, Trimethoprim/ Sulfamethoxazole (Cotrimoxazole), Cefotaxime, Piperacillin/Tazobactam, Cefuroxime, Ceftriaxone, Tobramycin. The cards were filled with inoculums (Prepared by transferring 200µL of culture suspension from the 0.5 McFarland culture suspension used for filling the identification cards into a fresh 3mL sterile saline solution obtaining a final turbidity of  $8 \times 10^6$  cfu/mL) in the filling chamber. The VITEK-2 System automatically processes the antimicrobial susceptibility cards until MIC's are obtained. The VITEK-2 compact system subsequently corrects, where necessary for MIC's or clinical category in accordance with the internal database of possible phenotypes for microorganism antimicrobial agent combinations. [14,15]

## RESULTS

Table 1: Specimen wise distribution of NFGNB

SPECIMEN	TOTAL	PERCENTAGE (%)
Blood	74	28.4 %
Tracheal aspirate	71	23.7 %
Pus/Wound Swab	50	19.2 %
Urine	36	13.8 %
Sputum	25	9.61 %
BAL	2	0.77 %
Pleural Fluids	1	0.38 %
Centerline Tip	1	0.38 %

A total of 260 strains collected from various clinical specimens were tested in VITEK-2 compact systems for identification of the isolate and for its antimicrobial susceptibility patterns. Out of the 260 strains, 74 strains were isolated from Blood samples, 71 from tracheal aspirates, 50 from pus/wound infections, 36 from urine, 25 from sputum, 2 from Bronchoalveolar lavage (BAL) and one each from central line tip and pleural fluid.

The NFGNB isolated from various clinical samples are presented in Table 1. *Pseudomonas aeruginosa* was the

commonest isolate accounting for 151 (58 %) followed by *Acinetobacter baumannii* 94 (36.1%), *Burkholderia cepacia* (1.92%), *Acinetobacter lwoffii* (1.15%), *Pseudomonas stutzeri* (1.15%) and one each of *Achromobacter xylosoxidans* (0.38%), *Sphingomonas Paucimobilis* (0.38%) were isolated.

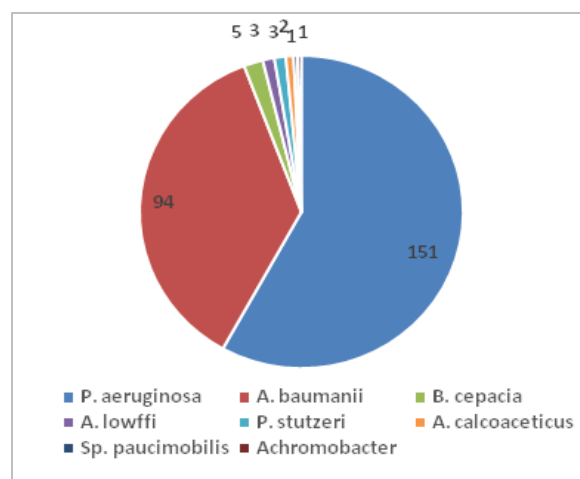


Figure 1: Species wise distribution of NFGNB

The sensitivity pattern of the NFGNB isolated is presented in Table 3. Most of the isolates of *Pseudomonas aeruginosa* were sensitive to Cefoperazone/Sulbactam (90%), Imipenem (70.2%), Meropenem (68.1%), Aztreonam (66.6%), Amikacin (66.6%), Netillin (66.6%), Ciprofloxacin (65.4%), Cefepime (61.9%), Gentamicin (57.5%) and Levofloxacin (55.5%). *Acinetobacter baumannii* isolates showed higher rate of resistance than *Pseudomonas aeruginosa* isolates. Out of 94 *Acinetobacter baumannii* isolates 77.7 % were sensitive to Colistin, 75% are sensitive to Cefoperazone/Sulbactam followed by Tigecycline (58.3%) and Trimethoprim / Sulfamethoxazole (53.3%). *Burkholderia cepacia* were found to be sensitive to Levofloxacin (100%), Meropenem (100%), Aztreonam (100%), Doripenem (100%), tigecycline (100 %) followed by Cefepime (75%), to Cefoperazone/ Sulbactam (75%). *Acinetobacter lwoffii* isolates were sensitive to Amikacin (75%), Ciprofloxacin (75%) and Imipenem (75%). *Acinetobacter*

*calcoaceticus* isolates showed sensitive to Amikacin (100%), Tigecycline (100%), Colistin (100%), Ertapenem (100%) followed by Cefoperazone/Sulbactam (50%). *Achromobacter xylosoxidans* was a

sensitive strain Sensitive to almost all antibiotics tested. While, *Sphingomonas paucimobilis* showed resistance to all the antibiotics tested.

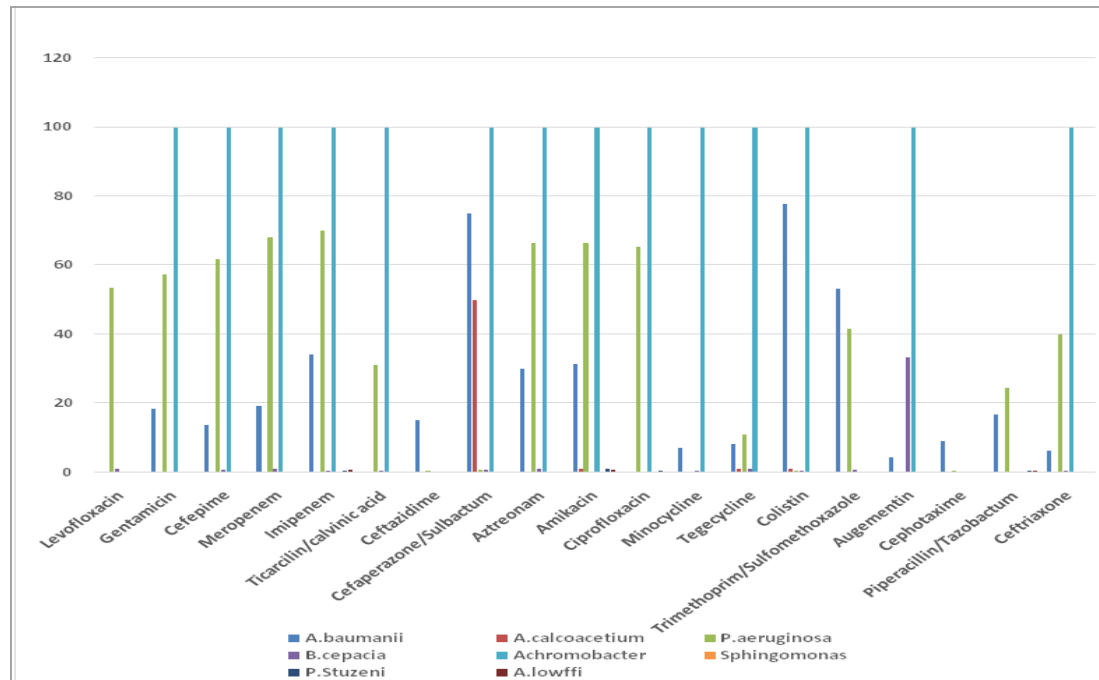


Figure 2: Sensitivity pattern of the Non-Fermenting Gram negative bacilli isolated in present study

## DISCUSSION

Non fermenting Gram negative bacilli were considered to be as a contaminants in the past but new emerges of the strain has shown an important impact on the health care facilities. [6,16]

In the present study *Pseudomonas aeruginosa* 151 (58%) was isolated predominantly followed by *Acinetobacter* spp, *Burkholderia cepacia*, *Pseudomonas stutzeri*, *Achromobacter xylosoxidans* and *Sphingomonas paucimobilis* which is in similarity with the study conducted by D Vijaya et al. [17] *Pseudomonas aeruginosa* was the commonest non-fermenter followed by *Acinetobacter* spp and this is in concordance with other studies. [1,17-21] Blood samples showed highest isolation of non-fermenters 28.4% (n=74) which is in correlation with studies conducted by D Vijaya et al and Yashodara et al. [17,22] From pus samples *Pseudomonas aeruginosa* (26.4%) was found to be most common isolate followed by *Acinetobacter* spp

(10.1%) while other previous conducted studies, [23,24] had found a varied prevalence from 13% to 38%. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* had accounted for 96.1% of the isolates that were used in the present study and are also considered to be the common nosocomial pathogens by many scientific observers. [6,16] A similar result has been reported from Kolar that 87% of all isolates comprises of *Pseudomonas* and *Acinetobacter* species. [25]

*Acinetobacter* is reported for about 10% of nosocomial infections in ICU patients. [26] In this study *Acinetobacter* was isolated in a significant proportion 38% from clinical samples in ICU infections and Multi drug resistant *Acinetobacter* isolates were found to be associated with almost all types of nosocomial infection like RTI s, BSI s, UTI s and wound infections in a earlier study by Prasanth et al [27] and Patwardan et al [28] reported multidrug resistant *Acinetobacter* responsible for majority of infection.



The other non fermenter isolates were minimal compared with *Pseudomonas* spp and *Acinetobacter* spp. This is similar to the study conducted by D Vijaya et al, Malini et al. [17,25]

Debilitated condition of the patient, invasive diagnostic and therapeutic procedures and more importantly use of contaminated fluids or life support equipment in ICU could be the source of infection. [29]

On observing the antimicrobial resistance pattern most of the isolates were seen to be resistant to 3 or more drugs. Resistant patterns among nosocomial bacterial pathogens may vary from country to country and also within the same country, over time. [26] Most of the isolates of *Pseudomonas aeruginosa* were sensitive to Cefoperazone/Sulbactam (90%), Imipenem (70.2%), Meropenem (68.1%), Amikacin (66.6%), Ciprofloxacin (65.4%) and Cefepime (61.9%). Earlier study stated Imipenem was found to be the most effective drug followed by Cefoperazone/Sulbactam. [29] Some workers reported Cefoperazone/Sulbactam and Carbapenem as the most effective drugs against NFGNB. [29-31] In a study of Taneja N et al, [32] from Chandigarh 42% of *Pseudomonas aeruginosa* isolates were found to be resistant to imipenem while in our study it is 30% only *Acinetobacter baumannii* isolates showed higher rate of resistance (77.7% were sensitive to Colistin, 75% were sensitive to Cefoperazone/Sulbactam followed by Tigecycline 58.3%) This is similar to the study conducted by Kalidas RIT et al, to aminoglycosides, Carbapenems, Amikacin, Ceftazidime compare to the study at Bangalore [33] and similar to the study conducted by Prasanth Parandekar et al. [34]

Most of our isolates of *Acinetobacter* spp showed high resistance to carbapenems and aminoglycosides which is similar to the study conducted by Nidhi Goel et al. [35] This is in contrast to the another study conducted by Gonlugur U et al and Gladstone P et al. [36,37]

*Acinetobacter baumannii* has the ability to acquire resistance to many major classes of antibiotics. Multiple antibiotic resistances in *Acinetobacter* spp were reported previously but plasmid borne nature of antibiotic resistance has been reported only in a few cases in India. [38]

Single isolate *Sphingomonas Paucimobilis* showed resistance to all the antibiotics tested is similar to the study conducted by Akhilesh et al. [39] *Sphingomonas Paucimobilis* which is having a lot of outbreaks recently reported in case of pediatric infections, neonatal intensive care units etc can be isolated from various clinical specimen and distilled water too. [39]

## CONCLUSION

*Pseudomonas aeruginosa* and *Acinetobacter baumannii* were the commonest NFGNB isolated in our study. NFGNB are emerging as important opportunistic pathogens and are resistant to commonly used antibiotics. Prevalence of NFGNB varies between communities, hospitals in the same community and among different patient populations in the same hospital. It is important for clinicians to be updated with occurrence and antibiotic susceptibility pattern of the pathogens.

These pathogens have a great potential to survive in a hospital environment so implementation of stringent antibiotic stewardship and strict infection control practices will be required to prevent or slow down there emergence and spread. Hand hygiene is essential when one comes in to contact with patients their secretions and the environment. Continuous awareness is needed to maintain good housekeeping, equipment decontamination and strict attention to hand washing. Emphasis must be laid on multidrug resistant species of NFGNB for further treatment and prevention of the disease.

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How to cite this article: Simgamsetty S, Yarlaga P, Myneni RB et al. Identification and antimicrobial susceptibility patterns of non-fermentative gram negative bacilli in a tertiary care hospital: our experience. Int J Health Sci Res. 2016; 6(8):105-111.

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