



RESEARCH ARTICLE

PREVALENCE OF NON-FERMENTING GRAM NEGATIVE BACILLI INFECTIONS AND THEIR  
ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN A TERTIARY CARE HOSPITAL

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ARTICLE INFO

Article History:

Received 09<sup>th</sup> September, 2017  
Received in revised form  
23<sup>rd</sup> October, 2017  
Accepted 12<sup>th</sup> November, 2017  
Published online 31<sup>st</sup> December, 2017

Key words:

Non-fermenting gram negative bacilli,  
Acinetobacter baumannii,  
Pseudomonas aeruginosa,  
Nosocomial pathogens.

ABSTRACT

**Background:** Aerobic non-fermenting gram negative bacilli [NFGNB] once considered as contaminants now associated with life threatening infections and emerging as multidrug resistant [MDR] nosocomial pathogens. AIM: Isolation and identification of NFGNB in various clinical samples and to determine their antibiotic susceptibility pattern.

**Materials and methods:** A retrospective study was conducted in the microbiology laboratory of a tertiary care hospital from July 2015 to December 2016. NFGNB were isolated from various clinical specimens on MacConkey agar. Further identification and antibiotic susceptibility testing [AST] was done by subjecting them to VITEK -2 system.

**Results:** Among 7654 clinical specimens, 325 yielded NFGNB accounting for an isolation rate of 4.24%. Blood was the most common specimen [34.77%] followed by tracheal aspirate [29.57%]. Acinetobacter baumannii was the most common isolate (98.6%) followed by Pseudomonas aeruginosa (78.1%). A high level of antibiotic resistance was recorded for most of the first and second line drugs. Thus confirming multidrug resistance. Colistin and tigecycline showed maximum activity with an overall susceptibility of (74.46%) and (57.85%) respectively. High carbapenem resistance in this study is of major concern which can cause outbreaks and limit therapeutic options due to MDR.

**Conclusion:** Identification of NFGNB and monitoring their susceptibility patterns will aid in proper management of infections caused by them. Improved antibiotic stewardship and infection control measures are needed to prevent emergence and spread of MDR NFGNB in the healthcare settings.

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Citation: Prudhivi Sumana et al. 2017. "Prevalence of non-fermenting gram negative bacilli infections and their antimicrobial susceptibility pattern in a tertiary care", International Journal of Current Research, 9, (12), 63427-63431.

INTRODUCTION

NFGNB are a diverse group of aerobic, non-sporing bacilli that either do not utilize glucose as a source of energy or utilize it oxidatively (Winn *et al.*, 2006). They are saprophytic in nature and were previously considered as contaminants or commensals of little significance (Vijaya *et al.*, 2000; Juyal *et al.*, 2013). However, recent literature review shows that they have emerged as important nosocomial pathogens causing life threatening infections particularly in hospitalised patients, immunocompromised hosts and patients with hematological malignancies (Rampal, 2008). They account for 15% of all bacterial isolates from clinical microbiological laboratory (Meherwal *et al.*, 2012). NFGNB are intrinsically resistant to many antibiotics and are known to produce ESBL's and metallo-β-lactamases (Gales *et al.*, 2001; Rubin *et al.*, 1985). The aim of the present study was to isolate, identify and characterise the prevalence of NFGNB along with their antimicrobial sensitivity pattern among the patients attending our tertiary care center.

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The heterogeneous group includes organisms like Pseudomonas spp, Acinetobacter spp, Alcaligenes spp, Stenotrophomonas maltophilia, Burkholderiaceae complex (BCC). Currently Pseudomonas aeruginosa and Acinetobacter baumannii are the most commonly isolated nonfermenters pathogenic for humans. Infections caused by other species are relatively infrequent.

MATERIAL AND METHODS

A retrospective study was conducted in the clinical microbiology laboratory of our hospital from July 2015 to December 2016. A total of 7654 clinical specimens were received in the laboratory which included blood (113), tracheal aspirates and sputum (96+20), urine (49), Pus (41), body fluids (5) and CSF (1). Samples were plated on blood agar and MacConkey agar. Respiratory samples were plated on chocolate agar in addition and incubated at 37° for 48 hrs before being reported as sterile. The isolates that showed non lactose fermenting colonies on MacConkey agar were provisionally considered as NFGNB. Further identification and antimicrobial susceptibility testing [AST] was performed by subjecting them to VITEK 2 compact system.

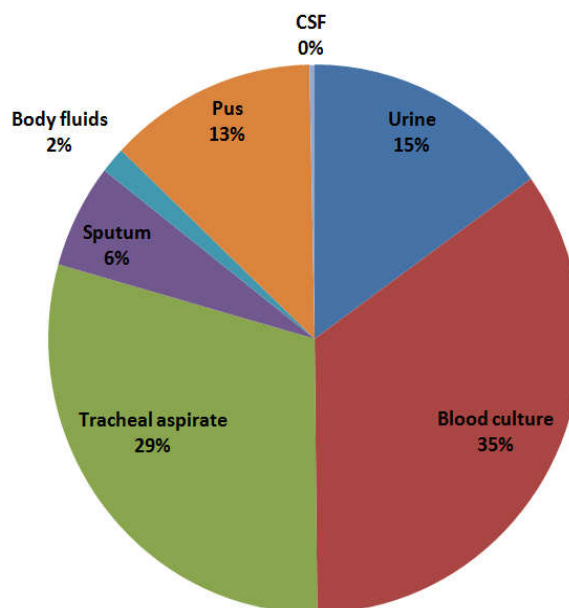


Fig. 1. Specimenwise distribution of total clinical isolates

### 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 DensiCheck instrument. 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). The culture suspension was inoculated into the GN card with the help of a vacuum device inside the filling chamber. The cards were later transferred into the loading chamber where the cards were sealed and were incubated in a rotating carousel at 37°C. Each loaded card was removed from the carousel for every 15 minutes, transported to the optical system for reaction readings and the returned to the carousel incubator until the next read time. Data was collected at 15-minute intervals during the entire incubation period.

### Quality control

The Vitek-2 compact machine was validated using the standard strains as per the manufacturer's instructions. *Pseudomonas aeruginosa* 27853, and *Stenotrophomonas maltophilia* 17666 were used. During the study period, the control strains were checked at regular intervals.

### Antimicrobial susceptibility testing

AST 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. A panel of twenty five antibiotics were tested in AST N281 card. The cards were filled with an inoculum (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.

### RESULTS

Among 7654 clinical samples, NFGNB were isolated from 325 samples accounting for an isolation rate of 4.24%. Monomicrobial growth was seen in 282 (86.76%) samples whereas 43 (13.23%) specimens showed polymicrobial growth, where nonfermenters were isolated with other organisms of which *S. aureus*, *E. coli*, *K. pneumonia* and *Citrobacter* species were common. Blood [113] was the most common specimen followed by tracheal aspirate [TA] [96], urine [49] and pus (41). Fig: 1 depicts the specimen wise distribution and percentage.

Nonfermenters were isolated from various clinical specimens. Majority were isolated from blood (34.77%), respiratory specimens [TA: 29.54%], sputum [6.15%], urine (15.08%) and pus (12.62%). Fig: 2 depicts the percentage of NFGNB. *Acinetobacter* species were the most common isolates accounting for 150 (46.15%) followed by *Pseudomonas* species 115 (35.38%). The emerging pathogens like *Achromobacter*, *Sphingomonas*, *Rhizobium*, *Raoultella* and *Stenotrophomonas* though few in number, were mostly isolated from blood and respiratory samples. The sensitivity pattern of the NFGNB isolated is presented in Fig: 3. Most of the isolates have shown high resistance to ciprofloxacin, gentamycin and carbapenems. Colistin and Tigecycline were found to be the most effective antibiotics.

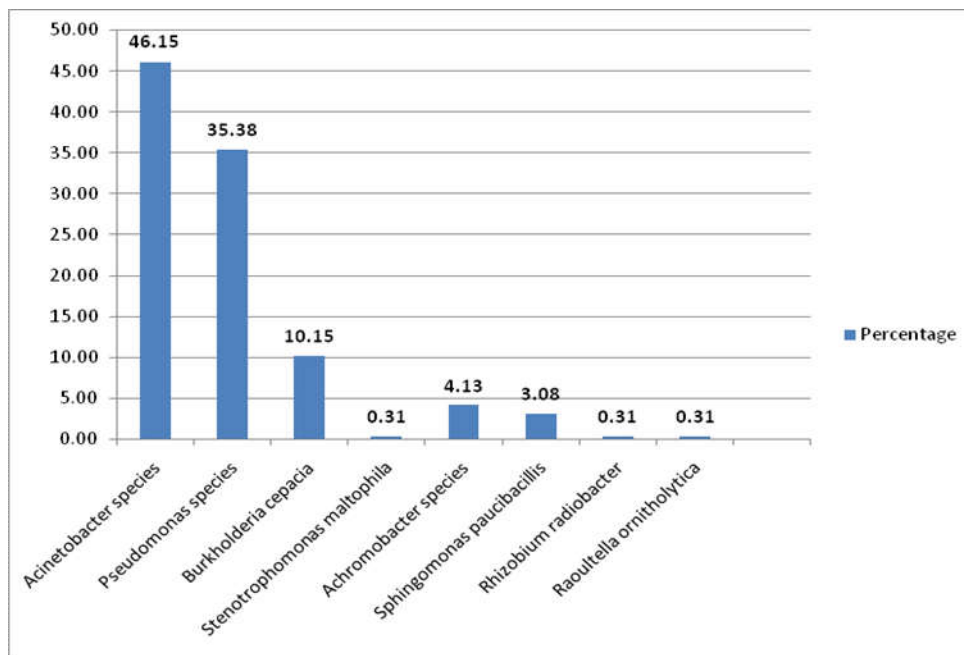


Fig. 2. Distribution of NFGNB species

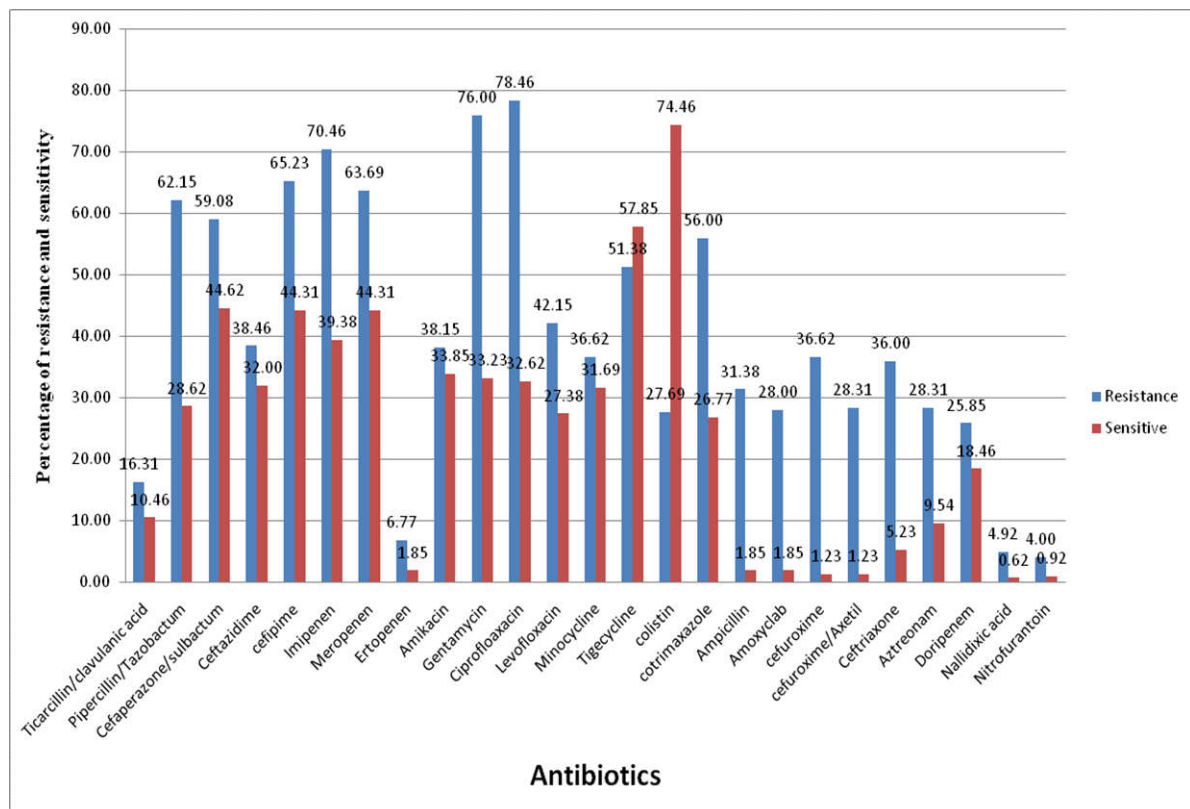


Fig. 3. Sensitivity and Resistance pattern of NFGNB

**DISCUSSION**

Nonfermenters are ubiquitous in environment. Although frequently they are considered as contaminants, the pathogenic potential of NFGNB has been established beyond doubt by their frequent isolation from clinical materials and their association with disease (Winn *et al.*, 2006; Prashanth and Badrinath, 2004). Earlier the identification of non-fermenters based on biochemical tests was cumbersome and many non fermenters were misidentified. But now with the availability of commercial systems like ViteK- 2 or API, the identification has become easier.

Studies carried out by different researchers have reported varied isolation rates. In our study the isolation rate of NFGNB was 4.24% which is in parallel with the studies conducted by Malini *et al.* (Malini *et al.*, 2009; Samanta *et al.*, 2011), Bruno *et al.* (2011) and Kirtilaxmi *et al.* (2014) whose isolation rates were 4.5%, 2.18% and 3.58% respectively. On the other hand various studies from Amritsar [45.9%] (Sidhuet *et al.*, 2010), Bangalore (21.80%) (Vijaya *et al.*, 2000) and Saudi Arabia ((16%) (Eltahawy and Khalaf, 2001) have reported high isolation rates. In our study most of the isolated NFGNB were from blood (34.77%) and tracheal aspirate samples (29.54%). This is in controversy with other studies which have shown

high prevalence in pus samples (Malini *et al.*, 2009; DipakBhargava *et al.*, 2015; Gokhale and Metgud, 2012; Malini *et al.*, 2009). In the present study, the most common NFGNB were *Acinetobacter* species and *Pseudomonas* species. *Acinetobacter* species accounting for (46.15%) [*A. baumannii* (98.6%), *A. lwoffii* (1.14%) and *A. sobria* (1-37%)] and *Pseudomonas* species accounting for (35.38%). [*P. aeruginosa* (74.78%), *P. stutzeri*, *P. putida*, *P. luteola* and other species (25.21%)]. This is in accordance with other studies (Deepak Juyal *et al.*, 2013; Kirtilaxmi K. Benachinmardi *et al.*, 2014; Bohra *et al.*, 2017). *Acinetobacter* species were the predominant organisms in respiratory specimens [TA:70.83%, Sputum 60%], pus (65.85%) and blood (20.2%) and *Pseudomonas* species were predominant in blood (31.85%) and urine (75.5%) samples. The role of both these organisms as health care associated pathogens is well established and they are associated with wound infection, UTI, septicemia, surgical site infections and VAP. The other emerging pathogenic NFGNB isolated in our study were *Burkholderia cepacia*, *Achromobacter denitrificans*, *Sphingomonas paucibacilis*, *Stenotrophomonas maltophilia*, *Rhizobium radiobacter* and *Raoultella ornitholytica*. Differentiation between colonization and infection by these pathogens is of utmost importance. Otherwise unnecessary institution of antibiotics will contribute to further increase in resistance. In our study *B. cepacia* was isolated in 10.2% of cases and majority of them were from blood. Other studies have shown isolation rates of 12.1% and 4.66% isolated from different types of specimens (Kiran Chawla *et al.*, 2013; Rahbar *et al.*, 2010). *B. cepacia* is another NFGNB colonizing and infecting patients with chronic respiratory illness. It is known to cause disease in cystic fibrosis (CF) patients and once infected it is very difficult to eradicate (Govan *et al.*, 2007). Reported prevalence rates of *Achromobacter* species have increased in recent years, although this may in part result from growing attention or improved microbiological techniques.

They are found in a variety of aquatic environments and has proved to survive on inanimate surfaces in hospital settings connecting its role as a nosocomial colonizer. It is considered as an opportunistic pathogen (Amoureux *et al.*, 2012). Our study has observed the isolation of *Achromobacter* species. [*A. denitrificans* (0), *A. xylosoxidans* (4)] in 4.13% cases. Kiranchawla *et al.* have shown the isolation rate of 9.1 % (Govan *et al.*, 2007). There were 10 (3.08%) isolates of *Sphingomonas paucimobilis* from the blood culture in our study. Bacteremia due to *sphingomonas* species was reported in literature (Steinberg and Rio, 2005; Marinella, 2002). One strain of *Stenotrophomonas maltophilia* was isolated from tracheal aspirate. Any NFGNB culture isolate should not be ignored as just contaminant but should be correlated clinically for its pathogenic potential and identified using standard methods, so as to institute appropriate and timely antibiotic coverage. All these non fermenters are known for their inherent resistance to multiple groups of antibiotics.

Hence correct identification of these non fermenters is very important for choosing correct antibiotic. NFGNB have shown high level of resistance to ciprofloxacin (78.46%), gentamycin (76%), imipenem (70.46%) cefepime (65.23%) meropenem (63.60%), cefoperazone/sulbactam (59%) and piperacillin/tazobactam (62.15%). Colistin and tigecycline showed maximum activity with an over all susceptibility of 74.46% and 57.85% respectively. They have shown wide variations not

only among various groups of drugs, but also various drugs within the same group. Table 3 depicts the antibiotic sensitivity pattern of NFGNB. In the present study from the antibiotic sensitivity pattern it is clear that most of the isolates showed high degree of resistance suggesting that majority of the first and second line drugs were ineffective and this further confirms the multi drug resistant attribute of NFGNB. Resistance pattern among nosocomial bacterial pathogens may vary widely from country to country at any given time and within the same country over time (Memish *et al.*, 2012). Because of these variations a surveillance of the nosocomial pathogens for resistograms in a given setup is needed in order to guide appropriate selection of empiric therapy. Various international authorities emphasize that every hospital should have its individual antibiotic pattern since the standard antibiotic sensitivity pattern may not hold true for every are (Vijaya *et al.*, 2000). High degree of resistance to almost all the antibiotics was seen and this finding is in line with the study from Chandigarh (Gupta *et al.*, 2002). High carbapenem resistance in this study is of major concern, as these strains may often cause outbreaks in the ICU setting and can limit therapeutic options due to the high degree of multidrug resistance. These organisms can also spread resistance to other susceptible bacteria (Deepak Juyal *et al.*, 2013). This is in accordance with the antibiotic sensitivity pattern of isolates from Bangalore (Vijaya *et al.*, 2000) and Chandigarh (Ugade *et al.*, 2012).

## Conclusion

The present study highlighted the fact that along with other NFGNB, *A. baumannii* and *P. aeruginosa* were the most common NFGNB isolated which have emerged as an important nosocomial pathogens exhibiting MDR. More significantly NFGNB have great potential to survive in hospital environment therefore improved antibiotic stewardship and infection control measures are needed to prevent emergence and spread of MDR NFGNB in health care settings.

## Acknowledgement

We sincerely extend our thanks to the teaching and technical staff of our department for supporting us throughout the study. We would also thank our management for their support.

**Funding:** No funding sources

**Conflict of interest:** None declared

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