

## Original Research Article

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## Identification and Antimicrobial Susceptibility testing of Non Fermenting Gram Negative Bacteria by Vitek 2 in a Teaching Hospital in Mahbubnagar

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### ABSTRACT

Non fermenting gram negative bacilli (NFGNB) are aerobic, non-spore forming bacteria. Although previously considered as contaminants, their pathogenic potential was proved by their frequent isolation from clinical samples and association with diseases. Aim: To identify NFGNB from various clinical samples and perform antimicrobial susceptibility testing (AST) by Vitek 2. Materials and methods: All the clinical samples received for routine bacterial culture in Department of Microbiology, SVS Medical College, from January 2017 to April 2018 were plated on agar plates and non-lactose fermenting colonies on MacConkey agar plates were further processed by Vitek 2. Results: A total of 126 NFGNB were isolated from various clinical samples and their AST was evaluated by Vitek 2. *Pseudomonas aeruginosa* (66.66%) and *Acinetobacter baumannii* (23.80%) were most common NFGNB isolated. High variability in antibiotic resistance pattern was noted in the present study. 47.61 % of NFGNB exhibited Multidrug resistance (MDR) and 3.37% *Pseudomonas aeruginosa* strains showed Pandrug resistance (PDR). Conclusion: Accurate and rapid identification and antimicrobial susceptibility testing of NFGNB by Vitek 2, help in early initiation of appropriate antimicrobial therapy and proper management of patients and will reduce emergence of MDR and PDR strains of NFGNB.

#### Keywords

NFGNB, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia*, *Burkholderia cepacia*, Vitek 2, MDR, PDR

#### Article Info

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### Introduction

Non fermenting gram negative bacteria are saprophytic in nature and were previously considered as contaminants or commensals of little significance (Prudhivi Sumana *et al.*, 2017). However their pathogenic potential has been proved beyond doubt by their frequent isolation from clinical material and their association with disease (Susmitha Simgamsetty *et al.*, 2016). Recent literature review also shows that they have emerged as important nosocomial pathogens causing life

threatening infections particularly in hospitalised patients, immunocompromised hosts and patients with hematological malignancies. NFGNB are intrinsically resistant to many antibiotics and are known to produce ESBL's and metallo- $\beta$ -lactamases. NFGNB consist of heterogeneous group of organisms like *Pseudomonas* spp, *Acinetobacter* spp, *Alkaligenes* spp, *Stenotrophomonas maltophilia*, *Burkholderia cepacia* (BCC). Currently *Pseudomonas aeruginosa*, *Acinetobacter baumannii* are the most commonly isolated non-fermenters

pathogenic for humans. Infections caused by other species are relatively infrequent (Prudhivi Sumana *et al.*, 2017).

In recent years due to liberal and empirical use of antibiotics, most of these organisms are now resistant to many routinely used antibiotics causing treatment failures. The emergence of resistance as a major problem has drawn attention to a need for better diagnostic techniques (Ridhima Wadhwa *et al.*, 2016). Vitek 2 is one such instrument which is fully automated system, designed to decrease the turnaround time for the identification of bacteria and determination of antimicrobial susceptibilities. The instrument also provides a more hands-off approach than the original Vitek instrument (Caroline M. O'Hara *et al.*, 2003). The purpose of present study is to identify non-fermenting gram negative bacteria from various clinical samples isolated in a teaching hospital and to evaluate their antimicrobial susceptibility patterns by Vitek 2 method.

### **Materials and Methods**

The present study was conducted in the Department of Microbiology, SVS medical college, Mahbubnagar during the period from January 2017 to April 2018. All the clinical samples received for bacterial culture were plated on blood agar and MacConkey agar and incubated at 37°C for 48hrs. The isolates that showed non lactose fermenting colonies on MacConkey agar were provisionally considered as NFGNB. Further identification and antimicrobial susceptibility testing was performed by subjecting them to Vitek 2 compact system (Prudhivi Sumana *et al.*, 2017).

### **Inoculum preparation**

From the isolated colonies grown on the media, a bacterial suspension was prepared in

3 ml of sterile saline in a 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 minutes. Identification with the Vitek 2 compact system was performed according to the manufacturer's instructions using the 64 well plastic Gram Negative (GN) card. 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 and transported to the optical system for reaction readings and data was collected (Prudhivi Sumana *et al.*, 2017).

### **Quality control**

The Vitek 2 compact machine was validated using the standard strain as per the manufacturer's instructions. *Pseudomonas aeruginosa* 27853 was used. During the study period, the control strain was 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. A panel of seventeen antibiotics in AST N281 card was tested. The cards were filled with 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 8x10<sup>6</sup> 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 in accordance with the internal database of possible phenotypes for microorganism antimicrobial agent combinations (Prudhivi Sumana *et al.*, 2017).

## Results and Discussion

A total of 126 non-fermenting gram negative bacteria were isolated from various clinical samples processed by Vitek 2 from January 2017 to April 2018 in SVS Medical College. Majority of NFGNB were isolated from pus/wound swabs (40.47%) followed by urine (21.42%), blood (19.04%), sputum (6.34%), body fluids (4.76%), bronchioalveolar lavage fluid (4.76%) and endotracheal tube tips (3.17%).

Among 126 NFGNB isolated in present study, 69.04% (87) were from patients above 50 years age group, 16.66% (21) from 20-55 years age group patients and 14.28% (18) from below 25 years age group patients.

NFGNB that were previously considered to be contaminants have emerged as important nosocomial pathogens. *Pseudomonas aeruginosa* and *Acinetobacter* species are known to be common among them (Susmitha Simgamshetty *et al.*, 2016). The Vitek 2 combines several advantages that may be of clinical interest for routine testing of gram-negative rods isolated from clinical samples:

rapid identification, simple methodology, a high level of automation and taxonomically updated databases (Providencia Joyanes *et al.*, 2001).

The Vitek 2 compact system identifies NFGNB along with their antibiotic susceptibility pattern within a time period of 8 to 16hrs (Susmitha Simgamsetty *et al.*, 2016). 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 system like Vitek 2, the identification has become easier (Prudhivi Sumana *et al.*, 2017).

In the present study majority of NFGNB were isolated from Pus/wound swabs (40.47%) followed by urine, blood, sputum which is similar to (Shilpa K. Gokale *et al.*, 2012) study and in contrary (Prudhivi Sumana *et al.*, 2017) study majority of NFGNB are isolated from blood and respiratory samples.

*Pseudomonas aeruginosa* (66.66%) was commonest NFGNB isolated in present study which is in accordance with (A Malini *et al.*, 2009) and (Susmitha Simgamshetty *et al.*, 2016) studies. A very high percentage of *Pseudomonas aeruginosa* (82.3%) are isolated in (Shilpa K. Gokale *et al.*, 2012) study. In contrast to present study, *Acinetobacter baumannii* (46.15%) is the commonest NFGNB isolated followed by *Pseudomonas aeruginosa* (12.62%) in (Prudhivi Sumana *et al.*, 2017) study (Table 1–6).

**Table.1** Distribution of isolated NFGNB Species in present study (n =126)

NFGNB	NO.OF ISOLATES	% ISOLATES
<i>Pseudomonas aeruginosa</i>	84	66.66 %
<i>Acinetobacter baumannii</i>	30	23.80 %
<i>Burkholderia cepacia</i>	6	4.76 %
<i>Stenotrophomonas maltophilia</i>	6	4.76 %

**Table.2** Comparison of commonest NFGNB isolates in different studies

Study (Year)	<i>Pseudomonas aeruginosa</i> (%)	<i>Acinetobacter baumannii</i> (%)
Malini <i>et al.</i> , (2009)	53.8%	22.2%
Shilpa K. Gokale <i>et al.</i> , (2012)	82.3%	15.4%
Susmitha Simgamshetty <i>et al.</i> , (2016)	56.45%	37.09%
Present study	<b>66.66%</b>	<b>23.80%</b>

**Table.3** Distribution of NFGNB isolated from samples sent from various areas in hospital

NFGNB (NO. OF ISOLATES)	% (no.) of NFGNB isolated from various areas in hospital			
	Wards	ICUs	ER	OPD
<i>Pseudomonas aeruginosa</i> (84)	28.57% (24)	64.28% (54)	3.57% (3)	3.57% (3)
<i>Acinetobacter baumannii</i> (30)	10% (3)	80% (24)	10% (3)	0%
<i>Burkholderia cepacia</i> (6)	0%	100% (6)	0%	0%
<i>Stenotrophomonas maltophilia</i> (6)	50% (3)	50% (3)	0%	0%
<b>TOTAL NFGNB (126)</b>	<b>23.80% (30)</b>	<b>69.04% (87)</b>	<b>4.7% (6)</b>	<b>2.38% (3)</b>

**Table.4** Sex-wise distribution of NFGNB

Study (Year)	Male %	Female%
Ridhima wadhwa <i>et al.</i> , (2016)	70 %	30 %
Kritilaxmi <i>et al.</i> , (2018)	68 %	32 %
Present study	<b>69.04 %</b>	<b>30.96 %</b>

**Table.5** Percentage (%) of NFGNB sensitive to antibiotics tested by Vitek 2 AST Panel

ANTIBIOTIC (code)	Antibiotic Sensitivity Percentage (%) of NFGNB			
	<i>Pseudomonas aeruginosa</i> (84)	<i>Acinetobacter baumannii</i> (30)	<i>Burkholderia cepacia</i> (6)	<i>Stenotrophomonas maltophilia</i> (6)
Amikacin (AN)	42.85%	0%	0%	0%
Aztreonam (ATM)	32.14%	0%	0%	0%
Cefepime (FEP)	50%	10%	0%	0%
Cefperazone/Sulbactam (SFP)	50%	10%	50%	0%
Ceftazidime (CAZ)	39.28%	0%	0%	0%
Ciprofloxacin (CIP)	25%	0%	0%	100%
Colistin (CS)	96.42%	100%	0%	100%
Doripenem (DOR)	53.57%	20%	100%	100%
Gentamicin (GM)	42.85%	10%	0%	0%
Imipenem (IPM)	57.14%	20%	50%	50%
Levofloxacin (LEV)	25%	0%	0%	100%
Meropenem (MEM)	53.57%	10%	100%	100%
Minocyclin (MNO)	21.42%	40%	100%	0%
Piperacillin/Tazobactam (TZP)	42.85%	0%	50%	0%
Ticarcillin/Clavulanic Acid (TCC)	25%	0%	0%	100%
Tigecycline (TGC)	25%	90%	0%	100%
Trimethoprim/Sulfamethoxazole(SXT)	28.57%	40%	100%	50%

**Table.6** Comparison of percentage (%) MDR NFGNB in Various studies

Study (Year)	% MDR NFGNB
Mathai <i>et al.</i> , (2012)	70%
Jayanthi <i>et al.</i> , (2012)	39.4%
Seema Solanki <i>et al.</i> , (2017)	48.5%
Present study	<b>47.61%</b>

\*Among 84 *Pseudomonas aeruginosa* isolates, 3 (3.57%) isolates showed Pan drug resistance (PDR).

In our study *Pseudomonas aeruginosa* strains were most susceptible to Colistin (96.42%) and least susceptible to Levofloxacin, Ciprofloxacin, Ticarcillin/Clavulanic acid, Tigecycline (25%) and Minocyclin (21%). In our study Imipenem resistance was seen in 42.86% of *Pseudomonas aeruginosa* strains which is accordance with (Gladstone *et al.*, 2005) study in which it is 42.8% and in contrast very less, that is 6% resistance was seen in (Malini *et al.*, 2009) study. In present study *Acinetobacter baumannii* strains were

most susceptible to Colistin (100%) and Tigecycline (90%) but about 76% of these strains showed high resistance to various drugs similar to (Kirtilaxmi *et al.*, 2014) study. *Burkholderia cepacia* and *Stenotrophomonas maltophilia* strains also showed high degree of resistance to various drugs tested. In present study about 47.61% NFGNB exhibited Multidrug resistance (among all NFGNB, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* constituted major percentage of MDRs) and 3.57% of

*Pseudomonas aeruginosa* strains exhibited Pan drug resistance. For this study, Multidrug resistant (MDR) are defined as those isolates resistant to more than three classes of antibiotics, Pan drug resistant (PDR) are those resistant to all the commonly used antibiotics (Minal Vasant Kusalkar *et al.*, 2018). The treatment of infections caused by these organisms is challenging because of high intrinsic and acquired resistance to all commonly used antibiotics (Vikas Gautam *et al.*, 2015). Hence accurate and timely identification and antimicrobial susceptibility testing of NFGNB is necessary for clinicians to choose correct antibiotics for treatment.

In present study, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are most common NFGNB isolated. Correct and rapid identification and antimicrobial susceptibility testing of NFGNB by Vitek 2 is very important because it will help in early initiation of appropriate antimicrobial therapy and proper management of patients. Different species of NFGNB isolated showed varied sensitivity patterns. Emergence and spread of Multidrug resistance and Pan drug resistance strains is an alarming situation and it emphasizes need for improved antibiotic stewardship and infection control measures to be implemented in hospital for better care and management of patients.

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