

## Prevalence and Antibigram of Non-Fermenting Gram Negative Bacilli in Hospital Acquired Infections with Multidrug Resistance Burden and Extended Spectrum Beta Lactamase Detection

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**Abstract:** Non-fermenting Gram negative bacilli (NFGNB) are taxonomically diverse group of pathogens that has emerged as a major cause of hospital acquired infections especially in immunocompromised hosts. Inappropriate antimicrobial therapy used to treat infections caused by these organisms, especially the multidrug resistant and ESBL-producing strains, often results in failure of clinical treatment. Therefore, timely detection of such infections can lead to a better selection of antibiotics and help to improve the outcome of such infections. The study aimed to determine the prevalence and antibiogram of non-fermenting Gram negative bacilli in hospital acquired infections (HAI) along with determination of multidrug resistance and extended spectrum beta lactamase (ESBL) enzymes amongst the same. The study was conducted in the department of Microbiology over a period of one year from January 2014 to December 2014. The various clinical specimens were collected from patients suspected to be suffering from HAI. These were cultured on blood agar and MacConkey agar. The growth was identified on the basis of colony morphology, Gram's stain and various biochemical reactions. The antibiotic susceptibility was tested on Mueller Hinton agar using antibiotics from different classes which included beta lactams, aminoglycosides, macrolides and fluoroquinolones. Multidrug resistance was defined as resistance of the isolate to three or more classes of antibiotics. Extended spectrum beta lactamase detection was done by the combined disc diffusion method. A total of 180 isolates of various organisms were isolated from different clinical samples. Out of these, 47 (26.11%) isolates were identified NFGNB. Among these 47 isolates, *Pseudomonas aeruginosa* accounted for 78.72% (37) and *Acinetobacter* for 21.28% (10). Antibiogram of *P. aeruginosa* showed maximum sensitivity towards Imipenem i.e. 64.86% while least towards Aztreonam (16.22%). In case of *Acinetobacter* also, sensitivity was maximum towards Imipenem (80%) and 0% towards cephalosporins, Ampicillin/Sulbactam and Ticarcillin/Clavulanic acid. ESBL production was found to be present in 37.84% isolates of *P. aeruginosa* and 20% of *Acinetobacter* while 40.54% and 30% strains of *P. aeruginosa* and *Acinetobacter* respectively were found to be multidrug resistant. Isolation of NFGNB and their antibiotic susceptibility pattern should be regarded with all seriousness in clinical practice and epidemiology because they are emerging nosocomial pathogens and by being resistant to multiple antibiotics, their prevalence not only limits the treatment options but also acts as a reservoir of drug resistant genes.

**Keywords:** Hospital acquired infections, *Pseudomonas aeruginosa*, *Acinetobacter*, Extended spectrum beta lactamase, Multidrug resistance.

### INTRODUCTION

Hospital acquired infections are one of the major causes of morbidity and mortality in hospitalized patients in the present scenario, leading directly or indirectly to an enormous increase in the cost of hospital care and to the emergence of new health hazards for the community. It has been observed that the majority of such infections emerge as a result of diagnostic and therapeutic interventions such as

intravenous cannulas, indwelling catheters, sophisticated life support, intravenous fluid therapy, prosthetic devices, immunosuppressive therapy, and the use of broad spectrum antibiotics [1]. The rate of hospital acquired infections varies from 2.8% to 34.6% among hospitalized patients [2]. A prevalence survey conducted under the auspices of WHO in 55 hospitals of 14 countries representing 4 WHO Regions (Europe, Eastern Mediterranean, South-East Asia and Western

Pacific) showed an average of 8.7% of hospitalized patients had hospital acquired infections.

At any time, over 1.4 million people worldwide suffer from infectious complications acquired in hospital [3]. The highest frequencies of hospital acquired infections were reported from hospitals in the Eastern Mediterranean and South-East Asia Regions (11.8% and 10.0% respectively) with a prevalence of 7.7% and 9.0% respectively in European and Western Pacific regions [4]. The organisms that cause hospital acquired infections are often multidrug-resistant which poses a major public health threat. Non-fermenting Gram negative bacilli (NFGNB) are taxonomically diverse group of pathogens that has emerged as a major cause of hospital acquired infections especially in immunocompromised hosts. Inappropriate antimicrobial therapy used to treat infections caused by these organisms and extended spectrum beta lactamases are an important cause of this resistance. Therefore, timely detection of such infections can lead to a better selection of antibiotics and help to improve the outcome of such infections. The detection of ESBL-producing organisms in laboratories is a critical requirement for appropriate management of patients, infection prevention and control efforts, as well for tracking these organisms in surveillance systems.

#### AIM

The study aimed to determine the prevalence and antibiogram of non-fermenting Gram negative bacilli in hospital acquired infections along with determination of multidrug resistance and extended spectrum beta lactamase (ESBL) enzymes amongst the same isolates.

#### MATERIALS AND METHODS

The study was conducted in the department of Microbiology over a period of one year from January 2014 to December 2014. Various clinical samples like urine, blood, pus, sputum, endotracheal secretions, and central venous line tips were collected from patients

suspected to be suffering from hospital acquired infections from various wards (including orthopaedics, surgery, medicine, gynaecology, paediatrics, ENT and ICUs) and were transported immediately to the laboratory. The specimens were processed according to standard bacteriological procedures available [5]. They were inoculated on blood agar and MacConkey agar plates and the growing organisms were identified by standard techniques. Ambiguous results were confirmed by automated VITEK 2-compact system (BioMerieux, France) following the manufacturer's instructions. Antibiotic sensitivity testing was performed on Mueller Hinton agar using antibiotics from different classes including beta lactams, glycopeptides, aminoglycosides, macrolides and fluoroquinolones. Multidrug resistance (MDR) was defined as resistance of the isolate to three or more classes of antibiotics. The CLSI recommended combined disk method involving ceftazidime (30 µg) and cefotaxime (30 µg) with and without the inhibitor clavulanic acid (10 µg) was used to confirm the presence of ESBL in NFGNB.

#### RESULTS

A total of 180 isolates of various organisms were isolated from different clinical samples. Out of these, 47 (26.11%) isolates were identified as NFGNB (Fig.1). Among these 47 isolates, *Pseudomonas aeruginosa* accounted for 78.72% (37) and *Acinetobacter* for 21.28% (10) as shown in fig.2. Antibiogram of *P. aeruginosa* showed maximum sensitivity towards Imipenem i.e. 64.86% while least towards Aztreonam (16.22%) which has been shown in table 1. In case of *Acinetobacter* also, sensitivity was maximum towards Imipenem (80%) and was found to be completely resistant towards cephalosporins, Ampicillin/Sulbactam and Ticarcillin/Clavulanic acid (table 2). ESBL production was found to be present in 37.84% isolates of *P. aeruginosa* and 20% of *Acinetobacter* (Fig.3) while 40.54% and 30% strains of *P. aeruginosa* and *Acinetobacter* respectively were found to be multidrug resistant (Fig.4).

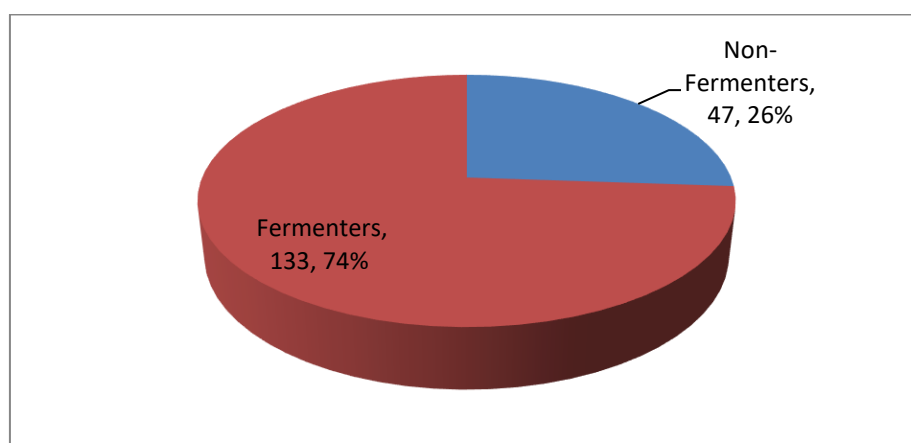
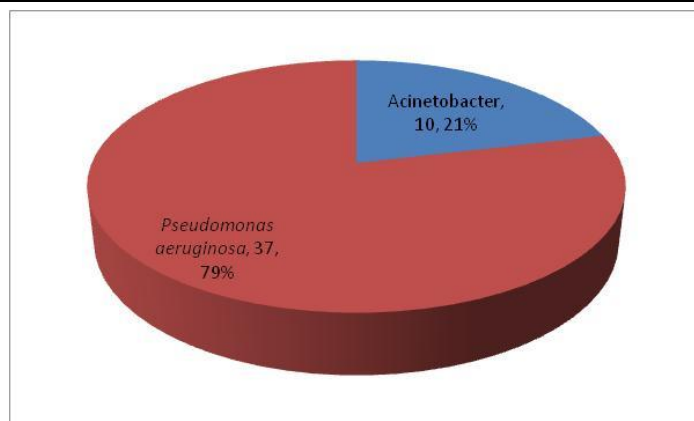


Fig-1: Percentage of Non Fermenters in HAI



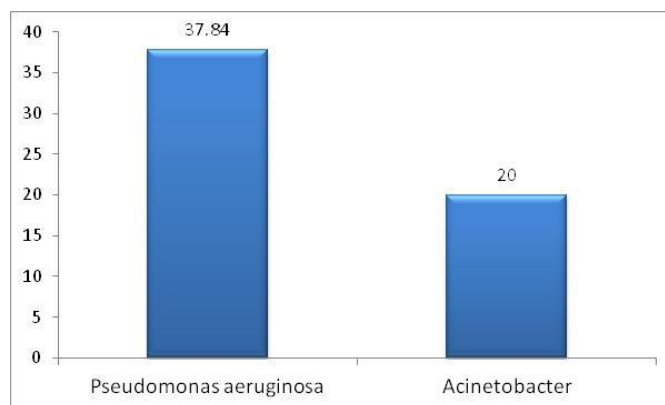
**Fig-2: Distribution of Non Fermenters**

**Table-1: Antibiotic susceptibility pattern of *Pseudomonas aeruginosa* (n=37)**

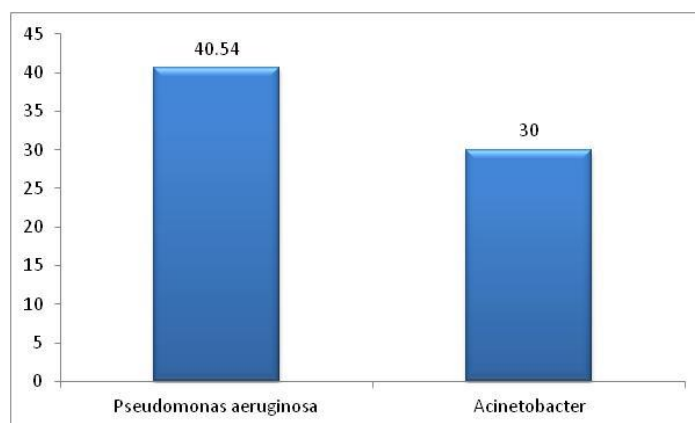
Antibiotic	Sensitive n (%)	Resistant n (%)
Aztreonam	6 (16.22)	31 (83.78)
Ceftazidime	10 (27.0)	27 (73.0)
Ciprofloxacin	11 (29.73)	26 (70.27)
Gentamicin	15 (40.54)	22 (59.46)
Imipenem	24 (64.86)	13 (35.14)
Levofloxacin	19 (51.35)	18 (48.65)
Piperacillin	15 (40.54)	22 (59.46)
Piperacillin/Tazobactam	20 (54.05)	17 (45.95)
Ticarcillin	16 (43.24)	21 (56.76)
Ticarcillin/Clavulanic acid	21 (56.76)	16 (43.24)
Tobramycin	13 (35.14)	24 (64.86)

**Table-2: Antibiotic susceptibility pattern of *Acinetobacter* (n=10)**

Antibiotic	Sensitive n (%)	Resistant n (%)
Ampicillin/Sulbactam	0 (0)	10 (100)
Amikacin	3 (30)	7 (70)
Cefotaxime	0 (0)	10 (100)
Ceftriaxone	0 (0)	10 (100)
Ceftazidime	0 (0)	10 (100)
Cefepime	0 (0)	10 (100)
Gentamicin	0 (0)	10 (100)
Imipenem	8 (80)	2 (20)
Levofloxacin	7 (70)	3 (30)
Tetracycline	0 (0)	10 (100)
Aztreonam	2 (20)	8 (80)
Ticarcillin/Clavulanic acid	0 (0)	10 (100)



**Fig-3: ESBL Producing Organisms (%)**



**Fig-4: Multidrug Resistance (%)**

## DISCUSSION

Hospital acquired infections occur worldwide, both in the developed and developing world. They are a significant burden to patients and public health and are a major cause of death and increased morbidity in hospitalized patients especially if caused by non fermenting Gram negative bacilli, being multidrug resistant. Our study therefore aimed to establish the local data on non-fermenters as the hospital acquired pathogens and detection of their multidrug resistance burden.

In the present study, 180 isolates were obtained from samples acquired from patients who showed signs and symptoms of infection after 48 hours of admission to the hospital. Out of these, 47 (26.11%) isolates were identified as NFGNB. This was in agreement with the study done by Zaveri *et al.* where non-fermenters constituted 29% of the hospital acquired pathogens [6]. However, in a study done by Candevir *et al.*, non-fermenters constituted around 40% of the total isolates [7].

In our study, *Pseudomonas aeruginosa* accounted for 78.72% (37) and *Acinetobacter* for 21.28% (10) of the non-fermenters as against Candevir *et al.* who reported around 36% isolates of *Pseudomonas aeruginosa* and 64% of *Acinetobacter* [7].

According to our study, 16% of the isolates of *Pseudomonas aeruginosa* were sensitive to aztreonam which was similar to that of other studies conducted by Mahmoud *et al.* Mahfoud *et al.* and Gill *et al.* who observed 17.5%, 17% 14.6% sensitivity respectively to aztreonam [8-10].

Our study showed that 27% and 54% strains were sensitive to ceftazidime and piperacillin/tazobactam. Similar results were shown by Mahfoud *et al.* who showed 28.6% and 54.5% sensitivity to ceftazidime and piperacillin/tazobactam respectively [9]. There was 65% sensitivity of *Pseudomonas*

*aeruginosa* to imipenem in our study which was similar to that observed by Mahmoud *et al.* (66.7%) whereas sensitivities observed by Mahfoud *et al.* (56.1%) and Gill *et al.* (9.7%) were lower than that of our study [8-10].

This difference may be due to difference in the antimicrobial policies which vary from hospitals to hospitals. We observed that all (100%) isolates of *Acinetobacter* were resistant to cefotaxime, ceftriaxone, ceftazidime, gentamicin and tetracycline which were in agreement with the study conducted by Sikka *et al.* who also showed 100% resistance of *Acinetobacter* against cefotaxime and gentamicin [11]. Another study conducted by Tripathi *et al.* also showed 100% resistance of *Acinetobacter* against ceftriaxone, ceftazidime and tetracycline which was also similar to our study [12]. However resistance of *Acinetobacter* to imipenem in our study was 20% which was lower than that observed by Tripathi *et al.* and Nahar *et al.* which was 43% and 66.7% respectively [12,13]. This difference may be because of lesser use of carbapenems in our hospital. This indicates that carbapenems should be kept as reserve drugs and should be used cautiously and only in case of resistance to other drugs. The patterns of organisms causing infections and their antibiotic resistance pattern vary widely from one country to another; as well as from one hospital to other and even among ICUs within one hospital [6]. In our study, we observed that 40.54% (15) isolates of *Pseudomonas aeruginosa* showed multidrug resistance which was similar to that observed by Basnet *et al.* (41.18%) [15]. However other studies done by Balkhair *et al.* and Zaveri *et al.* showed lower multidrug resistance in *Pseudomonas aeruginosa* i.e. 8.1% and 5.89% respectively [16,6]. Whereas other studies done by Mahmoud *et al.* and Unan *et al.* showed a higher percentage of multidrug resistance in *Pseudomonas aeruginosa* i.e. 52% and 60% respectively [8, 17].

We observed 30% (3) isolates of *Acinetobacter* to be multidrug resistant which was similar to that shown by Balkhair *et al.* i.e. 32.4% [16]. Basnet *et al.*

showed higher percentage (89.19%) whereas Zaveri *et al.* showed a lower percentage (10%) of multidrug resistance in Acinetobacter as compared to our study [15,6].

This difference in the multidrug resistance pattern of organisms in various studies may be due to difference in geographical distribution, as well as difference in antibiotic and infection control policies.

## CONCLUSION

Isolation of non-fermenting Gram negative bacilli and their antibiotic susceptibility pattern should be regarded with all seriousness in clinical practice and epidemiology because they are emerging nosocomial pathogens and by being resistant to multiple antibiotics, their prevalence not only limits the treatment options but also act as a reservoir of drug resistant genes.

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