



## Non fermenting gram negative bacilli as emerging pathogens - report from tertiary care hospital in Western India.

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### ABSTRACT

Nonfermenting gram-negative bacilli (NFGNB), which are saprophytic in nature, have emerged as important healthcare-associated pathogens. The main reason for concern about the prevalence of NFGNB is their tendency of being multidrug resistant. Keeping this in mind, this study was done to evaluate the NFGNB isolates from clinical samples. The nonfermenters were identified using a standard protocol that included tests for motility, oxidase production, oxidation-fermentation test, gelatin liquefaction, etc. Antibiotic susceptibility pattern (ABST) was evaluated using Kirby-Bauer disk diffusion method. Identification and ABST of some isolates was done using VITEK Automated system. The clinical significance was assessed by using various criteria. *Pseudomonas aeruginosa* and *Acinetobacter* spp were the most common isolates. Pus was the most common clinical sample. NFGNB are emerging as important opportunistic pathogens. Hence, proper management of infection is necessary to avoid emergence of drug resistance.

### KEYWORDS

*Acinetobacter* spp., *Pseudomonas aeruginosa*, carbapenemases, multidrug resistance

### Introduction

Non-fermenting Gram negative bacilli (NFGNB) are a diverse group of heterogeneous, aerobic, non-sporing Bacteria which are saprophytic in nature. These group of organisms do not utilize carbohydrate as a source of energy by fermentation and hence termed as 'Non-ferments'. However, they may utilize carbohydrate by oxidative method<sup>1</sup>. The NFGNBs have been defined as all aerobic Gram negative rods that show abundant growth within 24 hours on the surface of TSI medium but neither grows in nor acidifies the butt of the media.<sup>1</sup> This heterogeneous group includes organisms like *Pseudomonas* spp, *Acinetobacter* spp, *Alkaligenes* spp, *Stenotrophomonas maltophilia*, *Burkholderia cepacia* complex, etc.

Unlike, Enterobacteriaceae, the NFGNBs do not fit into a single family in a well characterized genera therefore the correct taxonomic placement of many NFGNB remains unresolved. However, DNA homology studies play an important role in the classification of bacteria. The major NFGNB are classified into 15 families. There are several schemes of identification of NFGNBs<sup>1, 2</sup>. The most important scheme of classification was given by King and Weaver-this was the first scheme for identifying aerobic GNB, developed by the late Elizabeth O' King. It uses simple and less complex media. It is economical and is the most commonly used scheme of identification. It follows utilization of glucose, ability to grow on MacConkey, oxidase activity, motility. Further grouping is based on colony morphological features, pigment production, Gram reaction and cellular morphology.

NFGNB can cause a wide variety of infections and account for approximately 15% of all gram negative bacilli cultured from clinical samples<sup>3</sup>. They are most commonly isolated from patients with serious underlying disease who has abusive use of wide spectrum antimicrobial agents, prolonged surgical procedures, prolonged hospital stay, inadequate mechanical instrumentation or tracheotomy, genitourinary instrumentation, in burns patients, low birth weight babies. The infection is observed in extreme age groups like neonates, children and geriatric age<sup>4</sup>. They are

opportunistic pathogens causing nosocomial infections in immunocompromised patients like pneumonia, meningitis, UTI & wound infections<sup>5</sup>. Among the species that are opportunist pathogens in immunologically compromised host either by disease or treatment, *Pseudomonas aeruginosa* is eminent, followed by *Acinetobacter baumannii*, *P.fluorescence*, *P.stutzeri*, *Stenotrophomonas maltophilia*, *P.putida*, *P.cepacia*<sup>2</sup>.

The medical and surgical advances in the management of seriously ill patients have increased the occurrence of NFGNB infections, previously believed to be non-pathogenic to man. The pattern of nosocomial infections dominated by resistant staphylococcus in 1950s has shifted to a predominance of infection due to GNB<sup>4</sup>.

NFGNBs are known to colonize first and then they subsequently invade the otherwise normally sterile site through trauma. It has been noted that disruption of natural barriers as an important route of entry of infections<sup>6,7</sup>. Rates of colonization increase in hospitalized patients particularly in those who have been hospitalized for extended periods or / and have received broad spectrum antimicrobial therapy/chemotherapy<sup>8</sup>.

Keeping these factors in mind, we had done this study to isolate and identify non fermenting gram negative bacilli from clinical samples and also to evaluate their antibiotic susceptibility pattern.

### Material and Methods

A retrospective study comprising of 130 NFGNB isolated from clinical samples, for a period of one year (January 2014 to December 2014) was carried out in department of Microbiology in a tertiary care hospital.

### Sample collection and processing:

Samples like respiratory sample, urine, blood, pus and body fluid were collected aseptically. Samples were processed as per standard microbiological techniques. All isolates of NFGNB were included in the study.

**Isolation and identification:**

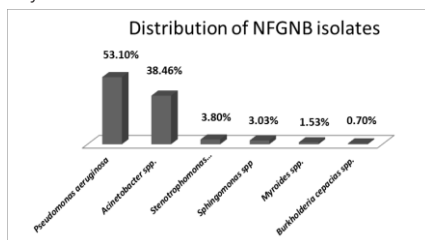
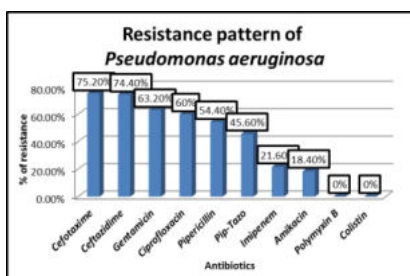
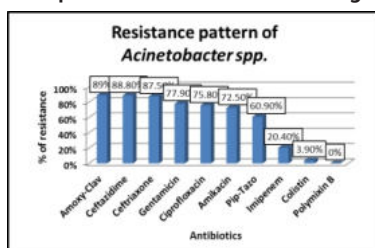
- All the samples were inoculated on Blood agar and MacConkey agar. NFGNB were identified as belonging to different genus by Hanging drop preparation for motility testing and Cytochrome oxidase. Other biochemical tests done to identify the NFGNB were:
- All organisms showing growth but no change in TSI (Triple Sugar Iron)
- Oxidative-Fermentation test using Hugh and Leifson medium
- Pigment production
- Indole production
- Citrate utilisation
- Nitrate reduction
- Esculin hydrolysis
- Decarboxylation tests
- Gelatin hydrolysis
- NFGNB were also identified by VITEK automated technique.

**Antibiotic susceptibility testing:**

Antibiotic sensitivity was done either by Kirby-Bauer disk diffusion method as per CLSI9 guidelines or by VITEK automated method. The various antibiotics used were Amoxycillin-Clavulinate 30 µg (Amoxy-Clav), Ceftazidime 30 µg, Ceftriaxone 30 µg, Gentamycin 10 µg, Amikacin 30 µg, Ciprofloxacin 5 µg, Piperacillin 100 µg, Piperacillin-Tazobactam 100/10 µg (Pip-Tazo), Imipenem 10 µg, Colistin 10 µg and Polymyxin B. *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as the control strains.

**Results**

Out of a total of 130 isolates of NFGNB, majority of them were isolated from patients belonging to age group of 60–80 years and majority of the patients were male (65%). Among various samples from which NFGNB were isolated, most common samples was pus (33.80%) followed by urine (30%), sputum (21.50%), body fluids (7.60%) and blood (6.90%). Surgery ward (36.90%) was the most common ward from where they were isolated. *Pseudomonas aeruginosa* and *Acinetobacter* spp were the most common isolates (Fig 1). The antibiotic susceptibility pattern of *Pseudomonas aeruginosa* and *Acinetobacter* spp are as shown in Fig 2 and Fig 3 respectively.

**Fig 1** Distribution of the NFGNB**Fig 2** Resistance pattern of *Pseudomonas aeruginosa***Fig 3** Resistance pattern of *Acinetobacter* spp.**Discussion**

Non fermenting gram negative bacilli are considered as important health care pathogens as they have emerged as multidrug resistant microorganisms. In this study, most of the isolates of NFGNB were isolated from pus and clinical conditions in which they were isolated were UTI, ventilator associated pneumonia and septicemia. Similar to our study, pus was the most common specimen in the study done by Patel et al<sup>10</sup> and Benachinmardi et al<sup>11</sup>.

Similar to our study, *Pseudomonas aeruginosa* and *Acinetobacter* spp. were the most commonly isolated NFGNB in several other studies like Patel et al<sup>10</sup>, Benachinmardi et al<sup>11</sup>, Bhargava et al<sup>12</sup>, Kalidas et al<sup>13</sup> and Nautiyal et al<sup>14</sup>. Other isolates in our study were *S. maltophilia*, *Myroides* spp, *Sphingomonas* spp and *Burkholderia cepacia*.

*Pseudomonas aeruginosa* showed maximum resistance to cefotaxime, ceftazidime and lower resistance to amikacin, colistin and polymyxin B. In studies like Kalidas et al<sup>13</sup>, Nautiyal et al<sup>14</sup> and Juyal et al<sup>15</sup>, *Pseudomonas aeruginosa* showed higher susceptibility to Amikacin and colistin. Unlike our study, Bhargava et al<sup>12</sup> shows higher resistance of *Pseudomonas aeruginosa* for Amikacin. *Acinetobacter* spp. showed maximum resistance to amoxclav, ceftazidime, ceftriaxone, aminoglycosides and lower resistance to Polymyxin B, Imipenem and Colistin. These results are similar to those shown by Kalidas et al<sup>13</sup> and Nautiyal et al<sup>14</sup>.

In our study, carbapenamase activity was shown by 20.4% and 21.6% of *Pseudomonas aeruginosa* and *Acinetobacter* spp. isolates respectively. In various studies across the world resistance to carbapenems varies between 4-60%<sup>15</sup>, with Imipenem resistant *P. aeruginosa* (42%) and *A. baumannii* (18.5%)<sup>8,16</sup>. In Sharan et al., only 70.3% and 62.1% of *Acinetobacter* spp. and *P. aeruginosa* isolates respectively, showed sensitivity for Imipenem while the remaining was resistant<sup>17</sup>.

The increasing concerns regarding the prevalence of NFGNB are mainly due to their multidrug resistance pattern. They exhibit resistance not only to beta lactam and the other groups of antibiotics, but also to carbapenems. In recent years due to the indiscriminate & irrational use of antimicrobials, NFGNB have emerged as important pathogens. Studies have confirmed a relation between antibiotic exposure and emergence of antibiotic resistant bacteria<sup>8</sup>. Studies show that due to increase usage of Imipenem in the past decade, Imipenem Resistance has emerged among the NFGNB<sup>18</sup>. Moreover, inherent resistance of these bacterial agents to commonly used disinfectants and their tendency to colonize various surfaces have played a pivotal role in their emergence as important nosocomial pathogens<sup>19</sup>. Development of resistance by NFGNB is multifactorial. More than one factor may be involved, like, mutations in genes encoding porins, efflux pump mechanism, Penicillin binding proteins, Chromosomal  $\beta$  lactamase<sup>20,21</sup>.

**Conclusion**

- NFGNB are emerging as important opportunistic pathogens. These bacilli are intrinsically resistance to many antibiotics and cause device related infections.
- NFGNB have potential to spread horizontally on fomites or hands of medical personnel and will continue to pose a challenge for clinicians in the future.
- Proper management of infection would avoid unnecessary usage of antibiotics and emergence of drug resistance.

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