

**Research Article**

**Non fermentative Gram negative bacilli infections in a tertiary care rural hospital**

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**Abstract**

**Introduction:** Non fermentative Gram negative bacilli (NF GNB) are being increasingly isolated from patients admitted in hospitals. Most of these isolates are multidrug resistant (MDR).

**Methods:** Over a period of one year, all clinical samples were processed and NF GNB was identified up to species level following conventional method. Antibiotic sensitivity test of the isolates were done following clinical and laboratory standard institute (CLSI) guidelines.

**Results:** Out of 1498 clinical specimens, 320 (21.36%) isolates were identified as NF GNB. Maximum number of samples was blood, 90(28.12%), followed by pus, 84 (26.25%). *Pseudomonas aeruginosa* (*P. aeruginosa*) was the commonest isolate, 192 (60%), followed by *Pseudomonas* species (*P. species*), 58 (18.12%) & *Acinetobacter baumannii* (*A. baumannii*), 46(14.37%). All isolates were MDR and were sensitive to polymyxin B, colistin and tigecycline.

**Conclusion:** Multidrug resistant NF GNB was not uncommon in our hospital. All isolates were sensitive to polymyxin B, colistin and tigecycline. Indiscriminate use of antibiotics against these organisms should be avoided.

**Keywords:** Non fermentative; Multi drug resistant; *Pseudomonas*; *Acinetobacter*

**1. Introduction**

The non fermentative Gram negative bacilli (NF GNB) are aerobic and non spore forming microorganisms. They do not utilize carbohydrates or breakdown them through oxidative metabolic pathway. Previously NF GNB were considered to be non pathogenic and of very little significance. Recently, rate of infection by NF GNB is rising, especially in hospitalized and immunocompromised patients. NF GNB infection constitutes about one – fifth of all Gram negative bacilli infections. These organisms can remain viable on medical devices and are resistant to many commonly used antibiotics. Therefore, they play an important role in hospital acquired infection. Although, rate of isolation of NF GNB from clinical specimens is increasing rapidly, very few laboratories in India identify these organisms routinely. Sometimes, NF – GNB is difficult to identify phenotypically. Most of them are multidrug resistant (MDR).<sup>1</sup> Malini *et al*<sup>2</sup> from Karnataka, India observed 4.5 % infection rate of NF GNB in their hospital.

Therefore, the present study was conducted to identify NF GNB, isolated from clinical specimens, up to species level and to find out suitable antibiotics to treat such patients.

## 2. Material & Methods

**2.1 Ethics committee approval** – The study was approved by the Institutional ethical committee (Reference no – PMT/RMC/RC/ 2010/522). All the clinical samples received in the microbiology department were processed without delay following conventional methods. Urine samples were screened for significant bacteriuria ( $\geq 10^5$  colonies/ mL). Gram staining and culture were done from pus samples. The clinical samples were cultured on blood agar and MacConkey's agar and incubated at 37° C for 24 – 48 hours aerobically. Battery of tests was done with the colony as per conventional methods.<sup>3</sup> Antibiotic sensitivity test was performed by using modified Kirby – Bauer disk diffusion method following clinical and laboratory standard institute (CLSI) guidelines.<sup>4</sup> The antibiotic disks used for this study were following – ceftazidime (10 µg), Amikacin (30 µg), Netilmycin (30 µg), ciprofloxacin (5µg), ticarcillin (75 µg), cefepime (30 µg), piperacillin/tazobactam (100/10µg), Imipenem (10µg), aztreonam (30 µg), colistin (10 µg), polymyxin B (50 µg) and tigecycline (15 µg). MIC (minimum inhibitory concentration) of imipenem was also detected in resistant isolates. All antibiotic disks were obtained from Himedia pvt ltd, India and the E strip for MIC detection from AB BioMerieux.

The isolates from patient's specimens were also clinically correlated by discussion with attending physician and by retrospective reference of the patient's records. The symptoms and signs of infections, hematological and radiological findings and repeated isolation of same organisms from patient's specimens were considered in support of clinical significance.

## 3. Observations & Results

A total of 1498 specimens, such as, blood, urine, pus etc were processed in the microbiology laboratory over a period of one year. Out of which, 320 (21.36%) NF GNB were isolated and identified up to species level. Organisms isolated were 192 (60%) *Pseudomonas aeruginosa* (*P. aeruginosa*), 58 (18.12%) *Pseudomonas* species (*P. species*), 11 (3.43%) *Pseudomonas fluorescence* (*P. fluorescence*), 46 (14.37%) *Acinetobacter baumannii* (*A. baumannii*), 7 (2.18%) *Acinetobacter lwoffii* (*A. lwoffii*), 2(0.62%) *Alkaligenes faecalis* (*A. faecalis*) and 1 (0.31%) *Stenotrophomonas maltophilia* (*S. maltophilia*). 3(0.93%) of the NF GNB isolates could not be identified up to species level. (Table 1)

**Table 1: Distribution of different non fermenting, Gram negative bacterial species from various clinical specimens.**

Samples (Total & %)	<i>P. aeruginosa</i>	<i>P. species</i>	<i>P. fluorescence</i>	<i>A. baumannii</i>	<i>A. lwoffii</i>	<i>A. faecalis</i>	<i>S. maltophilia</i>	Nil fermenter
Pus (84, 26.25%)	45	19	8	10	1	-	-	1
Blood (90, 28.12%)	71	5	-	13	1	-	-	-
Urine (58, 18.12%)	33	7	-	14	2	-	-	2
Cerebrospinal fluid (12, 3.75%)	2	4	1	3	1	-	1	-
Sputum (16, 5%)	10	2	1	2	1	-	-	-
Vaginal swab (16, 5%)	14	2	-	-	-	-	-	-
Pleural fluid (12, 3.75%)	2	8	-	2	-	-	-	-
Endotracheal tube (17, 5.31%)	7	6	1	2	1	-	-	-
Stool (3, 0.93%)	1	-	-	-	-	2	-	-
Catheter tip (5, 1.56%)	4	1	-	-	-	-	-	-
Throat swab (4, 1.25%)	2	2	-	-	-	-	-	-
Ascitic fluid (3, 0.93%)	1	2	-	-	-	-	-	-
Total	192 (60%)	58 (18.12%)	11 (3.43%)	46 (14.37%)	7 (2.18%)	2 (0.62%)	1 (0.31%)	3 (0.93%)

(n = 320)

Maximum number of isolates were from blood samples, i.e. 90 (28.12%), followed by 84 (26.25%) pus, 58 (18.12%) urine, 17 (5.31%) endotracheal tube aspiration, 16 (5%) sputum and vaginal swab each, 12 (3.75%) cerebrospinal fluid and pleural fluid each, 5 (1.56%) catheter tips, 4 (1.25%) throat swab and 3 (0.93%) stool and ascitic fluid each. (Table 1)

**Table 2: Antibiotic sensitivity pattern of various clinical isolates.**

Antibiotics	<i>P. aeruginosa</i> (n = 192)	<i>P. Species</i> (n = 58)	<i>P. Fluorescence</i> (n = 11)	<i>A. Baumannii</i> (n = 46)	<i>A. lwoffii</i> (n = 7)	<i>S. Maltophilia</i> (n=1)	<i>A. Faecalis</i> (n = 2)	Nil Fermenter (n = 3)
Ceftazidime	11 (5.72%)	8 (13.79%)	3 (27.27%)	10 (21.73%)	0	0	0	0
Amikacin	42 (21.87%)	10 (17.24%)	2 (18.18%)	12 (26.06%)	4 (57.14)	1 (100%)	1 (50%)	2 (66.66%)
Netilmycin	25 (13.02%)	13 (27.08%)	2 (18.18%)	11 (23.91%)	3 (42.85)	1 (100%)	2 (100%)	1 (33.33%)
Ciprofloxacin	21 (10.93%)	10 (17.24%)	3 (27.27%)	14 (30.43%)	3 (42.85%)	0	1 (50%)	0
Ticarcillin	9 (4.68%)	3 (5.17%)	2 (18.18%)	8 (17.39%)	2 (18.18%)	1 (100%)	0	1 (33.33%)
Piperacillin/ Tazobactam	47 (24.47%)	23 (39.65%)	7 (63.63%)	21 (45.65%)	4 (57.14%)	1 (100%)	1 (50%)	2 (66.66%)
Aztreonam	4 (2.08%)	4 (6.89%)	2 (18.18%)	2 (4.34%)	4 (57.14%)	1 (100%)	1 (50%)	2 (66.66%)
Imipenem	86 (44.79%)	39 (67.24%)	8 (72.72%)	28 (60.86%)	5 (71.42%)	1 (100%)	2 (100%)	3 (100%)
Polymyxin B	192 (100%)	58 (100%)	11 (100%)	46 (100%)	7 (100%)	1 (100%)	2 (100%)	3 (100%)
Colistin	192 (100%)	58 (100%)	11 (100%)	46 (100%)	7 (100%)	1 (100%)	2(100%)	3 (100%)
Tigecycline	192 (100%)	58 (100%)	11 (100%)	46 (100%)	7 (100%)	1 (100%)	2 (100%)	3 (100%)

All the isolates were MDR. All imipenem resistant NF GNB (by disk diffusion method) showed high MIC values to imipenem. All the NF GNB, isolated from clinical samples was found susceptible to polymyxin B, colistin and tigecycline. (Table 2)

#### 4. Discussion

NF GNB is rapidly emerging as important opportunistic pathogen, mainly in immunocompromised or hospitalized patients. These organisms are aerobic, non spore bearing and do not breakdown carbohydrates as a source of energy, other than fermentation.<sup>5</sup> They mainly cause hospital acquired infection.<sup>6</sup> Indiscriminate use of antibiotics play a major role in development of resistance to commonly used antibiotics.<sup>7</sup> Initial identification of NF GNB is done by absence of acid production in triple sugar iron (TSI) agar and no growth on MacConkey's agar. Major laboratory tests to identify NF GNB are – Gram stain, Hugh – Leifson's test, gelatin liquefaction, starch hydrolysis, urease production, nitrate reduction, indole test, hydrogen sulphide production, growth on 6.5% sodium chloride solution, pigment production, lysine decarboxylation and fermentation of glucose, lactose, maltose, Mannitol and xylose.<sup>8,9</sup> Over a period of one year, we isolated 320 (21.36%) NF GNB out of 1498 clinical specimens. The most common isolate was *P. aeruginosa*, i.e., 192 (60%) and *P. species*, 58 (18.12%) followed by *A. baumannii*, 46(14.37%). Siou Cling Su *et al*<sup>10</sup> in their study, isolated approximately 15% NF GNB out of all Gram negative bacilli isolates. They did oligonucleotide array based test to identify NF GNB from clinical specimens and found that *P. aeruginosa* was the commonest isolate, followed by *Acinetobacter* species. Our study was also similar to the study done by Malini *et al*<sup>2</sup>, i.e., 53.8% *P. aeruginosa* and 22.2% *A. baumannii* isolates. Upgade *et al*<sup>6</sup> isolated 43% *Pseudomonas* species and 21% *Acinetobacter* species in their study.

We isolated maximum number of NF GNB from blood, i.e., 90(28.12%). Some other researchers isolated majority of non fermenters from pus and urine samples.<sup>2</sup>

Multidrug resistance was considered when the organism was resistant to 3 or more classes of antibiotics.<sup>11</sup> Antibiotic susceptibility pattern of NF GNB vary from country to country and from also different places within the same country.<sup>12</sup> All of our NF GNB isolates were MDR. NF GNB has got a tendency for inherent or acquired drug resistance to the commonly used antibiotics.<sup>7</sup> Upgade et al<sup>6</sup> observed 80% resistance of NF GNB to major antibiotics. Nicasio et al<sup>13</sup> reported increasing resistance of NF GNB to commonly used antibiotics, including carbapenems, cephalosporin, penicillin, fluoroquinolones and amino glycosides. They also observed that isolates were mostly sensitive to polymyxin B. Some researchers reported that to treat NF GNB infection, some old antibiotics with more side effects were again in use.<sup>14</sup> All our isolates were sensitive to polymyxin B, colistin and tigecycline. Colistin and polymyxin B has got side effects like renal toxicity (27 – 58%).<sup>15</sup> Li et al<sup>16</sup> and Falaqas et al<sup>17</sup> also found usefulness of colistin and polymyxin B against MDR *P. aeruginosa*, *A. baumannii* and *Klebsiella pneumoniae* (*K. pneumoniae*) due to low resistance rate to this drug. They also advised judicious use of the antibiotics for infection caused by these 3 micro organisms.

Tigecycline (Gar 936) is a new glycopeptides derivative of tetracycline. It has a wide range of antibacterial activity, both against Gram positive and Gram negative bacteria.<sup>18</sup>

However, reports regarding resistance to colistin and tigecycline have been observed by some workers.<sup>19, 20</sup>

NF GNB is ubiquitously distributed in the environment. Their isolation from patient's samples should be clinically correlated to avoid unnecessary administration of antibiotics and thereby preventing the development of MDR strains. We included those cases in our study, in which, there were repeated isolation of same organism from repeat samples. Other inclusion criteria were presence of pus cells along with Gram negative bacilli in the stained smear of the clinical samples, evidence of mono microbial infection from sterile body fluid and radiological or hematological reports supporting infection. Symptoms and signs indicating infection were also taken into consideration.

## 5. Conclusion

NF GNB infection is emerging rapidly as major pathogens in health care settings. All infections by NF GNB should be clinically correlated. NF GNB develops resistance to commonly used antibiotics very fast. All our isolates were sensitive to polymyxin B; colistin and tigecycline. Careful administration of antibiotics should be applied, because these organisms have a tendency to develop resistance.

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