

## TO STUDY ANTIBIOTIC SENSITIVITY PATTERN OF NONFERMENTATIVE GRAM NEGATIVE BACILLI FROM VARIOUS CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL, JAIPUR.

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### Abstract

**Background:** Nonfermentative gram negative bacilli (NFGNB) frequently considered as commensals or contaminants but the pathogenic potential of nonfermenters has been proved beyond doubt. They are resistant to commonly used antimicrobials.

**Aim:** This study was undertaken to identify the nonfermenters isolated from various clinical samples and to know their Antibiotic sensitivity pattern.

**Materials and Methods:** The present study was carried out on 150 strains of Nonfermenters isolated from 1200 various non repetitive clinical samples received in Department of Microbiology, NIMS Jaipur. Nonfermenters were identified using a standard protocol and their antibiotic susceptibility testing was performed with the help of the modified Bauer disc diffusion method.

**Results:** Out of 150 nonfermenters isolated, *Pseudomonas aeruginosa* was the most common isolate 134 (89.33%) followed by *Acinetobacter baumannii* 16 (10.67%). Among all clinical samples Pus and Wound Discharge yield maximum isolates of NFGNB i.e. 54 (36%) % followed by sputum (39.0%). Most sensitive drug against NFGNB was Polymyxin-B (100%) followed by Imipenem (86 %) and Amikacin (71.33 %).

**Conclusion:** Nonfermenters have a great potential to survive in a hospital environment so implementation of antibiotic stewardship programs and strict infection control practices will be required to prevent or slow down their emergence and spread.

**Keywords:** Nonfermenters, Polymyxin-B, *Pseudomonas*, *Acinetobacter*.

### Introduction

The nonfermentative gram negative bacilli used in this study means all aerobic gram- negative rods that show abundant growth within 24 hours on the surface of Kleiger iron agar (KIA) or triple sugar iron medium, but neither grow in nor acidify the butt of these media.<sup>1</sup>

These organisms are invariably resistant to common disinfectants such as chlorhexidine and quaternary ammonium compounds.

Nonfermentative gram negative bacilli are known to be account for about 15% of all bacterial isolates of any clinical microbiology laboratory.<sup>1</sup> Some of the disease manifestations associated with non fermenting organisms are septicemia, meningitis, osteomyelitis, UTI, pneumonitis and wound infections usually following surgery or trauma. Multidrug resistance is a common problem in nonfermentative gram negative bacilli particularly *Pseudomonas aeruginosa*.

The increasing prevalence of infections due to pathogens is explained by several factors like their ability to produce enzymes capable of hydrolyzing broad spectrum beta lactams, A variety of resistant mechanisms, Non adherence to hospital antibiotics policy, Excessive and indiscriminate use of broad spectrum antibiotics.

Due to increasing prevalence of infection and increasing resistance to antibiotics of nonfermentative gram negative bacilli, it is important to know their antimicrobial drug susceptibility pattern.<sup>2</sup>

### Material and Methods

Present study was conducted in the department of Microbiology, National Institute of Medical Sciences (NIMS) Medical College and hospital, a tertiary care hospital, Jaipur from July 2013 to July 2014. A total 150 of strains of Nonfermenters were isolated from 1200 various non repetitive clinical samples. Present study was observational with a Cross Sectional design.

Specimens included were Pus, Wound discharge, Blood culture, Urine, Sputum, Ear discharge, Body fluid.

The clinical samples were processed immediately and cultured as per Standard protocol using battery of tests<sup>1</sup>

1. Cultural characters: Blood agar, MacConkey and Nutrient agar.
2. Pigment production: Blood agar and Nutrient agar
3. Morphology and Gram's stain
4. Motility: Hanging drop preparation.
5. Catalase test
6. Oxidase test
7. Indole, Methyl red, Voges Proskauer, Citrate utilization test, Urease test, and Triple sugar iron reaction.
8. Oxidation / fermentation (Hugh and Leifson's media) for glucose, lactose, xylose, mannitol and maltose.
9. Lysine, Ornithine decarboxylase and Arginine dihydrolase activity.
10. DNase test
11. ONPG tests
12. Esculin test.

Antimicrobial sensitivity testing was done by modified Kirby-Bauer disc diffusion method as per CLSI guidelines using commercially available (himedia) discs. The different antimicrobials tested were Piperacillin, Cefepime, Cefotaxime, Ceftazidime, Ceftriaxone, Amikacin, Gentamicin, Tobramycin, Netilmicin, Ofloxacin, Ciprofloxacin, Cotrimoxazole, Aztreonam, Imipenem, Polymyxin-B. *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were used as Quality Control strains.

## Results and Discussion

**Table 1:** Spectrum of Nonfermenters Isolates from Clinical Samples (n=150)

Species	No.	%
<i>Pseudomonas aeruginosa</i>	134	89.33
<i>Acinetobacter baumannii</i>	16	10.67

As shown in table 1: Most common isolate from clinical samples was *Pseudomonas aeruginosa* 89.33% followed by *Acinetobacter baumannii* about 10.67%.

**Table 2:** Types of Clinical Samples from which Nonfermenters were isolated

Specimens	<i>Pseudomonas aeruginosa</i>		<i>Acinetobacter baumannii</i>		Total	
	No.	%	No.	%	No.	%
Pus and Wound discharge	52	96.30	2	3.70	54	100.00
Sputum	33	84.62	6	15.38	39	100.00
Ear swab	28	96.55	1	3.45	29	100.00
Urine	16	84.21	3	15.79	19	100.00
Blood	4	57.14	3	42.86	7	100.00
CSF	0	0.00	1	100.00	1	100.00
Pleural fluid	1	100.00	0	0.00	1	100.00
Total	134	89.33	16	10.67	150	100.00

Chi-square = 21.874 with 6 degrees of freedom; P = 0.001

Table No. 2 show the maximum number of NFGNB was isolated from Pus and wound discharge followed by Sputum while minimum from the Pleural fluid and CSF.

**Table 3:** Antibiotic Sensitivity Pattern of NFGNB

Antibiotic Sensitivity Pattern	<i>Pseudomonas aeruginosa</i> (n=134)		<i>Acinetobacter baumannii</i> (n=16)	
	No.	%	No.	%
Piperacillin	46	34.33	4	25.00
Cefepime	84	62.69	9	56.25
Cefotaxime	NA	-	3	18.75
Ceftazidime	65	48.51	6	37.50
Ceftriaxone	NA	-	5	31.25
Amikacin	98	73.13	9	56.25
Gentamicin	74	55.22	5	31.25
Tobramycin	64	47.76	7	43.75
Netilmicin	38	28.36	4	25.00
Ofloxacin	79	58.96	3	18.75
Ciprofloxacin	84	62.69	6	37.50
Cotrimoxazole	NA	-	3	18.75
Aztreonam	64	47.76	7	43.75
Imipenem	118	88.06	11	68.75
Polymyxin-B	134	100.00	16	100.00

\*NA: Not applicable

As shown in Table No. 3: Most of the isolates of *Pseudomonas aeruginosa* showed higher

sensitivity to Polymyxin-B (100%) followed by Imipenem (88.06%) and least sensitivity against Piperacillin (34.33%) while *Acinetobacter baumannii* showed maximum sensitivity to Polymyxin-B (100%) and least sensitivity against Cefotaxime, Cotrimoxazole and Ofloxacin.

## Discussion

In the present study, a total 150 of strains of Nonfermenters were isolated from 1200 various non repetitive clinical samples. We isolated only *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *Pseudomonas aeruginosa* was most commonly isolated NFGNB about 89.33%. This result has correlated well with the study conducted Gokale et al (2012)<sup>3</sup> who isolated from 95.28% of cases.

In the present study the second commonest isolate was *Acinetobacter baumannii* 10.67%. The result correlated well with the study conducted by Arora et al. (2010)<sup>4</sup> who reported 8.4%.

In our study maximum number of NFGNB were isolated from Pus & Wound discharge 54 (36%) followed by Sputum 39 (26%), Ear swab 29 (19.33%), Urine 19 (12.67%), Blood 7(4.67%), CSF 1 (0.67%) and Pleural fluid 1 (0.67%). This was similar to other studies done by Mindoli P B et al. (2010)<sup>5</sup> and Malini A et al. (2010)<sup>6</sup> who reported maximum number of NFGNB from pus samples. It indicates that they are a common cause of suppurative pyogenic localized infections.

In our study *Pseudomonas aeruginosa* showed 34.33% sensitivity to Piperacillin while Chander A et al. (2013)<sup>7</sup>. In our study *Acinetobacter baumannii* showed a sensitivity of 25.00% to Piperacillin. In other studies conducted by Chander A et al (2013)<sup>7</sup> as 4.83%, Juyal D et al.<sup>8</sup>(2013) it showed a sensitivity of 30%.

In our study Cefepime was 62.69% sensitive to *Pseudomonas aeruginosa* comparable to the study of 36.17 % Juyal D et al. (2013)<sup>8</sup>. In our study Cefepime was 56.25% sensitive to *Acinetobacter baumannii*. It was comparable to the study of Mushtaq et al. (2013)<sup>9</sup> 70% and While other study show a lower sensitivity i.e Shareek et al. (2012)<sup>10</sup> 28%.and Juyal D et al. (2013)<sup>8</sup> 26.39%.

In our study 48.51% of *Pseudomonas aeruginosa* strains were sensitive to Ceftazidime which was comparable to other study Sharma et al. (2010)<sup>11</sup> to be 41%. The higher sensitivity were shown in the studies of D'Souza et al. (2014)<sup>12</sup>

to be 64. Lower sensitivity were shown by Franco et al. 14.5% in (2010)<sup>13</sup>.

In our study 37.50% *Acinetobacter baumannii* was sensitive to Ceftazidime, which was comparable to the study of Mushtaq et al. (2013)<sup>9</sup> 40%. The lower sensitivity was seen in the study of Juyal D et al. (2013)<sup>8</sup> 18.06%.

In present study, *Acinetobacter baumannii* was 31.25% sensitive to Ceftriaxone. It was comparable with the study of Mustaq S et al. (2013)<sup>9</sup> 44%, Shareek et al. (2012)<sup>10</sup> 10.5%.

In the present study, *Pseudomonas aeruginosa* showed 73.13% sensitivity against Amikacin. In other studies by Kumar V et al. (2011)<sup>14</sup> showed 68%. Other studies reported lower sensitivity to Amikacin against *Pseudomonas aeruginosa* such as Sharma et al. (2010)<sup>11</sup> 8.8%.

In present study, *Acinetobacter baumannii* was 56.25% sensitive to Amikacin. It was comparable with the study of Kumar E et al (2014)<sup>15</sup> showed 46.63%.

In the present study, Gentamicin was found to be 55.22% sensitive against *P. aeruginosa*. Higher sensitivity 53% was reported by Kumar V et al. (2011)<sup>14</sup>, 57.2%.. Lower sensitivity were shown in the studies of Ranjan R et al. (2001)<sup>15</sup> 29.1%, In our study, *Acinetobacter baumannii* was 31.25% sensitive to Gentamicin. It was comparable with other study of Kumar E et al. (2014)<sup>15</sup> 41.66% .

In our study, *Pseudomonas aeruginosa* was 47.76% sensitive to Tobramycin, it correlates with study Islahi S et al (2014)<sup>16</sup> 69.3%. In present study, *Acinetobacter baumannii* was 43.75% sensitive to Tobramycin.

In our study 28.36% isolates of *Pseudomonas aeruginosa* were sensitive to Netilmicin which was quite similar to the study of Javiya VA et al. (2008)<sup>17</sup> and Goel V et al. (2013)<sup>18</sup> i.e. 33.33% and 29.8% against *Pseudomonas aeruginosa*

respectively. In other study the maximum sensitivity 67.2%. To Netilmicin was shown by D'Souza et al. (2014).<sup>12</sup>

In our study 25% isolates of *Acinetobacter baumannii* were sensitive to Netilmicin .In the study of Kumar E et al. (2014)<sup>15</sup> showed higher sensitivity i.e. 41.02%

In our study, we found 62.69% isolates of *P. aeruginosa* to be sensitive to Ciprofloxacin. It was comparable to other studies that of Doosti M et al. (2013)<sup>19</sup> 58%. Lower sensitivity was found in other studies by Franco et al. (2010)<sup>13</sup> 14.5%.

Our study showed 47.76% *Pseudomonas aeruginosa* isolates were sensitive to Aztreonam. This was comparable to the study of Picao et al. (2008)<sup>20</sup> 44.1%. The study of Goel et al. (2013)<sup>18</sup> showed lower sensitivity 5.1%.

In present study, 43.75% *Acinetobacter baumannii* was sensitive to Aztreonam. The lower sensitivity was shown in other studies of Shareek et al. (2012)<sup>10</sup> 14% and Juyal D et al. (2013)<sup>8</sup> 8.33%. While Kumar E et al. (2014)<sup>15</sup> in their study showed that all *Acinetobacter baumannii* isolated were resistant to Aztreonam.

In the Carbapenem group, we tested Imipenem antibacterial spectrum to *Pseudomonas aeruginosa* isolates. The sensitivity of Imipenem in our study was 88.06%. Our study also correlate well with the study of D'Souza et al. (2014)<sup>12</sup> who showed 91.5% sensitivity to Imipenem and Hocquet et al. in (2007)<sup>21</sup> at France showing 82.5%. In other studies the while Picao et al. (2008)<sup>20</sup> reported a lower sensitivity 18.5 % of *Pseudomonas aeruginosa* isolates against Imipenem. Franco et al. (2010)<sup>13</sup> in contrast reported their all isolates resistant to Imipenem.

In our study *Acinetobacter baumannii* showed sensitivity 68.75% to Imipenem which is lesser as comparable to other studies Parandekar P K et al. (2012)<sup>22</sup> and Islahi S et al. (2014)<sup>16</sup> who showed sensitivity to Imipenem 86.3% and 80.43% respectively.

In our study *Acinetobacter baumannii* showed 18.75% sensitivity to Cotrimoxazole which was comparable to study Islahi S et al. (2014)<sup>16</sup> 8.69%.

In the present study, *Pseudomonas aeruginosa* were found to be 100% sensitive to Polymyxin B.

In present study, *Acinetobacter* was 100% sensitive to Polymyxin – B. It was comparable with the study of Shareek et al. (2012)<sup>10</sup> 100%.

## CONCLUSION

As there increasing frequency of human infections by Nonfermentative gram negative bacteria the diagnostic laboratory must be able to identify these organisms accurately. NFGNB are resistant to commonly used

antimicrobials. Therefore regular interaction between microbiologists and clinicians should be established to accustom them to the ongoing antibiotic susceptibility/resistance pattern of antibiotics prevailing among NFGNB. A functional hospital infection control committee should be constituted. Continued awareness of the need to maintain good housekeeping, equipment decontamination, strict attention to hand washing and isolation procedures and control of antibiotic usage especially in high risk areas is need of the hour to prevent spread of resistance among organisms.

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