Original article:

Phenotypic detection of ESBL and MBL in clinical isolates of Nonfermenters.


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Abstract:

Background: Non fermenting gram negative bacilli (NFGNB) producing Metallo β lactamases (MBL), extended spectrum β lactamases (ESBL) are an increasing cause of concern in the hospitals as they produce a therapeutic dilemma for the treating physician. The present study was undertaken to know the prevalence of ESBL and MBL producing non fermenting gram negative bacilli from clinical isolates and their antibiotic resistance pattern.

Materials and methods: A total of 103 non fermenting gram negative bacilli were collected from various clinical specimens. All the samples were processed for routine bacterial culture and antimicrobial susceptibility test done as per CLSI guidelines. Imipenem resistant isolates were selected for the detection of MBL production by Imipenem-EDTA combined disc test. ESBL production is done by phenotypic confirmatory double disk diffusion test using Ceftazidime with and without Clavulanic acid.

Results: Out of 103 Nonfermenter isolates, 37(38.3%) were ESBL producers and 16(18%) MBL producers. None of the isolates showed the coexistence of ESBL and MBL in the same isolate. ESBL and MBL production was observed in Pseudomonas, and Acinetobacter spp., isolated from various clinical samples.

Interpretation and conclusion: The study underlines problem of ESBL and MBL mediated resistance, which has created a therapeutic challenge for the clinicians and microbiologists. Maintenance of strict antibiotic policy in the hospital is a must to fight against antibiotic resistance.

Key words: NFGNB, MBL, ESBL, Pseudomonas, Acinetobacter.

Introduction:

NFGNB have emerged as important healthcare associated pathogens. They have been incriminated in infections such as, septicemia, meningitis, pneumonia, urinary tract infections (UTI), and surgical site infections (SSI)¹. NFGNB are innately resistant to many antibiotics and are known to produce extended spectrum β-lactamases and metallo β-lactamases²³. NFGNB causes serious infections in immunocompromised and hospitalized patients especially those admitted to intensive care units (ICU). These organisms further worsen the situation by virtue of their multidrug resistance and thus limit therapeutic options⁴. Carbapenems first introduced in 1980 are now frequently used as the last choice in treating serious infections caused by multidrug resistant gram negative bacilli. These antibiotics are stable to β-lactamases including the extended spectrum β-lactamases (ESBLs) and Amp C produced by gram negative bacilli. Unfortunately resistance to these antibiotics started emerging from 1990 and has been reported in non fermenting gram negative bacilli (NFGNB) worldwide over the years with varying frequencies.

Pseudomonas aeruginosa and Acinetobacter spp. in particular are most often associated with Carbapenem resistance. Unfortunately, there is paucity of data on the prevalence of Carbapenem resistance in the Indian literature⁵. Reportedly, several outbreaks due to Carbapenem resistant NFGNB have resulted with considerable morbidity
and mortality. Resistance to these antibiotics is due to decreased outer membrane permeability, increased efflux systems, alteration of penicillin binding proteins and Carbapenem hydrolyzing enzymes – Carbapenemases. They may be chromosomally or plasmid mediated and therefore pose a threat of spread of resistance by gene transfer among gram negative bacteria. The appearance of MBL genes and their spread among bacterial pathogens is a matter of concern. The present study was undertaken to find the prevalence of MBL and ESBL producing NFGNB isolates.

Materials and Methods
A total of 103 clinical isolates of Nonfermenters, which were isolated from various samples (blood, urine, sputum, pus) were identified by standard procedures. The susceptibility of isolates to antibiotics was determined by Kirby- Bauer disc diffusion method using Clinical and Laboratory Standard Institute (CLSI) guidelines. Antibiotics included were Aztreonam (30 µg), Cefipime (30 µg), Cefotaxime (30 µg), Cefoxitin (30 µg), Ceftazidime (30 µg), Imipenem (10 µg). Isolates resistant to the third generation Cephalosporins were tested for ESBL production and isolates showing resistance to Imipenem were tested for MBL production.

Detection of ESBL
This was performed by phenotypic confirmatory test as per the recommendations of CLSI. The Ceftazidime (30 g) discs alone and in combination with Clavulanic acid (Ceftazidime +Clavulanic acid, 30/10 g discs) were used. An increase of 5mm in zone of inhibition of the combination discs in comparison to the Ceftazidime disc alone was considered to be ESBL producer.

Detection of MBL
This was performed by Imipenem EDTA combined disc test. Two (10 g) Imipenem discs were placed on a plate inoculated with the test organism, and 10 l of 0.5 M EDTA solution was added to one disc. A zone diameter difference between the Imipenem and Imipenem + EDTA of 7 mm was interpreted as a positive result for MBL production.

Result:
NF GNBs were considered as a contaminant in the past but now emerged as an important health care pathogen. Pseudomonas aeruginosa and Acinetobacter sp., are known to be nosocomial pathogen. In the present study out of 103 non fermenters, Pseudomonas were 83.5% followed by Acinetobacter 15.5% species was the second commonest non-fermenter.

Distribution of different NFGNB various clinical specimens
Table-1

<table>
<thead>
<tr>
<th>Specimen</th>
<th>No of cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pus</td>
<td>50</td>
<td>48.6%</td>
</tr>
<tr>
<td>Sputum</td>
<td>34</td>
<td>33%</td>
</tr>
<tr>
<td>Urine</td>
<td>13</td>
<td>12.6%</td>
</tr>
<tr>
<td>Blood</td>
<td>06</td>
<td>5.8%</td>
</tr>
</tbody>
</table>
Distribution of various ESBL producing NFGNB

Table-2

<table>
<thead>
<tr>
<th>Pseudomonas</th>
<th>Acinetobacter</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>33</td>
<td>38.3%</td>
</tr>
<tr>
<td>O4</td>
<td>25%</td>
</tr>
</tbody>
</table>

Distribution of various MBL producing NFGNB

Table-3

<table>
<thead>
<tr>
<th>Pseudomonas</th>
<th>Acinetobacter</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>10</td>
<td>11.6%</td>
</tr>
<tr>
<td>O6</td>
<td>37.5%</td>
</tr>
</tbody>
</table>

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Discussion:
NF GNBs were considered as a contaminant in the past but now emerged as an important health care pathogen. Nonfermentative gram-negative bacilli (non-fermenters) cause a significant number of infections, particularly in the hospitalised patients and immunocompromised hosts. Pseudomonas aeruginosa and Acinetobacter baumanii are the most common nonfermenters pathogenic for humans. Infections caused by other species are relatively infrequent. This is similar to studies done by (Malini et al and Vijaya et al 2009). Pseudomonas isolation was predominant in the study done by Parimal et al 2013.

In our study highest number of NF GNBs were from pus samples followed by sputum. This is similar to study done by Malini et al and Parimal et al (2013). In our study NF GNBs were isolated (18.4%) from various clinical samples. In our study ESBL producing Pseudomonas aeruginosa were (38.3%) concordant with the study of Varun Goel et al (42.3%) and Agarwal et al (22.2%) where as Acinetobacter produces low prevalence of ESBLs.
as (25%). This similar study was concordant with Sinha et al\textsuperscript{12} (28%).

In the present study imipenem resistant strains of Pseudomonas aeruginosa were MBL producers, detected by phenotypic detection method of MBL production. In our study MBL producing strains of Pseudomonas were (11.6%) reasonably similar rate of MBL producers done by Nagaveni et al\textsuperscript{13} (24%) and (28%) by Anuradha et al\textsuperscript{14}. A total of 16 Acinetobacter spp. isolates were tested for the antibiotic susceptibility testing. Meropenem sensitivity of 90% has been reported in Shilpa .K Gokale et al\textsuperscript{15} study, & also well correlates with study conducted by Karlwosky et al\textsuperscript{16}(90%) and Taneja et al\textsuperscript{17} 12% of resistant which is concordant with our study(%). In our study, metallo beta lactamase was detected in 13% of the Meropenem resistant Acinetobacter baumanii isolates. Studies from the Indian subcontinent on the occurrence of metallo beta lactamase production by resistant Acinetobacter isolates are minimal. An Indian study on the Acinetobacter baumanii species stated that 70.9% of these isolates produced Metallo beta lactamase (Uma KR et al 2009)\textsuperscript{18}, while another study reported from Kerala, India, states that 21% of the Acinetobacter baumannii isolates were found to be Metallo-\( \beta \)-lactamase producers (Anil VK et al 2011)\textsuperscript{19}.

**Conclusion:**
A prospective study conducted to know the prevalence of different \( \beta \)-lactamases among 103 non-fermenting gram negative bacilli isolated from various clinical specimens. Majority of ESBL producers were sensitive to Imipenem, Piperacillin-tazobactam and Amikacin. All the isolates were susceptible to Polymyxin B. Monitoring and judicious usage of cephalosporins and Imipenem, periodic surveillance of antibiotic resistance patterns and efforts to decrease empirical antibiotic therapy would go a long way in addressing some of the problems associated with ESBL producers. Maintenance of strict antibiotic policy in the hospital is a must to fight against antibiotic resistance.

**Reference:**
**Pseudomonas** in nosocomially infected ICU patients, with special reference to metallo beta lactamase production. Ind J Pathol Microbiol2006; 49:44-48


