CLINICAL DISTRIBUTION AND ANTIBIOTIC RESISTANCE PATTERN OF NON-FERMENTING GRAM NEGATIVE BACILLI.

S.JAYANTHI AND M.JEYA*

Department of Microbiology, Chettinad Hospital and Research Institute, Chettinad University, Chennai, Tamilnadu, India.

ABSTRACT

Nonfermenting Gram negative bacilli (NFGNB) emerged as important nosocomial pathogen causing opportunistic infections. Aim of the study is to detect the clinical distribution and antibiotic resistant pattern of nonfermenting Gram negative bacilli isolated from the clinical samples and to detect Mettalobeta lactamase production by multidrug resistant isolates. A total of 6284 clinical samples were processed for the period of six months. The species of the NFGNB isolates were identified by API ID 32 GN system (bio merieux). Screening for Metallo beta lactamase (MBL) production by combined disc test method was done on the multidrug resistant isolates. Out of 6284 clinical samples 327 (5.2%) nonfermenting Gram negative bacilli were isolated. The split up of NFGNB were Pseudomonas aeruginosa 135(41.2%), Pseudomonas species other than P. aeruginosa were 91(27.8%), Acinetobacter species 88(26.9%) and others 13(3.9%). Multidrug resistant strains among these isolates were 129 (39.4%) and the metallo beta lactamase enzyme producers were 40 (31%).
KEY WORDS
Nonfermenting gram negative bacilli, drug resistance, metallo betalactamase

INTRODUCTION

Nonfermenting Gram negative bacilli (NFGNB) emerged as important nosocomial pathogen causing opportunistic infections. Pseudomonas aeruginosa and Acinetobacter baumanii are most common nonfermenters which are pathogenic to humans. They are generally saprophytic in nature. These isolates have also been responsible for serious infections such as septicaemia and pneumonia. Pseudomonas aeruginosa showing resistance to carbapenem which is currently the most effective treatment option and the number of reported incidences are gradually increasing. Resistance to carbapenems is often mediated by production of metallobetalactamase (MBL) a class of B type of beta-lactamase that require bivalent metal ions, usually zinc for their activity.

The emergence of multi drug resistant strains of Acinetobacter species, that suddenly causes an outbreak of infection involving several patients in clinical units are reported. Within the genus, Acinetobacter baumanii appears to be the species of greatest clinical importance. The Acinetobacter baumanii and Acinetobacter baumanii complex contains isolates that are multiresistant to antibiotics and that have been responsible for many outbreaks of infection throughout the world.

The process influenced by various risk factors like surgery, endotracheal tube, intravascular, ventricular and urinary catheters can result in colonization by this opportunistic bacilli. It gets disseminated via the hands of staff often remains undetected. This study gives the clinical distribution and antibiotic resistance pattern of the clinical isolates of nonfermenters in a tertiary care hospital.

MATERIALS AND METHODS

Source of bacterial strains:
Nonfermenters were isolated and identified from various clinical samples received during the period of six months from June to December 2010 in a tertiary care hospital. Total samples of 6284 were processed includes urine, blood, sputum, swabs from ear, nose, throat, wound, pus, CVP tip, pleural effusion and tracheal aspirates.

From 6284 samples, 328 NFGNB were isolated. They were identified by routine colony morphology and biochemical reactions as described in Bailey and Scotts diagnostic microbiology. The strains for which the species could not be identified by routine; tests were identified by using API ID 32 GN automated system (Biomeriux). All the nonfermentors isolated were subjected for antimicrobial susceptibility testing according to the CLSI guidelines and the results were interpreted. The isolates showing multidrug resistance (resistance to more than three classes of drugs) were again tested for MBL production.

The list of antibiotics tested were ampicillin (10µg) of the synthetic penicillin, amikacin (30µg), gentamicin (10µg) of the aminoglycosides, ciprofloxacin (5µg) ofloxacin (5µg), norfloxacin (10µg), of quinolones, cefotaxime (30µg), ceftazidime (30µg), cefepime (30µg), of cephalosporins, piperacillin tazobactum (100/10µg) of the betalactum inhibitor antibiotics, imipenem (10µg), meropenem (10µg) of carbapenems, aztreonam (30µg) of monobactum, and cotrimoxazole (23.75/1.25µg). Eight categories of antibiotics were tested.

Bacterial strains that demonstrated
resistance to three or more categories of antibiotics were defined as multidrug resistant isolates.

**MBL Screening Procedure (combined disc test)**

Screening for MBL production was done in multidrug resistant strains by combined disc test using imipenem (10µg) and imipenem with EDTA (10µg/750µg) discs. The imipenem–EDTA combined disk test was performed as described by Young et al. Test organisms were inoculated on to Muller Hinton agar plates. Two 10µg imipenem disks were placed with about 30mm distance apart on the plate and 10µL of EDTA solution was added to one of disks to obtain the desired concentration of EDTA (750µg). The inhibition zones of the imipenem and imipenem – EDTA disks were compared after 16-18 hrs of incubation at 35°C. In the combined disc test, if the zone of inhibition with the imipenem and EDTA disc was 7mm greater than plain imipenem disc, it was considered as positive MBL production. (Fig -1)

**RESULTS**

From 6284 clinical samples cultured, 327 (5.2%) nonfermenting Gram negative bacilli were isolated. The split up was Pseudomonas aeruginosa 135(41.2%), Pseudomonas species 91(27.8%), Acinetobacter species 88 (26.9%) and others 13 (3.9%). Among the NFGNB, Pseudomonas aeruginosa were more prevalent than Acinetobacter baumannii (Table1). The clinical samples from the inpatients yielded more number of the nonfermenting gram negative isolates than out patients (Table 2).
Clinical distribution of nonfermentors

The prevalence of NFGNB were higher from the exudate samples (43.4%), followed by the respiratory samples (35.4%), urine (11.6%) and blood samples (9.4%). The overall drug resistance to different classes of drugs were higher among Acinetobacter baumanii than Pseudomonas species. The split up of Pseudomonas species (27.8%) isolated other than P. aeruginosa were 10% P. stutzeri, 6.1% P. orizihabitans, 10.7% P. putida, 0.6% P. fluorescens. Other species isolated were 0.3% Ralstonia picketti, 1.2% Stenotrophomonas maltophilia, and 2.4% Burkholderia cepacia. All the Acinetobacter isolated from the clinical samples were Acinetobacter baumanii.

Table 1

<table>
<thead>
<tr>
<th>Clinical samples</th>
<th>Total no. of samples (6284)</th>
<th>Total no. of nonfermenters (327 5.2%)</th>
<th>Pseudomonas aeruginosa (135 41.2%)</th>
<th>Pseudomonas species (91 27.8%)</th>
<th>Acinetobacter species (88 26.9%)</th>
<th>Others (13 3.9%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>1786</td>
<td>31</td>
<td>12</td>
<td>8</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>Respiratory</td>
<td>484</td>
<td>116</td>
<td>44</td>
<td>31</td>
<td>35</td>
<td>6</td>
</tr>
<tr>
<td>Urine</td>
<td>2915</td>
<td>38</td>
<td>13</td>
<td>11</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>Exudates Pus/</td>
<td>1099</td>
<td>142</td>
<td>66</td>
<td>41</td>
<td>31</td>
<td>4</td>
</tr>
</tbody>
</table>

Clinical isolates

Out Patient isolates

<table>
<thead>
<tr>
<th>Clinical isolates</th>
<th>S</th>
<th>R</th>
<th>S</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>16</td>
<td>9</td>
<td>71</td>
<td>39</td>
</tr>
<tr>
<td>Pseudomonas species</td>
<td>10</td>
<td>4</td>
<td>49</td>
<td>28</td>
</tr>
<tr>
<td>Acinetobacter species</td>
<td>5</td>
<td>2</td>
<td>40</td>
<td>41</td>
</tr>
</tbody>
</table>

S-sensitive strains, R-resistant strains

Drug resistance pattern of P. aeruginosa

The resistant pattern of Pseudomonas isolates to specific anti - Pseudomonal drugs such as carbenicillin was 22%, followed by aztreonam 24.4% and tobramycin 27.4%. Among the aminoglycosides, gentamycin (30.3%) demonstrated higher resistance than amikacin (15.5%), in quinolones, ofloxacin (34 - 35.5%) than ciprofloxacin (24 - 31%), of the third generation cephalosporins the ceftriaxone (25.1%) showed less resistance than ceftazidime (34%) and ceftotaxime (37.7%). The betalactum inhibitors such as cefepemone sulbactum demonstrated 26.6% resistance and piperacillin tazobactum 22.9%. The resistance towards carbapenem drugs such as meropenem (14.8%) was higher than imipenem (6.6%). In-vitro resistance pattern of polymyxin (4.4%) was lesser compared to colistin (6.6%)(Fig 2).
**Drug resistance pattern of Acinetobacter**
The resistant pattern of Acinetobacter for ampicillin was 15%, cotrimoxazole was 7.8% only. Among aminoglycosides, gentamycin demonstrated higher resistance (39.3%) than amikacin (15.5%), in quinolones, ciprofloxacin (3.4%) showed significantly lesser resistance than ofloxacin (39.3%), of the cephalosporins, third generation showed higher resistance than other generations. The resistant pattern of betalactum inhibitors such as cefepirone sulbactum (34.8%) and piperacillin tazobactum (33.7%) were similar. The resistance towards carbapenem drugs were 21.3% for meropenem and 22.4% for imipenem. In vitro resistance pattern of polymyxin was 2.2% and to colistin 1.1%.(Fig 3)

Among the NFGNB 129 multidrug resistant isolates were tested for the metallo beta lactamase (MBL) production by combined disc diffusion method. Out of which 40(31%) (Table – 3) isolates exhibited MBL production.

**Table 3**

*Multi drug resistant Nonfermenting Gram negative bacilli and MBL producers.*

<table>
<thead>
<tr>
<th>Clinical isolates</th>
<th>MDR NFGNB (%)</th>
<th>MBL producers (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa (135)</td>
<td>48 (35.5%)</td>
<td>12 (25%)</td>
</tr>
<tr>
<td>Pseudomonas species (91)</td>
<td>32 (35.1%)</td>
<td>9 (28.1%)</td>
</tr>
<tr>
<td>Acinetobacter species (88)</td>
<td>49 (55.6%)</td>
<td>19 (38.7%)</td>
</tr>
<tr>
<td>Total (314)</td>
<td>129 (41.1%)</td>
<td>40 (31%)</td>
</tr>
</tbody>
</table>
DISCUSSION

Nonfermentors are ubiquitous in the environment. Of the total clinical samples tested 5.2% yielded nonfermenting gram negative bacilli. Among the NFGNB isolated Pseudomonas aeruginosa (41.2%) were higher than the Acinetobacter (26.9%). The prevalence of the multidrug resistant strains were 41.1 % which is in concordance with Behera et al, 2008. The prevalence of the metallo beta lactamase producers among the multidrug resistant strains were 31%. Resistance of nonfermentors to amikacin (15%) was remarkably lower than gentamycin (29-39%) which is similar to the studies of Taneja N et al, 2003.

Resistance to the mono ring beta lactam aztreonam for Pseudomonas (20-25 %) was less when compared to other studies (38%) Srinivasa rao et al, 2008. The resistance of Pseudomonas species to the fluorinated quinolones such as norfloxacin was nil ( for urinary isolates) and 23 to 35% for ciprofloxacin and 35 to 40% to ofloxacin. This is lesser when compared with other studies. The first generation (cephalexin 13%), second generation cefalosporins (Cefuroxime 15-16%) demonstrated less resistance than the third generation cephalosporins (Cefotaxime 37-41%, Ceftazidime 23-34 %, Ceftriaxone 21-25 %).Since third generation cephalosporins are most commonly prescribed by clinicians they show higher resistance than first and second generation cephalosporins. The resistance pattern towards the fourth generation Cefipime and Cefperome were 20 - 40% similar to Shobha et al, 2011. The overall resistance pattern for Acinetobacter isolates (55.6 %) was higher than, Pseudomonas isolates (35.5 %) which is concordance with other studies. Carbapenem has always been regarded as a dependable drug for treating Pseudomonas aeruginosa infection. Due to the increasing clinical use, the problem of resistance to
carbapenem is gradually getting worse.\textsuperscript{16,18} In our study carbapenem resistant strains exhibited significantly higher frequencies of resistance to other group of beta- lactam antibiotics (ceftazidime, cefepime, cefperazone sulbactum, Piperazine tazobactum, gentamycin, ofloxacin) than non-carbapenem resistant strains.\textsuperscript{14,15}

The other nonfermentors Stenotrophomonas maltophilia, Burkholderia cepacia, Ralstonia picketti were sensitive strains in our study. Even though the Burkholderia species was reported \textsuperscript{11} to have high intrinsic resistance to antimicrobial compounds, it did not correlate to our study. Over fifty percent of strains of Acinetobacter species isolated from the clinical samples, showed resistance to the various antibiotics similar to other studies \textsuperscript{15}. Acinetobacter baumanii is provoking more concentration because of potential ability to form biofilm might also explain its outstanding antibiotic resistance, survival properties, and increased virulence.

The multi drug resistant strains were of 48/135(35.5\%) Pseudomonas aeruginosa, 32/91(35.1\%) Pseudomonas species other than P.aeruginosa and 49/88 (55.6\%) Acinetobacter species similar to other studies \textsuperscript{20}. Combined disk method of detection of metallobetalactamase producers were cost effective and comfortable method in concordance with Lee et al, 2003. \textsuperscript{18} Great importance should be given to resistance surveillance and proper drug administration in accordance with the sensitivity test results, and following proper antibiotic policy is essential to minimize drug resistance.\textsuperscript{15}

REFERENCES


