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A total of 1310 consecutive strains of Pseudomonas aeruginosa were collected in 11 French hospitals in 1996. The percentages of susceptible isolates measured by the agar dilution method were: ticarcillin (53%), piperacillin (69%) (MIC ≤ 16 mg/L), ceftazidime (77%), cefepime (55%), cefpirome (40%), aztreonam (57.5%), imipenem (81.5%) (MIC ≤ 4 mg/L), amikacin (64.5%) (MIC ≤ 8 mg/L) and ciprofloxacin (58%) (MIC ≤ 1 mg/L). Resistance to β-lactams was linked to the production of transferable β-lactamases (30%), overproduction of cephalosporinase (29%) and to non-enzymic mechanisms (38%).

Introduction

Pseudomonas aeruginosa is one of the bacterial species most frequently responsible for nosocomial infection. The Groupe d’Etude de la Résistance de Pseudomonas aeruginosa aux Bétalactamines (GERPB) (Workgroup on the resistance of P. aeruginosa to β-lactam antibiotics) was created in the late 1980s and has monitored the evolution of the resistance of this bacterium in 11 French university hospitals since then. This report describes the results obtained for the year 1996.

Materials and methods

Bacterial strains

All the strains of P. aeruginosa isolated in the 11 collecting centres between May and October 1996 were sent to a central laboratory for investigation. The sources of these strains were recorded: hospital, ward and pathological specimen.

Determination of MICs

MICs for ticarcillin, ticarcillin plus clavulanic acid 4 mg/L, piperacillin, piperacillin plus tazobactam 4 mg/L, ceftazidime, cefepime, cefpirome, aztreonam, imipenem, amikacin and ciprofloxacin were determined by the agar dilution method in Mueller–Hinton medium (Sanofi Diagnostic Pasteur, Marnes-la-Coquette, France). Results were interpreted according to the recommendations of the Comité de l’Antibiogramme de la Société Française de Microbiologie.1,2

Detection and identification of β-lactamases

The β-lactamases were identified and cephalosporinase assayed in the isolates that showed decreased susceptibility or resistance to ticarcillin. The bacterial cells were cultured for 18 h in trypticase soy broth (bioMérieux, Marcy l’Étoile, France), sonicated and centrifuged at 1000 rpm for 20 min. The enzymes in the culture supernatant were separated by isoelectric focusing (Amersham Pharmacia Biotech, Orsay, France) on polyacrylamide gel3 and their
isolectric points identified using iodinated starch agar containing penicillin G. Isoelectric focusing was repeated using an oxacillin gel to visualize the OXA type enzymes for all the isolates whose phenotype indicated penicillinase production and for which no characteristic isoelectric point had been found. The \( \beta \)-lactamases with identical isoelectric points were distinguished by investigating their hydrolytic activity using a macro-acidimetric technique.\(^4\)

**Cephalosporinase assays**

Cephalosporinase was assayed by micro-acidimetry of the supernatant of sonicated cells. Hydrolysis of cephaloridine by the cephalosporinase increased the acidity of the medium and the shift in the coloured indicator was measured by spectrophotometry. \( \beta \)-Lactamase activity is expressed as micromoles of cephaloridine hydrolysed per minute per milligram protein. \( \beta \)-Lactamase activity was quantified by measuring the total protein concentration by the Bradford method.\(^5\) Preliminary assays on wild-type strains showed that cephalosporinase production never exceeded 100 milli-units (mU)/mg protein. Strains producing more than 100 mU were classified as cephalosporinase overproducers.\(^6\)

**Results**

**Frequency of isolation of \( P. \) aeruginosa**

\( P. \) aeruginosa was the bacterium most frequently isolated on cystic fibrosis wards (44%), pneumology (16%), burns (13%) and intensive care units (13%). \( P. \) aeruginosa was most frequently isolated from the sputum of patients with (50%) or without cystic fibrosis (23%), and from traumatic wounds (13%).

**Antibiotic susceptibility**

Over 75% of isolates were susceptible to imipenem, cefazidime and the association of piperacillin plus tazobactam (MICs below or equal to the lower breakpoint) (Table I). Fewer than 10% of strains were resistant to cefepime, aztreonam, cefazidime and piperacillin plus tazobactam (MICs higher than upper breakpoint).

**Mechanisms of resistance to \( \beta \)-lactam antibiotics**

Of the 564 strains resistant to ticarcillin, 30% produced a transferable \( \beta \)-lactamase, 29% overproduced a constitutive cephalosporinase and 38% had a non-enzymic mechanism. No extended-spectrum \( \beta \)-lactamase was found.

PSE-1 was the most frequent transferable \( \beta \)-lactamase, occurring in 92% of 171 strains. OXA \( \beta \)-lactamases were found in 8% of these isolates but no TEM-type \( \beta \)-lactamase.
**β-Lactam resistance in P. aeruginosa**

**Table II.** Mechanisms of β-lactam resistance and β-lactam geometric mean MIC (mg/L)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>S¹</th>
<th>PSE-1b</th>
<th>casec</th>
<th>NERd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ticarcillin</td>
<td>12</td>
<td>1700</td>
<td>87</td>
<td>65</td>
</tr>
<tr>
<td>Ticarcillin plus clavulanic acid</td>
<td>11.5</td>
<td>800</td>
<td>96</td>
<td>59</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>4</td>
<td>389</td>
<td>111</td>
<td>11</td>
</tr>
<tr>
<td>Piperacillin plus tazobactam</td>
<td>3</td>
<td>62.5</td>
<td>31.5</td>
<td>9.5</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>2</td>
<td>6</td>
<td>22.5</td>
<td>4</td>
</tr>
<tr>
<td>Cefepime</td>
<td>2</td>
<td>16</td>
<td>14</td>
<td>6.5</td>
</tr>
<tr>
<td>Cefpirome</td>
<td>4</td>
<td>18</td>
<td>39</td>
<td>10</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>3</td>
<td>12</td>
<td>17</td>
<td>12.5</td>
</tr>
</tbody>
</table>

¹S, isolates susceptible to β-lactams.
²PSE-1, PSE-1-producing isolates.
³Case, cephalosporinase hyperproduction ≥100 mU.
⁴NER, non-enzymic resistance.

was identified. Production of PSE-1 transferable β-lactamases produced a very high resistance to penicillins (ticarcillin, piperacillin). The β-lactamase inhibitors did not bring the MICs of these strains into the range of susceptibility. The MICs of cefepime, cefpirome and aztreonam for these isolates were four to eight times greater than those for susceptible strains. Overproduction of cephalosporinase caused a smaller increase in the MICs of penicillins than did transferable β-lactamases, and a greater increase in the MICs of cephalosporin; this was observed for ceftazidime, cefpirome, aztreonam and to a lesser extent for cefepime. Non-enzymic mechanisms of resistance conferred slight resistance to β-lactams. These isolates were less susceptible to ticarcillin, aztreonam, cefepime and cefpirome than to piperacillin and ceftazidime (Table II).

The isolates producing transferable β-lactamase were more frequently resistant to amikacin (81%), ciprofloxacin (92%) and imipenem (40%) than were the cephalosporinase-overproducing isolates (amikacin: 53%, ciprofloxacin: 79%, imipenem: 34%).

The non-enzymic resistance group includes all the strains without a transferable β-lactamase and/or producing low concentrations of cephalosporinase (<100 mU/mg protein without induction). This group is heterogeneous and includes different mechanisms of resistance, such as reduced permeability, hyperactivity of various efflux systems, low cephalosporinase production and altered penicillin-binding protein affinity.

Most of the strains with non-enzymic resistance were classified as having intermediate resistance to β-lactams. It is not possible to predict the outcome of treatment of infections caused by these strains. Systematic bacteriological and clinical comparisons are required to determine the significance and to identify the causes of this resistance.

The strains showing β-lactam multi-resistance were frequently resistant to amikacin and ciprofloxacin. Both imipenem and ceftazidime were efficient against PSE-1-producing strains. In contrast, cefepime was not active in vitro. The only β-lactam active against bacteria overproducing cephalosporinase was imipenem.

*P. aeruginosa* remains the pre-eminent bacterial cause of nosocomial infections in France. The seriousness of these infections is a consequence of strains frequently being very resistant to the principal antibiotics. This warrants the continued implementation of national monitoring networks. The therapeutic consequences of some resistance mechanisms are, however, poorly understood and prospective studies are needed to establish true bacteriological–clinical correlations. Imipenem, ceftazidime and piperacillin plus tazobactam were the more active β-lactams against *P. aeruginosa* in France, in 1996. Amikacin seems to be more useful than ciprofloxacin for combined treatment with β-lactams.

Discussion

**Frequency of P. aeruginosa**

The results of the present study are in good agreement with recently published data. Despite local epidemiological differences and variations in sampling with isolates from burns and patients with cystic fibrosis, *P. aeruginosa* is the second most frequently isolated bacterium from nosocomial infections. It is particularly frequent in intensive care units and in samples taken from the respiratory tract.⁷⁻⁸
References


Received 27 October 1999; accepted 2 February 2000