Gentamicin: Clinical Use with Carbenicillin and In-Vitro Studies with Recent Isolates of *Pseudomonas aeruginosa*

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The susceptibility to gentamicin of 33 isolates of *Pseudomonas aeruginosa* recovered at Memorial Sloan-Kettering Cancer Center before April, 1969, was compared to the susceptibility of 72 organisms recovered in the following 18 months. No significant difference in patterns of sensitivity was observed; 96 of 105 organisms (91%) were inhibited at or below 3.12 μg/ml. Twenty of 23 isolates from blood cultures were still inhibited at or below 3.12 μg/ml, despite the fact that they were recovered from patients receiving gentamicin for periods exceeding four days. Of 46 organisms isolated from blood cultures during 1969–1970, all but two were inhibited by concentrations of 1.56 μg/ml of gentamicin and 50 μg/ml of carbenicillin. Fifteen cases of bacteremia due to *Pseudomonas* were treated with a combination of these two drugs; infection was cured in seven and controlled in two. Success in therapy was associated with remission in leukemia or control of the underlying disease.

It has been well recognized for over a decade that infections due to *Pseudomonas aeruginosa* complicate the treatment of neoplastic diseases [1, 2]. Recent clinical and bacteriologic data further emphasize the persistence and severity of this problem. During the 18-month period from January, 1969 through June, 1970, *P. aeruginosa* was the etiologic agent in 10 of 12 cases (84%) of fatal bacteremia due to gram-negative rods observed among adult leukemic patients at Memorial Sloan-Kettering Cancer Center [3]. The deaths occurred despite the use of the polymyxins, gentamicin, and, more recently, carbenicillin in the treatment of pseudomonas infections.

To evaluate the possible role of emerging resistance to gentamicin that affects these clinical results, we have compared the in-vitro susceptibility of 33 strains of *Pseudomonas* isolated before April, 1969, with 72 others recovered during the following period, April, 1969 through November, 1970. Before April, 1969, gentamicin was used only in patients with infections documented as due to *Pseudomonas* or other gram-negative bacilli, but after this date, gentamicin became available for unrestricted use at our Center.

In this study, particular attention is devoted to the examination of the susceptibility of isolates of *Pseudomonas* from blood cultures of patients receiving gentamicin as contrasted to isolates from patients not treated with gentamicin. Because of previous reports of additive or synergistic antibacterial effects of gentamicin and carbenicillin in vitro [4, 5], results of studies performed on 46 isolates from blood cultures obtained in 1969 and 1970 using combinations of these drugs will be presented. Finally, the clinical results in 15 cases of bacteremia due to *Pseudomonas* that were treated with a combination of gentamicin and carbenicillin will be summarized.

**Materials and Methods**

*Isolates of P. aeruginosa.* Initial bacterial isolates were grown in pure cultures and identified as *P. aeruginosa* by standard taxonomic methods [6]. These isolates were grouped as follows. Group I consisted of 33 isolates (15 from blood, eight from urine, six from wounds, and
Study with \( \text{P. aeruginosa and Carbenicillin} \)

...four from sputum) obtained before April, 1969 from patients who had not previously received gentamicin. Group II consisted of 21 organisms isolated after April, 1969 from blood cultures of patients not being treated with gentamicin. Group III consisted of 23 isolates from blood cultures of patients who had received gentamicin for five or more days and were still being treated with the drug at the time when a positive blood culture was obtained. These isolates were from patients initially treated with gentamicin for localized or soft tissue pseudomonas infections which progressed despite this therapy. Finally, group IV consisted of 28 isolates from skin, saliva, and stools obtained after April, 1969 from patients who did not appear to be clinically infected with \text{Pseudomonas} and were not being treated with gentamicin. Each isolate was from a different patient.

Tests for susceptibility to gentamicin. Each isolate of \text{Pseudomonas} was streaked on Mueller-Hinton agar and incubated overnight at 37 C. The following morning several typical colonies were selected and subcultured to Mueller-Hinton broth and allowed to incubate for 3–5 hr until turbidity was visible. These suspensions contained approximately 10^7 organisms/ml and were then placed in the wells of the inoculum-replicating device of Steers, Foltz, and Graves [7]. Replicate inocula, containing approximately 5,000–10,000 organisms, were then delivered to a series of solidified Mueller-Hinton agar plates containing twofold increments of antibiotic (initially provided as the desiccated powder by Dr. George Arcieri, Schering Corporation). The MIC was determined as that which effected the greatest decrease in growth after 18 hr at 37 C as compared to a control. Because of the variability of results from the agar-dilution test using gentamicin and Mueller-Hinton agar [8, 9], four controls of known, reproducible MIC's (three isolates of \text{Pseudomonas} and one of \text{Staphylococcus aureus}, ATCC 6538P) were used in parallel, for each of the agar- and broth-dilution tests (referred to as the standardized agar-dilution technique).

Activity of combined antibiotics. Tests of the combined effect of gentamicin and carbenicillin used the two-dimensional agar-dilution method as described by Eickhoff [5]. (Carbenicillin was provided as the dessicated powder by Dr. Christopher Demos, Beecham Laboratories.) Mueller-Hinton agar plates were prepared containing 12.5, 6.25, 3.12, 1.56, 0.78, and 0.39 \( \mu \text{g/ml} \) of gentamicin alone and in combination with 100, 50, 25, and 12.5 \( \mu \text{g/ml} \) of carbenicillin. Inhibitory end points were recorded for the range of combinations, and the interpretive criteria for antibacterial synergy of Loewe [10] as described by Lacey [11] were used after constructing isobolograms summarizing the antibacterial activity against each strain.

Therapy with a combination of gentamicin and carbenicillin. Fifteen patients with bacteremia due to \text{Pseudomonas} were treated with combination therapy by the intravenous route. As we previously noted, an intravenous mode of administration for gentamicin has been necessitated by thrombocytopenia in patients receiving antineoplastic chemotherapy [12]. The underlying diseases were as follows. Thirteen patients had acute leukemia, one had a solid tumor, and one patient had methimazole-induced agranulocytosis. Gentamicin was administered in doses of 1 \( \mu \text{g/kg} \) every 6–8 hr as a pulsed intravenous infusion of drug dissolved in 50 ml of physiologic saline or 5% solution of glucose in water. Carbenicillin was given in total daily doses of 0.5 g/kg in six to eight pulsed, intravenous doses. In seven patients, therapy was begun with gentamicin alone, but carbenicillin was added within 72 hr.

Results

Susceptibility to gentamicin. Results are summarized in table 1. The geometric mean MIC of the 33 isolates in group I recovered before April, 1969, was 2.59 \( \mu \text{g/ml} \). Three isolates were inhibited at or above concentrations of 6.25 \( \mu \text{g/ml} \). Of the strains in group II isolated after April, 1969 from bacteremic patients who had not previously received gentamicin, the geometric mean MIC was 2.31 \( \mu \text{g/ml} \), with only one strain requiring 6.25 \( \mu \text{g/ml} \) for inhibition. Twenty-three strains (group III) were isolated from blood cultures of patients who had received gentamicin for five or more days before documented bacteremia. The geometric mean MIC of these isolates was 2.43 \( \mu \text{g/ml} \), with three isolates being inhibited by gentamicin at or above concentrations of 6.25 \( \mu \text{g/ml} \). Finally, the group of 28 organisms (group IV) isolated after April, 1969, which did not appear to be associated with clinical disease, had...
Table 1. Comparison of the susceptibility to gentamicin of strains of *Pseudomonas aeruginosa* isolated before and after April, 1969.

<table>
<thead>
<tr>
<th>Group</th>
<th>MIC (µg/ml)</th>
<th>Geometric mean of MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;1.56</td>
<td>1.56</td>
</tr>
<tr>
<td>I (33 patients)</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>II (21 patients)</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>III (23 patients)</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>IV (28 patients)</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

*Note.* Standardized agar-dilution technique with inoculum replicator of Steers, Foltz, and Graves [7] was used.

<table>
<thead>
<tr>
<th>Concentration of paired antibiotics (µg/ml)</th>
<th>Isolates inhibited</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin</td>
<td>Carbenicillin</td>
</tr>
<tr>
<td>≤0.78</td>
<td>≤ 25</td>
</tr>
<tr>
<td>1.56</td>
<td>50</td>
</tr>
<tr>
<td>3.12</td>
<td>100</td>
</tr>
<tr>
<td>&gt;3.12</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

*Note.* Two dimensional agar-dilution tests with the inoculum replicator of Steers, Foltz, and Graves [7] was used.
tion appeared to be controlled for two weeks, as follow-up blood cultures were sterile, but *Pseudomonas* was persistently cultured from locally infected sites. One of these patients died of superinfection with *Klebsiella*, and the second developed complicating pulmonary aspergillosis.

Six bacteremic patients died with persistent pseudomonas infection, although in two of them, life was sustained for seven and ten days, respectively. All six patients treated unsuccessfully had unremitting leukemia and granulocytopenia. Two patients in this last group also had meningitis, but intrathecal gentamicin was not used.

All isolates from blood cultures of these 15 patients were inhibited by concentrations of 1.56 μg/ml of gentamicin plus 50 μg/ml of carbenicillin.

**Discussion**

Gentamicin has been used in the treatment of life-threatening gram-negative bacillary infections at Memorial Sloan-Kettering Cancer Center since 1964 [12]. Until April, 1969, its use was limited to selected, bacteriologically-confirmed infections which had been unsuccessfully treated with other antibiotics. With the general availability of gentamicin since April, 1969, its systemic use has been unrestricted at our hospital, and it has become more commonly prescribed than kanamycin and the polymyxins combined. Accordingly, it seemed important to examine the susceptibility of strains of *Pseudomonas* recently isolated with those obtained before April, 1969.

The data summarized in Table 1 demonstrate that there has been no trend toward the emergence of resistant strains that require doses of gentamicin greater than 3.12 μg/ml for inhibition. Strains isolated from apparently saprophytic sources (group IV) showed no difference in susceptibility from those isolates obtained from patients who had been treated with gentamicin (group III). It should be noted that all organisms described in this report were simultaneously tested for susceptibility; reference organisms of known, consistent MIC were tested in parallel broth-dilution tests. The rationale for this is that we have observed the same variability in agar-dilution and disk-susceptibility tests using Mueller-Hinton agar as reported by others [8, 9], and this method of standardization appears to be a practical means of obtaining consistent results.

The most clinically relevant data appears to be the results of susceptibility tests with the isolates from group III. Two explanations seem to be plausible for the disturbing documentation of breakthrough pseudomonas bacteremia in patients already receiving gentamicin. Since the inception of clinical studies with gentamicin, in-vitro drug concentrations required for the inhibition of some bacterial species have appeared to be close to the mean levels in the blood achieved by gentamicin doses of up to 1.2 mg/kg [13]. Breakthrough bacteremia conceivably occurred during troughs in blood levels as suggested by data showing that serum concentrations 4–6 hr after intramuscular injection fall to below 3 μg/ml [14]. The second explanation is not easily quantitated but may be more significant—the role of the host. It has been observed previously that successful treatment of infections due to *Pseudomonas* in leukemic patients is associated with control of the underlying disease [2]. The critical factor for success of therapy in our experience has been induction of bone marrow remission, with repopulation of the peripheral blood with normal polymorphonuclear leukocytes. We have yet to document a clinical and bacteriologic cure of pseudomonas bacteremia in a patient with unremitting leukemia.

Unfortunately, some reports of the success of antimicrobial therapy in infections due to *Pseudomonas* in patients with cancer have not detailed the nature and success of concomitant antineoplastic therapy [15]. All of our isolates in group III were obtained from patients with one of the following: (1) marked, drug-induced granulocytopenia, (2) refractory hematologic neoplasms, (3) undrained abscesses, or (4) severe disease of the central nervous system which impaired mobilization of infected respiratory secretions. In this group it seems fair to implicate “patient failure” rather than “drug failure.”

Reports of the in-vitro synergistic effects of gentamicin combined with carbenicillin against *Pseudomonas* have appeared from several laboratories [4, 5, 16]. Although original studies on a limited number of organisms suggested striking synergistic activity [4, 16], synergistic activity could be demonstrated against only 35% of the strains in this series. Furthermore, the nature of the synergism is not striking, and in most cases
the effects of the two drugs are additive. What may be significant is that when all recent isolates from blood cultures are considered, 44 of 46 (96%) of the organisms were inhibited by a combination of 1.56 µg/ml of gentamicin plus 50 µg/ml of carbenicillin, which are blood levels that seem readily achievable and maintainable.

Less clear has been the clinical effect of combining gentamicin with carbenicillin compared with the use of either drug alone. Although nine of 15 (60%) bacteremic infections were cured or controlled, we had no concurrent group treated with a single drug. Historical comparisons with the results of treatment of infections due to Pseudomonas before combination therapy became available are open to criticism because of two developments. First, the improved success rate with initial induction of remission in leukemia (now exceeding 90% in acute lymphoblastic leukemia and exceeding 50% in acute myelogenous leukemia at this hospital), and secondly, the effect of platelet or blood-component transfusions in controlling another major complication of leukemia, hemorrhage. Since the therapy of the underlying neoplastic disease has improved, the results of treatment of infectious complications may now appear better.

References