IN VITRO ACTIVITY OF IMIPENEM AND CEFTAZIDIME AGAINST MUCOID AND NON-MUCOID STRAINS OF PSEUDOMONAS AERUGINOSA ISOLATED FROM PATIENTS IN IRAN

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Background – Mucoid strains of Pseudomonas aeruginosa are surrounded by a capsule composed of β-1, 4 linked D-mannuronic acid and L-guluronic acid. The capsule provides a protective barrier against antimicrobial agents and the immune system, and is an important pathogen in immunocompromised hosts. In this study, we compared the activities of imipenem and ceftazidime against mucoid and non-mucoid clinical strains of P. aeruginosa.

Methods – From April to September 2000, one-hundred and thirty-three P. aeruginosa cultures were isolated from hospitalized patients in Tehran. In addition to determination of the minimum inhibitory concentrations (MICs) of two antipseudomonal antibiotics, ceftazidime and imipenem, against these isolates, mucoid strains were selected by capsule staining. Mucoid phenotype was confirmed by precipitation of alginate in cold ethanol. Data were analyzed by the Chi-square method.

Results – More than 70.6% of the strains were sensitive to imipenem, which inhibited most strains at an MIC of 4 µg/mL. However, more than 72.9% of strains were resistant to ceftazidime, and only concentrations of 128 µg/mL or more inhibited their growth. There was a statistically significant relationship between the presence of the alginate capsule and resistance against ceftazidime. Such a relation did not exist for imipenem.

Conclusion – Considering the extensive use of ceftazidime in Iran, performing susceptibility tests, as well as determining the mucoid phenotype in strains of P. aeruginosa is strongly recommended prior to antimicrobial therapy in immunocompromised patients.

Keywords ● alginate ● ceftazidime ● imipenem ● Pseudomonas aeruginosa

Introduction

Pseudomonas aeruginosa is one of the most important opportunistic bacteria, causing a wide variety of infections especially in immunocompromised hosts such as burn patients, patients suffering from respiratory diseases like cystic fibrosis, and cancer chemotherapy patients.1-3 This organism is intrinsically resistant to many antibiotics, or can develop resistance during treatment.4 Although P. aeruginosa has an outer membrane with low permeability, this characteristic alone does not adequately explain its intrinsic antimicrobial resistance.5

An additional mechanism that may interfere with access of antibiotics to their targets is production of an alginate capsule by mucoid strains of P. aeruginosa.1-3 Alginate is a copolymer of O-acetylated β-1, 4 linked D-mannuronic acid and L-guluronic acid.6 In some previous studies, it was shown that mucoid strains are more resistant to certain antibiotics than non-
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Table 1. Susceptibility distributions of two antibiotics against 133 clinical isolates of P. aeruginosa.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (µg/mL)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0.125 0.25 0.5 1 2 4 8 16 32 64 128 &gt; 128</td>
</tr>
<tr>
<td>Imipenem (No.)</td>
<td>0 0 0 0 0 94* 20 9 5 5 0 0</td>
</tr>
<tr>
<td>Ceftazidime (No.)</td>
<td>0 0 0 0 8* 10* 18* 5 6 13 8 65</td>
</tr>
</tbody>
</table>

* Number of susceptible strains at minimum inhibitory concentration (MIC) breakpoints.

mucoid strains. In this study, we compared the activities of imipenem and ceftazidime against mucoid and non-mucoid clinical strains of P. aeruginosa. We selected ceftazidime which is extensively used for the treatment of pseudomonal infections in Iran and imipenem which is one of the newest antipseudomonal antibiotics with no data about its efficacy in Iran.

Materials and Methods

Bacterial strains
A total of 133 P. aeruginosa isolates, cultured from patients treated between April and September 2000, were collected from specimens submitted to the clinical microbiology laboratories of selected hospitals in Tehran. The obtained specimens were from blood, wound swab, urine, sputum, stool and cerebrospinal fluid. The type of infection, source of each specimen, and patient characteristics were not important to the study design and are not presented here. All clinical isolates were identified and stored in skimmed milk at –70°C until use. Strains were subcultured twice on Mueller-Hinton agar (Difco Laboratories, Detroit, MI, USA) prior to testing. P. aeruginosa ATCC 27853 was used as a quality control strain for the susceptibility test.

Antimicrobial agents and MIC determination
Ceftazidime and imipenem-cilastatin were provided from Sigma-Tau, Italy. Agar dilution susceptibility tests used, were those outlined by the National Committee for Clinical Laboratory Standards (NCCLS).

MICs were determined on plates of Mueller-Hinton agar containing serial two-fold dilutions of each antibiotic. Bacterial suspensions of 10^6 colony-forming units (CFU)/mL were inoculated onto the surface of the plates, and results were recorded after overnight incubation at 35°C in an aerobic atmosphere. The MIC was defined as the lowest antibiotic concentration with no visible growth. For imipenem, NCCLS breakpoints of ≤ 4 µg/mL (susceptible), 8 µg/mL (intermediate) and ≥ 16 µg/mL (resistant) were applied. For ceftazidime, NCCLS breakpoints of ≤ 8 µg/mL (susceptible), 16 µg/mL (intermediate), and ≥ 32 µg/mL (resistance) were applied.

Differentiation of mucoid and non-mucoid strains
Using the Muir method, a thin film of bacterial suspension was prepared and dried in air. The film was covered with a piece of filter paper, cut to the size of the smear, and the slide was flooded with Ziehl-Neelsen carbolfuchsin. The slide was heated to steaming for 30 seconds using a low Bunsen flame. The slide was gently rinsed with 95% ethanol and then with distilled water. Mordant solution was added for 20 seconds followed by a wash with distilled water. Decolorization was performed by ethanol. For counter stain, 0.3% methylene blue was used for 30 seconds before examination under oil immersion lens. The cells stained red, and the capsule blue. Precipitation of alginate was used for confirmation of capsule staining. For each isolate, a 48-hour culture was prepared in 100 mL nutrient broth (shaking at 34 – 35°C) and alginate was precipitated by the addition of 50 mL cold ethanol. This mixture was kept at –20°C for 30 min until formation of the alginate precipitate was complete.

Statistical analysis
Antibiotic susceptibility results of mucoid and non-mucoid strains of P. aeruginosa were compared with Chi-square analysis using MS-TAT-C software (version 1.42, R.D. Freed, Michigan State University, USA).

Results
Of 133 clinical isolates, 43 isolates had mucoid phenotype by Muir staining and precipitation of alginate. More than 70.6% of all strains were sensitive to imipenem with an MIC equal to 4 µg/mL. However, more than 72.9% of all strains
were resistant to ceftazidime, and only concentrations equal to 128 µg/mL or more, inhibited their growth.

Distribution of antimicrobial susceptibility of clinical isolates according to NCCLS susceptibility breakpoints are shown in Table 1.

Comparative in vitro activities of the two antibiotics (according to MIC determination) against mucoid and non-mucoid strains of P. aeruginosa are shown in Table 2.

While statistical analysis showed that mucoid strains were significantly more resistant than non-mucoid strains to ceftazidime (p < 0.01), this was not the case for imipenem.

Discussion

We designed the present study to examine the difference in susceptibility patterns of mucoid and non-mucoid clinical isolates of P. aeruginosa to imipenem and ceftazidime in vitro. Several investigators have shown that imipenem possesses an activity superior to that of β-lactams against P. aeruginosa, including strains resistant to third-generation cephalosporins. The narrow range of the MICs of imipenem against our P. aeruginosa isolates is comparable to that reported by other investigators.\(^{10,16,17}\) This property of imipenem is believed to be due to its stability against the β-lactamases and its rapid and lethal penetration of target sites and molecular structure.\(^{16,17}\)

In earlier studies, we have shown that inhibitory concentrations of gentamicin reduced alginate production in mucoid strains of P. aeruginosa.\(^{18–20}\) In the present study, we observed that the alginate capsules of mucoid strains of P. aeruginosa could not act as a barrier against imipenem. This finding is comparable to the results of Slack and Nichol’s studies, in which alginate impeded the penetration of all antibiotics except the β – lactams.\(^{20,21}\) However, the alginate glycocalyx provides a barrier against penetration of ceftazidime, and this antibiotic was clearly inferior to imipenem against our P. aeruginosa strains. In addition, this reduced susceptibility may be related to the more extensive use of ceftazidime in Iran. On the other hand, additional resistance mechanisms—especially production of extended-spectrum β-lactamases (ESBLs) and other enzymes—may contribute to ceftazidime resistance.\(^4\)

References


Table 2. Comparative in vitro activity of imipenem and ceftazidime against mucoid and non-mucoid strains of P. aeruginosa according to minimum inhibitory concentration.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>M</th>
<th>NM</th>
<th>M</th>
<th>NM</th>
<th>M</th>
<th>NM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible</td>
<td></td>
<td>Intermediate</td>
<td></td>
<td>Resistant</td>
<td></td>
</tr>
<tr>
<td>Imipenem</td>
<td>28 (65.12)</td>
<td>66 (73.33)</td>
<td>71 (16.27)</td>
<td>13 (14.45)</td>
<td>8 (18.61)</td>
<td>11 (12.22)</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>6 (13.95)</td>
<td>30 (33.33)</td>
<td>1 (2.32)</td>
<td>4 (4.44)</td>
<td>36 (83.73)</td>
<td>56 (62.23)</td>
</tr>
</tbody>
</table>

M = mucoid; NM = non-mucoid.

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