The VITEK 2 (bioMérieux Inc., Hazelwood, MO) automated system employs an advanced expert system (AES) analyzing antimicrobial susceptibility test results according to the bacterial species (2). According to the AES, the piperacillin susceptibilities of all Klebsiella spp. are converted to resistance even though the automated MICs determined for their isolates are low. In contrast, other automated systems do not convert the piperacillin susceptibilities of Klebsiella spp. to resistance. In addition, there is no explanation in the Clinical and Laboratory Standards Institute (CLSI) guideline in this regard, and the U.S. FDA product (http://www.fda.gov) package insert lists Klebsiella as an organism for which piperacillin use is indicated [Pipracil (piperacillin) for injection, package insert; Wyeth Pharmaceuticals Inc., Philadelphia, PA]. In this study, we intended to evaluate the appropriateness of this reporting system in comparison with results for other Enterobacteriaceae, for which the susceptibility results are not converted to resistance to piperacillin.

Between January and June 2007, 135 consecutive, nonduplicate Klebsiella spp. (120 Klebsiella pneumoniae isolates and 15 Klebsiella oxytoca isolates) were collected. The piperacillin MICs were determined by the agar dilution method according to the CLSI guidelines (7) and compared with VITEK 2 automated MIC determinations and interpretative susceptibilities determined by AES. For 107 isolates susceptible to piperacillin as determined by VITEK 2, PCRs for the blaTEM (6) blaSHV (3), and blaOXA (1) genes were performed using whole DNAs extracted by boiling at 95°C for 10 min and a PTC-100 thermal cycler (MJ Research Inc., Watertown, MA). For 50 members of the family Enterobacteriaceae (10 Enterobacter cloacae isolates, 6 Enterobacter aerogenes isolates, 19 Escherichia coli isolates, 7 Serratia marcescens isolates, and 8 Proteus mirabilis isolates) susceptible to piperacillin by VITEK 2, agar dilution MICs and PCRs for the above genes were also performed. For 20 selected Klebsiella isolates susceptible to piperacillin according to both VITEK 2 automated MICs and agar dilution MICs and for 50 members of the family Enterobacteriaceae, the inoculum effect (more than three twofold increases in MIC when tested with 10⁶ CFU/spot instead of 10⁴ CFU/spot) was investigated.

The results of VITEK 2 automated MIC determinations, agar dilution MIC determinations, and AES interpretative reading regarding piperacillin for Klebsiella spp. are listed in Table 1. The concordance rate between automated MICs and agar dilution MICs for 135 Klebsiella isolates was 96.3%, and all 20 selected Klebsiella isolates revealed an inoculum effect. Of the 95 K. pneumoniae isolates found to be susceptible to piperacillin by VITEK 2, 92 (96.8%) harbored blaSHV genes and the remaining 3 isolates did not harbor any of the blaTEM, blaSHV, or blaOXA genes. In contrast, none of the 12 K. oxytoca isolates harbor any of these genes. Agar dilution MICs were within the susceptible range for 49 members of the family Enterobacteriaceae, and 1 E. coli isolate had a MIC of 32 μg/ml.

The inoculum effect was found for all of them, but only blaTEM was detected in four isolates.

Livermore et al. reported that the use of any penicillin except temocillin against Klebsiella spp. should be discouraged, because Klebsiella spp. have classical low-level SHV-1 or K1 β-lactamase (4), and the inoculum effect was observed with piperacillin and other ureidopenicillins tested against them (5). However, in this study, the inoculum effect for piperacillin was not related to the species or the presence of blaSHV, blaTEM, or blaOXA. Although more studies including use of an animal infection model are needed, conversion of piperacillin susceptibility to resistance for Klebsiella spp. must be reconsidered or used with caution.

### Table 1. Determination of piperacillin resistance in Klebsiella spp. by using agar dilution MICs, automated MICs, and AES interpretative results by VITEK 2

<table>
<thead>
<tr>
<th>MIC result</th>
<th>PIP auto MIC</th>
<th>PIP AES result</th>
<th>Agar dilution MIC result</th>
<th>No (%) of isolates with results</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>R</td>
<td>S</td>
<td>107 (79.3)</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>R</td>
<td>S/I/R</td>
<td>2/2/3 (1.5/1.5/2.2)</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>R</td>
<td>R</td>
<td>21 (15.6)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>135 (100)</td>
<td></td>
</tr>
</tbody>
</table>

* S, susceptible (MIC of ≤16 μg/ml); I, intermediately resistant (MIC of ≥32 μg/ml and ≤64 μg/ml); R, resistant (MIC of ≥128 μg/ml). PIP, piperacillin; auto, automated.

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