Mutant prevention concentration of tigecycline for Acinetobacter baumannii and Klebsiella pneumoniae clinical isolates

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Sir,

Tigecycline is a therapeutic option for multidrug-resistant Acinetobacter baumannii and Klebsiella pneumoniae infections.1 Recently, tigecycline-non-susceptible A. baumannii and K. pneumoniae have been reported in patients receiving tigecycline.2 Despite several reports concerning in vitro and in vivo susceptibility of bacterial pathogens to tigecycline, there is a paucity of data regarding its effect on the development of resistant single-step mutants within the mutant selection window (MSW) during clinical tigecycline dosing.3

In this study, we investigated the mutant prevention concentrations (MPCs) of tigecycline along with the effect of efflux pumps such as AdeABC and AcrAB-ToIC on the emergence of tigecycline-resistant mutants in A. baumannii and K. pneumoniae, respectively. Thirty A. baumannii and 30 K. pneumoniae isolates (15 colistin-resistant and 15 colistin-susceptible, respectively), obtained from six Korean hospitals, were investigated. The MICs of tigecycline were determined by a broth microdilution method according to CLSI 2013 guidelines.4 The clinical isolates of A. baumannii and K. pneumoniae were considered susceptible to tigecycline at breakpoints of 1 mg/L according to EUCAST.5 The MPC of tigecycline was determined as described previously and is defined as the lowest antimicrobial concentration at which the growth of colonies on an agar plate is completely inhibited after 24 h of incubation.6 Single-step mutants were selected by plating the wild-type strain onto LB agar plates containing tigecycline within the concentration range of the MSW (0.5–4 mg/L). Amino acid substitutions in the AdeABC efflux pump system or the AcrAB-ToIC efflux pump system and their respective local repressor genes, such as adeRS or acrR, were examined in single-step mutants of five A. baumannii and K. pneumoniae isolates.5–8 The expression levels of adeB or acrA were also investigated using quantitative real-time PCR in seven pairs of isogenic A. baumannii and K. pneumoniae strains, respectively.

The MICs for A. baumannii and K. pneumoniae isolates ranged from 0.5 mg/L and from 0.5 to 1 mg/L, respectively. For A. baumannii isolates, tigecycline MPCs ranged from 1 to 4 mg/L (Table 1) and the MPC/MIC ratio was in the range of 4–16. Higher MPC values (>8 mg/L) were observed only in four colistin-resistant A. baumannii isolates, which suggests that careful consideration should be given to use tigecycline as an alternative for the treatment of colistin-resistant A. baumannii infections. With one exception, in all other K. pneumoniae isolates the tigecycline MPCs were between 4 and 16 mg/L and the MPC/MIC ratio was in the range 4–16. The MPCs and MPC/MIC for K. pneumoniae isolates were unaffected by their colistin susceptibility. Tigecycline MICs for all single-step mutants exhibited a 2- to 16-fold increase compared with the parental isolates of both A. baumannii and K. pneumoniae. A similar phenomenon was described previously for fluoroquinolones against Escherichia coli.9 adeABC and adeRS were not detected in some of the seven isolates of A. baumannii under investigation. adeABC and adeRS was not identified in 07AC-029, adeC was identified in only three isolates: E07-612, E10-93 and H09-504. Four isolates harboured adeRS (Table 1). Although the AcrAB-ToIC efflux pump and acrR were identified in all seven K. pneumoniae isolates, no amino acid alterations were found. Quantitative real-time PCR analysis revealed that the expression of adeB or acrA in single-step mutants of A. baumannii and K. pneumoniae was different between isolates that had high and low MPCs (Table 1). Compared with their parental isolates, in A. baumannii the expression of adeB in single-step mutants with MPC of ≥4 mg/L or MPC/MIC of ≥8 was 2.4- to 71.6-fold higher. However, a single-step mutant, E07-612 (MPC/MIC, ≥8), showed no increase in adeB expression. 07AC-029, which possessed no AdeABC efflux pump, showed a low MPC and a low MPC/MIC. 06AC-108, with no adeRS, showed a high expression level of adeB, and adeC may affect the increased adeB expression in E10-93 and H09-504, compared with 06AC-23 and 06AC-66. However, further studies may be required. Five out of the seven single-step mutants of K. pneumoniae, which had MPC ≥8 mg/L or MPC/MIC ratios ≥8, showed a 3.3- to 17.2-fold increase in acrA expression compared with their parental isolates. In the other two single-step mutants, K01-08-10058 and LJA (MPC/MIC, 2 and 4, respectively), acrA expression increased by only 1.7-fold.

These results are consistent with the notion that up-regulation of efflux pumps is associated with tigecycline resistance in clinical isolates.6,7 A plasma Cmax of 0.87 mg/L tigecycline in the recommended dose regimen (loading dose of 100 mg followed by multiple doses of 50 mg every 12 h) is within the MSW in most
A. baumannii and K. pneumoniae isolates, which indicates that the current clinical dosage regimen may be involved in the development of tigecycline-resistant mutants. Given the possibility of development of tigecycline resistance, continuous monitoring of the emergence of resistant isolates and responsiveness of patients to tigecycline treatment would be required.

Acknowledgements
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Transparency declarations
None to declare.

References

Table 1. Change in adeB or acrA mRNA transcript levels and MICs of tigecycline for adeRS or acrR mutants, parental strains and first-step mutants

<table>
<thead>
<tr>
<th>Species</th>
<th>Isolate</th>
<th>MIC (mg/L)</th>
<th>Presence of AdeAB/AdeRS efflux systems</th>
<th>Fold change in adeB or acrA&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Mutation of adeRS or acrR in single-step mutant&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>parent</td>
<td>mutant</td>
<td></td>
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<tr>
<td>A. baumannii</td>
<td>E07-612</td>
<td>0.5</td>
<td>1</td>
<td>2</td>
<td>B, C</td>
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<tr>
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<td>07AC-029</td>
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<td>1</td>
<td>2</td>
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<td></td>
<td>06AC-23</td>
<td>1</td>
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<td>16</td>
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<tr>
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<td>64</td>
<td>32</td>
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<td>4</td>
<td>4</td>
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<tr>
<td></td>
<td>E10-93</td>
<td>1</td>
<td>16</td>
<td>8</td>
<td>A, B, C, R, S</td>
</tr>
<tr>
<td></td>
<td>H09-504</td>
<td>1</td>
<td>16</td>
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<td>K. pneumoniae</td>
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<td>0.5</td>
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<td>2</td>
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<td>LJA</td>
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<td>16</td>
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<tr>
<td></td>
<td>K08-Bact-08-039</td>
<td>0.5</td>
<td>8</td>
<td>8</td>
<td>16</td>
</tr>
</tbody>
</table>

ND, not detected.
<sup>a</sup>AdeAB/AdeRS efflux systems: A, adeA; B, adeB; C, adeC; R, adeR; S, adeS.
<sup>b</sup>Fold change in adeB or acrA mRNA transcripts in A. baumannii or acrA mRNA transcripts in K. pneumoniae isolates.
<sup>c</sup>Mutation in adeRS in first-step mutants of A. baumannii or acrR in first-step mutants of K. pneumoniae compared with their parental strains.
<sup>d</sup>ISaba1 disrupted adeS at the site of 379 nucleotides.