Discrepant susceptibility to gentamicin despite amikacin resistance in *Klebsiella pneumoniae* by VITEK 2 represents false susceptibility associated with the armA 16S rRNA methylase gene

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**Abstract**

Because we experienced gentamicin failure in *Klebsiella pneumoniae* bacteraemia that was susceptible to gentamicin despite amikacin resistance, as determined by VITEK 2, we evaluated the true susceptibility and mechanism of resistance. We screened 2818 *K. pneumoniae* isolates during a 1-year period at a university hospital and reviewed anti-microbial susceptibility reports using the VITEK 2 system. The minimum inhibitory concentration was substantiated by broth microdilution (BMD), and the presence of 16S rRNA methylase genes and aminoglycoside-modifying enzymes was also investigated. A total of 131 amikacin-resistant isolates from 19 patients were gentamicin non-resistant according to the VITEK 2 system. Among these, we were able to collect isolates from 12 patients (63.2 %), and a single isolate from each patient was tested. Eleven of the gentamicin non-resistant isolates (91.7 %) showed high-level resistance to both amikacin and gentamicin by BMD in association with the armA gene. Gentamicin is not an adequate treatment option for amikacin-resistant *K. pneumoniae*, even if VITEK 2 reports susceptibility.

Because of the increasing β-lactam resistance of Enterobacteriaceae, aminoglycoside antibiotics are becoming important treatment options for multi-drug resistant Enterobacteriaceae [1]. Although the VITEK 2 automated system (bioMérieux Inc., Marcy l’Etoile, France) plays a major role in clinical practice for the measurement of antibiotic susceptibility, there have been reports that high-level resistance to aminoglycosides can be falsely detected as susceptibility [2–4]. Since we also experienced gentamicin treatment failure in bacteraemia caused by *Klebsiella pneumoniae* isolates that had been reported from VITEK 2 to be susceptible to gentamicin despite resistance to amikacin, we evaluated the true aminoglycoside susceptibility and mechanism of resistance of *K. pneumoniae* with this discrepant aminoglycoside susceptibility pattern, as determined by VITEK 2.

We screened *K. pneumoniae* clinical isolates from blood, urine and stool from January 2015 through February 2016 at a 1950-bed university hospital. The minimum inhibitory concentration (MIC) values reported by VITEK 2 were reviewed, and isolates that showed gentamicin non-resistance despite amikacin resistance according to the criteria of Clinical and Laboratory Standards Institute (CLSI) were collected [5]. If multiple isolates with same susceptibility pattern were obtained from a single patient, only one isolate was selected. Isolates were maintained in brain heart infusion broth (BD Diagnostics, Sparks, MD, USA) with 50 % glycerol and stored at −70 °C until use. Isolates were subcultured a minimum of three times prior to experimentation. We substantiated the MICs of selected isolates by broth microdilution (BMD). *E. coli* (ATCC 25922) and *Enterococcus faecalis* (ATCC 29212) were used as control strains. The presence of 16S rRNA methylase genes, including rmtA, rmtB, rmtC, rmtD, rmtE, rmtF and armA, and common aminoglycoside-modifying enzyme (AME) genes, including AAC(6’)-Ib, AAC(3)-IV, ANT(2’”)-Ia and APH
During the study period, a total of 2818 isolates of *K. pneumoniae* from 1056 patients were identified, 359 (12.7 %) of which were reported as resistant to amikacin by VITEK 2. Among these 359 amikacin-resistant isolates, 131 isolates (36.5 %) from 19 patients were susceptible (101 isolates) or intermediate (30 isolates) to gentamicin. Among these amikacin-resistant gentamicin non-resistant isolates, we were able to collect isolates from 12 patients (63.2 %), and a single isolate from each patient was tested (Table 1). Eleven out of 12 gentamicin non-resistant isolates (91.7 %) showed high-level resistance to both gentamicin and amikacin by BMD (MIC >256 and >128 µg ml⁻¹, respectively) and harboured both *armA* and *AAC(6')-Ib* genes, while other 16S rRNA methylase and AME genes were not detected. Nine of these isolates (81.8 %) harboured New Delhi metallo-β-lactamase 1 (NDM-1) and seven (63.6 %) co-produced OXA-232, a variant of OXA-48 β-lactamase [9]. On the other hand, only one gentamicin non-resistant isolate (8.3 %) was actually susceptible to gentamicin and intermediate to amikacin by BMD (MIC=2 and 32 µg ml⁻¹, respectively), and it did not harbour the *armA* gene, although the *AAC(6')-Ib* gene was detected.

For further evaluation of the association of gentamicin false susceptibility with the discrepant aminoglycoside susceptibility pattern presented by VITEK 2, we also screened *E. coli*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* blood isolates during the study period. None of 1005 *E. coli* blood isolates showed such a discrepant aminoglycoside susceptibility pattern. Among two of 133 *P. aeruginosa* blood isolates showed discrepant gentamicin non-resistance despite amikacin-resistance, these isolates were intermediate to gentamicin by BMD and negative for 16S rRNA methylase genes. We observed that four of 119 *A. baumannii* blood isolates showed gentamicin false-susceptibility by VITEK 2 and harboured the *armA* 16S rRNA methylase gene, while amikacin susceptibility was not reported by the VITEK 2 test card.

Decreased accuracy of the VITEK 2 automated system in aminoglycoside susceptibility reporting has been observed [2–4], and the antimicrobial susceptibility of aminoglycosides is becoming more problematic, as the increased β-lactam resistance of Enterobacteriaceae may require the use of aminoglycosides as alternatives to β-lactam antibiotics [1, 10]. Although additional susceptibility testing, such as disk diffusion or BMD, is recommended for accurate aminoglycoside susceptibility results [2, 4], it is not feasible to perform additional tests routinely for all clinical isolates. However, as such errors in *K. pneumoniae* have been observed exclusively as false gentamicin susceptibility, in association with the *armA* 16S rRNA methylase gene [4], our findings will be helpful for selecting *K. pneumoniae* isolates for additional susceptibility tests according to the VITEK 2 reports. This will be especially important in carbapenem-resistant Enterobacteriaceae, since *armA* was frequently reported in association with carbapenemases such as NDM-1 or KPC [11–15].

To investigate the possible association of aminoglycoside false-susceptibility reports by VITEK 2 with other 16S rRNA methylase genes, we searched PubMed with the keywords ‘16 s RNA methylase’, or ‘rmtA’ to ‘rmtF’. However, most reports for other 16S rRNA methylase genes have not been investigated by polymerase chain reaction (PCR) [6, 7]. Isolates with carbapenem resistance were also tested for carbapenemase genes by the PCR method [8].

### Table 1. Antimicrobial susceptibility and resistance genes of the 12 *K. pneumoniae* isolates with discrepant gentamicin susceptibility by VITEK 2

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Specimen</th>
<th>VITEK 2</th>
<th>Broth microdilution</th>
<th>Antibiotic resistance genes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AMK</td>
<td>GEN</td>
<td>AMK</td>
</tr>
<tr>
<td>1</td>
<td>Blood</td>
<td>≥64</td>
<td>4</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>Blood</td>
<td>≥64</td>
<td>4</td>
<td>&gt;128</td>
</tr>
<tr>
<td>3</td>
<td>Blood</td>
<td>≥64</td>
<td>4</td>
<td>&gt;128</td>
</tr>
<tr>
<td>4</td>
<td>Blood</td>
<td>≥64</td>
<td>8</td>
<td>&gt;128</td>
</tr>
<tr>
<td>5</td>
<td>Blood</td>
<td>≥64</td>
<td>8</td>
<td>&gt;128</td>
</tr>
<tr>
<td>6</td>
<td>Urine</td>
<td>≥64</td>
<td>4</td>
<td>&gt;128</td>
</tr>
<tr>
<td>7</td>
<td>Urine</td>
<td>≥64</td>
<td>4</td>
<td>&gt;128</td>
</tr>
<tr>
<td>8</td>
<td>Urine</td>
<td>≥64</td>
<td>4</td>
<td>&gt;128</td>
</tr>
<tr>
<td>9</td>
<td>Urine</td>
<td>≥64</td>
<td>2</td>
<td>&gt;128</td>
</tr>
<tr>
<td>10</td>
<td>Urine</td>
<td>≥64</td>
<td>4</td>
<td>&gt;128</td>
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<tr>
<td>11</td>
<td>Urine</td>
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<td>&gt;128</td>
</tr>
<tr>
<td>12</td>
<td>Stool</td>
<td>≥64</td>
<td>8</td>
<td>&gt;128</td>
</tr>
</tbody>
</table>

*Presented by the VITEK 2 system.*
not presented MIC values from the VITEK 2 automated system. Since a case of *K. pneumoniae* with the rmtB 16S rRNA methylase gene has been reported from our centre [9], we reviewed the antimicrobial susceptibility report by VITEK 2 and laboratory test results. However, the isolate was resistant to gentamicin and amikacin according to both BMD and the VITEK 2 system. Although the purpose of this study is not to identify the mechanism through which aminoglycoside false-susceptibility is reported by VITEK 2, we observed that this false susceptibility was not observed by MicroScan (Siemens Healthcare Diagnostics, Deerfield, IL, USA) when the isolate from patient 3 was tested. The mechanism of aminoglycoside false-susceptibility might be related to the MIC-determining algorithms of VITEK 2, which are based on kinetic analyses of growth data [16].

We think that these findings cannot be applied generally to other species yet. We could not find an association of this discrepant aminoglycoside pattern with aminoglycoside false susceptibility in *P. aeruginosa*. *E. coli* isolates from our centre did not show such a discrepant pattern. Although we found that gentamicin false susceptibility was associated with armA in *A. baumannii*, false susceptibility for amikacin had also been observed previously in *A. baumannii* when using VITEK 2 [2]. In addition, since we could not test all gentamicin- or amikacin-susceptible isolates, we cannot exclude the possibility that other false-susceptibility patterns might exist among *K. pneumoniae* isolates, and this requires further evaluation.

In conclusion, the discrepant gentamicin susceptibility presented by the VITEK 2 system for *K. pneumoniae*, despite amikacin resistance, predicted gentamicin false-susceptibility associated with the armA 16S rRNA methylase gene. Based on this finding, gentamicin is not an adequate treatment option for amikacin-resistant *K. pneumoniae* isolates, even if VITEK 2 reports susceptibility to gentamicin.

**Funding information**

This study was supported by a grant from the Korean Health Technology R&D Project, Ministry of Health and Welfare, Republic of Korea (grant no. HI12C0756). The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**References**